The behaviour and energetics of macaroni penguins (*Eudyptes chrysolophus*)

by

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Abstract

Heart rate (f_H) and rate of oxygen ($\dot{V}_{O_2}$) consumption were recorded from adult macaroni penguins while exercising on a treadmill. No differences were found in the relationship between f_H and $\dot{V}_{O_2}$ in breeding and moulting female penguins, but a significant difference was found between male and female penguins. These relationships were used to estimate field metabolic rate (FMR) for free-ranging female penguins, which were implanted with heart rate and temperature data loggers. While foraging to provision their chick, FMR was 8.92 ± 0.44 W kg^{-1} and 9.07 ± 0.42 W kg^{-1} respectively while at-sea during the brood and crèche phases of the breeding season. While on-shore, the FMR was 6.08 ± 0.43 W kg^{-1} and 5.64 ± 0.40 W kg^{-1} respectively for the brood and crèche phases. During their moult fast, male and female penguins showed a pattern of increasing and then decreasing FMR and females had a mean FMR of 5.25 ± 0.88 W kg^{-1}. The peak of energy expenditure was associated with maximum feather loss, probably due to increased costs of thermoregulation. During natural diving, penguins showed complex fluctuations in heart rate. Abdominal temperature fell during dive bouts with the magnitude of this decline increasing with bout length. Put together, these adjustments in heart rate and circulation may be enough to enable all natural dives to be aerobic in nature.

Dedication

This thesis and the work described within it is dedicated to my Mum and Dad, who I think always knew I would do this, to Fiona who was always right next to me, even when I was 8000 miles away, to the penguins who made it possible and to my Grandfather, who didn't get the chance to see me finish it.

“Whatever a penguin does has individuality, and he lays bare his whole life for all to see. He cannot fly away. And because he is quaint in all that he does, but still more because he is fighting against bigger odds than any other bird, and fighting always with the most gallant pluck, he comes to be considered as something apart from the ordinary bird – sometimes solemn, sometimes humorous, enterprising, cheeky – and always (unless you are driving a dog team) a welcome and, in some ways, an almost human friend.”

from ‘The Worst Journey In The World’ by Apsley Cherry-Garrard, Zoologist and Polar Explorer, 1916.

“We men of action who serve science serve only a reflection in a mirror. The tasks are difficult, the objectives remote; but scholars sitting in bookish surroundings tell us where to go, what to look for, and even what we are apt to find. Likewise, they pass dispassionate judgment on whatever we bring back. We are nothing more than glamorous middlemen between theory and fact, materialists jobbing in the substance of universal truths.”

from ‘Alone’ by Admiral Richard E. Byrd, Polar Explorer, 1938.

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Contents

List of figures

List of tables

Chapter 1

General Introduction

The measurement of physiological parameters in free ranging animals has applications in several fields, not just for the study of comparative physiology *per se.* In the present study, heart rate and body temperature were measured in free-ranging macaroni penguins in an attempt to answer questions about aspects of their ecology, behaviour and physiology.

Heart rate as a tool for answering questions in ecology

The role of seabirds in marine ecosystems

Seabirds are an essential and integral part of many marine ecosystems. As predators they are responsible for the consumption of large numbers of prey species such as fish or marine invertebrates, while they themselves are prey for higher predators (Walker et al. 1998). They are involved in nutrient recycling by depositing nitrogen-rich guano on the land around their nesting sites (Löfgren 1984, Furness and Monaghan 1987) and their eggs, chicks and carcasses are a food source for terrestrial predators and scavengers (Young 1994). In addition, the behaviour of seabirds is a good indicator of perturbations to marine ecosystems from sources such as pollutants and depletion and movement of fish stocks (Löfgren 1984, Furness and Monaghan 1987, Cairns 1992, Monaghan 1996).

Seabirds tend to be long-lived, with a low average annual breeding output and delayed maturity (Croxall 1987, Furness and Monaghan 1987). As such, their populations are relatively stable and resilient in the face of the normal short term variability found within marine systems. However, large scale perturbations such as the destruction of breeding habitats or removal of prey species, can have dramatic effects on such populations and they can be very slow to recover. In addition it is often difficult to predict the outcome of such perturbations and whether or not they may be reversible (Furness and Monaghan 1987). Humans are responsible for a variety of threats to seabird populations. For example, through

hunting for sport, food or oil (Furness and Monaghan 1987, Williams, T.D. 1995), egg collection (Krasnov and Barrett 1995), pollution (Furness and Camphuysen 1997), disturbance of breeding areas (Löfgren 1984, Furness and Monaghan 1987, Williams, T.D. 1995) and commercial fishing (Schaefer 1970).

If populations of seabirds are to persist in the face of the likely continuation of threats and exploitation then it is essential to have an understanding of their ecology and role in the marine ecosystem. In the 21st century the most likely source of such threats is through competition with large commercial fisheries. There is increasing emphasis among those responsible for the regulation of fisheries on investigating the foraging patterns and requirements of the predators that "compete" with fisheries. These predators include seabirds as well as fish at higher trophic levels and marine mammals and reptiles.

In order to make an accurate assessment of the food requirements of predators then certain key data are required (Croxall 1987, 1995):

1) Composition of the diets, in terms of age class, numbers, size and energy content of food items.
2) Population sizes of predators and prey with demographic data.
3) Distribution in space and time of predator and prey populations.
4) Energy requirements of predators.

In addition, these data should if possible be ascertained for all age and sex classes of individuals, at all times of the year and for different years if conditions vary on that scale. Also, in complex ecosystems with more than one predator dependent on a particular prey resource, these data must be collected for all key predators and added to the overall model of the system. However, obtaining all of these data is extremely difficult and labour intensive and has not been achieved in any investigation of this type. Such a list of requirements should be viewed as an ideal in such studies, with the knowledge that it will be difficult to achieve.

Despite these difficulties, continuing research by some groups has led to the beginnings of an understanding of the role of seabirds and their dependence on prey resources in several marine ecosystems and in particular how established fisheries have affected seabird populations and behaviour. Particularly well studied areas include the Barents Sea (Krasnov

and Barrett 1995, Mehlum and Gabrielsen 1995), Prydz Bay, Antarctica (Cooper and Woehler 1994, Woehler 1997), the North Sea (Monaghan 1996, Monaghan et al. 1996) and Scotia Sea, Sub-Antarctica (Croxall et al. 1984, 1999, Croxall and Prince 1987). These studies have integrated data from fisheries with data from top predators including prey intake rates (Garthe et al. 1999), composition of diets (Granadeiro et al. 1998) population sizes (Woehler 1997), energy expenditure (Konarzewski et al. 1993, Gabrielsen 1996) and location of foraging areas (Culik and Luna-Jorquera 1997) to derive spatially explicit rates of prey consumption by the whole population. These studies started to model how prey consumption rates might vary in response to changes in prey density due either to natural fluctuations or the influence of fisheries.

These studies and models are subject to development and improvement to increase their accuracy and usefulness. A common weakness or limitation in such studies is in the reliance on allometric equations to estimate energy expenditure in free-ranging predators. Estimates of population energy expenditure are especially sensitive to the use of these equations (Brown 1989), and can generate results with 95% confidence intervals of between 30% and 50% of the mean, depending on the equations used (Furness 1978). The equations also do not take account of different activity budgets. The use of actual measurements of energy expenditure has the potential to reduce the errors associated with the use of allometric equations. However, these measurements have only been made on a narrow range of species and individuals. The aim of this study is to estimate energy expenditure in free-ranging macaroni penguins (*Eudyptes chrysolophus*), the dominant predator in the Scotia Sea around South Georgia.

The role of macaroni penguins in the Scotia Sea around South Georgia

Penguins are thought to make up 80% of the numbers of birds in the Subantarctic with 50% of these being macaroni penguins (Croxall 1984). The most recent estimation of population size gave a minimum of 11.8 million pairs on Subantarctic islands and the Antarctic peninsula with approximately 5.4 million pairs breeding at South Georgia (Woehler 1993) (Fig. 1.1), although there are large and unknown levels of error associated with all these estimates.

Macaroni penguins feed predominantly on Antarctic krill (*Euphasia superba*), which can form 98% by mass of their diet during the breeding season (Croxall et al. 1997).

Other species of predators breeding at South Georgia and foraging in the Scotia Sea are also dependent on Antarctic krill as their primary food source. These include Antarctic fur seal (*Arctocephalus gazella*) (Reid 1995, Reid and Arnould 1996), Antarctic prion (*Pachyptila desolata*) (Reid et al. 1997a), diving petrels (*Pelacanoides georgicus, P. urinatrix*) (Reid et al. 1997b), gentoo penguin (*Pygoscelis papua*) (Croxall et al. 1988, Williams, T.D. 1991) and white chinned petrel (*Procellaria aequinocitialis*) (Croxall et al. 1995, Berrow and Croxall 1999). Pelagic species including cetaceans (Reid et al. 2000) and mackerel icefish (*Champsocephalus gunnari*) (Kock et al. 1994) are also important predators of krill in the Scotia Sea and the icefish are also consumed by Antarctic fur seals (Reid and Arnould 1996)and gentoo penguins (Williams, T.D. 1991). At present, the large krill fishery (approx 100 000 tonnes per year) in the Scotia Sea is under the management of the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR). There are no immediate signs of expansion in this fishery (Everson 2000). However, expansion cannot be ruled out and one of the problems identified by krill fishing nations, which may hinder the development of a fishery model (and hence catch limits and schedules), is a lack of understanding of the functional relationships between krill and predators (Everson 2000) (Fig. 1.2).

The characteristics of this ecosystem, with short food chains, reduced complexity of trophic interactions and pronounced yet regular seasonality provides an opportunity to improve our understanding of the dynamics of pelagic systems (Reid et al. 1999). Much of the variability of this system has now been described and documented (Sahrhage 1988), yet the causes and consequences of this variability are poorly understood. Krill are the keystone species and their population size, structure and location are influenced by the interactions of a variety of large-scale biological and physical factors at a range of scales and trophic levels (Reid et al. 1999). These include the direct and indirect effects of cycles in sea-ice duration and extent (Priddle et al. 1988, Murphy et al. 1998). These factors combine to produce a reasonably predictable cycle in the abundance of krill available to the South Georgia krill predators. This is characterised by a season of relatively low krill abundance every 3-4 years (Reid et al. 1999).

Adaptations by krill predators to the constraints imposed by this season of low abundance by increasing the diversity of prey species taken and reducing overlap between predator species (McCafferty et al. 1998, Croxall et al. 1999). As a result, provisioning rates to offspring decrease as meal sizes decrease and foraging trip durations increase, leading to a reduction in fledgling mass and breeding success (McCafferty et al. 1998, Croxall et al. 1999). At a larger scale, longer term trends in the abundance of krill may have negatively affected krill predators in terms of population sizes and reproductive output (Reid and Croxall 2001).

Macaroni penguins seem better adapted to withstand the periodic years of low krill abundance than the other krill predators at South Georgia. Even though the mass of fledglings is reduced, their overall breeding success is reduced by only 10%, compared with 90% in gentoo penguins (Croxall et al. 1999). This apparent ability in macaroni penguins to withstand variability in food availability can probably be attributed to their capacity to maintain their productivity after switching to an alternative diet of amphipods (Croxall et al. 1999). Alternatively, Antarctic fur seals were found to increase their rates of energy expenditure in years of low krill availability (Boyd et al. 1994). Investigations of the role of predators and models of the ecosystem of which they are a component must be able to include this variation in environmental conditions and the response of predators to it. If the understanding of the dynamic ecosystem in the Scotia Sea is to be improved then the impact of macaroni penguins on marine resources must be estimated at all temporal and spatial scales and this relies upon accurate measurements of energy expenditure. In order to achieve this, a method is required which can measure energy expenditure at a fine timescale, for long periods and in different age and sex classes.

The measurement of energy expenditure in free-ranging vertebrates

1. Time-energy budgets (TEB)

This method involves the collection of data about the time spent in different activities by animals in the field. These activities are then assigned a metabolic cost, determined either from studies in the laboratory or from allometric equations. The sum of the probability of each activity occurring multiplied by its energetic cost gives the total energy expenditure

(equation 1.1).

$$TEE = p_1r_1 + p_2r_2 + p_3r_3 + \ldots\ldots\ldots p_nr_n \quad (1.1)$$

(Where TEE = total energy expenditure, p = the proportion of time engaged in a particular activity, r = the rate of energy expenditure of that activity.)

Perhaps due to its relatively low financial cost and general simplicity and accessibility, this method is used commonly (Goldstein 1988), and has proved successful and superior to other methods under certain circumstances (Grémillet et al. 1995). However, the major disadvantages of the TEB method are that it can be extremely time consuming to implement and it is necessary to assume that behaviour is discrete and that every activity observed can be assigned to one of the categories created by the investigator. Such categorisation is very subjective and a lack of consensus in methodology in TEB studies restricts their accuracy and comparative value (Buttemer et al. 1986). In addition, it may be impossible to calculate costs for all categories of activity in the field. One important source of inaccuracy occurs because of the sensitivity of animals to variations in their thermal environment (Nagy 1989). This applies in particular to smaller homeothermic animals. Variations in ambient temperature outside its thermo-neutral zone lead to thermoregulatory costs by the animal, which are difficult to estimate.

Validation studies have shown that energy expenditure as calculated by TEBs may, when compared with estimates from other techniques such as doubly labelled water, yield estimates too low by as much as 44% or too high by as much as 57% (Nagy 1989). This suggests that this method can have a relatively low level of accuracy. However, these studies only compare one potentially inaccurate technique against another and as such are of limited value. Almost all TEB studies have been restricted to birds as they are relatively easy to observe in the field and tend to behave predictably. The technique would be inappropriate for example, for a nocturnal or diving animal unless automated equipment were used to record activity.

2. Doubly labelled water (DLW)

This technique is based on the observation that oxygen atoms in metabolically produced carbon dioxide (CO_2) freely equilibrate with the oxygen atoms of water via the action of carbonic anhydrase in the blood (Lifson and McClintock 1966). When known amounts of ^3H and ^{18}O labelled water are injected into an animal, the ^{18}O water equilibrates with both the CO_2 and water pools, and declines as a function of water influx and CO_2 production. In contrast the ^3H water equilibrates only with the water pool and dilutes as a function of water influx. The initial dilution of these isotopes after an equilibration period allows determination of the total body water (TBW) volume. As CO_2 is produced by metabolism, ^{18}O water is diluted while ^3H water does not change . Therefore, the difference between ^{18}O turnover and ^3H turnover across the duration of the experiment is a measure of the animal's rate of CO_2 production ($\dot{V}_{CO_2}$).

In order to derive the equations for $\dot{V}_{CO_2}$, a number of assumptions must be made and adhered to (Lifson and McClintock 1966):

1) The animal is in a steady state of body composition, i.e. TBW remains constant.
2) All rates of intake and output of water and CO_2 remain constant.
3) All body water is uniformly labelled and ^3H and ^{18}O are not incorporated into body tissues.
4) H only leaves the body as water, and O only leaves the body as water or CO_2.
5) Specific activities of H and O lost from the body are the same as those of the body water.
6) No water or CO_2 enters the body with inspired air or through the skin.

Unfortunately in real-life biological systems it is almost impossible for all of these criteria to be satisfied and examples exist to show that each of the above assumptions can be invalidated (Nagy 1980, Nagy and Costa 1980, Speakman and Racey 1986, Haggarty and McGaw 1988, Haggarty et al. 1991, Midwood et al. 1993, Thomas et al. 1994). Isotopic fractionation of labelled water, input of CO_2 and inaccuracy in measuring isotope levels are the factors most likely to introduce large variability in estimates of $\dot{V}_{CO_2}$.

Despite the potential for some problems and inaccuracies of estimates, the technique has been widely employed in a range of different species (Speakman 1997). By their very nature,

techniques to estimate metabolic rate (MR) in the field (FMR) are difficult to validate. A comparison of MR measured using TEB and DLW in free-ranging phainopeplas (*Phainopepla nitens*) concluded that the TEB technique underestimated MR by 40% (Weathers and Nagy 1980), whereas a similar study of savannah sparrows (*Passerculus sandwichensis*) found that MR estimated by DLW was only 5% above that estimated by TEB (Williams, J.B. and Nagy 1984). Again, these studies only compare one potentially inaccurate technique against another and as such are of limited value. Validation studies from the laboratory give a better understanding of the accuracy of this method. Such studies have show MR estimated to within 11% (Westerterp et al. 1988, Nagy 1989), though most of these earlier validation studies involved resting animals. A more recent, more rigorous validation study of gentoo penguins (Bevan et al. 1995c) gave an algebraic error of only 1.6%, and results that were not significantly different to those obtained by respirometry. However, the range of estimates from DLW was considerable, and the absolute error, which ignores the sign, was 48.2%. Other studies have shown that though DLW can be used to estimate FMR, it is possible that FMR can be overestimated by as much as 36% in aquatic animals (Bevan et al. 1995b, Boyd et al. 1995). Similarly, a validation study on black-browed albatrosses (*Diomedea melanophris*) (Bevan et al. 1994) concluded that though DLW estimates of metabolic rate were not significantly different from those obtained by respirometry (within 4%), the range of individual errors was considerable, and the technique could not, therefore, be used to determine the energy expenditure of individuals. This pattern has been shown in other validation studies (Gales 1989, Nolet et al. 1992).

The DLW technique is also subject to other sources of error. Oxygen consumption is calculated from CO_2 production using RE (respiratory exchange ratio), the ratio between O_2 consumed and CO_2 produced. RE is dependent on the substrate used for metabolism (carbohydrate, lipid or protein) which depends on dietary content. Suitably detailed data on diet and RE for some groups (including seabirds) are limited and values from other species have been used when estimating MR (Gales 1989). There is also a suggestion that the production of CO_2 will vary depending on the energy balance status of the individual (Kam and Degen 1997), which may depend on, for example, the phase of the breeding season.

In addition, several equations exist for the interpretation of isotope data and conversion to rates of CO_2 production and there is a certain amount of subjectivity in the selection of an appropriate equation. Though body mass is an important factor, it is probably always necessary to perform a validation study in the laboratory for the species under investigation (Nolet et al. 1992, Speakman 1993, Bevan et al. 1995c), in order to ensure that the correct equation is used. However, in a validation study of black browed albatrosses (Bevan et al. 1994), it was suggested that the equation chosen to calculate energy expenditure is not an important factor in determining the error in the results. Perhaps the biggest problem with the variation in accuracy in estimates of MR using DLW is that the errors are not easily quantifiable.

Advantages of the technique are simplicity of use and despite some evidence of deleterious effects to seabirds used in experiments in terms of reduced nest attendance (Birt-Friesen et al. 1989) and chick feeding (Uttley et al. 1994), it is relatively non-invasive. For example it is possible to undertake a DLW study of metabolic rate in both members of a breeding pair without causing undue disturbance (Nagy et al. 1984). The results of the technique are instantly usable, without the need to do further calibration studies in the laboratory before results can be interpreted. The technique can be used on very small animals, for example shrew-tenrecs (*Microgale dobsoni, M. talazaci*) (Stephenson, P.J. et al. 1994) and long-eared bats (*Plecotus auritus*), which demonstrated no change in behaviour during DLW experiments (Entwistle et al. 1994).

The high cost of stable isotopes means that, especially for large animals, the technique can be very expensive. In addition to this, the DLW method has two main disadvantages. Firstly, the duration of an experiment is limited by the rate of turnover of ^{18}O. Animals must be recaptured and sampled within a specific window of time which allows the ^{18}O level to decline sufficiently to detect a difference from initial levels, but not too far such that the ^{18}O is not different to background levels. The length and timing of this window will vary with the size of the animal, its MR and the initial enrichment of ^{18}O but is typically several days from the start of the experiment (Speakman 1997). This imposes limits on the design of experiments and subject animals used. Even when this has been taken into account, it is possible that animals will behave unpredictably and not return to have samples of blood taken

before isotope enrichments fall to background levels (Arnould et al. 1996a). When this does happen, then resources are wasted and results weakened as sample size decreases. Even re-sampling animals too soon can create difficulties (Boyd et al. 1995). DLW studies involve disturbing the animals several times in a few days, as in most studies background isotope enrichment levels are taken from experimental animals before injection with isotopes and subsequent sampling. This repeated disturbance could increase stress levels and this could influence energy expenditure, although it is sometimes possible to reduce these effects by, for example, taking background samples from other individuals (Arnould et al. 1996b).

The second significant disadvantage with the DLW technique is that the value for energy expenditure obtained is simply an average for the number of days of the experiment between the two sampling points. It is not possible to assign metabolic costs to different activities. It is still necessary to construct time budgets, and/or make assumptions about resting metabolic rates (Nagy et al. 1984, Ballance 1995, Arnould et al. 1996a, b), in order to interpret the energetic data, and at best all this can produce is an overall value for FMR. Resolution can be improved by blood sampling individuals throughout the duration of the experiment (Nagy et al. 1984), but this is not always possible, and increases disturbance. Multiple experiments may be performed in order to determine energy expenditures, for example, at different stages of a breeding season (Davis et al. 1989). However, in order to minimise disturbance, different individuals are used, which increases variation.

3. Heart-rate (f_H)

The heart rate method for estimating MR has become better developed and more widely used in recent years as it is facilitated by advances in technology for measuring and recording heart rate in the field. It is underpinned in principle by the link between heart rate and rate of oxygen consumption given by the Fick Equation (equation 1.2).

$$\dot{V}_{O_2} = f_H \times \dot{V}_s (C_a O_2 - C_{\bar{v}} O_2) \quad (1.2)$$

(Where $\dot{V}_{O_2}$ = rate of oxygen consumption, f_H = heart rate, $\dot{V}_s$ = cardiac stroke volume (amount of blood pumped during one heart beat), C_aO_2 = oxygen content of arterial blood and $C_{\bar{v}}O_2$ = oxygen content of mixed venous blood.)

If $\dot{V}_s (C_a O_2 - C_{\bar{v}} O_2)$, the oxygen pulse, remains constant or varies systematically then it is possible to demonstrate a simple relationship between rate of oxygen consumption ($\dot{V}_{O_2}$) and heart rate (f_H) and hence calculate the former from the latter (Butler 1993). This relationship has been demonstrated in several species of birds including blue-winged teal (*Anatidae discors*) (Owen 1969), black duck (*Anas rubripes*) (Wooley and Owen 1977), pigeon (*Columba livia*) (Flynn and Gessaman 1979), American kestrel (*Falco sparverius*) (Gessaman 1980), marabou stork (*Leptoptilos crumeniferus*) (Bamford and Maloiy 1980), emu (*Dromaius novahollandiae*) (Grubb et al. 1983), tufted duck (*Aythya fuligula*) (Woakes and Butler 1983, Bevan and Butler 1992), barnacle goose (*Branta leucopsis*) (Nolet et al. 1992), black browed albatross (Bevan et al. 1994), gentoo penguin (Bevan et al. 1995c), common eider (*Somateria mollissima*) (Hawkins et al. 2000) and king penguin (*Aptenodytes patagonicus*) (Froget et al. 2001). Relationships have also been demonstrated in a number of mammals including pine marten (*Martes americana*) (Fisher et al. 1987), red squirrel (*Scirius vulgaris*) (Pauls 1980) bottlenose dolphin (*Tursiops truncatus*) (Williams, T.M. et al. 1993) and Californian sea-lion (*Zalophus californianus*) (Butler et al. 1992). Heart rate can be detected and recorded, either by radio transmitter, or more appropriately for free-ranging animals, by miniature data loggers. It is the recent development of these miniature data loggers that has contributed to the development of this technique for free-ranging animals (Woakes et al. 1995).

Validation studies, in conjunction with DLW and or respirometry indicate that f_H does indeed allow an accurate estimate of $\dot{V}_{O_2}$, with in general, less error than the DLW method (Livingstone et al. 1990, Nolet et al. 1992, Bevan et al. 1994, 1995c, Boyd et al. 1995). However, these validation studies conclude, that as with DLW, a relatively wide range of

errors demonstrates that f_H cannot be used to estimate the metabolic rate of individual animals, unless each animal is individually calibrated. This is normally logistically impractical. Ideally, a laboratory calibration should be performed with as large a sample size as possible (Bevan et al. 1995c), and preferably on as many days as possible (Pauls 1980). This calibration must cover the full range of f_H and $\dot{V}_{O_2}$ levels (Nolet et al. 1992) and behaviours (Bevan et al. 1995c) likely to occur in the wild. With the f_H method, it should be possible to quantify the magnitude of errors in estimation, which will depend on the number of calibration animals and data points. Few previous studies using f_H have attempted accurately to model this source of error, which is something the current project will address.

Some inconsistencies have been detected in the relationship between f_H and $\dot{V}_{O_2}$. The relationship has been found to vary during different seasons of the year in white-tailed deer (*Odocoileus virginianus*) (Holter et al. 1976) or if measured on different dates in American kestrels (Gessaman 1980). However, in tufted ducks, f_H was a consistent predictor of $\dot{V}_{O_2}$ in two sets of experiments on different animals, separated by nine years (Woakes and Butler 1983, Bevan and Butler 1992). Social interactions were found to modify the relationship in pigeons (Flynn and Gessaman 1979). Different relationships might be necessary for periods of exercise and rest (Gessaman 1980, Froget et al. 2001), or modes of locomotion (Hawkins et al. 2000) though this is not always the case (Nolet et al. 1992, Bevan et al. 1995c). It appears that determination of the $f_H/\dot{V}_{O_2}$ relationship when animals are in different physiological states is essential and may explain some of the temporal differences observed in several studies (Holter et al. 1976, Gessaman 1980). All potential problems with the relationship must be considered in a rigorous calibration procedure and interpretation of results must also account for these factors. The present study will attempt to determine the $f_H/\dot{V}_{O_2}$ relationship for exercising macaroni penguins, while taking into account different physiological states of animals.

The principle advantage of the f_H method is that it can provide estimates of MR at a far greater temporal resolution than other techniques; the resolution being limited only by the calibration procedure and method of recording of heart rate. The data loggers record heart rate measurements as a series and so, in conjunction with behavioural data, individual activities

can be assigned an energy cost. The behavioural data can be in the form of observations or commonly collected by other automated recording devices such as time-depth recorders (Bevan et al. 1997), speedometers (Boyd et al. 1999), salt water switches (Bevan et al. 1995a) and satellite transmitters (Butler et al. 1998). There can be problems with using these externally mounted devices as they can impose an additional energetic cost by increasing drag when swimming or flying which can effect foraging and breeding success (Wilson et al. 1986, Ward and Flint 1995, Carbone et al. 1996, Hull 1997). In an effort to counter this and to increase the compatibility of the different sets of data, the data loggers used in the present study were implanted into the animals abdominal cavity and were able to monitor heart rate, pressure (diving depth) and abdominal temperature on a single time scale.

The disadvantages associated with the f_H method may include the internal implantation of data loggers under general anaesthetic (Stephenson, R. et al. 1986). Although this is a relatively simple procedure, not all investigators will be able to perform it, as it can be quite time consuming, and adequate facilities must be in place in the field. It could also represent a considerable trauma to the subject animal, although evidence suggests that after a recovery period of about two days, implanted penguins behaved quite normally both during and after experiments (Bevan et al. 1995c). However, the trauma and risk of desertion of the nest in birds is still sufficiently great that both members of a breeding pair would not be implanted simultaneously. The surgical procedure and size of the data loggers imposes a minimum size limit on experimental species. Common guillemots (*Uria aalge*) at 800-1000g are the smallest birds to have been successfully implanted with data loggers (Hawkins 1995), though in one set of experiments all six experimental animals deserted their nests (Hawkins 1995). Heart rate radio transmitters can be much smaller and have been deployed in smaller birds and mammals such as laboratory mice and mongolian gerbils (*Meriones unguiculatus*) (Stöhr 1988).

Heart rate as a tool for answering questions in physiology and behaviour

The physiology of diving vertebrates

Investigations of diving physiology have progressed considerably since early work on forced submergence of diving and non-diving animals (Scholander 1940, Bond et al. 1961, Andersen 1966). These studies elicited the theory of the "classic dive response" where an intense bradycardia (reduction of heart rate to below resting levels) was observed upon submersion followed by a further progressive decline in heart rate. After emerging from such a "dive" there would then be a period of tachycardia (acceleration of heart rate). The slowing of heart rate while submerged was indicative of circulatory adjustments. In an effort to conserve oxygen during a "dive" of unknown duration, a common finding was reduction in muscle perfusion, while blood flow to the central nervous system and heart was maintained or increased as these organs are unable to withstand oxygen deprivation. Reduction in perfusion to the locomotor muscles led investigators to believe that these muscles switched to anaerobic metabolism during diving (Butler and Jones 1982, 1997).

More recently, such ideas have been reconsidered. Most divers are thought to maintain aerobic metabolism during dives. The cardiovascular adjustments associated with breath-hold diving in vertebrates are also being see as a trade off between the "classic dive response" and the response to exercise (Butler 1988, Castellini 1988). Animals diving voluntarily under laboratory (Butler and Woakes 1979, 1984, 1987, Stephenson et al. 1992), semi-natural (Kooyman et al. 1992, Thompson and Fedak 1993, Williams, T.M. et al. 1999) and natural (Fedak et al. 1988, Thompson and Fedak 1993, Hindell and Lea 1998, Le Beouf et al. 2000) conditions showed quite a different response from that seen in forcibly submerged animals. Not all responses are identical in all animals studied, but in general, before diving, animals show tachycardia and tachypnoea suggesting that oxygen stores are loaded before submersion. Upon submersion there is a brief bradycardia before f_H increases to a level close or slightly below resting values. There is another period of tachycardia as the animals surface and replenish their oxygen stores. Heart rate then declines steadily before the next cycle commences. Extended bradycardia below resting rates has been observed in diving birds (Kooyman et al. 1992), but is more common in marine mammals (Thompson and Fedak

1993). These adjustments show that blood flow to non-essential tissues is reduced (Bevan and Butler 1992) to conserve oxygen stores and prolong the duration of dives. Heart rates during diving in birds are typically below those when exercising in air despite the fact that work is being done under water (Millard et al. 1973, Woakes and Butler 1983). Clearly the circulatory responses are a trade-off between oxygen conservation as seen in forced dives and increased f_H as seen during exercise. Animals exercising in air show similar circulatory adjustments to those seen in diving animals, including a redistribution of blood flow away from inactive tissues (Butler et al. 1988) and increase of blood flow to active leg muscles (Bevan and Butler 1992). Interestingly, artificially extending dives by preventing the birds from surfacing at the end of a voluntary dive leads birds to switch from this exercise modulated response to a "classic" response, presumably in order to conserve O_2 stores and therefore preserve life in a dive of unpredictable duration (Stephenson et al. 1986, 1992).

Considerations of conservation of oxygen stores while submerged have led to the concept of the aerobic dive limit (ADL) (Kooyman et al. 1980, 1983). The ADL, also referred to as the diving lactate threshold (DLT) (Butler and Jones 1997), is defined as the diving duration beyond which blood lactate (a product of anaerobic metabolism) increases above resting levels. To date, DLT has only been measured in four species (Kooyman and Ponganis 1998), Weddell seals (adults and pups) (*Leptonychotes weddelli*) (Kooyman et al. 1980), Baikal seals (*Phoca sibirica*) (Ponganis et al. 1997a), California sea lions (Ponganis et al. 1997c) and emperor penguins (*Aptenodytes forsteri*) (Ponganis et al. 1997b). In the absence of measured values, ADL has been calculated (cADL) based on body oxygen stores and diving metabolic rate for a variety of species including southern elephant seals (*Mirounga leonina*) (Hindell et al. 1992), thick-billed murres (*Uria lornvia*) (Croll et al. 1992), blue-eyed shag (*Phalacrocorax atriceps*) (Croxall et al. 1991), king penguins (Culik et al. 1996a), Adélie (*Pygoscelis adeliae*) (Chappell et al. 1993), chinstrap (*Pygoscelis antarctica*) and gentoo penguins (Culik et al. 1994). In these studies, researchers have concluded that many dives (up to 90%) exceed the cADL (Boyd and Croxall 1996). This seems unlikely in the context of the recovery times between dives, which should be associated with such dives and observed patterns of diving behaviour and surface interval duration (Boyd 1997, Ponganis and Kooyman 2000). The logical conclusion following this is that there must be errors in the

calculation of either O_2 stores and/or $\dot{V}_{O_2}$ while diving (Boyd 1997, Ponganis and Kooyman 2000).

Oxygen stores are relatively easy to determine through experimentation (Lydersen et al. 1992), dissection or allometry, and researchers have discovered that diving animals have particular adaptations including decreased respiratory system volumes and higher oxygen stores in the blood and muscle (Kooyman and Ponganis 1998). Meanwhile, estimation of $\dot{V}_{O_2}$ while submerged is widely accepted to be difficult (Ponganis and Kooyman 2000). There are errors with estimating metabolic rate of individual activities and in particular diving, with all the methods used thus far. Doubly labelled water studies give only an average metabolic rate for the period of deployment. This makes it impossible to partition energy expenditure into resting, surface swimming, surface intervals and submerged swimming (Costa 1988). Respirometry of animals swimming in flumes has been used to estimate metabolic rate while diving but these are not necessarily analogous to animals swimming while diving due to the absence of pressure effects on buoyancy and the effect of accelerating and decelerating. In the present study, the use of the heart rate method should allow the estimation of $\dot{V}_{O_2}$ for individual dive cycles and give some insight into energy use while submerged.

In addition, the present study might give an insight into other mechanisms which have been suggested to lower $\dot{V}_{O_2}$ while submerged and allow dive durations to be extended. These mechanisms include minimizing the buoyancy of air trapped in feathers and lung volumes (Lovvorn and Jones 1991), and the use of gliding at low metabolic cost (Williams, T.M. et al. 2000). The devices used to measure heart rate in the present study also record abdominal temperature. Extreme declines in body temperature (over $10^{O}C$) have been observed in diving birds, including king cormorants (*Phalacrocorax albiventer*) (Kato et al. 1996), king penguins (Culik et al. 1996b, Handrich et al. 1997), blue-eyed shags (Bevan et al. 1997) and gentoo penguins (Bevan et al. 1998). It is proposed that this drop in temperature can also lead to reduced $\dot{V}_{O_2}$ while diving since metabolism is reduced at lower body temperatures through the effect of temperature on enzyme activity (the Q_{10} effect (Heldmaier and Ruf 1992)). In addition, these regions will have lowered thermoregulatory costs, leading to an apparent Q_{10}. The precise cause of this drop in body temperature is still unknown. The ingestion of cold

food is no doubt partly responsible (Ancel et al. 1996), but in foraging penguins, temperature recorded in the abdomen was even lower than that recorded simultaneously in the stomach (Handrich et al. 1997). It is still unclear whether these temperature drops are facilitated and heat "dumped" to the water by perfusion of extremities, whether the temperature drops are simply a consequence of changes in circulation or whether some other mechanism is involved. Simultaneous measurement of heart rate, diving depth and body temperature in the present study might help to investigate this question.

The behaviour of diving vertebrates

The behaviour of animals which depend on diving in order to feed can be modelled as a particular case of the "marginal value theorem" (Charnov 1976). This theory predicts the behaviour of animals that travel to feed on a patchy food resource at which the rate of energy (food) intake decreases with time. It predicts that as travel time increases to the resource then longer should be spent feeding at the resource, even though the rate of energy gain decreases. This theory is reasonably well tested and supported with experimental evidence (Krebs and Davies 1993).

Other authors (Kramer 1988, Houston and Carbone 1992) have used the marginal value theorem to develop models of diving behaviour in air-breathing vertebrates. In this case it is assumed that animals are attempting to maximise the rate of delivery of oxygen to the foraging area, which is usually assumed to be at the bottom of the dive. It is also assumed that this will maximise the mean rate of energy intake. A prediction from these models is that many of these dives will not use all of their oxygen reserves during any particular dive. If, by returning to the surface to breathe, the diver can increase the total time spent in the foraging area of the dive when averaged across dives, then dives will be terminated before all oxygen has been used up. Similarly, while at the surface these models predict that the surface will be left before O_2 stores are fully replenished. This is because of the shape of the oxygen-loading curve, where the rate of oxygen gain decreases as the saturation point of O_2 in the blood is approached.

This application of the theory to diving generates several predictions which have been observed in freely diving animals. As dive duration increases, the time spent at the surface between dives increases (Gentry et al. 1986, Kramer 1988, Croll et al. 1992, Chappell et al. 1993, Wanless et al. 1993, Costa and Gales 2000), dive duration increases with increasing dive depth (Gentry et al. 1986, Feldkamp et al. 1989, Croll et al. 1992, Chappell et al. 1993, Wanless et al. 1993) and animals will regularly feed at depths far shallower than their maximum, or for durations well within their ADL (Gentry et al. 1986, Kooyman et al. 1986, Feldkamp et al. 1989, Williams, T.D. et al. 1992).

Such cost-benefit considerations based on prey availability are probably as important as the physiology of diving in governing observed behaviours (Fedak and Thompson 1993, Thompson et al. 1993). The location, density and energetic value of prey in the water column are probably the major factors influencing diving behaviour. What explicit tests there have been of the Houston and Carbone type of models show that animals do appear to dive with regard to food intake rather than maximising dive depths or durations (Carbone and Houston 1994). In addition, these models predict that animals should able to modify their behaviour and switch to more inefficient anaerobic metabolism if this is advantageous in terms of gaining food during a diving (Ydenberg and Clark 1989). This theory has still to be tested rigorously but appears to be consistent with observations of some animals including Weddell seals (Kooyman et al. 1980, 1983). Other restrictions or influences on such behaviours may be due to environmental factors, multiple searching methods or prey types or strategies to avoid surface predators. For example, some birds are limited to foraging during the day time as they are dependent on minimum light levels (Wilson et al. 1993, Wanless et al. 1999) or by the amount of sea-ice cover in foraging areas (Watanuki et al. 1997).

Clearly a full understanding of observed patterns of diving will be based on a combination of the animals physiology, behavioural strategies and intentions, prey characteristics and environmental variation. Only detailed studies attempting to include all of these variables will be able to explain observed patterns in diving. Though not all of these variables will be measured in the present study, an inclusive approach will be taken towards analysing behavioural data on diving with simultaneously measured physiological data.

Study species and sites

Macaroni penguins

Macaroni penguins are the most numerous of all the penguins. In 1993, there were thought to be at least 11.8 million pairs (Woehler 1993) breeding on subantarctic islands. These birds are distributed on both sides of the Antarctic convergence zone (where the Southern Ocean meets temperate oceanic waters (Fig. 1.1). In addition, approximately 850 000 pairs of royal penguins (*Eudyptes schlegeli*) breed at Macquarie Island. This species is very similar in appearance and behaviour to the macaroni penguin and some authors have described the royal and macaroni penguins as sub-species (Williams, T.D. 1995). Also, (unsuccessful) attempts by royal penguins to breed with macaroni penguins have been observed (pers. obs.). Macaroni penguins often breed in large colonies of up to 100 000 pairs (Williams, T.D. 1995), on steep rocky ground on headlands and on level rocky ground, typically devoid of vegetation. Nesting sites may be several hundred metres away from the sea, and penguins will travel between the two along well worn paths (Marchant and Higgins 1990, Williams, T.D. 1995). Macaroni penguins are monogamous with long-lasting pair bonds. They also show a high level of fidelity to the breeding site. Breeding within colonies is largely synchronous with 95% of clutches initiated within 4-6 days (Williams, T.D. and Croxall 1991). The breeding season is characterised by long alternating periods of fasting on land and foraging at sea (Williams, T.D. and Croxall 1991) (Fig. 1.3).

The population of macaroni penguins at South Georgia has been the subject of several investigations (Williams, A.J. 1981, Davis et al. 1983, 1989, Croxall et al. 1988, 1993, Williams, T.D. 1989, Ghebresmeskel et al. 1991, Reid et al. 1999) and is the only population from south of the Antarctic Convergence that has had its breeding biology comprehensively studied (Williams, T.D. and Croxall 1991) (Fig. 1.3). At South Georgia, males arrive towards the end of October, and are joined approximately 9 days afterwards by females. A nest is made from stones, bones and other bits of debris. Two eggs are laid, though the smaller first egg rarely hatches or fledges. Incubation takes 35 days and comprises three long shifts; the first is shared between the two partners, the second is undertaken by the female and the third by the male. After the egg hatches, the male continues to guard the chick at the nest until the

chicks form crèches at 23-25 days of age, he then goes off to sea. During this period, the female provisions the chick at regular intervals of 1-2 days. When the male returns from the post-crèching foraging trip, provisioning duties are shared between the male and female until the chicks fledge at 60 days of age. Once chicks have fledged, adults will depart to sea for a foraging trip of 12-14 days before returning to the colony to moult. Moult lasts 24 ± 0.2 days after which adults depart to sea. All adults have departed from the colony by the end of April. Nothing is known about the behaviour and location of animals during the winter, but there are no observations of their coming ashore in large numbers (Marchant and Higgins 1990, Williams, T.D. 1995).

During the chick rearing period of the breeding season, parents forage at-sea and bring back food, mostly antarctic krill (Croxall et al. 1997), for their chicks. Most foraging trips commence at dawn (05:00 - 07:00) and finish during the afternoon or evening of the same day (11:30 - 22:30). However some trips are longer and animals will remain at sea overnight (Brown and Klages 1987, Croxall et al. 1993). Macaroni penguins dive to feed and data from previous studies suggests that, for animals from South Georgia, most diving occurs during the day to a median depth of 29 m (Croxall et al. 1988).

South Georgia and Bird Island

South Georgia is a mountainous island 160 km long and up to 50 km wide rising to 2935 m. It is situated approximately 250 km south of the Antarctic Convergence between latitudes 54^{o} to 55^{o} S and longitudes 36^{o} to 38^{o} W (Fig. 1.1). Bird Island lies off the north-western tip of South Georgia at 54^{o}00'S 38^{o}02'W and is 6.5 km long and up to 1.5 km wide with a surface area of 400 hectares (Hunter et al. 1982). The long axis of Bird Island is orientated approximately E-W (Fig. 1.4). The northern coast consists of high cliffs rising to 365 m, and is precipitous and devoid of beaches. The southern coast however has numerous gently sloping rocky beaches (Bonner and Croxall 1988). Most of the gentler slopes below about 100 m are clothed with tussock grass (*Paradiochloa flabellata*), but in places relatively level meadows of shorter grass and other plants occur. These are dotted with small pools. Lying well to the South of the Antarctic Convergence the climate at South Georgia shares

characteristics of the sub-Antarctic and maritime Antarctic. Extensive meteorological data from Bird Island is limited. However, the weather is predominantly damp and cloudy with frequent high winds. Mean daily temperatures vary from -2^{o}C to 9^{o}C in summer and from -10^{o}C to 3^{o}C in winter (Richards and Tickell 1968). Snow occurs in all months of the year but there is no permanent snow or ice on the island, although late snow patches may extend until January.

Most of the animals used in field deployments were taken from the small macaroni penguin colony at Fairy Point on the northern side of Bird Island (Fig. 1.4). The birds nest on a gently sloping smooth rocky patch surrounded by tussock grass and with a southerly aspect. The penguins enter and leave the sea from a flat rock shelf (the 'landing stage') and ascend to the colony via a steep and well-worn rocky path (the 'ladder'). At a point where the path narrows and must be crossed in order for access for the colony to be gained, there is an automatic device to read passive implanted transponder (PIT) tags. During the 1997/98 season, 580 pairs of macaroni penguins nested at this colony. Other birds were taken from a larger colony at nearby Goldcrest Point. This colony is also on the north side of the island and there were approximately 26 307 pairs of penguins nested here during the 1997/98 season. Surgical operations and experiments with captive birds were performed at the British Antarctic Survey base at Jordan Cove on the south side of the island.

Summary

The principle aims of the present study and the chapters in which these objectives are addressed, are as follows:

1. Derive a relationship between heart rate and rate of oxygen consumption in macaroni penguins, allowing for differences in physiological status (chapter 2).
2. Quantify and evaluate the errors associated with estimating rate of oxygen consumption from heart rate in the field (chapter 2).
3. Estimate metabolic rate from free-ranging macaroni penguins (chapters 3 and 4).
4. Estimate metabolic rates associated with different activities and phases of the breeding season (chapters 3 and 4).

5. Investigate energy expenditure and metabolism during diving in free-ranging macaroni penguins (chapter 5).
6. Examine changes in abdominal temperature associated with diving and evaluate their effect on metabolism and diving behaviour while foraging (chapter 5).

1. South Georgia
2. Bouvetoya
3. Prince Edward Islands
4. Crozet Islands
5. Kerguelen Islands
6. Heard Island
7. McDonald Islands
8. Macquarie Island

Figure 1.1. Map of Southern Ocean from 40^{o} South, showing location of South Georgia and other Sub-Antarctic islands with populations of macaroni or royal penguins. Dotted line represents the approximate position of the Antarctic Convergence.

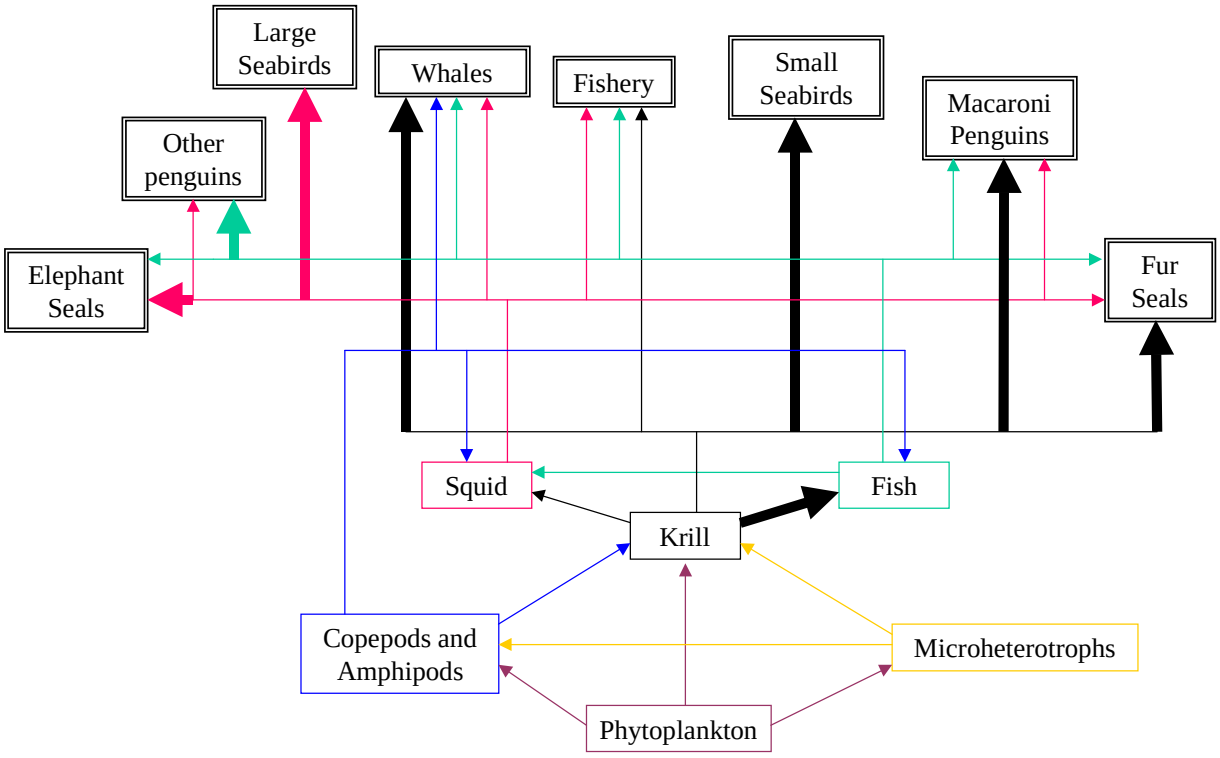

Figure 1.2. Diagram showing major features of the foodweb of the Scotia Sea around South Georgia and the central role of Antarctic krill. Thick arrows depict particularly strong preferences/dependencies.

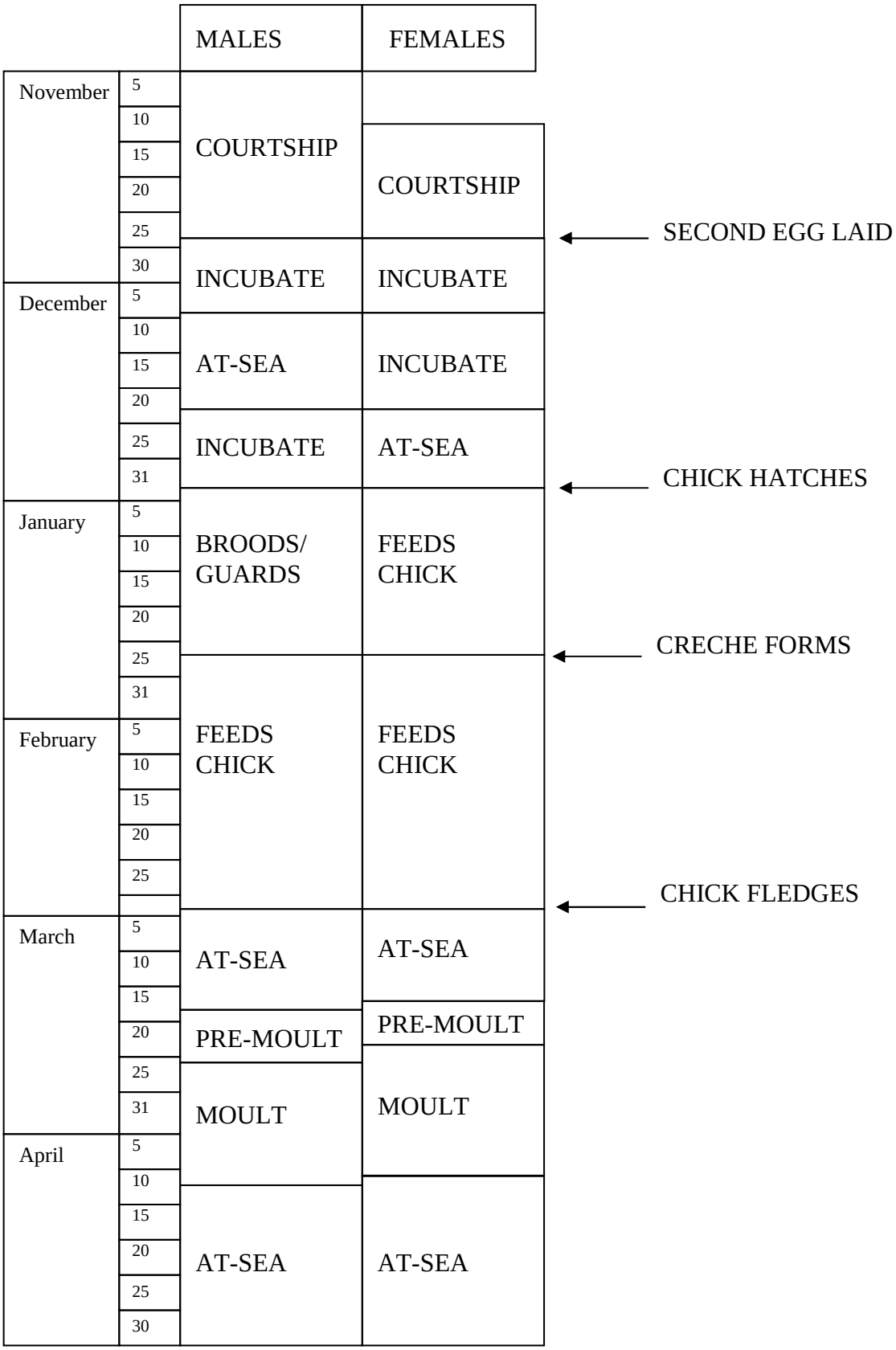

Figure 1.3. Timetable and division of responsibilities in macaroni penguins breeding at South Georgia.

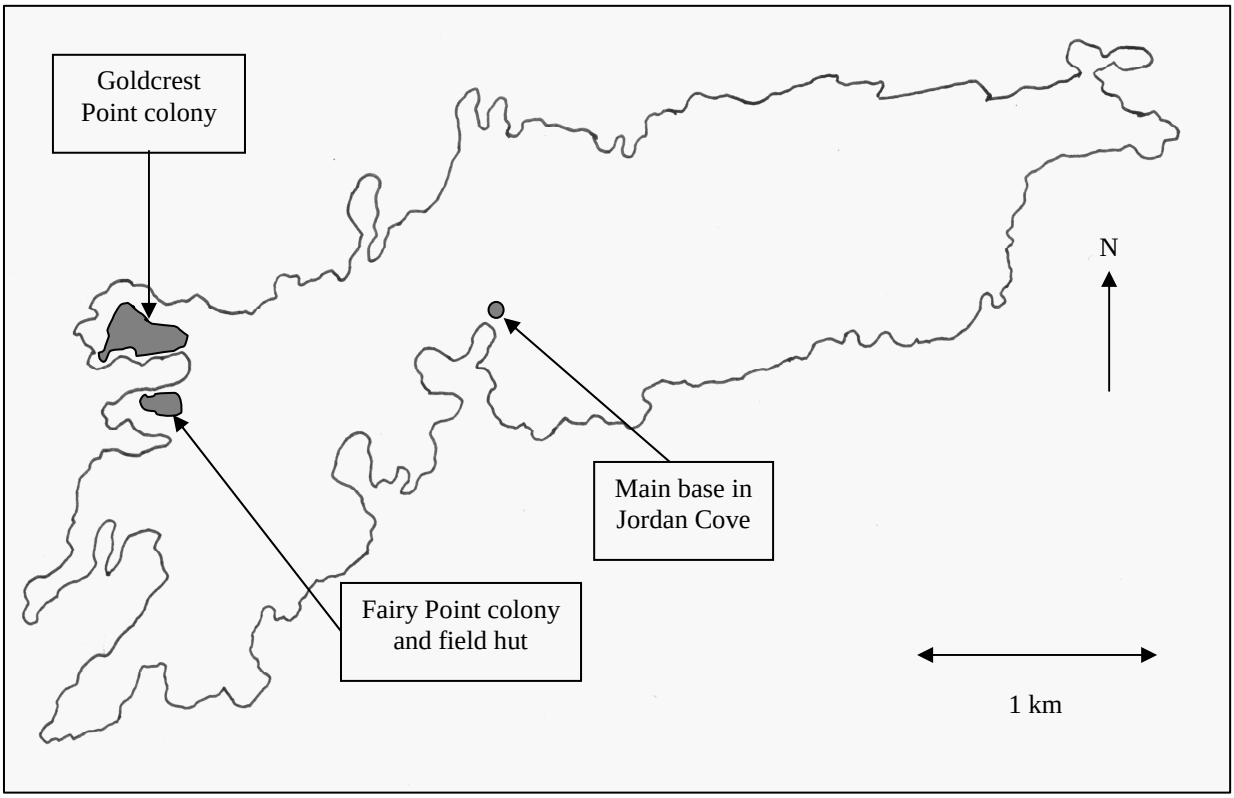

Figure 1.4. Map of Bird Island showing location of main base and study sites.

References

Ancel A., Horning M., and Kooyman G.L. (1996). Prey ingestion revealed by oesophagus and stomach temperature recordings in cormorants. Journal of Experimental Biology **200**: 149-154

Andersen H.T. (1966). Physiological adaptations in diving vertebrates. Physiological Reviews **46**: 212-243

Arnould J.P.Y., Boyd I.L., and Speakman J.R. (1996a). The relationship between foraging behaviour and energy expenditure in Antarctic fur seals. Journal of Zoology **239**: 769-782

Arnould J.P.Y., Briggs D.R., Croxall J.P., Prince P.A., and Wood A.G. (1996b). The foraging behaviour and energetics of wandering albatrosses brooding chicks. Antarctic Science **8**: 229-236

Ballance L.T. (1995). Flight energetics of free-ranging red-footed boobies (*Sula sula*). Physiological Zoology **68**: 887-914

Bamford O.S., and Maloiy G.M.O. (1980). Energy metabolism and heart rate during treadmill exercise in the Marabou stork. Journal of Applied Physiology **49**: 491-496

Berrow S.D., and Croxall J.P. (1999). The diet of white-chinned petrels *Procellaria aequinoctialis* Linnaeus 1758, in years of contrasting prey availability at South Georgia. Antarctic Science **11**: 283-292

Bevan R.M., and Butler P.J. (1992). Cardiac output and blood flow distribution during swimming and voluntary diving of the tufted duck (*Athya Fuligula*). Journal of Experimental Biology **168**: 199-217

Bevan R.M., Woakes A.J., and Boyd I.L. (1994). The use of heart rate to estimate oxygen consumption of free ranging black-browed albatrosses *Diomedea Melanophrys*. Journal of Experimental Biology **193**: 119-137

Bevan R.M., Butler P.J., Woakes A.J., and Prince P.A. (1995a). The energy expenditure of free-ranging black-browed albatrosses. Philosophical Transactions of the Royal Society of London B **350**: 119-131

Bevan R.M., Speakman J.R., and Butler P.J. (1995b). Daily energy expenditure of tufted ducks:a comparison between indirect calorimetry, doubly labelled water and heart rate. Functional Ecology **9**: 40-47

Bevan R.M., Woakes A.J., Butler P.J., and Croxall J.P. (1995c). Heart rate and oxygen consumption of exercising gentoo penguins. Physiological Zoology **68**: 855-877

Bevan R.M., Boyd I.L., Butler P.J., Reid K., Woakes A.J., and Croxall J.P. (1997). Heart rates and abdominal temperatures of free-ranging South Georgian shags, *Phalocrocorax georgianus*. Journal of Experimental Biology **200**: 661-675

Bevan R.M., Bishop C.M., and Butler P.J. (1998). The physiology of polar birds. In: Portner H.O. and Playle R.C. (eds) Cold Ocean Physiology. Society of Experimental Biology seminar series, 66. Cambridge University Press, Cambridge

Birt-Friesen V.L., Montevecchi W.A., Cairns D.K., and Macko S.A. (1989). Activity-specific metabolic rates of free-living northern gannets and other seabirds. Ecology **70**: 357-367

Bond C.F., Douglas S.D., and Gilbert P.W. (1961). Effects of submergence on cardiac cycle and rate in aquatic and terrestrial birds. American Journal of Physiology **200**: 723-726

Bonner W.N., and Croxall J.P. (1988). An assessment of environmental impacts arising from scientific research and its logistic support at Bird Island, South Georgia. British Antarctic Survey, Cambridge

Boyd I.L. (1997). The behavioural and physiological ecology of diving. Trends in Evolution and Ecology **12**: 213-217

Boyd I.L., and Croxall J.P. (1996). Dive durations in pinnipeds and seabirds. Canadian Journal of Zoology **74**: 1696-1705

Boyd I.L., Arnould J.P.Y., Barton T., and Croxall J.P. (1994). Foraging behaviour of Antarctic fur seals during periods of contrasting prey abundance. Journal of Animal Ecology **63**: 703-713

Boyd I.L., Woakes A.J., Butler P.J., Davis R.W., and Williams T.M. (1995). Validation of heart rate and doubly labelled water as measures of metabolic rate during swimming in California Sea Lion. Functional Ecology **9**: 151-160

Boyd I.L., Bevan R.M., Woakes A.J., and Butler P.J. (1999). Heart rate and behaviour of fur seals: implications for measurement of field energetics. American Journal of Physiology **276**: H844-H857

Brown C.R. (1989). Energy requirements and food consumption of *Eudyptes* penguins at the Prince Edward Islands. Antarctic Science **1**: 15-21

Brown C.R., and Klages N.T.W. (1987). Seasonal and annual variation in diets of Macaroni (*Eudyptes chrysopholus*) and Southern rockhopper (*E. chrysocome chrysocome*) penguins at sub-antarctic Marion Island. Journal of Zoology **212**: 7-28

Butler P.J. (1988). The exercise response and the "classical" diving response during natural submersion in birds and mammals. Canadian Journal of Zoology **66**: 29-39

Butler P.J. (1993). To what extent can heart rate be used as an indicator of metabolic rate in free-living marine mammals. Symposium of the Zoological Society of London **66**: 317-332

Butler P.J., and Jones D.R. (1982). The comparitive physiology of diving in vertebrates. Advances in Comparitive Physiology and Biochemistry **8**: 179-364

Butler P.J., and Jones D.R. (1997). Physiology of diving of birds and mammals. Physiological Reviews **77**: 837-899

Butler P.J., and Woakes A.J. (1979). Changes in heart rate and respiratory frequency during natural behaviour of ducks, with particular reference to diving. Journal of Experimental Biology **79**: 283-300

Butler P.J., and Woakes A.J. (1984). Heart rate and aerobic metabolism in humboldt penguins, *Spheniscus Humboldti*, during voluntary diving. Journal of Experimental Biology **108**: 419-428

Butler P.J., and Woakes A.J. (1987). Heart rate in humans during underwater swimming with and without breath-hold. Respiration Physiology **69**: 387-399

Butler P.J., Turner D.L., Al-Wassia A., and Bevan R.M. (1988). regional distribution of blood flow during swimming in the tuffted duck (*Athya Fuligula*). Journal of Experimental Biology **135**: 461-472

Butler P.J., Woakes A.J., Boyd I.L., and Kanatous S. (1992). Relationship between heart rate and oxygen consumption during steady-state swimming in California sea lions. Journal of Experimental Biology **170**: 35-42

Butler P.J., Woakes A.J., and Bishop C.M. (1998). Behaviour and physiology of Svalbard Barnacle Geese *Branta leucopsis* during their autumn migration. Journal of Avian Biology **29**: 536-545

Buttemer W.A., Hayworth A.M., Weathers W.W., and Nagy K.A. (1986). Time-budget estimates of avian energy expenditure: Physiological and meteorological considerations. Physiological Zoology **59**: 131-149

Cairns D.K. (1992). Bridging the gap between ornithology and fisheries science:use of seabird data in stock assessment models. The Condor **94**: 811-824

Carbone C., and Houston A.I. (1994). Patterns in the diving behaviour of the pochard, *Aythya ferina*: a test of an optimality model. Animal Behaviour **48**: 457-465

Carbone C., De Leeuw J J, and Houston A.I. (1996). Adjustments in the diving time budgets of tufted duck and pochard: is there evidence for a mix of metabolic pathways? Animal Behaviour **51**: 1257-1268

Castellini M.A. (1988). Visualizing metabolic transitions in aquatic mammals: does apnea plus swimming equal "diving"? Canadian Journal of Zoology **66**: 40-44

Chappell M.A., Shoemaker V.H., Janes D.N., Bucher T.L., and Maloney S.K. (1993). Diving behaviour during foraging in breeding Adelie penguins. Ecology **74**: 1204-1215

Charnov E.L. (1976). Optimal Foraging, the Marginal Value Theorem. Theoretical Population Biology **9**: 129-136

Cooper J., and Woehler E.J. (1994). Consumption of Antarctic krill (*Euphausia superba*) by seabirds during summer in the Prydz Bay region, Antarctica. In: El-Sayed S.Z. (ed) Southern Ocean ecology: the BIOMASS perspective. Cambridge University Press, Cambridge

Costa D.P. (1988). Methods for studying the energetics of freely diving animals. Canadian Journal of Zoology **66**: 45-52

Costa D.P., and Gales N.J. (2000). Foraging energetics and diving behaviour of lactating New Zealand sea lions, *Phocarctos hookeri*. Journal of Experimental Biology **203**: 3655-3665

Croll D.A., Gaston A.J., Burger A.E., and Konnoff D. (1992). Foraging behaviour and physiological adaptation for diving in thick-billed murres. Ecology **73**: 344-356

Croxall J.P. (1984). Seabirds. In: Laws R.M. (ed) Antarctic Ecology. Academic Press, London

Croxall J.P. (ed) (1987). Seabirds: Feeding ecology and role in marine ecosystems. Cambridge University Press, Cambridge

Croxall J.P. (1995). Remote-recording of foraging patterns in seabirds and seals for studies of predator-prey interactions in marine systems. In: Skjoldal H.R., Hopkins C., Erikstad K.E. and Leinaas H.P. (eds) The Ecology of Fjords and Coastal Waters. Elsevier Science B.V., Amsterdam

Croxall J.P., and Prince P.A. (1987). Seabirds as predators on marine resources, especially krill, at South Georgia. In: Croxall J.P. (ed) Seabirds: Feeding ecology and role in marine ecosystems. Cambridge University Press, Cambridge

Croxall J.P., Ricketts C., and Prince P.A. (1984). Impact of seabirds on marine resources, especially krill, of South Georgia waters. In: Whittow G.C. and Rahn H. (eds) Seabird Energetics. Plenum Press, New York

Croxall J.P., Davis R.W., and O'Connell M.J. (1988). Diving patterns in relation to diet of gentoo and macaroni penguins at South Georgia. The Condor **90**: 157-167

Croxall J.P., Naito Y., Kato A., Rothery P., and Briggs D.R. (1991). Diving patterns and performance in the Antarctic blue-eyed shag *Phalacrocorax atriceps*. Journal of Zoology **225**: 177-199

Croxall J.P., Briggs D.R., Kato A., Naito Y., Watanuki Y., and Williams T.D. (1993). Diving pattern and performance in the macaroni penguin *Eudyptes chrysolophus*. Journal of Zoology **230**: 31-47

Croxall J.P., Hall A.J., Hill H.J., North A.W., and Rodhouse P.G. (1995). The food and feeding ecology of the white-chinned petrel *Procellaria aequinoctialis* at South Georgia. Journal of Zoology **237**: 133-150

Croxall J.P., Prince P.A., and Reid K. (1997). Dietary segregation of krill-eating South Georgian seabirds. Journal of Zoology **242**: 531-536

Croxall J.P., Reid K., and Prince P.A. (1999). Diet, Provisioning and productivity responses of marine predators to differences in availability of Antarctic krill. Marine Ecology Progress Series **177**: 115-131

Culik B.M., and Luna-Jorquera G. (1997). Satellite tracking of Humboldt penguins (*Sphesniscus humboldti*) in northern chile. Marine Biology **128**: 547-556

Culik B.M., Wilson R.P., and Bannasch R. (1994). Underwater swimming at low energetic costs by pygoscelid penguins. Journal of Experimental Biology **197**: 65-78

Culik B.M., Pütz K., Wilson R.P., Allers D., Lage J., Bost C.-A., and Le Maho Y. (1996a). Diving energetics in king penguins (*Aptenodytes patagonicus*). Journal of Experimental Biology **199**: 973-983

Culik B.M., Pütz K., Wilson R.P., Bost C.-A., Le Maho Y., and Verselin J.-L. (1996b). Core temperature variability in diving king penguins (*Aptenoydytes patagonicus*) a preliminary analysis. Polar Biology **16**: 371-378

Davis R.W., Kooyman G.L., and Croxall J.P. (1983). Water Flux and estimated metabolism of free-ranging gentoo and macaroni penguins at South Georgia. Polar Biology **2**: 41-46

Davis R.W., Croxall J.P., and O'Connell M.J. (1989). The reproductive energetics of gentoo (*Pygoscelis papua*) and macaroni (*Eudyptes chrysolophus*) penguins at South Georgia. Journal of Animal Ecology **58**: 59-74

Entwistle A.C., Speakman J.R., and Racey P.A. (1994). Effect of using doubly labelled water techniques on long-term recapture in the brown long-eared bat (*Plecotus auritus*). Canadian Journal of Zoology **72**: 783-785

Everson I. (ed) (2000). Krill: Biology, Ecology and Fisheries. Blackwell Science, Oxford

Fedak M.A., and Thompson D. (1993). Behavioural and physiology options in diving seals. Symposium of the Zoological Society of London **66**: 333-348

Fedak M.A., Pullen M.R., and Kanwisher J. (1988). Circulatory responses of seals to periodic breathing: heart rate and breathing during exercise and diving in the laboratory and open sea. Canadian Journal of Zoology **66**: 53-60

Feldkamp S.D., Delong R.L., and Antonelis G.A. (1989). Diving patterns of Californa sea lions. Canadian Journal of Zoology **67**: 872-883

Fisher R., Gilbert F.F., and Robinette J.D. (1987). Heart rate as an indicator of oxygen consumption in the pine marten (*Martes americana*). Canadian Journal of Zoology **65**: 2085-2089

Flynn R.K., and Gessaman J.A. (1979). An evaluation of heart rate as a measure of daily metabolism in pigeons (*Columba livia*). Comparative Biochemistry and Physiology A **63**: 511-514

Froget G., Butler P.J., Handrich Y., and Woakes A.J. (2001). Heart rate as an indicator of oxygen consumption: influence of body condition in the king penguin. Journal of Experimental Biology **204**: 2133-2144

Furness R.W. (1978). Energy requirements of seabird communities: a bioenergetics model. Journal of Animal Ecology **47**: 39-53

Furness R.W., and Camphuysen C.J. (1997). Seabirds as monitors of the marine environment. ICES Journal of Marine Science **54**: 726-737

Furness R.W., and Monaghan P. (1987). Seabird Ecology. Blackie, Glasgow

Gabrielsen G.W. (1996). Energy expenditure of breeding Common Murres. In: Montevecchi W.A. (ed) Studies of high latitude seabirds 4: trophic relationships and energetics of endotherms in cold oceanic systems. Canadian Wildlife Service Occasional Paper No. 91, Ottawa

Gales R. (1989). Validation of the use of tritiated water, doubly labelled water and 22Na for estimating food, energy, and water intake in little penguins, *Eudyptula minor*. Physiological Zoology **62**: 147-169

Garthe S., Grémillet D., and Furness R.W. (1999). At-sea activity and foraging efficency in chick-rearing northern gannets *Sula bassana*: a case study in Shetland. Marine Ecology Progress Series **185**: 93-99

Gentry R.L., Kooyman G.L., and Goebel M.E. (1986). Feeding and diving behaviour of Northern fur seals. In: Gentry R.L. and Kooyman G.L. (eds) Fur seals: maternal strategies on land and at sea. Princeton University Press, Princeton, NJ

Gessaman J.A. (1980). An evaluation of heart rate as an indirect meaure of daily energy metabolism of the american kestrel. Comparative Biochemistry and Physiology A **65**: 273-289

Ghebresmeskel K., Williams T.D., Williams G., Gardner D.A., and Crawford M.A. (1991). Plasma metabolite in macaroni penguins (*Eudyptes chrysolophus*) arriving on land for breeding and moulting. Comparative Biochemistry and Physiology A **99**: 245-250

Goldstein D.L. (1988). Estimates of daily energy expenditure in birds: The time-energy budget as an integrator of laboratory and field studies. American Zoologist **28**: 829-844

Granadeiro J.P., Monteiro L.R., and Furness R.W. (1998). Diet and feeding ecology of Cory's shearwater *Calonectris diomedea* in the Azores, north-east Atlantic. Marine Ecology Progress Series **166**: 267-276

Grémillet D.J.H., Schmid D., and Culik B.M. (1995). Energy requirements of breeding great cormorants *phalacrocorax carbo sinensis*. Marine Ecology Progress Series **121**: 1-9

Grubb B., Jorgensen D.D., and Conner M. (1983). Cardiovascular changes in the exercising emu. Journal of Experimental Biology **104**: 193-201

Haggarty P., and McGaw B.A. (1988). Non-restrictive methods for measuring energy expenditure. Proceedings of the Nutrition Society **47**: 365-374

Haggarty P., McGaw B.A., Fuller M.F., Christie S.L., and Wong W.W. (1991). Water hydrogen incorporation into body fat in pigs: effect on double/triple-labelled water method. American Journal of Physiology **260**: R627-R634

Handrich Y., Bevan R.M., Charrassin J.-B., Butler P.J., Pütz K., Woakes A.J., Lage J., and Le Maho Y. (1997). Hypothermia in foraging king penguins. Nature **388**: 64-67

Hawkins P.A.J. (1995) . Ph.D. Thesis, University of Birmingham.

Hawkins P.A.J., Butler P.J., Woakes A.J., and Speakman J.R. (2000). Estimation of the rate of oxygen consumption of the common eider duck (*Somateria mollissima*) with some measurements of heart rate during voluntary dives. Journal of Experimental Biology **203**: 2819-2832

Heldmaier G., and Ruf T. (1992). Body Temperature and metabolic rate during natural hypothermia in endotherms. Journal of Comparative Physiology B **162**: 696-706

Hindell M.A., and Lea M.-A. (1998). Heart rate, swimming speed, and estimated oxygen consumption of a free-ranging southern elephant seal. Physiological Zoology **71**: 74-84

Hindell M.A., Slip D.J., Burton H.R., and Bryden M.M. (1992). Physiological implications of continuous, prolonged, and deep dives of the southern elephant seal (*Mirounga leonina*). Canadian Journal of Zoology **70**: 370-379

Holter J.B., Urban W.E., Hayes H.H., and Silver H. (1976). Predicting metabolic rate from telemetered heart rate in white-tailed deer. Journal of Wildlife Management **40**: 626-629

Houston A.I., and Carbone C. (1992). The optimal allocation of time during the diving cycle. Behavioral Ecology **3**: 255-265

Hull C.L. (1997). The effect of carrying devices on breeding royal penguins. The Condor **99**: 530-534

Hunter I., Croxall J.P., and Prince P.A. (1982). The distribution and abundance of burrowing seabirds (Procelliariiformes) at Bird Island, South Georgia: I. Introduction and methods. British Antarctic Survey Bulletin **56**: 49-67

Kam M., and Degen A.A. (1997). Energy budgets in free-living animals: a novel approach based on the doubly labelled water method. American Journal of Physiology **272**: R1336-R1343

Kato A., Naito Y., Watanuki Y., and Shaughnessy P.D. (1996). Diving pattern and stomach temperature of foraging king cormorants at subantarctic Macquarie Island. The Condor **98**: 844-848

Kock K.-H., Wilhelms S., Everson I., and Gröger J. (1994). Variations in the diet composition and feeding intensity of mackerel icefish *Champsocephalus gunnari* at South Georgia (Antarctic). Marine Ecology Progress Series **108**: 43-57

Konarzewski M., Taylor J.R.E., and Gabrielsen G.W. (1993). Chick energy requirments and adult energy expenditure of dovekies (*Alle alle*). The Auk **110**: 343-353

Kooyman G.L., and Ponganis P.J. (1998). The physiological basis of diving to depth: Birds and mammals. Annual Review of Physiology **60**: 19-32

Kooyman G.L., Wahrenbrock E.A., Castellini M.A., Davis R.W., and Sinnett E.E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. Journal of Comparative Physiology B **138**: 335-346

Kooyman G.L., Castellini M.A., Davis R.W., and Maue R.A. (1983). Aerobic dive limits in immature Weddell seals. Journal of Comparative Physiology b **138**: 335-346

Kooyman G.L., Davis R.W., and Croxall J.P. (1986). Diving behavior of Antarctic fur seals. In: Gentry R.L. and Kooyman G.L. (eds) Fur seals: maternal strategies on land and at sea. Princeton University Press, Princeton, NJ

Kooyman G.L., Ponganis P.J., Castellini M.A., Ponganis E.P., Ponganis K.V., Thorson P.H., Eckert S.A., and Le Maho Y. (1992). Heart rate and swim speeds of emperor penguins diving under sea ice. Journal of Experimental Biology **165**: 161-180

Kramer D.L. (1988). The behavioral ecology of air breathing by aquatic animals. Canadian Journal of Zoology **66**: 89-94

Krasnov J.V., and Barrett R.T. (1995). Large-scale interactions among seabirds, their prey and humans in the Barents Sea. In: Skjoldal H.R., Hopkins C., Erikstad K.E. and Leinaas H.P. (eds) Ecology of Fjords and Coastal Waters. Elsevier Science B.V., Amsterdam

Krebs J.R., and Davies N.B. (1993). An introduction to behavioural ecology. Blackwell Scientific Publications, Oxford

Le Beouf B.J., Crocker D.E., Grayson J., Gedamke J., Webb P.M., Blackwell S.B., and Costa D.P. (2000). Respiration and heart rate at the surface between dives in northern elephant seals. Journal of Experimental Biology **203**: 3265-3274

Lifson N., and McClintock R. (1966). Theory of use of the turnover rates of body water for measuring energy and material balance. Journal of Theoretical Biology **12**: 46-74

Livingstone M.B.E., Prentice A.M., Coward W.A., Ceesay S.M., Strain J.J., McKenna P.G., Nevin G.B., Barker M.E., and Hickey R.J. (1990). Simultaneous measurement of free-living energy expenditure by the doubly labelled water method and heart-rate monitoring. American Journal of Clinical Nutrition **52**: 59-65

Löfgren L. (1984). Ocean birds: their breeding, biology and behaviour. Croom Helm, London

Lovvorn J.R., and Jones D.R. (1991). Body mass, volume, and bouyancy of some aquatic birds, and their relation to locomotor strategies. Canadian Journal of Zoology **69**: 2888-2892

Lydersen C., Ryg M.S., Hammill M.O., and O'Brien P.J. (1992). Oxygen stores and aerobic dive limit of ringed seals (*Phoca hispida*). Canadian Journal of Zoology **70**: 458-461

Marchant S., and Higgins P.J. (eds) (1990). Handbook of Australian, New Zealand and Antarctic Birds, vol 1. Oxford University Press, Australia, Melbourne

McCafferty D.J., Boyd I.L., Walker T.R., and Taylor R.I. (1998). Foraging responses of Antarctic fur seals to changes in the marine environment. Marine Ecology Progress Series **166**: 285-299

Mehlum F., and Gabrielsen G.W. (1995). Energy expenditure and food consumption by seabird populations in the Barents Sea region. In: Skjoldal H.R., Hopkins C., Erikstad K.E. and Leinaas H.P. (eds) Ecology of Fjords and Coastal Waters. Elsevier Science B.V., Amsterdam

Midwood A.J., Haggarty P., and McGaw B.A. (1993). The doubly labelled water method: errors due to deuterium exchange and sequestration in ruminants. American Journal of Physiology **264**: R561-R567

Millard R.W., Johansen K., and Milsom W.K. (1973). Radiotelemetry of cardiovascular responses to exercise and diving in penguins. Comparative Biochemistry and Physiology A **46**: 227-240

Monaghan P. (1996). Relevance of the behaviour of seabirds to the conservation of marine environments. Oikos **77**: 227-237

Monaghan P., Wright P.J., Bailey M.C., Uttley J.D., Walton P., and Burns M.D. (1996). The influence of changes in food abundance on diving and surface-feedin seabirds. In: Montevecchi W.A. (ed) Studies of high latitude seabirds 4: trophic relationships and energetics of endotherms in cold oceanic systems. Canadian Wildlife Occasional Paper, No. 91, Ottawa

Murphy E.J., Watkins J.L., Reid K., Trathan P.N., Everson I., Croxall J.P., Priddle J., Brandon M.A., Brierley A.S., and Hofman E. (1998). Interannual variability of the South Georgia marine ecosystem: biological and physical sources of variation in the abundance of krill. Fisheries Oceanography **7**: 381-390

Nagy K.A. (1980). CO2 production in animals: analysis of potential errors in the doubly labeled water method. American Journal of Physiology **238**: R466-R473

Nagy K.A. (1989). Field bioenergetics: Accuracy of models and methods. Physiological Zoology **62**: 237-252

Nagy K.A., and Costa D.P. (1980). Water flux in animals: analysis of potential errors in the tritiated water method. American Journal of Physiology **238**: R454-R465

Nagy K.A., Siegfried W.R., and Wilson R.P. (1984). Energy utilization by free-ranging jackass penguins, *Spheniscus demersus*. Ecology **65**: 1648-1655

Nolet B.A., Butler P.J., Masman D., and Woakes A.J. (1992). Estimation of the daily energy expenditure from heart rate and doubly labeled water in exercising geese. Physiological Zoology **65**: 1188-1216

Owen R.B.J. (1969). Heart rate, a measure of metabolism in blue-winged teal. Comparative Biochemistry and Physiology A **31**: 431-436

Pauls R.W. (1980). Heart rate as an index of energy expenditure in red squirrels (*Tamiasciurus hudsonicus). Comparative Biochemistry and Physiology A **67**: 409-418*

Ponganis P.J., and Kooyman G.L. (2000). Diving physiology of birds: a history of studies on polar species. Comparative Biochemistry and Physiology a **126**: 143-151

Ponganis P.J., Kooyman G.L., Baranov E.A., Thorson P.H., and Stewart B.S. (1997a). The aerobic submersion limit of Baikal seals, *Phoca sibirica*. Canadian Journal of Zoology **75**: 1323-1327

Ponganis P.J., Kooyman G.L., Starke L.N., Kooyman C.A., and Kooyman T.G. (1997b). Post-dive blood lactate concentrations in emperor penguins, *Aptenodytes forsteri*. Journal of Experimental Biology **200**: 1623-1626

Ponganis P.J., Kooyman G.L., Winter L.M., and Starke L.N. (1997c). Heart rate and Plasma lactate response during submerged swimming and trained diving in California sea lions, *Zapholus californianus*. Journal of Comparative Physiology B **197**: 9-16

Priddle J., Croxall J.P., Everson I., Heywood R.B., Murphy E.J., Prince P.A., and Sear C.B. (1988). Large-Scale Fluctuations in Distribution and Abundance of Krill - A Discussion of Possible Causes. In: Sahrhage D. (ed) Antarctic Ocean and Resources Variability. Springer-Verlag, Berlin

Reid K. (1995). The diet of Antarctic fur seals *Arctocephalus gazella* Peters 1875) during winter at South Georgia. Antarctic Science **7**: 241-249

Reid K., and Arnould J.P.Y. (1996). The diet of Antarctic fur seals *Arctocephalus gazella* during the breeding season at South Georgia. Polar Biology **16**: 105-114

Reid K., and Croxall J.P. (2001). Environmental response of upper trophic-level predators reveals a system change in an Antarctic marine ecosystem. Proceedings of the Royal Society London B **268**: 1-8

Reid K., Croxall J.P., and Edwards T.M. (1997a). Interannual variation in the Diet of the Antarctic Prion *Pachyptila desolata* at South Georgia. The Emu **97**: 126-132

Reid K., Croxall J.P., Edwards T.M., Hill H.J., and Prince P.A. (1997b). Diet and feeding ecology of the diving petrels *Pelecanoides georgicus* and *P. urinatrix* at South Georgia. Polar Biology **17**: 17-24

Reid K., Barlow K.E., Croxall J.P., and Taylor R.I. (1999). Predicting changes in the Antarctic krill, *Euphausia superba*, population at South Georgia. Marine Biology **135**: 647-652

Reid K., Brierley A.S., and Nevitt G.A. (2000). An initial examination of relationships between the distribution of whales and Antarctic krill *Euphausia superba* at South Georgia. Journal of Cetacean Research and Management **2**: 143-149

Richards P.A., and Tickell W.L.N. (1968). Comparison between the weather at Bird Island and King Edward Point, South Georgia. British Antarctic Survey Bulletin **15**: 63-69

Sahrhage D. (ed) (1988). Antarctic ocean and resources variability. Springer-Verlag, Berlin

Schaefer M.B. (1970). Men, birds and anchovies in the Peru current - dynamic interactions. Transactions of the American Fish Society **9**: 461-467

Scholander P.F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. Hvalrådets Skrifter **22**: 1-131

Speakman J.R. (1993). How should we calculate CO2 production in doubly labelled water studies of animals? Functional Ecology **7**: 746-750

Speakman J.R. (1997). Doubly Labelled Water. Chapman & Hall, London

Speakman J.R., and Racey P.A. (1986). Measurement of CO2 production by the doubly labelled water technique. American Journal of Physiology **61**: 1200-1202

Stephenson P.J., Speakman J.R., and Racey P.A. (1994). Field metabolic rate in two species of shrew-tenrec, *Microgale dobsoni* and *M. talazaci*. Comparative Biochemistry and Physiology A **107**: 283-287

Stephenson R., Butler P.J., and Woakes A.J. (1986). Diving behaviour and heart rate in tufted ducks *Aythya fuligula*. Journal of Experimental Biology **126**: 341-359

Stephenson R., Hedrick M.S., and Jones D.R. (1992). Cardiovascular responses to diving and involuntary submergence in the rhionceros auklet (*Cerorhinca monocerata* Pallas). Canadian Journal of Zoology **70**: 2203-2210

Stöhr W. (1988). Longterm heart rate telemetry in small mammals: A comprehensive appraoch as a prerequisite for valid results. Physiology & Behavior **43**: 567-576

Thomas D.W., Martin K., and Lapierre H. (1994). Doubly labelled water measurements of field metabolic rate in White-tailed Ptarmigan: variation in background isotope abundances and effect on CO2 production estimates. Canadian Journal of Zoology **72**: 1967-1972

Thompson D., and Fedak M.A. (1993). Cardiac responses of grey seals during diving at sea. Journal of Experimental Biology **174**: 139-164

Thompson D., Hiby A.R., and Fedak M.A. (1993). How fast should I swim? Behavioural implications of diving physiology. Symposium of the Zoological Society of London **66**: 349-368

Uttley J., Tatner P., and Monaghan P. (1994). Measuring the Daily Energy Expenditure of Free-living Arctic Terns (*Sterna paradisaea*). The Auk **111**: 453-459

Walker T.R., Boyd I.L., McCafferty D.J., Huin N., Taylor R.I., and Reid K. (1998). Seasonal occurrence and diet of leopard seals *Hydrurga leptonyx) at Bird Island, South Georgia. Antarctic Science **10**: 75-81*

Wanless S., Corfield T., Harris M.P., Buckland S.T., and Morris J.A. (1993). Diving behaviour of the shag *Phalacrocorax aristotelis* (Aves: Pelecaniformes) in relation to water depth and prey size. Journal of Zoology **231**: 11-25

Wanless S., Finney S.K., Harris M.P., and McCafferty D.J. (1999). Effect of the diel light cycle on the diving behaviour of two bottom feeding marine birds: the blue-eyed shag *Phalacrocorax atriceps* and the European shag *P. aristotelis*. Marine Ecology Progress Series **188**: 219-224

Ward D.H., and Flint P.L. (1995). Effect of harness-attached transmitters on premigration and reproduction of brant. Journal of Wildlife Management **59**: 39-46

Watanuki Y., Kato A., Naito Y., Robertson G., and Robinson S. (1997). Diving and foraging behaviour of Adélie penguins in areas with and without fast sea-ice. Polar Biology **17**: 296-304

Weathers W.W., and Nagy K.A. (1980). Simultaneous doubly lablled water (3HH18O) and time-budget estimates of daily energy expenditure *Phainopepla nitens*. The Auk **97**: 861-867

Westerterp K.R., Brouns F., Saris W.H.M., and Foppe T.H. (1988). Comparison of doubly labelled water with respirometry at low- and high-activity levels. American Journal of Physiology **65**: 53-56

Williams A.J. (1981). The clutch size of macaroni and rockhopper penguins. The Emu **81**: 87-90

Williams J.B., and Nagy K.A. (1984). Daily energy expenditure of savannah sparrows: comparison of time-energy budget and doubly-labelled water estimates. The Auk **101**: 221-229

Williams T.D. (1989). Agression, incubation behaviour and egg-loss in macaroni penguins, *Eudyptes chrysolophus*, at South Georgia. Oikos **55**: 19-22

Williams T.D. (1991). Foraging ecology and diet of Gentoo Penguins *Pygoscelis papua* at South Georgia during winter and an assessment of their winter prey consumption. Ibis **133**: 3-13

Williams T.D. (1995). The Penguins. Oxford University Press, Oxford

Williams T.D., and Croxall J.P. (1991). Annual variation in breeding biology of macaroni penguins, *Eudyptes chrysolophus*, at Bird Island, South Georgia. Journal of Zoology **223**: 189-202

Williams T.D., Briggs D.R., Croxall J.P., Naito Y., and Kato A. (1992). Diving pattern and performance in relation to foraging ecology in the gentoo penguin, *Pygoscelis papua*. Journal of Zoology **227**: 211-230

Williams T.M., Friedl W.A., and Haun J.E. (1993). The physiology of bottlenose dolphins (*Tursiops truncatus*): heart rate, metabolic rate and plama lactate concentration during exercise. Journal of Experimental Biology **179**: 31-46

Williams T.M., Haun J.E., and Friedl W.A. (1999). The diving physiology of bottlenose dolphins (*Tursiops truncatus*) I. Balancing the demands of exercise for energy conservation at depth. Journal of Experimental Biology **202**: 2739-2748

Williams T.M., Davis R.W., Fuiman L.A., Francis J., Le Bouef B.J., Horning M., Calambokidis J., and Croll D.A. (2000). Sink or swim: strategies for cost-efficient diving by marine mammals. Science **288**: 133-136

Wilson R.P., Grant W.S., and Duffy D.C. (1986). Recording devices on free ranging marine animals: Does measurement affect performance? Ecology **67**: 1091-1093

Wilson R.P., Pütz K., Bost C.A., Culik B.M., Bannasch R., Reins T., and Adelung D. (1993). Diet dive depth in penguins in relation to diel vertical migration of prey: whose dinner by candlelight? Marine Ecology Progress Series **94**: 101-104

Woakes A.J., and Butler P.J. (1983). Swimming and diving in tufted ducks, *Aythya fuligula*, with particular reference to heart rate and gas exchange. Journal of Experimental Biology **107**: 311-329

Woakes A.J., Butler P.J., and Bevan R.M. (1995). Implantable data logging system for heart rate and body temperature: Its application to the estimation of field metabolic rates in Antarctic predators. Medical & Biological Engineering & Computing **33**: 145-151

Woehler E.J. (1993). The distribution and abundance of Antarctic and subantarctic penguins. Scientific Committee of Antarctic Reseach (SCAR) bird Biology Sub-Committee, Cambridge, UK

Woehler E.J. (1997). Seabird abundance, biomass and prey consumption within Prydz Bay, Antarctica, 1980/1981-1992/1993. Polar Biology **17**: 371-383

Wooley J.B.J., and Owen R.B.J. (1977). Metabolic rates and heart rate-metabolism relationships in the Black Duck (*Anas rubipes*). Comparative Biochemistry and Physiology A **57**: 363-367

Ydenberg R.C., and Clark C.W. (1989). Aerobiosis and anaerobiosis during diving by western grebes: an optimal foraging approach. Journal of Theoretical Biology **139**: 437-449

Young E. (1994). Skua and penguin: predator and prey. Cambridge University Press, Cambridge

Chapter 2

Heart rate and rate of oxygen consumption of exercising macaroni penguins

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Summary

Twenty-four Macaroni penguins (*Eudyptes chrysolophus*) from three groups: breeding males (n=9), breeding females (n=9) and moulting females (n=6) were exercised on a variable speed treadmill. Heart rate (f_H) and mass specific rate of oxygen consumption ($s\dot{V}_{O_2}$) were recorded from the animals, and both f_H and $s\dot{V}_{O_2}$ were found to increase linearly with increasing treadmill speed. A linear regression equation described the relationship between f_H and $s\dot{V}_{O_2}$ for each individual. There were no significant differences in these regressions between breeding and moulting females. There were significant differences in these relationships between females and breeding males. f_H and $s\dot{V}_{O_2}$ were recorded from five of these animals for a total of 24 hours. When f_H was used to predict $s\dot{V}_{O_2}$ for the 24 hours with the derived regressions, the estimate was not significantly different from the measured values with an average error of –2.1%. When f_H was used to predict $s\dot{V}_{O_2}$ for the five minute intervals used for the calibration in all 24 birds, the estimate was not significantly different from the observed values, and the average error only +0.47%., Since the $f_H / s\dot{V}_{O_2}$ relationship was the same during periods of the annual cycle when animals were inactive/fasting and active/foraging, it seems reasonable that as long as sex differences are taken into account, that f_H can be used to predict metabolic rates of free ranging macaroni penguins all year round.

Introduction

Approximately 80% of the avian biomass in the Antarctic region is made up of penguins (Croxall 1984). In the Southern Ocean around the island of South Georgia, large resident populations of penguins (Woehler 1993) are thought to be significant consumers of marine resources (Croxall et al. 1997). These resources include Antarctic krill (*Euphausia superba*) which is the major food item for several species including Antarctic fur seal (*Arctocephalus gazella*) (Reid 1995, Reid and Arnould 1996) and gentoo penguin (*Pygoscelis papua*) (Croxall et al. 1997) and also has the potential to form a major fishery. Most models that estimate population food consumption are based on energetics (Croxall 1995). Thus, accurate estimates of energy expenditure are essential. The energy budgets of individuals that are used to construct population energy budgets and food consumption rates should, ideally, be sensitive to changes in behaviour so that different classes of individuals (e.g. adults, juveniles, breeders, non-breeders) can be reflected in the energy budget. To avoid the need to measure energy expenditures in all of these classes of individuals it is important to be able to construct energy budgets from activity budgets. This requires the measurement of the energy expenditures associated with the major classes of activity, such as swimming, diving, feeding, walking, incubating and moulting.

Several studies have attempted to obtain the energy costs of specific activities in penguins, either in the laboratory (Pinshow et al. 1976, Butler and Woakes 1984, Baudinette and Gill 1985, Culik and Wilson 1991), or in the field through the use of doubly labelled water (DLW) (Davis et al. 1983, Nagy et al. 1984, Costa et al. 1986, Nagy and Obst 1992). DLW can give a reasonably accurate estimate of energy expenditure for an animal, and is relatively simple to use in the field. However, the estimate obtained is only an average for the duration of the experiment, which is limited to a few days in penguins by the biological half-life of the injected stable isotopes (Speakman 1997). Despite refinements to this technique (Davis et al. 1989, Culik and Wilson 1991, Gales et al. 1993) it is still difficult to obtain estimates of rates of energy expenditure for specific activities such as foraging, sustained swimming or bouts of diving.

More recently heart rate (f_H) has been used as a proxy for the measurement of metabolic rate (Owen 1969, Nolet et al. 1992, Bevan et al. 1994, 1995c, Boyd et al. 1995, Butler et al. 1995). Heart rate and rate of oxygen consumption ($\dot{V}_{O_2}$) are related to each other, as illustrated by the Fick equation (equation 2.1).

$$\dot{V}_{O_2} = f_H \times \dot{V}_s (C_a O_2 - C_{\bar{v}} O_2) \quad (2.1)$$

(where $\dot{V}_{O_2}$ = rate of oxygen consumption, f_H = heart rate, $\dot{V}_s$ = cardiac stroke volume (amount of blood pumped during one heart beat), C_aO_2 = oxygen content of arterial blood and $C_{\bar{v}}O_2$ = oxygen content of mixed venous blood.)

If $\dot{V}_s(C_aO_2 - C_{\bar{v}}O_2)$, the oxygen pulse, remains constant or varies systematically, then it is possible to demonstrate a simple relationship between $\dot{V}_{O_2}$ and f_H and hence calculate the former from the latter (Butler 1993). Studies have shown that this method is at least as robust as DLW for most species, including gentoo penguins (Bevan et al. 1995c). Recent developments in technology with regard to electronic miniaturisation have made it possible to monitor and record f_H in free-ranging animals over long time periods (Woakes et al. 1995). Heart rate is then a feasible and useful technique for estimating metabolic rate for extended periods of up to a year at a resolution of just a few minutes. However, the success of this technique depends on the robustness of the relationship between f_H and $\dot{V}_{O_2}$. Ideally, validation is required at different times of year (Flynn and Gessaman 1979) under differing social conditions (Holter et al. 1976), for different sexes and for all types of activities (Woakes and Butler 1983) so that any appropriate corrections can be made.

The present study tests the hypothesis that heart rate can be used as a proxy of metabolic rate in macaroni penguins (*Eudyptes chrysolophus*). The aims are to: (1) measure f_H and $\dot{V}_{O_2}$ of penguins walking on a treadmill and determine the relationship between these two variables; (2) develop and refine the statistical methods used to describe this relationship and the errors associated with its use; (3) determine whether the relationship is valid over an extended period in the laboratory; (4) determine whether this relationship is significantly different at

different stages of the annual cycle when the animals may be experiencing different physiological stresses.

Materials and methods

Animals

Although the United Kingdom Animal (Scientific Procedures) Act 1986 does not apply to South Georgia where this study was conducted, we were meticulous in following its provisions, especially those set out by the Home Office in the Official Guidance on the operation of the Act. As our benchmark, we followed guidance to researchers using similar methods in the United Kingdom. Our procedures also conformed to the Code of Ethics of Animal Experimentation in Antarctica.

All experiments were performed at the British Antarctic Survey base at Bird Island, South Georgia, during the austral summer of 1998/99. Twenty-four macaroni penguins were caught from the colonies on the north side of the island and transported back to the base. Animals were sexed using bill size measurements, a reliable method for this species (Williams and Croxall 1991). Nine male (mean mass = 3.89 kg) and nine female penguins (mean mass = 3.26 kg) were used during the early part of the breeding season (late November to early January) and six further females (mean mass = 4.25 kg) were used during the middle of their moult (late March). All penguins were kept overnight prior to the experiment, in an outdoor enclosure, without food, but with access to water, to ensure that they were post-absorptive.

Experimental apparatus

An open circuit respirometry system similar to that used on gentoo penguins (Bevan et al. 1995c) was used to measure rates of oxygen consumption and carbon dioxide production. A Perspex respirometer was fixed to a variable-speed treadmill (model EG10, Powerjog, Sports Engineering Ltd). The respirometer was equipped with three fans in a side compartment that ensured good and rapid mixing of air. Brush-style draught excluders ensured a good fit between a wooden frame, fixed to the treadmill frame, and the treadmill belt, while foam

rubber seals ensured an air tight junction between the respirometer and wooden frame. Air was drawn through the respirometer using an air pump (B105, Charles Austen) at about 60 l min^{-1}, measured using an electronic flowmeter (100 Flo-Sen, McMillan Co.) which was calibrated at the beginning, middle and end of the series of experiments using two 40 l min^{-1} variable area flowmeters (Fisher Controls 1100). A subsample of the outlet air flow was passed, via a container of drying agent (silica gel), to an infrared CO_2 analyser (Servomex 1410) and then to a paramagnetic O_2 analyser (Servomex 570A). A solenoid valve (RS Components Ltd.) switched between sampling outlet and ambient air. The atmospheric pressure was measured using an electonic barometer pressure transducer (Farnell Electronic Services) and the humidity and temperature of both the outlet and ambient air were continuously monitored using suitable sensors (Farnell Electronic Services). The O_2 and CO_2 analysers were calibrated with atmospheric air, nitrogen and a specially prepared 1% CO_2 in N_2 mixture (Air Products Ltd.).

The output signals from the O_2 and CO_2 analysers, humidity and temperature sensors, barometer and flowmeter were passed to a purpose built interface box that amplified the signals to a standard range of –10 V to +10 V. The amplified output voltages were passed to an analogue to digital converter unit (DAQPad-1200, National Instruments), then to a desktop computer (Viglen Genie Professional). The computer sampled the outputs at 1000 Hz, took a mean of these values and saved them to a file every 10 s with a program developed using a software package for automatic instrumentation (LabVIEW, National Instruments). The penguins were monitored on the treadmill using a closed circuit TV system, to avoid any disturbance caused by observers.

Heart rate was sampled using a miniature heart rate data logger (HRDL) designed for abdominal implantation (Woakes et al. 1995). This device samples and stores heart rate every 15 s. For animals during the breeding season, the data logger was mounted externally to avoid the post operative recovery time associated with implantation of these devices. Moulting birds were implanted, as the large amount of subcutaneous fat deposited as reserves for the moult made it impossible to obtain an electrocardiogram (e.c.g.) free of interference if the device was mounted externally. The birds were implanted 10 days before the calibration experiments which allowed a more than adequate recovery time (Bevan et al. 1995a). For external

deployment, the electrodes of the HRDL were replaced with 20 G hypodermic needles and the complete unit weighed 25 g. The HRDL was attached to the dorsal feathers using adhesive tape and the two needles inserted subcutaneously. The most satisfactory position was for one electrode to be positioned at the back of the neck, and the other underneath the left flipper. This position gave the best e.c.g. signal, free of interference caused by other electrical activity such as that associated with muscle contraction. As well as storing the heart rate data, the HRDL also emitted a radio signal when an e.c.g. was detected and this was picked up using a receiver (877R, International) tuned to 115 MHz. This signal was monitored at all times and could be counted, using a hand tally counter, to calculate a heart rate as a back up to the HRDL.

Protocol

Once equipped with a HRDL, the penguin was introduced into the respirometer. The bird was initially left for at least an hour to attain a resting metabolic rate, which was judged to occur when a stable value of rate of oxygen consumption had been observed for 25 min. After the resting measurement, the bird then walked on the treadmill at nine speeds, every 0.2 km h^{-1} from 0.2 to 1.8 km h^{-1}. The sequence of workloads was randomly assigned, though not every bird could walk at the highest speed. The penguins undertook three exercise periods, with 30 min rest between them. In each period, the penguins walked for 30 min at each of three different speeds. At each speed, limb frequency was determined by counting the number of steps taken by the right leg during three minutes. Measurements of heart rate, O_2, CO_2, humidity, temperature, pressure and flow were taken continuously throughout the duration of the experiment, but for calibration calculations the values from only the last five minutes of each rest and exercise period (when steady state conditions had been achieved) were used. Heart rate was also counted from the radio signal for this five minute period. After the final exercise period, the penguin was kept in the respirometer for at least one more hour to take a final resting rate in darkness. Five of the penguins remained in the respirometer overnight, for a total of 24 hours, in order to gather more data to validate the findings from the calibration runs. These penguins walked for a further hour at 1 km h^{-1} the next morning. The room used for respirometry had large windows and adequate ventilation and therefore conditions of light and temperature were very similar to the natural state in the colony. The average temperature

inside the respirometer from all experiments was 7.88 ± 0.25°C which was only slightly higher than the average temperature of 5.78 ± 0.39 °C outside the room used for respirometry, from where the ambient air was sampled.

Analysis and calculations

Rate of oxygen consumption was calculated using the equations of Depocas and Hart (Depocas and Hart 1957), modified by Withers (Withers 1977) (equations 2.2 and 2.3).

$$\dot{V}_{CO_2} = \dot{V}_{STPD} \times \frac{F_{CO_2Out} - F_{CO_2Amb}}{1-(1-\frac{F_{CO_2Out} - F_{CO_2Amb}}{F_{O_2Amb} - F_{O_2Out}} \times F_{CO_2Amb})} \quad (2.2)$$

$$\dot{V}_{O_2} = \frac{\dot{V}_{STPD} \times (F_{O_2Amb} - F_{O_2Out}) - \dot{V}_{CO_2} \times F_{O_2Amb}}{1 - F_{O_2Amb}} \quad (2.3)$$

(where $\dot{V}_{STPD}$ is the flow rate of air through the respirometer in ml min^{-1} corrected for standard temperature and pressure, dry and F_{O_2Out}, F_{O_2Amb}, F_{CO_2Out} and F_{CO_2Amb} are the fractional concentrations of O_2 and CO_2 in outlet and ambient air respectively.)

Rate of oxygen consumption $\dot{V}_{O_2}$ is also commonly expressed as mass specific $\dot{V}_{O_2}$ ($s\dot{V}_{O_2}$), in order to correct for the potential effects of individual differences in the mass of animals. A function based on the relationship between resting $\dot{V}_{O_2}$ and mass is used to modify $\dot{V}_{O_2}$ to give $s\dot{V}_{O_2}$.

The data obtained during the calibration experiments were used to derive an equation to estimate $s\dot{V}_{O_2}$ from heart rate. During the validation experiments, this equation was used to derive predicted values of $s\dot{V}_{O_2}$ over two time-scales. Firstly, $s\dot{V}_{O_2}$ was estimated for 30 min periods throughout the whole 24 h, to see whether short term changes in metabolic rate

could be accurately predicted. Secondly $s\dot{V}_{O_2}$ was estimated over the whole 24 h period to see whether longer term estimates of metabolic rate were accurate.

Statistics

Statistical testing was performed with the spreadsheet package Excel (Microsoft) and the statistical packages Minitab (Minitab Inc.) and Systat (SPSS Inc.). Least squares regressions were used to determine the relationships between two variables (e.g., f_H and $s\dot{V}_{O_2}$ for individual calibrations). Regression equations were compared using an analysis of variance general linear model (GLM) (Zar 1999). Student's *t*-test was used to compare the significance of any difference between the means of two populations. Analysis of variance (ANOVA) with Tukey post-hoc testing was used when more than two populations were compared. Results were considered significant at $P < 0.05$, and are quoted at the level at which they were found to be significant. All mean values are given ± S.E.

Results

Effect of mass

Resting $\dot{V}_{O_2}$ increased with body mass in kg. Over the range of values for mass in this experiment, the relationship was best described by a linear equation (equation 2.4).

$$\dot{V}_{O_2} = (\text{mass} \times 12.11) - 5.99, \qquad n = 24 \; r^2 = 0.53 \; (P < 0.001) \quad (2.4)$$

(where mass is in kg and $\dot{V}_{O_2}$ is in ml min-^{1})

A similar coefficient of determination was obtained when using the logarithms of both axes to investigate the power relationship between the two variables (r^2 = 0.52). The relationship between mass and resting $\dot{V}_{O_2}$ is often described by a power relationship (Schmidt-Nielsen 1984), especially where the range of body mass is great (typically several orders of

magnitude). In the present study however, the ranges of body mass and resting $\dot{V}_{O_2}$ were relatively small and since the aim is merely to correct for the potential effects of variation in mass then the linear relationship can be used to derive $s\dot{V}_{O_2}$. As such, $s\dot{V}_{O_2}$ is merely $\dot{V}_{O_2}$ divided by the mass of the animal in kg.

Calibrations

During rest periods, some penguins would preen or investigate the respirometer but most would usually stand hunched, a typical behaviour which was observed in the colony, or occasionally lie prone (on the belly). For all individuals the minimum values of f_H and $s\dot{V}_{O_2}$ occurred during these rest periods. If all resting values from all individuals were pooled into groups (breeding females, breeding males, moulting females), then the mean values of resting f_H and $s\dot{V}_{O_2}$ were significantly different (ANOVA; for f_H $F_{(2, 21)} = 10.39$, $P < 0.001$, for $s\dot{V}_{O_2}$ $F_{(2, 21)} = 3.47$, $P < 0.05$) (Table 2.1). Further post-hoc Tukey tests showed that resting f_H of the breeding males was significantly less than those for both breeding and moulting females ($P < 0.05$). However there was no difference in resting f_H between breeding and moulting females ($P > 0.05$). Resting $s\dot{V}_{O_2}$ of males was significantly less than that for moulting females ($P < 0.05$) but there were no significant differences in resting $s\dot{V}_{O_2}$ between breeding and moulting females and between breeding males and females ($P > 0.05$).

The fastest walking speed attained by the birds was usually 1.8 km h^{-1} except for two of the moulting birds that could only sustain 1.6 km h^{-1} Maximum values of f_H and $s\dot{V}_{O_2}$ occurred at either the fastest or second fastest walking speed. If the maximum values from each individual were pooled into groups and these mean maximum values were compared, there was no significant difference between maximum $s\dot{V}_{O_2}$ (ANOVA $F_{(2, 21)} = 0.97$, $P > 0.05$) (Table 2.1), but there was a significant difference between maximum f_H (ANOVA $F_{(2, 21)} = 4.72$, $P < 0.05$). Further post-hoc Tukey tests showed there was no difference in mean maximum f_H between breeding males and females, but both of these groups had a mean maximum f_H that was lower than that of moulting females. There was consistent variation

between groups in factorial increases in f_H and $s\dot{V}_{O_2}$ from resting to maximum activity (Table 2.2). For both f_H (ANOVA $F_{(2, 21)}$.= 4.33, P <0.05 with Tukey test) and $s\dot{V}_{O_2}$ (ANOVA $F_{(2, 21)}$.= 4.08, P <0.05 with Tukey test) the factorial increase from resting to maximum activity was not significantly different between breeding males and females but was significantly different between breeding males and moulting females

With the results from all birds pooled together, both, f_H and $s\dot{V}_{O_2}$ increased linearly with treadmill speed (Fig. 2.1). These relationships were both significant (P <0.001) (Equations 2.5 & 2.6).

$$f_H = (\text{treadmill speed} \times 33.48) + 109.15, \qquad n = 24 \; r^2 = 0.99 \; (P < 0.001) \quad (2.5)$$

(where f_H is in beats min^{-1} and treadmill speed is in km h^{-1})

$$s\dot{V}_{O_2} = (\text{treadmill speed} \times 10.66) + 14.02, \quad n = 24 \; r^2 = 0.98 \; (P < 0.001) \quad (2.6)$$

(where $s\dot{V}_{O_2}$ is in ml min^{-1} kg^{-1} and treadmill speed is in km h^{-1})

Heart rate and $s\dot{V}_{O_2}$ were well correlated in each individual (Fig. 2.2). These relationships were best described by a linear function, (Table 2.3 - mean $r^2 = 0.85 \pm 0.024$), and the fit was not improved by $\log_e$-transforming the data (mean $r^2 = 0.81 \pm 0.030$). ANCOVA (P >0.05) was used to compare the values of the constants a (the intercept) and b (the slope) of the individual regressions within each group. This analysis showed no significant differences between the slopes but did show significant differences between the intercepts within each group. Because the penguins selected were a random sample from a wider population, the intercepts were regarded as a random sample from a distribution of intercept values and a random effects model was adopted. An additional random term was therefore introduced into the model by allowing the intercept to be entered as a random effect in an appropriate analysis of covariance. The equations derived for each of the groups in the present study are:

Breeding males: $s\dot{V}_{O_2} = (0.33 \times f_H) - 18.77$ n = 9 $r^2 = 0.91$ (2.7)

Breeding females: $s\dot{V}_{O_2} = (0.30 \times f_H) - 17.40$ n = 9 $r^2 = 0.84$ (2.8)

Moulting females: $s\dot{V}_{O_2} = (0.27 \times f_H) - 20.94$ n = 6 $r^2 = 0.81$ (2.9)

(where $s\dot{V}_{O_2}$ is in ml min^{-1} kg^{-1} and f_H is in beats min^{-1}.)

The standard deviation (σ_0) of an estimate made using a regression equation can be used to calculate confidence intervals for the regression line [see Zar (1984) p 273 for definition]. However, in the present case, the presence of intercept as a random factor leads to the introduction of an additional error term in the calculation of σ_0 and the equation used to calculate it must be modified (equation 2.10).

$$\sigma_0 = \sqrt{d^2\left[\frac{1}{n_1}\right] + e^2\left[\frac{1}{n_2} + \frac{(X_i - \bar{X})^2}{\sum x^2}\right]} \qquad (2.10)$$

(where d^2 = the error associated with the variation between penguins, n_1 = the number of penguins used in the calibration process, e^2 = the error associated with the scatter around the regression lines, n_2 = the total number of data points in the regression, $\bar{X}$ = the mean value of heart rate used in the regression, X_i = the value of heart rate for which σ_0 is to be estimated, $\sum x^2$ = the sum of all the squared values of heart rate used in the regression.)

The value of d^2, the variance associated with the random distribution of intercepts, and e^2, the variance associated with the scatter of points around the individual regressions would tend to decrease if more penguins were used in the calibration process.

If the equation is to be used to estimate $s\dot{V}_{O_2}$ from an average value of f_H measured in the field, then a further additional error term is added [see Zar (1984) p 275 for definition] to account for the variation within the new individuals selected randomly from the field. In the case of this model, another error term is once again introduced to account for the variation

between the individuals selected randomly from the field. The standard error σ_1 of an estimate of $s\dot{V}_{O_2}$ made from an average value of f_H taken from the field is given by equation 2.11.

$$\sigma_1 = \sqrt{d^2\left[\frac{1}{n_1}+\frac{1}{n_3}\right]+e^2\left[\frac{1}{n_2}+\frac{1}{n_4}+\frac{\left(X_i - \overline{X}\right)^2}{\sum x^2}\right]} \qquad (2.11)$$

(where d^2 = the error associated with the variation between calibration penguins, n_1 = the number of penguins used in the calibration process, n_3 = the number of penguins from which the field value of heart rate was obtained, e^2 = the error associated with the scatter around the regression lines, n_2 = the total number of data points in the regression, n_4 = the number of data points from which the field value of heart rate was obtained, $\overline{X}$ = the mean value of heart rate used in the regression, X_i = the mean value of heart rate from the field from which σ_1 is to be estimated, $\sum x^2$ = the sum of squares (SS) of the values of heart rate used in the regression.)

If the values of n_3 and n_4 are set to 1, then equation 2.11 can be used to produce 95% prediction intervals for the regression (Fig. 2.3). As the values of n_1, n_3 and n_4 increase then σ_1 will tend to decrease as a proportion of the estimate (Fig. 2.4). The value of n_4 is dependent on the number of data points used to derive the average f_H and since f_H can be recorded over periods of five minutes for several days at a time in the field, the value of the error term involving n_4 will tend towards zero, increasing the accuracy of the estimate of $s\dot{V}_{O_2}$. This also illustrates the importance of recording f_H from as many individuals as possible in the field so that n_3 is high and the value of that error term will also tend towards zero (Fig. 2.4).

Random effects analysis of covariance can also be used to compare the three common equations and determine whether they are similar. If the intercepts are treated as random effects, and hence would potentially take different values if the whole experiment were to be repeated, there was a significant difference between the slopes for breeding males and breeding or moulting females ($P < 0.01$). However there was no significant difference in the slope between breeding and moulting females ($P > 0.05$). The individuals from breeding and moulting females were therefore considered together and a new common regression equation

for all females was derived (equation 2.12) which is still significantly different from the regression equation for males (P <0.01). Since σ_0 of all females is significantly greater than σ_0 of males, (F-test P <0.05), then the variance components for both groups must be calculated separately. If there had been no significant difference, then the data from all the individuals could have been used to calculate the variance for both common regressions.

All Females: $s\dot{V}_{O_2} = -18.88 + 0.29 \times f_H$ n = 15 $r^2 = 0.83$ (2.12)

(where $s\dot{V}_{O_2}$ is in ml min^{-1} kg^{-1} and f_H is in beats min^{-1}.)

Validations

Breeding female penguins were used to validate the procedure and determine its accuracy in estimating rate of oxygen consumption from heart rate for macaroni penguins. The data obtained from the five penguins kept in the respirometer for 24 h were divided into 30 min segments. The mean f_H for each 30 min segment for each individual bird was used to estimate $s\dot{V}_{O_2}$ for that segment using a version of equation 2.12, modified by not including the calibration data for this individual (Fig. 2.5). Predicted $s\dot{V}_{O_2}$ was plotted against observed $s\dot{V}_{O_2}$ and the regression line between the two was significantly different to the line of equality. However, the mean of these estimates of $s\dot{V}_{O_2}$ was 15.29 ± 0.51 ml min^{-1} kg^{-1}, which was not signifcantly different from the observed mean $s\dot{V}_{O_2}$ of 15.70 ± 0.48 (paired-sample *t*-test, P >0.05). Percentage error for these estimates is calculated by dividing the difference between the estimated and observed values by the observed value and multiplying by 100. The average percentage algebraic error of all the 30 min segments, which takes the sign of the difference into account was 0.74%. However, the range of this error was from –59.90% to +114.52% and so the average absolute error, which ignores the sign of the difference between the observed and estimated values, was 30.02%.

However, in a study of this nature, it is more appropriate to look at the average oxygen consumption and error for the whole 24-hour period for each individual bird (Table 2.4).

There was again no significant difference between the means of the observed and predicted $s\dot{V}_{O_2}$ (paired-sample *t*-test, P >0.05), and the average algebraic error was -2.13%. In this case the average absolute error was 24.91%. If equations 2.12 and 2.10 are used to calculate $s\dot{V}_{O_2}$ and σ_0 from the mean value of f_H obtained from the validation then the estimate of $s\dot{V}_{O_2}$ is 15.54 ± 1.24. The averaged measured value of $s\dot{V}_{O_2}$ is within the 95% prediction intervals for this estimate and the algebraic error of this estimate is –1.03%.

As a further check on the validity of the f_H method, the data used for the calibrations were examined. For each animal, $s\dot{V}_{O_2}$ was estimated using modified versions of equations 2.7 and 2.12 for males and all females respectively, where the constants for the slope and intercept were calculated using the data from the other members of its group. This was repeated for each animal, and average observed and predicted values of $s\dot{V}_{O_2}$ calculated for each animal. Again if predicted $s\dot{V}_{O_2}$ is plotted against observed $s\dot{V}_{O_2}$ then the regression line between the two variables is significantly different from the line of equality. However, within both groups and for all the individuals there was no significant difference between the estimated and measured $s\dot{V}_{O_2}$ (paired-sample *t*-test P >0.05 Table 2.5).

Discussion

The accuracy and usefulness of f_H for determining metabolic rate in free-ranging predators has already been demonstrated for a number of different species. Previous studies have shown that f_H is at least as accurate as DLW (Nolet et al. 1992, Bevan et al. 1994, 1995c, Boyd et al. 1995) and that for gentoo penguins the relationship between f_H and $s\dot{V}_{O_2}$ is the same when exercising by walking or by swimming (Bevan et al. 1995c). Following on from that work, the present study has three main aims: firstly to determine and validate the $f_H/s\dot{V}_{O_2}$ relationship for macaroni penguins to determine if f_H can be used as a technique for measuring the rate of energy expenditure of these animals in the field; secondly to find out whether this relationship is valid for different sexes and stages of the penguins' yearly physiological cycle; finally to develop further the statistical methods used to derive these relationships and

determine and minimise the errors associated with using them to estimate field metabolic rates (FMR).

Resting $s\dot{V}_{O_2}$ determined in the present study is 15% lower than the FMR of 12.24 ml O_2 min^{-1} kg^{-1} for incubating macaroni penguins estimated from DLW (Davis et al. 1989). This seems reasonable since conditions inside the respirometer are likely to be less stressful than those in the colony. Even though the temperature in the respirometer was only 2.1 ^{o}C higher than the external temperature, the penguins were protected from wind chill factors so thermoregulation could be partly responsible for raised $s\dot{V}_{O_2}$ in the field. Comparison with resting $s\dot{V}_{O_2}$ of other penguin species, obtained by respirometry suggests that the resting rates of macaroni penguins in the present study are comparable to those from other species (Fig. 2.6). Resting metabolic rate (RMR) decreases exponentially with increasing body mass (equation 2.13) and the values from the present study fall very close to this line.

$$\mathrm{RMR} = 14.79(\mathrm{mass})^{0.24} \qquad (r^2 = 0.65,\ P < 0.001) \qquad (2.13)$$

FMR using DLW for the moult was 20 ml O_2 min^{-1} kg^{-1}, (Davis et al. 1989) which is 73% higher than the value for resting metabolic rate of moulting birds in the present study, but the value of FMR was taken from the field and hence is again likely to be higher than that measured in the laboratory.

Maximum rates of $s\dot{V}_{O_2}$ measured in the present study are nearly 50% lower than estimates made of FMR using DLW while foraging (65.67 ml O_2 min^{-1} kg^{-1}, (Davis et al. 1989)), and also up to 30% lower than estimates of MR while swimming at average speed made using respirometry (4.2 x RMR, (Culik et al. 1994)). This supports the proposal that estimates of energy expenditure made using DLW for diving and swimming birds and mammals may not be of acceptable accuracy (Bevan et al. 1995b, Boyd et al. 1995). Analysis of f_H data from free ranging macaroni penguins will indicate whether this is a possibility. DLW is a useful technique but there is potential for bias in estimates made using it if the assumptions inherent in its implementation are not upheld, and in order to use it to give activity-specific energy expenditures, further assumptions must be made.

The maximum walking speed of the penguins in the present study was 1.8 km h^{-1}. When compared with data for other penguins walking on treadmills, this value is lower than those reported for Adélie (3.7 km h^{-1}), emperor (2.8 km h^{-1}), white flippered (2.7 km h^{-1}) (Pinshow et al. 1976) and king penguins (2.6 km h^{-1}) (Froget et al. 2001), but faster than the 1.5 km h^{-1} recorded for gentoo penguins in a very similar experimental situation (Bevan et al. 1995c). The reasons for this are unclear. It is possible that the penguins used in the earlier studies were trained to walk on the treadmill but more likely that these species are in general better adapted to walking than macaroni penguins. Emperor and Adélie penguins may have to walk up to 100 km over ice to reach their colonies (Pinshow et al. 1976) and king penguins may also have to walk large distances to their colonies. Macaroni penguins typically have to walk no further than a few hundred meters from landing areas on the shore to their nest sites (Marchant and Higgins 1990). This journey is usually over steep rocky ground and the penguins tend to hop rather than walk. On the treadmill, however, the macaroni penguins could not be induced to hop, even at high speeds or inclines of 22º, and seemed untroubled when walking steadily. Gentoo penguins walk only slightly greater distances than macaroni penguins (Marchant and Higgins 1990), so their lower maximum speed may have a similar explanation.

As with emperor, Adélie and white flippered penguins, $s\dot{V}_{O_2}$ and f_H both increased linearly with treadmill speed. There was no initial sharp increase from resting rates, followed by a steady increase as seen in other studies (Nolet et al. 1992, Bevan et al. 1994, 1995c). It is suggested that this initial increase is due to the change in attitude from lying or sitting to standing and walking. Gentoo penguins, geese and albatrosses tend to sit when resting whereas macaroni penguins usually remain standing. The starting of the treadmill and accompanying noise may startle some species, while others may never be comfortable on the treadmill, so leading to raised $s\dot{V}_{O_2}$ and f_H. The macaroni penguins seemed relatively untroubled on the treadmill, and rarely tried to escape or jump out of the respirometer.

The relationships between f_H and $s\dot{V}_{O_2}$ for all individuals were well described by both linear and curvilinear regressions. However, the linear regressions provided a slightly better fit. A

curvilinear relationship between f_H and $s\dot{V}_{O_2}$ has been found in other species (e.g., emu, (Grubb et al. 1983), gentoo penguin (Bevan et al. 1995c) and black-browed albatross (Bevan et al. 1994)) though in these cases a linear relationship also gave a reasonable fit. Within each group, analysis of covariance showed that the slopes were the same but the intercepts significantly different. In this case, random effects analysis of covariance was used to derive group relationships and standard errors. However, if the slopes as well as intercepts are significantly different within a group then all the data should be pooled and a simple regression derived from this pooled data (Hawkins et al. 2000). This represents a case where the individual variation is even greater than that in the present study, and hence the regressions will be less accurate, with more error associated with their implementation in estimation of $s\dot{V}_{O_2}$.

In the present study, random effects analysis of covariance was also used to show that, despite a great deal of individual variation, there was no significant difference in the relationship between f_H and $s\dot{V}_{O_2}$ between breeding and moulting females. However, there was a significant difference in the relationship between f_H and $s\dot{V}_{O_2}$ between breeding males and females. Differences in the $f_H/s\dot{V}_{O_2}$ relationship between sexes have not been demonstrated previously. However, it is not always clear whether the sexes of the animals used in similar experiments had been determined. Failing to determine the relationship in different sex classes and at different times of the breeding season has often been cited as a possible weakness in the f_H technique (Holter et al. 1976, Flynn and Gessaman 1979, Woakes and Butler 1983). This present study shows that these are legitimate concerns and, wherever possible, sex differences must be taken into account when using the heart rate method. Male macaroni penguins have a significantly greater body mass and body size. It is possible that this sexual dimorphism in body mass and size is involved in creating the difference in the relationship between f_H and $s\dot{V}_{O_2}$. However, it seems unlikely that the difference is due simply to body mass and size itself. Mass was corrected for in the analysis and moulting female penguins weigh significantly more than breeding female penguins and no difference in the relationship between f_H and $s\dot{V}_{O_2}$ was found when these two groups were compared.

Moulting penguins undergo extreme physiological stress (Brown 1985) and even though values of f_H were significantly higher both at rest and at maximum speed, and $s\dot{V}_{O_2}$ was significantly higher at rest than those in breeding penguins, there was no significant difference in the relationship between f_H and $s\dot{V}_{O_2}$. Moulting a complete set of feathers is an energetically costly exercise and metabolic rates are expected to be elevated (Brown 1985).

Analysis of the errors associated with estimating $s\dot{V}_{O_2}$ from f_H gives a more quantitative description of the assertion that increasing the numbers of animals and data points in both the calibration process and field work is essential in minimising the error of the estimate (Nolet et al. 1992, Bevan et al. 1994, 1995c, Boyd et al. 1995). Figure 2.4 shows that increasing the number of animals from the field continues to increase the accuracy of the estimate up to approximately 20 animals, but beyond this gain is minimal. Similarly, if the number of data points per field animal for 10 animals increases above approximately 10 then there is very little improvement in accuracy. This means that in this example, with 10 animals from the field it would be possible to estimate the cost of an activity with only 10 data points. This would mean that the animals need only perform an activity once for 50 min or 10 times for 5 min on each occasion, representing a very fine scale of resolution for energetic measurements. Figure 2.4 also shows that benefits of increasing the number of calibration animals used. We assume that the animals used in the calibration accurately represent the variability in the population, but the more animals used the more we can accurately model this variability. One restriction of the f_H technique is that in order to use a regression relationship it is only statistically sound to use values of f_H from the field from within the range of values found in the calibration procedure. To extrapolate the relationship beyond these boundaries is not valid since other physiological changes could be occurring of which we are unaware.

Validation

The equation derived to relate f_H to $s\dot{V}_{O_2}$ was applied to two sets of data to assess the accuracy of the relationship for macaroni penguins. When the data from the 24 h validations were examined, the relationship between observed and estimated $s\dot{V}_{O_2}$ was significantly

different from equality, but using f_H to estimate $s\dot{V}_{O_2}$ for the whole 24 hour period underestimated $s\dot{V}_{O_2}$ by only 2.13%. When the data from the calibration runs only were examined, the relationship between observed and estimated $s\dot{V}_{O_2}$ was again significantly different from equality, tending to overestimate $s\dot{V}_{O_2}$ at the highest levels of activity (Fig. 2.5), as was also seen with gentoo penguins (Bevan et al. 1995c). However since the magnitude of these overestimations can be quantified then it is possible to compensate for it when considering activities recorded from the field which have a corresponding high average heart rate. When the full range of heart rates from the calibration runs is considered, then f_H overestimated $s\dot{V}_{O_2}$ by only 0.47%. It seems, therefore, that f_H can provide a good estimate of mean $s\dot{V}_{O_2}$ over a range of activities in macaroni penguins. The absolute errors in these validations are relatively large (25% and 21% respectively) but this is to be expected since there is considerable individual variation, which we have modelled and accounted for. Validations of the DLW technique show a similar pattern where the technique is as accurate as f_H but the mean absolute errors can be as large as 42% (Bevan et al. 1995c).

The regressions derived can be used to predict values of $s\dot{V}_{O_2}$ of birds in the field from values of f_H which are the average of many data points from several animals. It is easy to compare averages of heart rates for different activities, but less obvious how the values of $s\dot{V}_{O_2}$ predicted from the regression equations may be compared. One way in which this could be achieved is by examining the distribution of differences between the averages for each activity for each animal. For example, an average f_H is recorded from each of n penguins for two activities a and b, and for each mean value of f_H a value of $s\dot{V}_{O_2}$ is calculated using equation 2.7 or 2.12 as appropriate. s^2_1 for each estimate should be calculated, but omitting the error term involving n_3 since in this case each penguin is considered individually and is not representing the whole population. For each individual, the difference between the $s\dot{V}_{O_2}$ value for a and b (D), and s^2_D, the variance of D which is the sum of the two variances for the two $s\dot{V}_{O_2}$ values are calculated (Table 2.6). Next $\overline{D}$, which is simply the mean of all n values of D and $\sigma^2_{\overline{D}}$ (equation 2.14) are calculated then the value of $\overline{D}$ is compared to zero

using equation 2.15 to obtain a Z statistic. A simple approximate normal test will determine whether $\overline{D}$ is significantly different from zero. If it is then the mean estimates of $s\dot{V}_{O_2}$ for the two activities are significantly different.

$$\sigma^2_{\overline{D}} = \left(\frac{1}{n}\right)^2 \left(\sum_1^n \sigma^2_D\right) \tag{2.14}$$

(where $\sigma^2_{\overline{D}}$ = variance of $\overline{D}$, the mean difference, n = number of individuals, σ^2_D = variance of D for each individual.)

$$Z = \frac{\overline{D} - 0}{\sqrt{\sigma^2_{\overline{D}}}} \tag{2.15}$$

(where $\overline{D}$ = the mean of the differences for each individual, $\sigma^2_{\overline{D}}$ = the variance of $\overline{D}$ (equation 2.14).)

In conclusion, the present study has shown for the macaroni penguin that, provided data are obtained from as large a group as possible and it is not applied to individual animals, f_H can be used accurately to estimate mean metabolic rate, even for animals in different physiological states. There were differences between the sexes in the way that f_H and $s\dot{V}_{O_2}$ are related and this should be taken into account during any calibration process for this species. Provided this precaution is taken, then through the long term monitoring of f_H, it is possible to determine the metabolic rates of free ranging animals over several months or years, yet still with a fine enough time resolution to determine the energetic costs of different activities.

Group	Mean Resting f_H (beats min^{-1})		Mean Resting $s\dot{V}_{O_2}$ (ml min^{-1} kg $^{-1}$)		n
Breeding Males	85.36 ± 4.41	†	9.45 ± 0.43	Φ	9
Breeding Females	96.61 ± 2.27	ω	10.72 ± 0.47		9
Moulting Females	124.87 ± 11.58	† ω	11.59 ± 0.87	Φ	6

Group	Mean Maximum f_H (beats min^{-1})		Mean Maximum $s\dot{V}_{O_2}$ (ml min^{-1} kg $^{-1}$)	n
Breeding Males	162.97 ± 4.68	§	35.13 ± 1.00	9
Breeding Females	166.20 ± 6.50	‡	33.58 ± 0.91	9
Moulting Females	193.00 ± 10.93	§ ‡	33.63 ± 0.58	6

Table 2.1. Mean resting and maximum values of rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) for the three groups of macaroni penguins used in the calibration procedure. Significant differences between pairs of groups are represented by the following symbols: † ω Φ §‡

Group	Mean factorial increase in f_H		Mean factorial increase in $s\dot{V}_{O_2}$		n
Breeding Males	1.95 ± 0.10	†	3.77 ± 0.19	ω	9
Breeding Females	1.72 ± 0.06		3.20 ± 0.20		9
Moulting Females	1.58 ± 0.09	†	2.98 ± 0.22	ω	6

Table 2.2. Mean factorial increases in rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) from resting to maximum activity in three groups of macaroni penguins. Significant differences between pairs of groups are represented by the following symbols: † ω.

Bird	Group	Mass (kg)	a	b	r^2	*P*
cal04	BM	3.86	-19.795	0.308	0.972	<0.001
cal05	BM	3.70	-22.422	0.341	0.947	<0.001
cal06	BM	3.48	-16.514	0.303	0.938	<0.001
cal08	BM	3.23	-25.981	0.379	0.834	<0.001
cal09	BM	3.24	-14.857	0.351	0.966	<0.001
cal10	BM	3.20	-11.464	0.328	0.898	<0.001
cal12	BF	3.32	-22.499	0.298	0.858	<0.001
cal13	BF	3.23	-22.207	0.323	0.834	<0.001
cal16	BF	3.14	-11.618	0.230	0.958	<0.001
cal18	BF	3.23	-28.207	0.375	0.832	<0.001
cal19	BF	2.98	-17.530	0.312	0.924	<0.001
cal20	BF	2.88	-14.370	0.257	0.783	<0.001
cal21	BF	2.96	-25.247	0.415	0.928	<0.001
cal24	BM	4.56	-18.104	0.321	0.886	<0.001
cal25	BM	4.22	-19.925	0.321	0.627	<0.005
cal26	BF	3.62	-13.038	0.277	0.776	<0.001
cal27	BF	4.00	-20.146	0.354	0.709	<0.001
cal28	BM	4.34	-24.055	0.371	0.945	<0.001
n05	MF	3.99	-20.927	0.246	0.812	<0.001
n07	MF	4.57	-24.417	0.410	0.818	<0.001
x02	MF	3.91	-38.576	0.324	0.843	<0.001
n09	MF	4.10	-35.459	0.341	0.472	<0.05
h83	MF	4.40	-17.434	0.267	0.958	<0.001
c13	MF	4.56	-14.618	0.232	0.936	<0.001

(Note: The form of the equation is $\mathrm{s}\dot{V}_{\mathrm{O}_2} = \mathrm{a} + \mathrm{b} \times f_{\mathrm{H}}$ *)*

Table 2.3. Individual regression equations of mass specific rate of oxygen consumption ($\mathrm{s}\dot{V}_{\mathrm{O}_2}$*, ml min*$^{-1}$ *kg*$^{-1}$*) against heart rate (*f_{H}*, beats min*$^{-1}$*) of the twenty-four macaroni penguins used in the calibration procedure. The three groups are breeding males (BM), breeding females (BF) and moulting females (MF).*

Bird	Observed 24 h f_H (beats min^{-1})	Observed 24 h $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Predicted 24hr $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Δ (Predicted $s\dot{V}_{O_2}$ - Observed $s\dot{V}_{O_2}$)	Algebraic % Error	Absolute % Error
Cal12	128.56	14.33	18.01	3.68	25.69	25.69
Cal19	108.89	14.35	11.99	-2.35	-16.41	16.41
Cal20	144.77	17.13	22.47	5.35	31.23	31.23
Cal26	109.50	16.28	12.06	-4.22	-25.95	25.95
Cal27	110.50	16.45	12.30	-4.15	-25.24	25.24
Mean	120.45	15.71	15.37	-0.34	-2.13	24.91
S.E.M.	7.11	0.58	2.11	2.03		

Table 2.4. Mean rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) obtained from five macaroni penguins over 24 hours, with estimates of $s\dot{V}_{O_2}$ derived from a previously calibrated relationship between $s\dot{V}_{O_2}$ and f_H.

Group	Average Observed f_H (beats min^{-1})	Average Observed $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Average Predicted $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Average Δ (Predicted $s\dot{V}_{O_2}$ - Observed $s\dot{V}_{O_2}$)	Average Algebraic % Error	Average Absolute % Error	n
Males	119.91 ± 2.56	20.99 ± 0.72	21.00 ± 0.98	0.01 ± 1.42	1.44	15.12	9
Females	139.92 ± 5.95	21.11 ± 0.24	21.12 ± 1.80	0.01 ± 1.76	-0.11	24.44	15
All	132.42 ± 4.19	21.06 ± 0.30	21.08 ± 1.13	0.01 ± 1.17	0.47	20.94	24

Table 2.5. Mean rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) obtained from 24 macaroni penguins kept in a respirometer for an 8 hour session of rest and exercise, with estimates of $s\dot{V}_{O_2}$ derived from a previously calibrated relationship between $s\dot{V}_{O_2}$ and f_H.

Bird	$\dot{V}O_2$ estimate of activity a	$\dot{V}O_2$ estimate of activity b	Difference (D)	Variance of difference (σ^2_D)
1	$V_a^1 \pm \sigma^2{}_a^1$	$V_b^1 \pm \sigma^2{}_b^1$	$D^1 = V_a^1 - V_b^1$	$\sigma^2{}_D^1 = \sigma^2{}_a^1 + \sigma^2{}_b^1$
2	$V_a^2 \pm \sigma^2{}_a^2$	$V_b^2 \pm \sigma^2{}_b^2$	$D^2 = V_a^2 - V_b^2$	$\sigma^2{}_D^2 = \sigma^2{}_a^2 + \sigma^2{}_b^2$
3	$V_a^3 \pm \sigma^2{}_a^3$	$V_b^3 \pm \sigma^2{}_b^3$	$D^3 = V_a^3 - V_b^3$	$\sigma^2{}_D^3 = \sigma^2{}_a^3 + \sigma^2{}_b^3$
n	$V_a^n \pm \sigma^2{}_a^n$	$V_b^n \pm \sigma^2{}_b^n$	$D^n = V_a^n - V_b^n$	$\sigma^2{}_D^n = \sigma^2{}_a^n + \sigma^2{}_b^n$

Table 2.6. Method for detection of significant differences between two estimates of $s\dot{V}_{O_2}$ *associated with different activities in the same group of animals.*

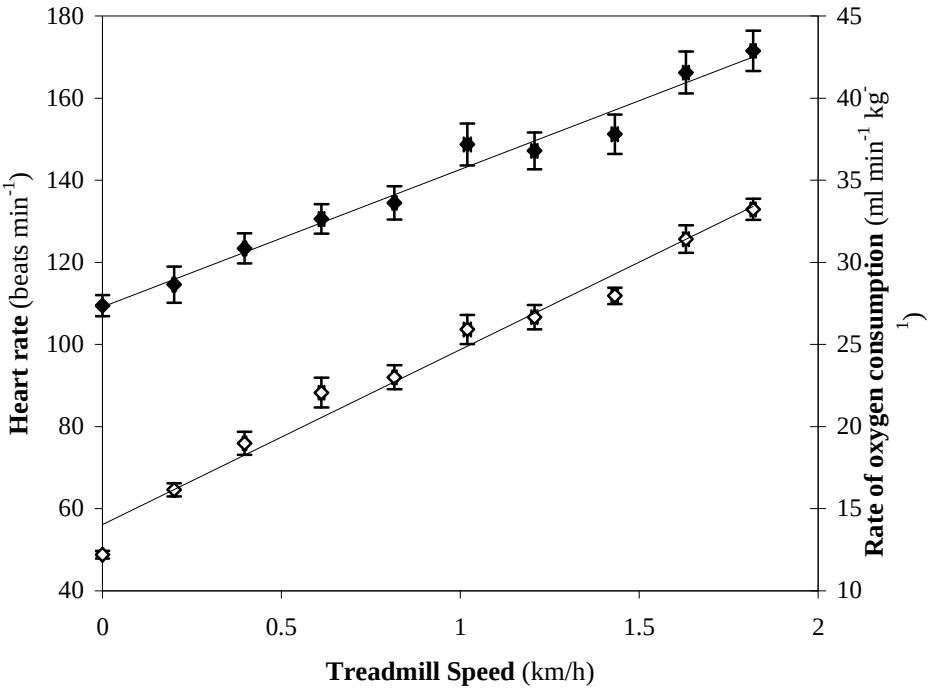

Figure 2.1. Mean heart rate (open symbols) and mass specific rate of oxygen consumption (filled symbols) of twenty-four macaroni penguins walking at different speeds on a treadmill. The error bars represent 1 S.E.

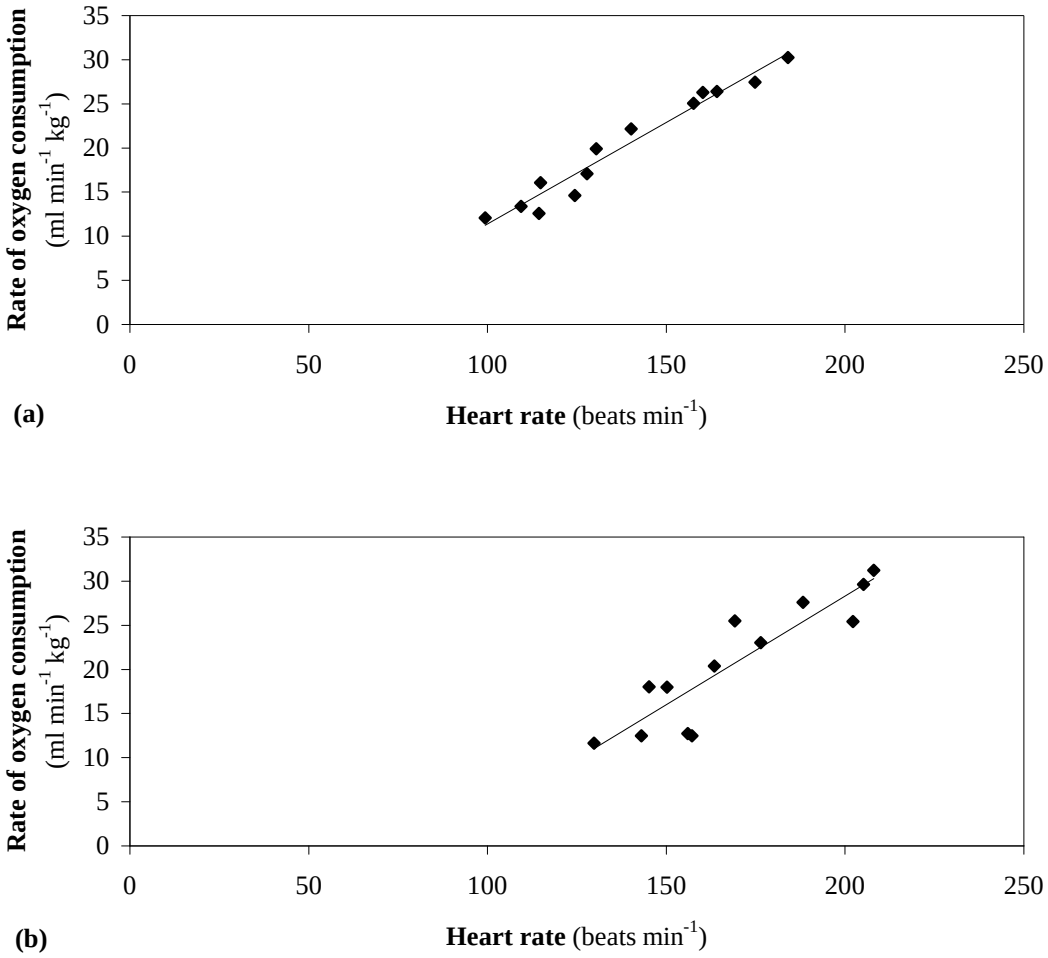

Figure 2.2. Rate of oxygen consumption ($s\dot{V}_{O_2}$) as a function of heart rate (f_H) in two macaroni penguins, (a) a breeding female of mass 3.14 kg ($s\dot{V}_{O_2}$ = (0.23 x f_H) – 11.62) and (b) a moulting female of mass 3.99 kg ($s\dot{V}_{O_2}$ = (0.25 x f_H) – 20.93).

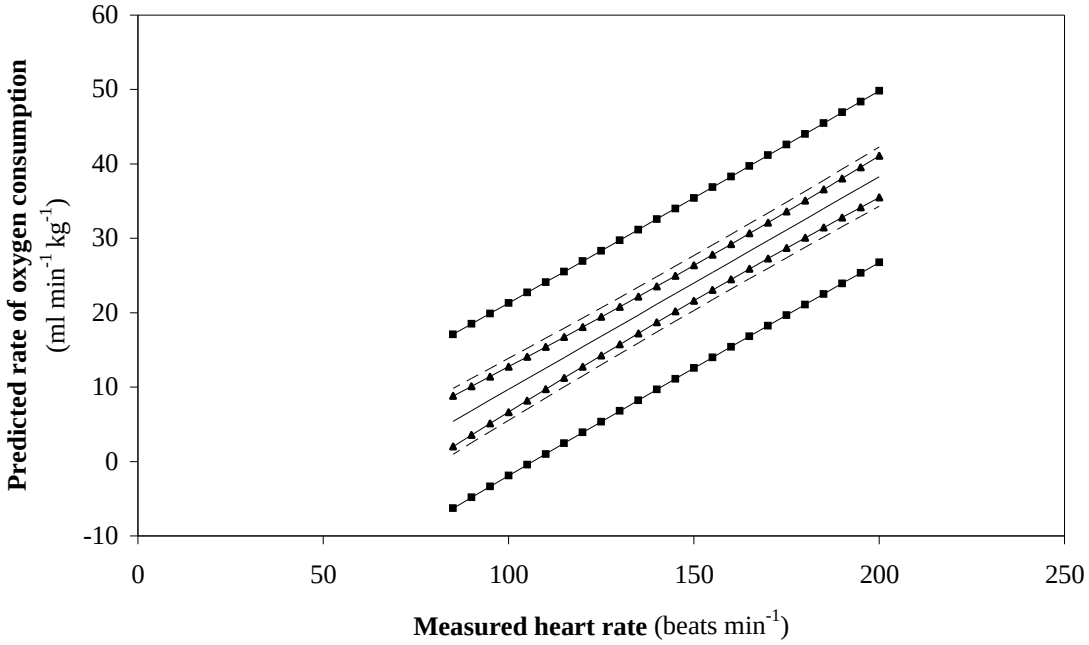

Figure 2.3. Predicted rate of oxygen consumption as a function of measured heart rate, obtained from 15 female macaroni penguins, showing 95% confidence intervals (triangular symbols) and 95% prediction intervals (square symbols) for the predictions. The short dashed lines represent the 95% prediction intervals when rate of oxygen consumption is estimated from 10 000 measurements of heart rate spread between 10 individuals.

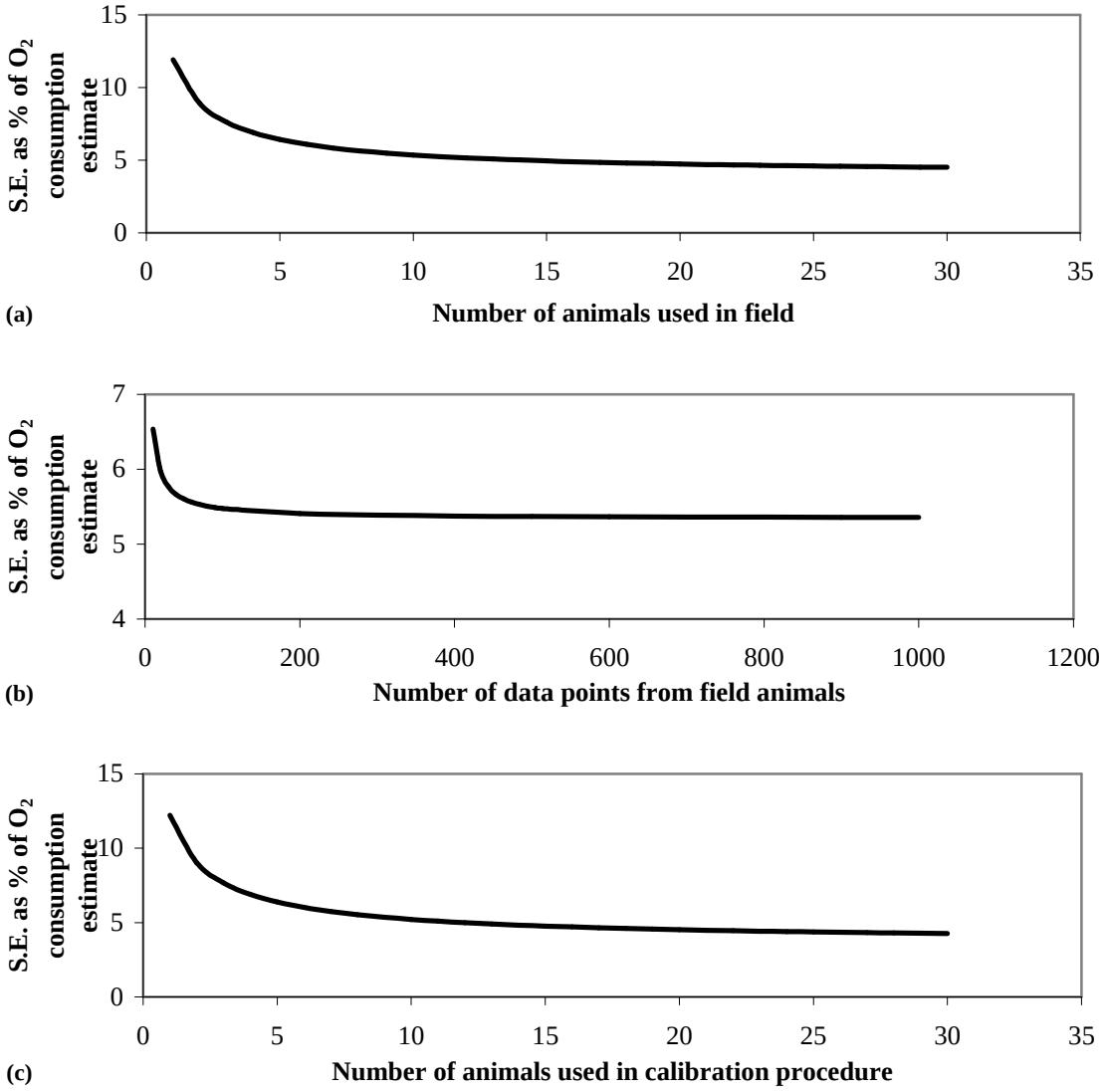

Figure 2.4. Graphic representation of the effect on the standard error (S.E.) of the estimate of oxygen consumption, of changing four parameters; n_1 (number of calibration animals), n_2 (number of data points from calibration animals), n_3 (number of field animals) and n_4 (number of data points from field animals). $s\dot{V}_{O_2}$ *and standard error were calculated with the heart rate arbitrarily fixed at 150 beats min^{-1} using equation 8 for breeding females. (a) n_1 = 9, n_2 = 117, n_3= variable, n_4 = 100 per field animal. (b) n_1 = 9, n_2 = 117, n_3 = 10, n_4 = variable. (c) n_1 = variable, n_2 = 13 per calibration animal, n_3= 10, n_4 = 1000. Changing the value of heart rate, or the equation used to calculate* $s\dot{V}_{O_2}$ *had no effect on the shape of the curves.*

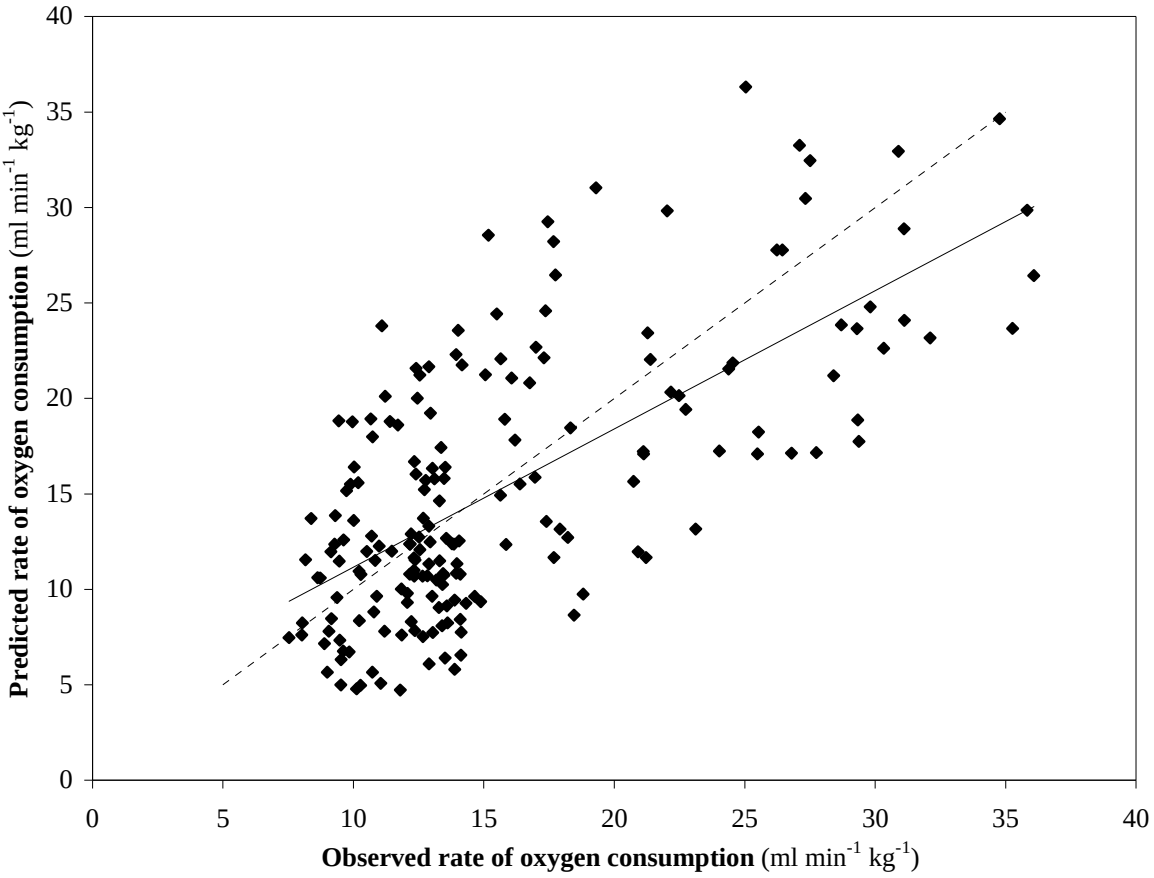

Figure 2.5 Rate of oxygen consumption ($s\dot{V}_{O_2}$) estimated from heart rate as a function of measured rate of oxygen consumption at different exercise levels in 5 macaroni penguins. The dotted line is the line of equality and the solid line is the regression equation between the two variables described by the equation: Estimated $s\dot{V}_{O_2}$ = 3.3904 + 0.3238 (Measured $s\dot{V}_{O_2}$) (r^2 = 0.46, P <0.001). The two lines are significantly different.

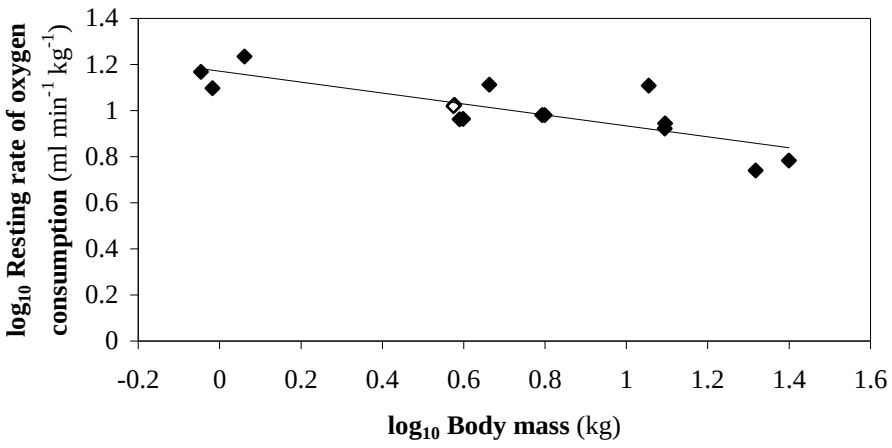

Figure 2.6. Log$_{10}$ of resting rate of oxygen consumption ($s\dot{V}_{O_2}$, ml min^{-1} kg^{-1}) as a function of log$_{10}$ of body mass of different penguin species (closed symbols). The equation of the regression line is log$_{10}$ (resting $s\dot{V}_{O_2}$) = 1.17 - 0.24 x log$_{10}$ (body mass) (r^2 = 0.65, P <0.001). Data are from gentoo (Dumonteil et al. 1994, Bevan et al. 1995c), Humboldt (Spheniscus humboldti), *White flippered* (Eudyptyla) *(Pinshow et al. 1976), little blue* (Eudyptyla minor) *(Stahel and Nicol 1982, Stahel et al. 1984), king* (Aptenodytes patagonicus) *(Barré and Roussel 1986, Froget et al. 2001), Adélie* (Pygoscelis adeliae) *(Pinshow et al. 1976) emperor* (Aptenodytes forsteri) *(Le Maho et al. 1976, Pinshow et al. 1976) and macaroni (Brown 1984) penguins. The open symbol is from the macaroni penguins in the present study.*

References

Barré H., and Roussel B. (1986). Thermal and metabolic adaptation to first cold-water immersion in juvenile penguins. American Journal of Physiology **251**: R456-R462

Baudinette R.V., and Gill P. (1985). The energetics of 'flying' and 'paddling' in water: locomotion in penguins and ducks. Journal of Comparative Physiology B **155**: 373-380

Bevan R.M., Woakes A.J., and Boyd I.L. (1994). The use of heart rate to estimate oxygen consumption of free ranging black-browed albatrosses *Diomedea Melanophrys*. Journal of Experimental Biology **193**: 119-137

Bevan R.M., Butler P.J., Woakes A.J., and Prince P.A. (1995a). The energy expenditure of free-ranging black-browed albatrosses. Philosophical Transactions of the Royal Society of London B **350**: 119-131

Bevan R.M., Speakman J.R., and Butler P.J. (1995b). Daily energy expenditure of tufted ducks:a comparison between indirect calorimetry, doubly labelled water and heart rate. Functional Ecology **9**: 40-47

Bevan R.M., Woakes A.J., Butler P.J., and Croxall J.P. (1995c). Heart rate and oxygen consumption of exercising gentoo penguins. Physiological Zoology **68**: 855-877

Boyd I.L., Woakes A.J., Butler P.J., Davis R.W., and Williams T.M. (1995). Validation of heart rate and doubly labelled water as measures of metabolic rate during swimming in California Sea Lion. Functional Ecology **9**: 151-160

Brown C.R. (1984). Resting metabolic rate and energetic cost of incubation in macaroni penguins (*Eudyptes chrysopholus*) and rockhopper penguins (*E. chrysocome*). Comparative Biochemistry and Physiology A **77**: 345-350

Brown C.R. (1985). Energetic cost of moult in macaroni penguins (*Eudyptes chrysolophus*) and rockhopper penguins (*E. chrysocome*). Journal of Comparative Physiology B **155**: 515-520

Butler P.J. (1993). To what extent can heart rate be used as an indicator of metabolic rate in free-living marine mammals. Symposium of the Zoological Society of London **66**: 317-332

Butler P.J., and Woakes A.J. (1984). Heart rate and aerobic metabolism in humboldt penguins, *Spheniscus Humboldti*, during voluntary diving. Journal of Experimental Biology **108**: 419-428

Butler P.J., Bevan R.M., Woakes A.J., Croxall J.P., and Boyd I.L. (1995). The use of data loggers to determine the energetics and physiology of aquatic birds and mammals. Brazilian Journal of Medical and Biological Research **28**: 1307-1317

Costa D.P., Dann P., and Disher W. (1986). Energy requirements of free-ranging little penguins, *Eudyptyla minor*. Comparative Biochemistry and Physiology a **85**: 135-138

Croxall J.P. (1984). Seabirds. In: Laws R.M. (ed) Antarctic Ecology. Academic Press, London

Croxall J.P. (1995). Remote-recording of foraging patterns in seabirds and seals for studies of predator-prey interactions in marine systems. In: Skjoldal H.R., Hopkins C., Erikstad K.E. and Leinaas H.P. (eds) The Ecology of Fjords and Coastal Waters. Elsevier Science B.V., Amsterdam

Croxall J.P., Prince P.A., and Reid K. (1997). Dietary segregation of krill-eating South Georgian seabirds. Journal of Zoology **242**: 531-536

Culik B.M., and Wilson R.P. (1991). Energetics of under-water swimming in Adélie penguins (*Pygoscelis adeliae*). Journal of Comparative Physiology B **161**: 285-291

Culik B.M., Wilson R.P., and Bannasch R. (1994). Underwater swimming at low energetic costs by pygoscelid penguins. Journal of Experimental Biology **197**: 65-78

Davis R.W., Kooyman G.L., and Croxall J.P. (1983). Water Flux and estimated metabolism of free-ranging gentoo and macaroni penguins at South Georgia. Polar Biology **2**: 41-46

Davis R.W., Croxall J.P., and O'Connell M.J. (1989). The reproductive energetics of gentoo (*Pygoscelis papua*) and macaroni (*Eudyptes chrysolophus*) penguins at South Georgia. Journal of Animal Ecology **58**: 59-74

Depocas F., and Hart J.S. (1957). Use of Pauling oxygen analyser for measurement of oxygen consumption of animals in open-circuit systems and in a short-lag, closed-circuit apparatus. Journal of Applied Physiology **10**: 388-392

Dumonteil E., Barré H., Rouanet J.-L., Diarra M., and Bouvier J. (1994). Dual core and shell temperature regulation during sea acclimatization in gentoo penguins (*Pygoscelis papua*). American Journal of Physiology **266**: R1319-R1326

Flynn R.K., and Gessaman J.A. (1979). An evaluation of heart rate as a measure of daily metabolism in pigeons (*Columba livia*). Comparative Biochemistry and Physiology A **63**: 511-514

Froget G., Butler P.J., Handrich Y., and Woakes A.J. (2001). Heart rate as an indicator of oxygen consumption: influence of body condition in the king penguin. Journal of Experimental Biology **204**: 2133-2144

Gales R., Green B., Libke J., Newgrain K., and Pemberton D. (1993). Breeding energetics and food requirements of gentoo penguins (Pygoscelis papua*) at Heard and Macquarie Islands. Journal of Zoology **231**: 125-139*

Grubb B., Jorgensen D.D., and Conner M. (1983). Cardiovascular changes in the exercising emu. Journal of Experimental Biology **104**: 193-201

Hawkins P.A.J., Butler P.J., Woakes A.J., and Speakman J.R. (2000). Estimation of the rate of oxygen consumption of the common eider duck (*Somateria mollissima*) with some

measurements of heart rate during voluntary dives. Journal of Experimental Biology **203**: 2819-2832

Holter J.B., Urban W.E., Hayes H.H., and Silver H. (1976). Predicting metabolic rate from telemetered heart rate in white-tailed deer. Journal of Wildlife Management **40**: 626-629

Le Maho Y., Delclitte P., and Chatonnet J. (1976). Thermoregulation in fasting emperor penguins under natural conditions. American Journal of Physiology **231**: 913-922

Marchant S., and Higgins P.J. (eds) (1990). Handbook of Australian, New Zealand and Antarctic Birds, vol 1. Oxford University Press, Australia, Melbourne

Nagy K.A., and Obst B.S. (1992). Food and energy requirements of Adélie penguins (*Pygoscelis adeliae*) on the Antarctic peninsula. Physiological Zoology **65**: 1271-1284

Nagy K.A., Siegfried W.R., and Wilson R.P. (1984). Energy utilization by free-ranging jackass penguins, *Spheniscus demersus*. Ecology **65**: 1648-1655

Nolet B.A., Butler P.J., Masman D., and Woakes A.J. (1992). Estimation of the daily energy expenditure from heart rate and doubly labeled water in exercising geese. Physiological Zoology **65**: 1188-1216

Owen R.B.J. (1969). Heart rate, a measure of metabolism in blue-winged teal. Comparative Biochemistry and Physiology A **31**: 431-436

Pinshow B., Fedak M.A., and Schmidt-Nielsen K. (1976). Terrestrial locomotion in penguins: It costs more to waddle. Science **195**: 592-594

Reid K. (1995). The diet of Antarctic fur seals *Arctocephalus gazella* Peters 1875) during winter at South Georgia. Antarctic Science **7**: 241-249

Reid K., and Arnould J.P.Y. (1996). The diet of Antarctic fur seals *Arctocephalus gazella* during the breeding season at South Georgia. Polar Biology **16**: 105-114

Schmidt-Nielsen K. (1984). Scaling. Cambridge University Press, Cambridge

Speakman J.R. (1997). Doubly Labelled Water. Chapman & Hall, London

Stahel C.D., and Nicol S.C. (1982). Temperature regulation in the little penguin, *Eudyptyla minor*, in air or water. Journal of Comparative Physiology b **154**: 83-100

Stahel C.D., Megirian D., and Nicol S.C. (1984). Sleep and metabolic rate in the little penguin, *Eudyptula minor*. Journal of Comparative Physiology B **154**: 487-494

Williams T.D., and Croxall J.P. (1991). Annual variation in breeding biology of macaroni penguins, *Eudyptes chrysolophus*, at Bird Island, South Georgia. Journal of Zoology **223**: 189-202

Withers P.C. (1977). Measurements of VO2, VCO2, and evaporative water loss with a flow through mask. Journal of Applied Physiology **42**: 120-123

Woakes A.J., and Butler P.J. (1983). Swimming and diving in tufted ducks, *Aythya fuligula*, with particular reference to heart rate and gas exchange. Journal of Experimental Biology **107**: 311-329

Woakes A.J., Butler P.J., and Bevan R.M. (1995). Implantable data logging system for heart rate and body temperature: Its application to the estimation of field metabolic rates in Antarctic predators. Medical & Biological Engineering & Computing **33**: 145-151

Woehler E.J. (1993). The distribution and abundance of Antarctic and subantarctic penguins. Scientific Committee of Antarctic Reseach (SCAR) bird Biology Sub-Committee, Cambridge, UK

Zar J.H. (1999). Biostatistical Analysis, 4th edn. Prentice-Hall International Inc., New Jersey

Chapter 3

The energy cost of breeding in macaroni penguins at South Georgia

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Chapter 4

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Chapter 5

Heart rate, metabolic rate and abdominal temperature during diving in macaroni penguins

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Summary

Heart rate, abdominal temperature and diving depth were measured in thirteen free-ranging breeding female macaroni penguins. Measurement of these parameters allowed estimation of the rate of oxygen consumption $\dot{V}_{O_2}$ while diving and investigation of the physiological adjustments that might facilitate the diving behaviour observed in this species. In common with other diving birds, macaroni penguins showed bradycardia and tachycardia associated with diving and along with body temperature, these parameters accounted for 41% of the variation in dive duration within a multiple regression. When $\dot{V}_{O_2}$ was calculated for dives of different durations, all dives measured were within the calculated aerobic dive limit (cADL) for this species. Significant changes in abdominal temperature were not detected within individual dives though the time constant of the device used may not have been low enough to record these changes if they were present. Abdominal temperature did decline consistently during diving bouts of all durations and there was a linear relationship between bout length and the magnitude of this temperature drop. However, there was no commensurate increase in dive duration during dive bouts. We conclude that changes in abdominal temperature may facilitate lower metabolic rates while submerged or alternatively may simply be a consequence of circulatory adjustments made to restrict blood flow and conserve oxygen stores during diving bouts. Further investigations of these changes in circulation and body temperature during natural diving are required.

Introduction

Penguins are among the most accomplished of avian divers. Numerous studies of their diving behaviour have shown that penguins have remarkable dive performances (Kooyman et al. 1992a, Williams, T.D. et al. 1992, Bengston et al. 1993). The emperor penguin (*Aptenodytes forsteri*), the largest species at 22 kg can reach depths of 524 m (Kooyman and Kooyman 1995) for durations of up to 22 min (Ponganis and Kooyman 2000). Even the considerably smaller (3.6 kg) Adélie penguin (*Pygoscelis adéliae*) can dive to 98 m for up to 160 s (Chappell et al. 1993).

Further investigations have examined how physiological and behavioural adjustments might permit such extreme diving behaviour (Butler and Jones 1997, Kooyman and Ponganis 1998). The extent to which diving animals balance the use of aerobic and anaerobic metabolism during natural dives is unclear. Anaerobic metabolism may be used in some circumstances (Kooyman et al. 1980, Ponganis et al. 1997b) but within any dive, there must be oxygen available for the CNS, heart and active muscles even after lactate begins to accumulate. Most evidence suggests however, that most dives are essentially aerobic (Butler and Jones 1997). Anaerobic metabolism is energetically expensive, yielding only 2 moles of ATP from 1 mole of glucose whereas aerobic metabolism yields 38 moles of ATP per mole of glucose (Maughan et al. 1997). In addition, lactate is accumulated as a by-product of anaerobic metabolism and must be recycled or oxidised at the surface or during subsequent dives (Fedak and Thompson 1993). The marginal value theorem applied to diving behaviour predicts that diving animals will maximise their foraging efficiency if they minimise the time spent at the surface (Kramer 1988, Houston and Carbone 1992). A consequence of this would be predominantly aerobic metabolism during diving with rapid replacement of O_2 stores while at the surface.

Observations of diving behaviour confirm that most dives are within bouts of repeated diving with relatively low ratios of post-dive surface interval duration to dive duration (dive pause ratio). However, under certain circumstances, for example if maintaining contact with a rich food resource, behavioural studies predict that partial anaerobic metabolism or mixed aerobic and anaerobic strategies could permit higher net energy gain (Ydenberg and Clark 1989,

Carbone and Houston 1996, Mori 1998). However, a necessary consequence of anaerobic diving is that more time must be spent at the surface between dives and high dive pause ratios following anaerobic diving have been observed in seals (Castellini et al. 1988, Fedak and Thompson 1993).

The aerobic dive limit (ADL), the diving duration beyond which blood lactate levels increase above resting values, was first determined experimentally in Weddell seals (*Leptonychotes weddellii*) (Kooyman et al. 1980) and coined by Kooyman et al (Kooyman et al. 1983). Since then, ADL or diving lactate threshold (DLT) (Butler and Jones 1997) has been determined in two more species of seal (Ponganis et al. 1997a, d) under captive conditions and in freely-diving emperor penguins (Ponganis et al. 1997c). In emperor penguins the DLT was 5 – 7 mins and this agreed quite closely with an ADL of 8 mins estimated from observations of natural diving behaviour (behavioural ADL), as indicated by the dive pause ratio (Kooyman and Kooyman 1995). Some dives were longer than the behavioural ADL but it was concluded that most were aerobic.

ADL has also been calculated (cADL) for many diving animals, including several penguin species, by dividing usable body oxygen stores by an estimate of the rate of oxygen consumption ($\dot{V}_{O_2}$) while submerged. When compared to observed patterns of diving in different penguin species, these studies have found that between 2 and 50% of dives exceed the cADL (Chappell et al. 1993, Culik et al. 1994, 1996a, Boyd and Croxall 1996, Bethge et al. 1997). In these studies, examination of the dive pause ratio suggests that it is unlikely that so many dives use anaerobic metabolism. In order for a large proportion of natural dives by penguins to be aerobic, the cADL must be greater. Both usable oxygen stores and $\dot{V}_{O_2}$ while submerged are difficult to measure and other pathways such as the metabolism of phosphocreatine might provide energy while submerged (Butler and Jones 1997). Submerged $\dot{V}_{O_2}$ in particular is difficult to measure (Costa 1988). If usable oxygen stores are approximately correct then $\dot{V}_{O_2}$ during diving needs to be as low as that recorded from penguins at rest on the water surface (Butler 2000).

Two principle methods have been used to estimate $\dot{V}_{O_2}$ during diving and both predict that it would be greater than $\dot{V}_{O_2}$ while resting on the water surface. Doubly labelled water (DLW) is a commonly used method for the estimation of field metabolic rate (FMR) (Speakman 1997), and some studies have equated FMR estimated from DLW to $\dot{V}_{O_2}$ while diving (Chappell et al. 1993). DLW provides a single estimate of the rate of carbon dioxide production ($\dot{V}_{CO_2}$), which is then converted to $\dot{V}_{O_2}$ This one estimate covers the entire period of measurement by this technique (typically a few days), not only from bouts of diving and while the animals is submerged. The second commonly used method involves respirometry to measure $\dot{V}_{O_2}$ from penguins surfacing after submerged swims in a static water canal (Culik et al. 1994). This method does measure $\dot{V}_{O_2}$ which is a direct result of diving but the dives in these studies do not accurately reflect conditions in the field. The animals are constrained by the dimensions of the canal, there are no effects of depth and pressure and the animals do not dive for the durations observed in the field (Culik and Wilson 1991). Neither of these methods therefore accurately reflect $\dot{V}_{O_2}$ during natural dives.

Detailed studies of the physiology and behaviour of diving animals have begun to outline a suite of adjustments and adaptations that contribute to the maximising of cADL while submerged. These include variation of heart rate and circulation (Butler and Woakes 1979, Fedak et al. 1988, Kooyman et al. 1992b, Davis and Kanatous 1999), regional hypothermia (Handrich et al. 1997) and the use of passive gliding while swimming (Williams, T.M. et al. 2000). In the present study we measured heart rate (f_H) abdominal temperature (T_{ab}) and depth in macaroni penguins (*Eudyptes chrysolophus*) diving freely while foraging in their natural environment, using purpose built implantable data loggers (DL) (Woakes et al. 1995). Heart rate can be used to estimate $\dot{V}_{O_2}$ (Butler 1993) and a relationship between heart rate and $\dot{V}_{O_2}$ has been established for macaroni penguins (Green, J.A. et al. 2001). Thus these measurements enabled us to determine whether metabolic and circulatory adjustments can explain observed patterns of diving behaviour

The present study, therefore, has three main aims. 1) To examine heart rate changes on a fine scale (measured every 2 s) in order to provide evidence for circulatory adjustments made

during diving which might extend dive duration (Butler and Jones 1997). 2) To estimate the energy cost of free-ranging diving behaviour in a diving bird from heart rate. 3) To measure abdominal temperature and investigate the hypothesis that lowered body temperature contributes to the extension of diving duration (Culik et al. 1996b, Handrich et al. 1997).

Materials and Methods

Study animals

Although the United Kingdom Animal (Scientific Procedures) Act 1986 does not apply to South Georgia, where this study was conducted, we were meticulous in following its provisions, especially those set out by the Home Office in the Official Guidance on the operation of the Act. As our benchmark, we followed guidance to researchers using similar methods in the United Kingdom. Our procedures also conformed to the Code of Ethics of Animal Experimentation in Antarctica.

The study was undertaken at the British Antarctic Survey (BAS) base on Bird Island, South Georgia during the austral summer of 1998/99. The macaroni penguins used in the study were breeding females from the colony at Fairy Point on the north side of the island. The population at this colony has been monitored for many years (Williams, T.D. and Croxall 1991) and has also been the subject of more intensive studies (Davis et al. 1983, 1989, Croxall et al. 1988, 1993, 1997, Williams, T.D. 1989). All birds used in the present study were engaged in provisioning a growing chick. Where possible, birds were caught for implantation away from the nesting area of the colony after they had fed their chick. After capture, the birds were removed to the surgical facility and kept in an outdoor enclosure for 2-3 h before the surgery to allow digestion of food. Fifteen birds were selected for implantation with DLs.

Implantation procedure

Implantation of the DL into the abdominal cavity allows data to be recorded without compromising the swimming, foraging and breeding performance of animals, as has been

observed with the use of externally mounted devices on the morphometrically identical royal penguin (Hull 1997). The implantation procedure was basically the same as described for similar studies (Bevan et al. 1995). Briefly, the sterilised DL was implanted into the abdominal cavity via a mid-line incision made in the skin and body wall muscle in the brood patch while the bird was anaesthetised with halothane. The logger design incorporates a low power radio frequency transmitter which emits a short pulse on each QRS wave of the electrocardiogram (ECG). Detection of this signal on a radio receiver was used to indicate when the DL was in the correct position. Once in position, the body wall and skin were sutured, antibiotic powder (Woundcare, Animalcare Ltd.) applied to the wound and a long acting antibiotic (LA Terramycin, Pfizer) and analgesic (Vetergesic, Reckitt and Colman Products Ltd.) injected intramuscularly. Aseptic conditions were maintained wherever possible. The time at which the DL was implanted was noted to the nearest second.

All birds were weighed immediately before surgery using a spring balance (10 ± 0.1 kg Pesola) and a passive implanted transponder (PIT) tag, mounted on a plastic cable tie was secured around their ankle. Birds were put into a large darkened box to recover from the surgery. Once the birds were alert and responsive, usually after 1-2 h, they were returned to the colony where behaviour varied between individuals. Some would go swimming within a few hours, whereas others made their way to the nest site or stood alone elsewhere in the colony.

Around the time at which the DL memory was predicted to be full, implanted birds were recaptured after returning from a foraging trip and having fed their chicks. The DL was removed using the same procedure as during implantation, and the bird was released back in to the colony once it had recovered. The time of removal of the DL was noted and the precise times of implantation and removal were later used to establish the time base of the data downloaded from the DL. The heart rate, abdominal temperature and depth data from within the DL memory were downloaded onto a computer using purpose designed software.

Heart rate data loggers

The DLs could record heart rate, hydrostatic pressure (diving depth) and abdominal temperature every two seconds and, at this sampling rate, could store data over 30.3 days. Before use, the devices were encased in paraffin wax and encapsulated in silicon rubber to provide waterproofing and biocompatability. The temperature sensor of the DL was calibrated by immersing the device in water baths of known temperature. This procedure was also used to determine the time constant (τ) of the temperature sensor, which was 74 s. The time constant was the time taken for the logger to register 67% of the change in temperature when the logger was moved between two environments of different temperatures. The hydrostatic pressure sensor in the DL could detect diving depth to a resolution of 1.2 m.

Data analysis

The data were prepared and analysed using purpose-written computer programs within the SAS statistical package (version 6.11, SAS institute) on a UNIX workstation. Further analyses were performed with the statistical packages Minitab 12 (Minitab Inc.) and Excel 97 (Microsoft). The recovery period following the implantation procedure was excluded from the analysis by ignoring data collected during the period from implantation to the start of the first foraging trip. In the present study this period had a mean duration of 55.5 ± 5 (S.E.M) hours.

Time at-sea on foraging trips was estimated from the depth data, supported with data from field observations and a PIT tag recorder (FSI ltd.) situated in a gate at the edge of the colony. Each record of heart rate, abdominal temperature and dive depth was also marked with the daylight conditions (light or dark). These were calculated using the times for civil sunrise and sunset calculated for the longitude and latitude of Bird Island (54^{o} 00’ S 38^{o} 02’ W). In examining dive records, dives with maximum depths of <2.4 m were counted but otherwise ignored, since wave action and recorder noise degraded depth accuracy for shallower dives. In all analyses, dives are treated as independent events. While accepting that this assumption may not be correct, it is necessary in order to perform further statistical analyses.

Bouts of dives were defined following the iterative statistical method of Boyd et al. (Boyd et al. 1994) which relies on searching the dive sequence for a change in behaviour which differs significantly from the previous set of behaviours since the last significant change. A minimum dive bout was formally defined as a group of at least three dives occurring within a period of 10 min. The dive record for each penguin was searched sequentially from the start and once a group of dives had satisfied this minimum requirement, a search was made through the subsequent dives to find the end of the diving bout. This was done by calculating the mean and standard deviation of the surface intervals between dives, within the diving bout, and comparing these with the next surface interval in the sequence. If the next surface interval was significantly greater than the previous surface intervals in the bout (*t*-test, $P < 0.01$) then the bout was deemed to have ended. If the duration of the surface interval was not significantly different from those in the current bout, then the dive was included within the bout, the mean and standard deviation of the surface intervals for the bout were recalculated, and the analysis then moved onto the next dive in the sequence.

The f_H data were used to estimate rate of oxygen consumption using the relationship between f_H and mass specific oxygen consumption ($s\dot{V}_{O_2}$) obtained from macaroni penguins walking on a treadmill (Green, J.A. et al. 2001). In the current study $\dot{V}_{O_2}$ is used interchangeably with $s\dot{V}_{O_2}$, with the units quoted where it is necessary to highlight a distinction. For breeding female penguins, which were the subjects of the present study, the equation is:

$$s\dot{V}_{O_2} = (0.297 * f_H) - 17.40 \quad r^2 = 0.84 \qquad (5.1)$$

(where $s\dot{V}_{O_2}$ is in ml min^{-1} kg^{-1}, and f_H is in beats min^{-1})

This technique is normally validated when the animals' metabolism is in steady state and hence can not be used to estimate $\dot{V}_{O_2}$ while the animal is submerged. However, if f_H and $\dot{V}_{O_2}$ are averaged over a number of complete dive/surface cycles then f_H is an accurate and reliable predictor of $\dot{V}_{O_2}$ in aquatic birds and mammals (Fedak 1986, Bevan et al. 1992, Butler 1993). The standard deviation of an estimate made using equation 5.1 was calculated

using equation 11 of Green et al. (2001) and is quoted in the text where estimates have been made. Oxygen stores have not been measured in macaroni penguins, so calculations of cADL were made using the assumption that all penguins have oxygen stores of 58 ml kg^{-1} (Kooyman and Ponganis 1990, Butler 2000).

Results were considered significant at $P < 0.05$ and the significance level is quoted in the text. Mean values are quoted ± 1 S.E.M. Percentage values were arcsine transformed before comparisons were made (Zar 1999). All times are given in local time unless otherwise stated.

Results

Deployments and large-scale variation

Data were obtained from 13 penguins. Failure in the encapsulation led to battery failure in the other two deployments. Table 5.1 shows details of the 13 birds from which data were obtained, including a total of 44022 dives. Figure 5.1 shows heart rate (f_H), temperature (T_{ab}) and dive depth averaged every hour for six days from one individual. Heart rate and abdominal temperature appear to vary synchronously with diving activity. Low mean T_{ab} and f_H appear to be associated with increased average diving depth. In order to investigate this in more detail, average hourly f_H and T_{ab} were compared to diving activity in terms of hourly proportion of time spent submerged, using least squares linear regression. In each individual, there was a significant negative relationship between T_{ab} and diving activity (Table 5.2), with an average r^2 of 0.44 ± 0.04. Similarly, in each individual there was a significant negative relationship between T_{ab} and hourly cumulative diving depth, but in this case the average $r^2$was only 0.39 ± 0.02. There were significant positive relationships between f_H and diving activity in six individuals and no significant relationship in the other seven (Table 5.2). The average r^2 value of the significant relationships was 0.11 ± 0.04, but only 0.05 ± 0.02 for all 13 relationships. This implies that diving activity is a reasonable predictor of T_{ab} but a poor predictor of f_H, and therefore of $\dot{V}_{O_2}$ and metabolic rate (MR). Diving activity was greater during the day (Fig. 5.2) when dives were deeper, (2-way ANOVA, $F_{(23,276)} = 45.19$, $P < 0.001$), more frequent (2-way ANOVA, $F_{(23,276)} = 15.43$, $P < 0.001$) and longer (2-way ANOVA, $F_{(23,276)} = 51.38$, $P < 0.001$).

Diving behaviour and dive bouts

When dives were classified into bouts, 98% of all dives were part of a bout consisting of at least three dives. Only dives within bouts were considered for further analyses. When considering post-dive surface intervals, the last dive of a bout was discarded. Individuals varied slightly in their diving behaviour (Table 5.3). Figure 5.3 shows the relationship between dive depth, dive duration and frequency for all dives. For each individual, there was a highly significant positive linear relationship between dive depth and duration (mean r^2 = 0.64). Figure 5.4 shows the relationship between dive duration, post-dive surface time and frequency for all dives. This indicates the large amount of variation in surface intervals, especially at shorter dive durations. There were significant positive relationships between dive duration and post-dive surface interval in five animals, significant negative relationships in seven animals and no relationship in the other. However, the average r^2 value of these was only 0.01 ± 0.04 so these relationships are not very instructive. The distributions of dive depths and dive durations averaged over the 13 animals are shown in Figure 5.5. The distribution of depths was not normal (Fig. 5.5a) with twenty one percent of all dives to a maximum depth of 4.8 m, and then descending frequencies to the maximum dive depth recorded of 94.8 m. This dive was recorded by penguin H79, which was responsible for most of the deeper and longer dives, including all those deeper than 70 m. Dive durations were more normally distributed (Fig. 5.5b), though skewed slightly towards dives of a shorter duration.

Abdominal temperature during diving

The mean T_{ab} during diving bouts was 34.8 ± 1.2 ^{o}C while the mean T_{ab} while at-sea but not diving and while on-shore was 39.5 ± 0.9 ^{o}C and 40.1 ± 0.8 ^{o}C respectively. Analysis of variance with Tukey post-hoc testing ($F_{(2, 36)}$ = 8.7, P <0.001) showed that there was no significant difference between on-shore and non-diving T_{ab}, but both were significantly greater than T_{ab} while diving. Further analyses were performed to further investigate this decline in T_{ab} associated with diving and what effects it might have. Linear regressions were used to determine whether mean diving T_{ab}, dive duration and mean diving f_H varied

progressively during the course of each diving bout (Table 5.4). 63.4 % of all dive bouts showed a significant change in T_{ab} through the course of the bout and 76% of these were significant declines with a mean r^2 of 0.76 (Table 5. 4). However, only 35.0% and 35.4% of bouts showed a significant change in dive duration and f_H over the course of the bout, and the average r^2 of these relationships was only 0.37 and 0.34 respectively. The magnitude of the decline in T_{ab} was calculated as the difference between the maximum and minimum average temperature during diving for each dive bout. The mean magnitude of this decline was 2.32 ± 0.20 ^{o}C while the mean maximum decline was 13.52 ± 1.10 ^{o}C. The magnitude of the drop in T_{ab} increased with the length of the diving bout for each individual (mean r^2 =0.55, all P <0.001) and for all diving bouts pooled (r^2 = 0.46, P <0.001, Fig. 5.6).

Linear regression was also used to determine whether T_{ab} tended to be lower during dives with longer durations. There was no significant relationship between dive duration and mean T_{ab} during the dive in 3 animals. The other 10 animals did show significant relationships between T_{ab} and dive duration, but the mean r^2 of these relationships was only 0.1. Paired t-tests were used to compare abdominal temperature at the beginning and end of dives in all individuals in dives of all durations to determine whether abdominal temperature varied within dives. Table 5.5 shows the results of these comparisons. In general, there were no significant changes, though it is possible that the time constant of the DL was too large in comparison to the duration of dives to detect small or rapid changes. Again, there was a great deal of variation between individuals with some penguins showing several significant drops in body temperature during dives, some showing none and three showing a significant increase in body temperature at some durations. The mean drop in temperature of all the significant declines was 0.07 ^{o}C.

Heart rate and rate of oxygen consumption while diving

Mean heart rate while the penguins were at-sea but not diving, calculated from f_H between diving bouts, was 132 ± 4 beats min^{-1}. This was not significantly different (t-test $t_{(12)}$ = 1.27 P >0.05) to the mean f_H while penguins were on-shore (127 ± 5 beats min^{-1}). Relative to these values, macaroni penguins showed bradycardia and tachycardia associated with dives of all durations. The extent of the bradycardia and tachycardia associated with diving were related

to dive duration. Table 5.6 shows mean, maximum and minimum f_H at different stages of the diving cycle for dives of different durations while Figure 5.7 shows how heart rate and abdominal temperature varied during dives of 102-110 seconds in duration, the most frequently observed category of dive duration (Fig 5.5b). A similar pattern was observed in dives of both longer and shorter durations and can be described as follows: 1) f_H increased prior to diving and started to decrease just before leaving the water surface. 2) Upon submersion, f_H immediately dropped before recovering slightly and then decreasing more slowly. 3) As the dive depth reached a plateau then so did f_H. 4) As the penguin started to ascend to the surface, f_H increased. 5) f_H then increased more rapidly after the penguin surfaced. 6) The subsequent tachycardia would then be followed immediately by another dive, if the dive was part of a dive bout, otherwise f_H would decrease to non-diving levels. For dives of all durations, pre-dive and post-dive f_H were higher than f_H while not diving. In addition, the mean minimum f_H during dives was lower than f_H while not diving for all dives and mean f_H while diving was lower than non-diving f_H for all dives greater than 70 s in duration (Table 5.5).

The effects that the different changes in f_H and T_{ab} associated with diving had on dive duration were investigated using a multiple regression analysis. Two multiple regressions were performed; the first using f_H measurements from the dive cycle, the second using differences in f_H between different phases of the dive cycle. The independent variables in the first were: mean diving f_H, minimum diving f_H, minimum f_H within the first 10s of submersion, pre-dive mean f_H, pre-dive maximum f_H, post-dive mean f_H, post-dive maximum f_H and abdominal temperature. The independent variables used in the second were: the drop in f_H from mean pre-dive to mean during diving, the drop in f_H from maximum pre-dive to minimum during diving, the drop in f_H from maximum pre-dive to minimum within 10s of submersion and abdominal temperature. Each regression was performed for each animal and the results are shown in Table 5.7. The first analysis indicated that 41% of the variation in dive duration can be explained by the adjustments in f_H and T_{ab}. There was a great deal of variation between individuals, but the most consistent influences on dive duration were minimum f_H while submerged, followed by T_{ab}, minimum f_H shortly after submersion and mean pre-dive f_H. The second analysis examining the magnitude of heart rate changes from pre-dive to within the dive did not explain as much of the variation (28%) and is thus less useful than the first

analysis. The regressions were repeated without T_{ab} as a variable. This had little effect other than to lower the mean r^2 values for the first and second analyses to 36% and 22% respectively.

In order to determine whether diving within bouts was aerobic in nature, the aerobic dive limit was calculated (cADL) and compared to observed dive durations. To calculate cADL, $\dot{V}_{O_2}$ while submerged was estimated from f_H using equation 5.1. The use of f_H to estimate $\dot{V}_{O_2}$ has been validated for completed dive cycles, but it is not possible to measure $\dot{V}_{O_2}$ while submerged and examine how this relates to f_H while submerged. However, it seems likely that $\dot{V}_{O_2}$ while submerged lies somewhere between the values for $\dot{V}_{O_2}$ if estimated using (a) mean f_H from the completed dive cycle and (b) f_H during submersion only. Since mean f_H varied with dive duration (Table 5.6), it was necessary to estimate $\dot{V}_{O_2}$ while submerged at each different dive duration for the full range observed by macaroni penguins (Fig. 5.8). As mean f_H during diving decreased with dive duration (Table 5.6) then so did estimated $\dot{V}_{O_2}$ (Fig. 5.8). cADL was also calculated for each dive duration using oxygen stores of 58 ml kg^{-1} and increased with increasing dive duration as $\dot{V}_{O_2}$ decreased. When submerged $\dot{V}_{O_2}$ was estimated from f_H for the completed dive cycle, all dives up to 138 s in duration (Fig 5.5; 95.3% of all dives) were of a shorter duration than the cADL for that duration. When submerged $\dot{V}_{O_2}$ was estimated from f_H while submerged only, all dives were of a shorter duration than the cADL (Fig. 5.8). Assuming that the true diving $\dot{V}_{O_2}$ lies somewhere between these two estimates, it seems reasonable to assume that all dives within bouts by macaroni penguins were aerobic.

Discussion

Diving behaviour

Two previous studies have investigated the diving behaviour of macaroni penguins breeding at Bird Island, using externally mounted devices (Croxall et al. 1988, 1993). The first of these

studies used a simple depth histogram recorder (DHR) on 8 breeding males. The second used a more sophisticated dive depth recorder, but this was heavy, bulky and only used on two female penguins. Another more comprehensive study was completed on breeding males and females at Heard Island (Green, K. et al. 1998), and used time depth recorders measuring depth every three seconds to give more detailed dive profiles. Despite these differences in methodology and location, the patterns in diving behaviour shown by these studies were similar and these, in turn, are similar to the patterns observed in the present study. Similarities were observed in distributions of dive depth and duration, with many short dives to less than 5 m and other longer dives to around 40-50 m. In all four studies there was considerable individual variation in diving behaviour and the comparison suggests that the macaroni penguins were no more inconvenienced, in terms of diving performance, by carrying an implanted heart rate data logger than they were by carrying one of the other externally mounted depth recording devices.

In all four studies, macaroni penguins tended to dive predominantly in daylight. Dives at night were less frequent, to shallower depths and of shorter duration. For macaroni penguins foraging in waters around Bird Island, a suggested cause for this was the diurnal migration of Antarctic krill (Croxall et al. 1993). Krill are found near the top of the water column at night but are more widely dispersed through the water column during daylight. For penguins feeding near Heard Island, the reasons are less clear, since the myctophid icefish they feed on has no clear diurnal migration. Instead, a reliance on visual foraging was suggested as the explanation for decreased diving at night (Green, K. et al. 1998). Such a reliance on daylight for successful foraging has also been proposed in other penguin species feeding on a variety of prey in different locations (Wilson et al. 1993).

Heart rate changes within dives

Figure 5.7 shows the average change in heart rate associated with dives of between 102-110 seconds. Although individual dives were more variable than the mean, the pattern was reasonably consistent between individuals. Heart rate during diving has been recorded previously in diving birds, but only within laboratory conditions (Butler and Woakes 1979, 1984, Stephenson et al. 1986), semi-natural conditions (Kooyman et al. 1992b) or in the field

at a lower resolution (Bevan et al. 1997). These studies showed similar patterns in the change of heart rate before during and after dives with tachycardia followed by bradycardia then tachycardia. Such a response is now widely accepted to be a trade-off between the "classic dive response" which conserves oxygen stores while the animal is deprived of access to air and the "exercise response" which prioritises blood flow and oxygen uptake to active muscles when exercising (Butler 1988).

In the present study, the mean pre-dive and post-dive f_H were higher than f_H when the birds were on-shore or at-sea and not diving, and this was consistent for dives of all durations. In addition, the mean minimum f_H during the dive was lower than non-diving f_H for dives of all durations and the mean diving f_H was lower than non-diving f_H in all dives longer than 70 s duration (Fig 5.5; 69% of all dives). In emperor penguins diving from man-made ice holes, diving f_H was on average less than f_H while resting ashore at night, though no distinction was made between dives of different durations (Kooyman et al. 1992b). Adjustments in f_H allow dive duration to be extended by ensuring full loading of oxygen stores before the dive, then by reducing metabolism during the dive (Butler and Jones 1997) and ensuring the full and effective use of oxygen stores while submerged (Davis and Kanatous 1999).

The multiple regression analysis indicated that the most important physiological influences on dive duration were minimum heart rate during the dive and abdominal temperature. Including abdominal temperature within the multiple regressions improved the accuracy of the models, but when considered alone, there was only a very poor correlation between T_{ab} and dive duration. This implies that the physiological adjustments interact with each other to increase dive durations. The multiple regression analysis is instructive, but it is difficult to determine whether dive duration is dependent on the metabolic adjustments or vice versa. What can be stated with certainty is that in macaroni penguins, the cardiac adjustments become more exaggerated as dive duration increases.

$\dot{V}_{O_2}$ was estimated from f_H recorded while the penguins were submerged and from f_H averaged over completed dive cycles. It is not possible to measure the $\dot{V}_{O_2}$ while submerged, nor is it apparent how this relates to f_H while submerged. Indeed, f_H while submerged has not

been related to $\dot{V}_{O_2}$ for completed dive cycles in diving birds. However it does seem likely that $\dot{V}_{O_2}$ while submerged is lower than the average of the completed dive cycle as birds consume oxygen on the surface and restock stores, and may be as low as that predicted by submerged f_H. In the present study, using f_H from the completed dive cycle, 95.3% of dives had durations which were less than the cADL, whereas using f_H while submerged only, all dive durations were less than the cADL. This implies that all dives within bouts by macaroni penguins are aerobic in nature. In addition, $\dot{V}_{O_2}$ calculated from non-diving f_H of 132 beats min^{-1} would be 21.8 ± 1.3 ml min^{-1} kg^{-1}. The resultant cADL would be 160 s with a range of 142 – 182 s if the error of estimation of $\dot{V}_{O_2}$ is taken into account. This would translate to 98.9% of observed dives being within the cADL with a range of 96.5% - 99.7%. Similar calculations of cADL using $\dot{V}_{O_2}$ while resting on the water have been made for other penguin species (Butler 2000). In emperor penguins 96% of foraging dives in the field would be within the cADL whereas in king (*Aptenodytes patagonicus*) and gentoo (*Pygoscelis papua*) penguins, only 80% of dives in the field would be within the cADL.

It is not clear why there should be this discrepancy between species in the balance of aerobic and anaerobic dives. It is possible that errors in estimation, measurement and assumption could have lead to erroneous conclusions. Alternatively, macaroni and emperor penguins could be diving comfortably within their physiological limits whereas gentoo and king penguins work harder and perform more anaerobic dives while foraging. Gentoo penguins foraging at South Georgia with a mass of approximately 6 kg spent the same proportion of foraging trips submerged (52%) but dived to much greater depths (mean approx. 55m) and for greater durations (mean approx. 125 s) (Williams, T.D. et al. 1992) than macaroni penguins. Ecological differences between the two species have been described previously (Croxall et al. 1997) and these combined with the vulnerability of gentoo penguins to variations in their food availability (Croxall et al. 1999) suggest that this species is performing much closer to its physiological limits. Similarly, despite significant differences in body mass and size between king and emperor penguins (means approx. 13 and 22 kg respectively) (Pütz et al. 1998), their diving performance is not very different (Kooyman et al. 1992a, Kooyman and Kooyman 1995). Though emperor penguins are capable of superior maximum dive depth and duration

than king penguins, the distributions of the bulk of foraging dives are similar between the two species (Kooyman et al. 1992a, Kooyman and Kooyman 1995). This implies that emperor penguins are operating well within their physiological limits whereas king penguins are pushing themselves to dive for as long and as deep as possible.

Changes during dive bouts

Abdominal temperature showed a progressive decline during most dive bouts. Similar declines in body temperatures have been observed in other diving birds including king penguins (Culik et al. 1996b, Handrich et al. 1997), gentoo penguins (Bevan et al. 1998), king cormorants (Kato et al. 1996) and blue-eyed shags (Bevan et al. 1997) as well as in marine mammals (Hill et al. 1987). The cause of this decline is still uncertain (Kooyman et al. 1980, Hill et al. 1987, Kooyman 1989, Handrich et al. 1997). It may simply be the consequence of conduction to cold water from exposed surfaces on the feet and flippers, or of ingesting cold food. Animals may attempt to halt this process or simply allow it to continue. Alternatively it may be the result of an increase of blood flow to the periphery to intentionally lose the heat energy. Indeed, these explanations may not be mutually exclusive and the subject is still under investigation. Data from king penguins suggest that the process is in some way facilitated and not just the consequence of ingesting cold food, as the temperature in the abdomen of foraging king penguins was lower than that in the stomach (Handrich et al. 1997). It has been proposed that this reduction in body temperature leads to lowered metabolic rates in diving birds (Culik et al. 1996b) through the effect of cold temperatures on metabolically active tissues and by reducing the cost of thermoregulation (Boyd and Croxall 1996, Butler 2000). This lowering of metabolic rate is suggested to be sufficient to bring most dives observed in the field within the ADL (Boyd and Croxall 1996, Butler 2000). In king penguins, fluctuations in temperature in localised parts of the body were found to vary between consecutive dives (Culik et al. 1996b). In the present study, consistent variation within dives could not be detected. However, this may be attributed to the relatively long time constant and hence slow response time of the data loggers and/or the short dive duration of macaroni penguins. The variation between individuals may be explained by slight differences in the positioning of the DL within the abdominal cavity. It is possible that there was variation of temperature within individual dives leading to a reduction in metabolic costs during the dive.

However, it was not possible to detect this change with the DL used in the present study. Further work involving more sensitive and faster responding temperature sensors at multiple locations around the body may cast more light on the importance of this mechanism in extending dive durations.

Though it was not possible to detect differences in T_{ab} within individual dives in the present study, T_{ab} did decline progressively during diving bouts. The shape and gradient of this temperature decline varied between individuals (which may also be attributable to DL position) and between bouts performed by the same individual, but in each case the decline was progressive throughout the bout, and abdominal temperature only increased after or at the very end of the bout. The magnitude of the temperature drop did, however, increase consistently with the duration of diving bouts (Fig. 5.6). If diving behaviour was determined only by physiological capacity and lowered abdominal temperature was essential in facilitating increased diving duration, then we might expect to see dive duration or mean f_H increasing progressively through bouts as abdominal temperature decreases. However, as Table 5.5 shows, nearly as many diving bouts showed a decrease in dive duration during bouts as showed an increase and over 64% showed no significant change at all. In addition, all dives were within the cADL. This supports the suggestion that for macaroni penguins, though physiology will limit maximum dive duration, other factors are likely to be more important in determining diving behaviour. Such factors could include progressive satiation during dive bouts and the location and density of patches of food within the water column, especially since Antarctic krill are found in swarms (Everson 2000). In gentoo and king penguins which may be pushing the physiological limits of diving more than macaroni penguins, such patterns of changing dive duration or mean f_H within bouts might be observed.

The progressive decline in abdominal temperature may be the result of many smaller declines associated with individual dives. The abdomen may not have sufficient time to return to its initial temperature during the surface interval between dives and the overall decline in temperature may be the result of an accumulation of these cycles. Alternatively, we suggest that circulatory adjustments are made at the onset of diving bouts, which restrict blood flow to the abdomen, and as the bout continues temperature drops steadily through conduction to the colder seawater. This would allow stomach temperature to be higher as, even though cold

food is ingested, the stomach is closer to the core of the body and the warm pectoral muscles. It is possible that the major influence in extending dive duration could be the restriction of blood flow and the consequent reduction in heart rate, and that the drop in abdominal temperature is a consequence of this rather than a direct influence on metabolic suppression.

Changes in blood flow and perfusion during diving have been proposed ever since the early physiological experiments on forcibly submerged animals (Scholander 1940) and have subsequently been observed in freely diving penguins (Millard et al. 1973) and other diving birds (Bevan and Butler 1992). Blood flow is reduced to areas of the body not used while diving including the inactive limbs (legs in penguins) and gastrointestinal tract, while it is maintained in the active and heat generating muscles and the oxygen dependent heart and brain. Data on these circulatory adjustments are limited (Kooyman and Ponganis 1998) but they could have a very great effect on reducing metabolic rate and maximising the effective use of oxygen stores (Davis and Kanatous 1999). The kidneys, splanchnic organs and heart account for 40% of resting $\dot{V}_{O_2}$ (Kooyman and Ponganis 1998) and the metabolic rate of all of these could decrease significantly with reduced perfusion (Kooyman and Ponganis 1998). In diving tufted ducks, after the flow to the heart, brain and active leg muscles had been subtracted there was very little of the total cardiac output to share around the rest of the body, suggesting that most other supplies were cut off or severely limited (Bevan and Butler 1992).

In most individuals there was a significant relationship between abdominal temperature and dive duration but the r^2 value of these correlations was very low. There would be some additional metabolic advantages in a reduction in temperature in some regions of the body. However if very little blood is reaching the regions where perfusion is reduced then any advantages of the effect of lowered temperatures on metabolism (Heldmaier and Ruf 1992), reduced thermoregulatory costs and the decreased affinity for oxygen (Schmidt-Nielsen 1997) in cold tissues are likely to be minimal.

Conclusions

The present study does not fully explain how metabolic and circulatory adjustments permit the diving behaviour exhibited by penguins and other birds. However, it does indicate that

most dives by macaroni penguins within bouts of repeated dives are likely to be aerobic. Circulatory adjustments and the consequent reduction of heart rate during dives are sufficient to permit a sufficiently low level of oxygen consumption such that even the longest observed dives performed by these animals may be supported by aerobic metabolism. A result of this is a reduction in abdominal temperature, which may further reduce metabolism in its own right. Further work is necessary to investigate these circulatory adjustments; how often they are made and to what extent is blood flow is reduced, how tissues are affected by prolonged periods of hypothermia and reduced blood supply, and exactly which parts of the body are affected by these adjustments. Only by investigating further will we understand the extent to which physiological adjustments enable these birds to undertake their impressive underwater foraging behaviour.

Bird	Mass (kg)	Duration of data record (d)	Proportion of time at-sea	Proportion of at-sea time submerged	Number of dives recorded
H02	3.6	8.26	0.61	0.61	2656
H15	3.3	12.95	0.80	0.58	6367
H17	3.1	4.65	0.78	0.58	1926
H25	3.9	11.56	0.69	0.56	4466
H29	3.6	6.17	0.79	0.47	2341
H53	3.3	28.01	0.49	0.52	7182
H59	4.0	1.29	0.83	0.48	466
H61	3.4	6.29	0.70	0.50	2339
H69	3.8	4.18	0.73	0.17	639
H73	3.8	3.48	0.54	0.42	857
H79	4.1	26.81	0.51	0.58	6932
H93	3.6	9.09	0.54	0.62	3316
H95	3.4	13.26	0.58	0.64	4535
Mean ± S.E.M.	3.6 ± 0.3	10.46 ± 2.32	0.67	0.52	3386 ± 6 49

Table 5.1. Deployment details and simple parameters of diving for 13 breeding female macaroni penguins from which data were obtained.

	(a) Abdominal temperature vs proportion of time spent				(b) Heart rate vs proportion of time spent			
Bird	Gradient	Intercept	*P*	r^2	Gradient	Intercept	*P*	r^2
H02	-4.82	44.29	< 0.001	0.33	32.24	140.86	< 0.001	0.14
H15	-14.54	38.32	< 0.001	0.35	3.25	1230.58	> 0.05	0.00
H17	-10.17	38.91	< 0.001	0.34	12.29	113.45	> 0.05	0.04
H25	-12.62	38.82	< 0.001	0.42	16.064	146.01	< 0.01	03.03
H29	-7.15	44.26	< 0.001	0.43	9.49	136.47	> 0.05	0.01
H53	-2.96	38.82	< 0.001	0.54	5.32	171.02	> 0.05	03.00
H59	-2.67	39.82	< 0.001	0.40	13.40	152.15	> 0.05	0.06
H61	-4.90	44.40	< 0.001	0.59	10.24	116.85	> 0.05	0.01
H69	-3.94	33.06	< 0.001	0.28	35.74	148.64	> 0.05	0.04
H73	-5.92	39.59	< 0.001	0.73	38.83	117.13	< 0.01	0.24
H79	-3.36	38.46	< 0.001	0.28	14.19	104.27	< 0.01	0.02
H93	-10.55	38.95	< 0.001	0.53	55.35	148.21	< 0.01	0.17
H95	-9.59	39.76	< 0.001	0.53	7.30	122.90	> 0.05	0.01
Mean ± S.E.	-7.17 ± 1.09	39.81 ± 0.85		0.44 ± 0.04	19.41 ± 4.40	135.50 ± 5.27		0.06 ± 0.02

Table 5.2. Characteristics of the relationships between (a) hourly mean abdominal temperature and hourly proportion of time spent underwater (b) hourly mean heart rate and hourly mean proportion of time spent underwater, recorded from 13 breeding female macaroni penguins.

Bird	Number of diving bouts	Number of dives within bouts	Number of dives not within bouts	Average number of dives per bout	Mean dive depth (m)	Mode dive depth (m)	Median dive depth (m)	Maximum dive depth (m)	Mean dive duration (s)	Mode dive duration (s)	Median dive duration (s)	Maximum dive duration (s)
H02	53	2615	41	49.3	21.7 ± 0.3	7.2	19.2	69.6	100.3 ± 0.7	124	102	180
H15	171	6251	116	36.6	17.0 ± 0.1	2.4	14.4	57.6	81.4 ± 0.4	78	82	224
H17	43	1879	47	43.7	18.1 ± 0.2	15.6	16.8	45.6	95.4 ± 1.4	108	102	164
H25	141	4371	95	31.0	19.0 ± 0.2	2.4	18.0	60.0	88.1 ± 0.5	112	96	168
H29	66	2281	60	34.6	18.3 ± 0.2	2.4	20.4	42.0	85.6 ± 0.6	102	94	142
H53	186	7091	91	38.1	14.9 ± 0.1	2.4	12.0	69.6	84.5 ± 0.4	109	86	178
H59	24	456	10	19.0	13.0 ± 0.4	2.4	10.8	54.0	95.4 ± 1.4	109	98	186
H61	66	2262	77	34.3	16.2 ± 0.2	2.4	14.4	51.6	83.5 ± 0.7	114	90	156
H69	20	618	21	30.9	18.1 ± 0.5	2.4	19.2	44.4	68.6 ± 1.6	104	82	180
H73	35	845	12	24.2	14.2 ± 0.4	2.4	12.0	44.4	80.3 ± 1.1	100	84	172
H79	216	6814	118	31.5	23.4 ± 0.3	2.4	13.2	94.8	95.4 ± 0.6	58	92	232
H93	71	3277	39	46.1	16.1 ± 0.2	2.4	15.6	46.8	79.8 ± 0.6	68	82	174
H95	98	4484	51	45.8	17.5 ± 0.1	2.4	18.0	58.8	87.8 ± 0.5	112	92	174
Mean ± S.E.	91.54 ± 19.20	3326 ± 640	59.9 ± 10.2	35.6 ± 2.5	17.5 ± 0.8	3.8 ± 1.0	15.7 ± 0.8	56.9 ± 4.1	86.6 ± 2.4	99.8 ± 5.4	90.9 ± 2.0	179.2 ± 6.8

Table 5.3. Characteristics of diving behaviour within diving bouts in 13 breeding female macaroni penguins.

	Dive bouts with decreasing abdominal temperature, heart rate or dive duration			Dive bouts with increasing abdominal temperature, heart rate or dive duration			Dive bouts with no change in abdominal temperature, heart rate or dive duration		
Variable	Mean proportion of bouts	Mean r^2	Mean number of dives/bout (± S.E.M.)	Mean proportion of bouts	Mean r^2	Mean number of dives/bout (± S.E.M.)	Mean proportion of bouts	Mean r^2	Mean number of dives/bout (± S.E.M.)
Abdominal temperature	48.3	0.76 ± 0.04	54 4	15.1	0.64 ± 0.04	27 3	36.6	0.33 ± 0.03	14 1
Heart rate	12.0	0.31 ± 0.04	72 9	23.3	0.36 ± 0.02	60 7	64.6	0.16 ± 0.01	21 1
Dive duration	16.1	0.36 ± 0.02	80 6	18.9	0.38 ± 0.03	45 4	65.0	0.02 ± 0.01	22 2

Table 5.4. Characteristics of the linear regressions describing changes in abdominal temperature, heart rate and dive duration within diving bouts.

Bird	Dive duration: 1 – 20 s	21 – 30 s	31 – 40 s	41 – 50 s	51 – 60 s	61 – 70 s	71 –80 s	81– 90 s	91– 100 s	101 – 110 s	111 – 120 s	121 – 130 s	131 – 140 s	141 – 150 s	151 – 160 s	161 – 170 s	171 – 180 s	181 + s
H02	n/s	n/s	n/s	n/s	*	*	*	n/s	n/s	n/s	n/s	**	n/s	n/s	**	n/s	n/s	
H15	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	**	n/s	n/s			n/s
H17	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	***	n/s	n/s			
H25	n/s	n/s	***	n/s	*	***	n/s	***	**	***	***	***	***	**	*	n/s		
H29	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s					
H53	n/s	n/s	n/s	*	n/s	n/s	*	n/s	n/s	n/s	n/s	*	n/s	n/s	n/s	n/s	n/s	
H59	n/s	n/s			*	n/s	n/s	n/s	**	***	n/s	**	n/s	**	n/s	n/s		
H61	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s		
H69	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s			
H73	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s			n/s	
H79	n/s	n/s	n/s	n/s	n/s	***	***	*	***	**	**	**	n/s	n/s				
H93	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	*	n/s	n/s	n/s	n/s	n/s	n/s	n/s	***
H95	n/s	n/s	n/s	**	**	***	***	***	***	***	***	*	*	***	n/s			

Table 5.5. Results of paired t-tests to examine the change in abdominal temperature during dives of different durations. One asterisk represents a significant decrease at P <0.05, two asterisks a significant decrease at P <0.01 and three asterisks a significant decrease at P <0.001. n/s represents a non-significant change and the shaded boxes represent significant increases in abdominal temperature.

Dive duration (s)	Number of penguins	Number of dives	Mean f_H during pre-dive (beats min^{-1})	Max f_H pre-dive (beats min^{-1})	Mean f_H during dive (beats min^{-1})	Mean min f_H during dive (beats min^{-1})	Min f_H during 1st 10s of dive (beats min^{-1})	Mean f_H post-dive (beats min^{-1})	Max f_H post-dive (beats min^{-1})	Max f_H pre-dive – min during dive (beats min^{-1})	Mean f_H during pre-dive - mean during dive (beats min^{-1})
0 – 20	13	1913	185 ± 11	242 ± 9	156 ± 8	105± 9	107 ± 8	179 ± 10	263 ± 10	30 ± 7	137 ± 8
21 – 40	13	3048	194 ± 12	255 ± 10	143 ± 8	89 ± 9	107 ± 8	186 ± 11	264 ± 10	51 ± 7	166 ± 8
41 – 60	13	5253	203 ± 15	269 ± 11	139 ± 7	81 ± 8	109 ± 8	200 ± 14	274 ± 12	65 ± 11	188 ± 8
61 – 80	13	7111	209 ± 16	273 ± 12	133 ± 5	71 ± 7	105 ± 7	207 ± 16	276 ± 12	76 ± 12	203 ± 9
81 – 100	13	8206	214 ± 17	278 ± 12	129 ± 4	62 ± 6	95 ± 7	212 ± 17	278 ± 12	84 ± 14	215 ± 9
101 – 120	13	9005	216 ± 18	281 ± 12	127 ± 4	56 ± 5	89 ± 6	214 ± 18	281 ± 12	89 ± 15	225 ± 10
121 – 140	13	5126	217 ± 17	289 ± 12	125 ± 3	53 ± 5	93 ± 8	215 ± 16	288 ± 10	91 ± 15	236 ± 11
141 – 160	12	1562	209 ± 19	284 ± 11	122 ± 4	51 ± 6	941 ± 11	208 ± 19	282 ± 12	887 ± 19	233 ± 9
161 – 180	7	554	208 ± 26	264 ± 26	119 ± 11	53 ± 8	93 ± 14	266 ± 35	319 ± 25	89 ± 22	211 ± 20
180 +	2	220	210 ± 30	246 ± 8	153 ± 54	47 ± 11	156 ± 60	182 ± 11	283 ± 23	57 ± 24	201 ± 19

Table 5.6. Average (± S.E.M.) mean, maximum and minimum values of heart rate (f_H) associated with different stages of the diving cycle, recorded from 13 breeding female macaroni penguins.

Bird	Mean f_H during dive	Min f_H during dive	Mean f_H post-dive	Max f_H post-dive	Min f_H during 1st 10s	Mean f_H pre-dive	Max f_H pre-dive	T_{ab}	r^2	Max pre- f_H dive – min f_H during 1st 10s	Max pre-dive f_H – min f_H during dive	Mean pre-dive f_H – mean f_H during dive	T_{ab}	r^2
H02	n/s	***	***	n/s	**	***	n/s	***	0.46	n/s	***	***	***	0.3
H15	***	***	***		***	***	n/s	***	0.34	***	***	***	***	0.21
H17	n/s	***	n/s	n/s	**	n/s	**	n/s	0.18	***	***	***	*	0.06
H25	***	***	***		***	***	***		0.43	***	***	***	***	0.28
H29	**	***	n/s	**	n/s	n/s	n/s	***	0.21	n/s	n/s	***	***	0.13
H53	***	***	***	**	***	***	***	***	0.42	***	***	***	***	0.35
H59	n/s	***	***	n/s	n/s	***	n/s	***	0.35	n/s	***	***	***	0.24
H61	***	***	n/s	n/s	***	***	n/s	***	0.27	***	***	***	***	0.2
H69	***	***	***	n/s	***	***	n/s	***	0.72	***	***	***	***	0.63
H73	***	***	***	n/s	**	n/s	***	***	0.63	***	***	***	***	0.5
H79	***	***	**	***	***	***	***	***	0.34	***	***	***	***	0.28
H93	***	***	***	***	***	***	***	***	0.49	***	***	***	***	0.37
H95	***	***	***	***	n/s	n/s	***	***	0.44	***	***	***	***	0.19
Mean ± S.E.									0.41 ± 0.04					0.29 ± 0.04
All dives	***	***	***	n/s	***	***	***	***	0.21	***	***	***	***	.0.09

Table 5.7. Different aspects of the changes in heart rate (f_H) and abdominal temperature (T_{ab}) during diving in female macaroni penguins, and their relative influence on diving duration in two multiple linear regressions. One asterisk represents a significant influence at P <0.05, two asterisks a significant influence at P <0.01 and three asterisks a significant influence at P <0.001. n/s represents a non-siganificant influence.

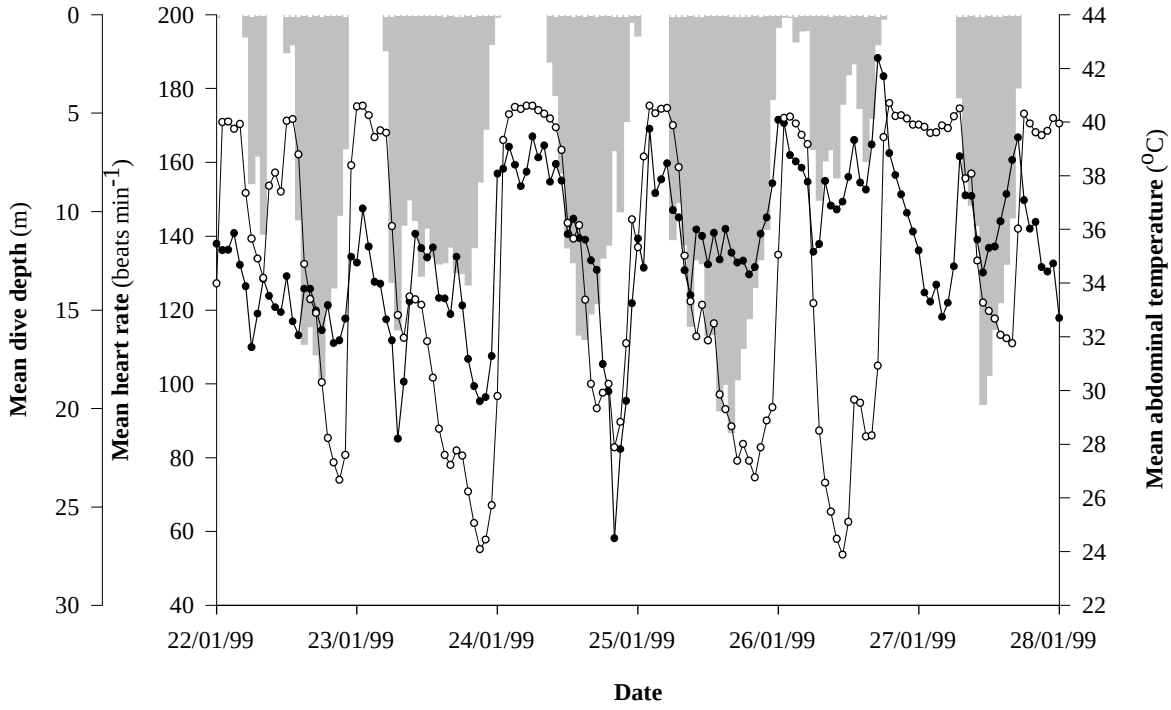

Figure 5.1. Hourly means of heart rate, body temperature and diving depth recorded over six days from penguin H95. The open circles are abdominal temperature, the closed symbols heart rate and the grey bars diving depth.

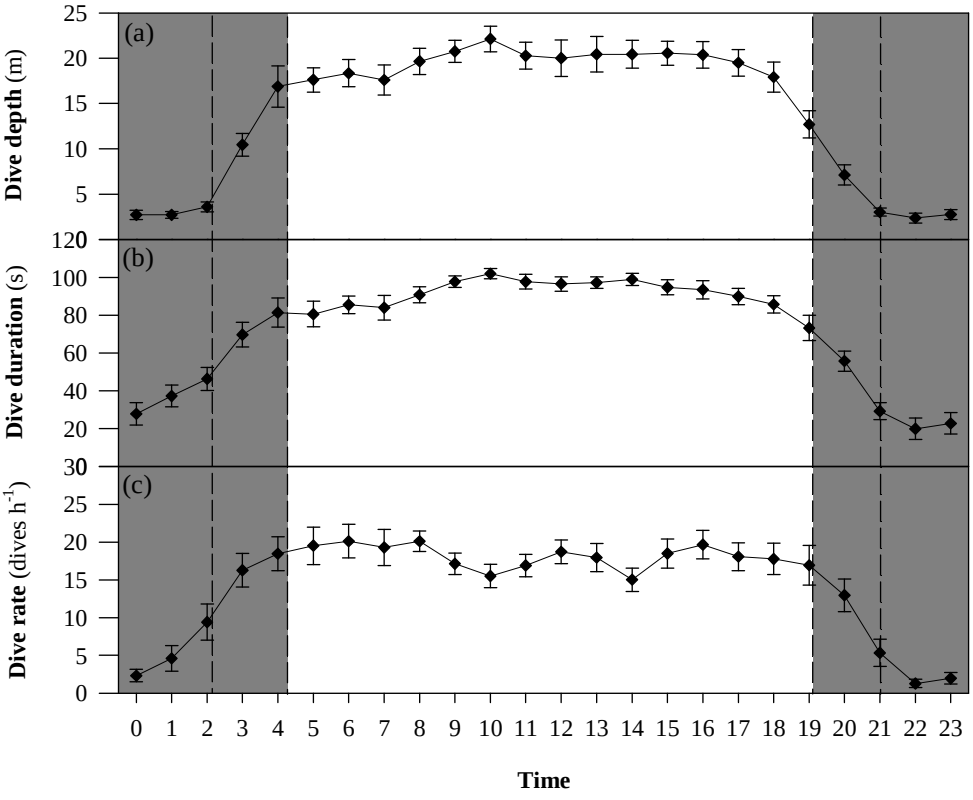

Figure 5.2. Influence of time of day on a) mean dive depth, b) mean dive duration and c) mean dive rate, recorded from 13 breeding female macaroni penguins. All values given as mean ± S.E.M. Dashed lines represent dawn and dusk, which moved during the season. The darker shaded area represents the hours of darkness.

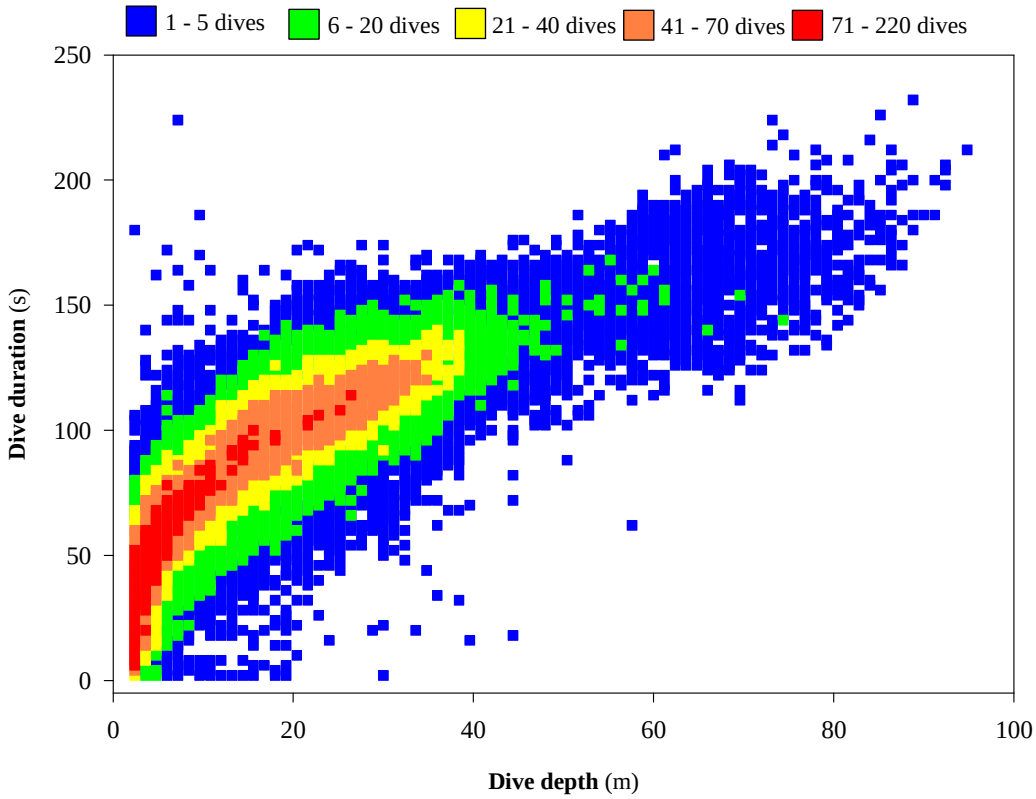

Figure 5.3. Relationship between dive depth, dive duration and frequencies of combinations of the two, for 43244 dives recorded from 13 breeding female macaroni penguins.

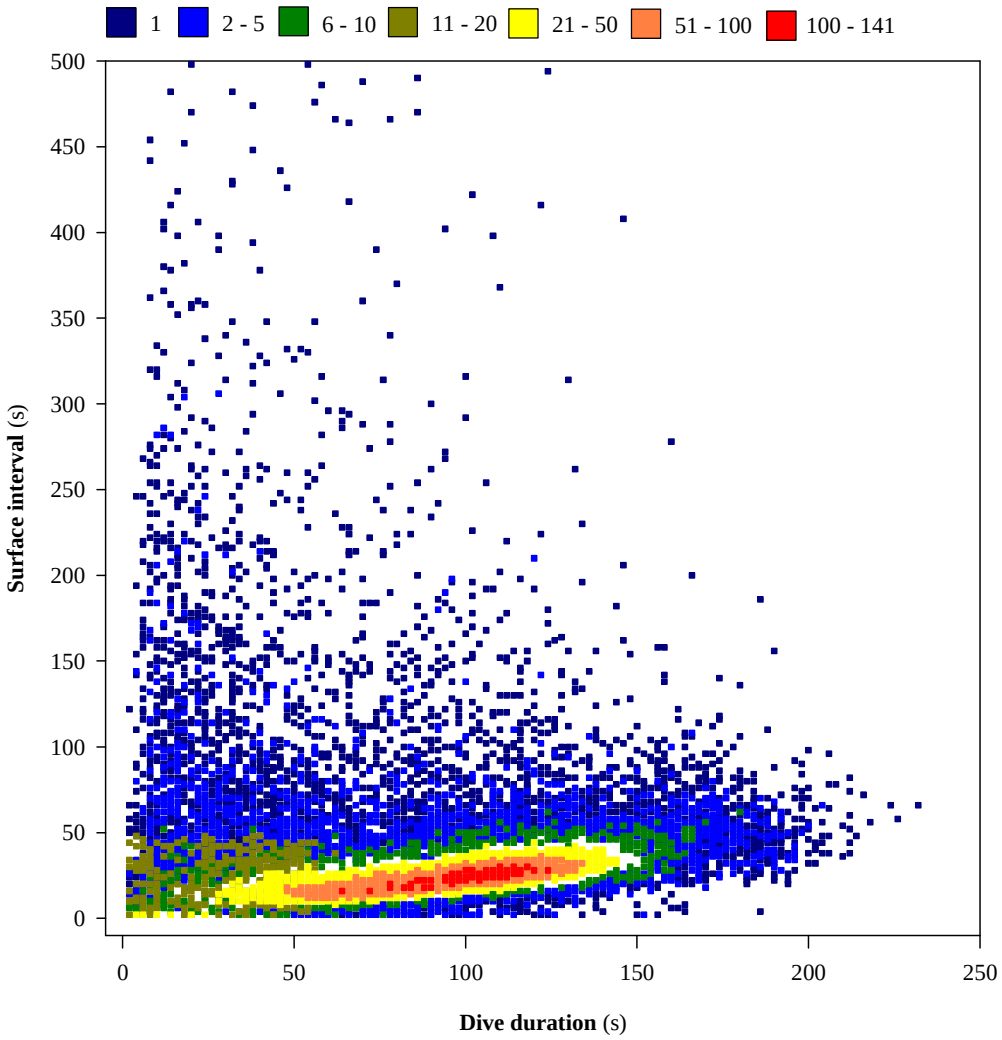

Figure 5.4. Relationship between dive duration, post dive surface interval duration and frequencies of combinations of the two, for 43244 dives recorded from 13 breeding female macaroni penguins.

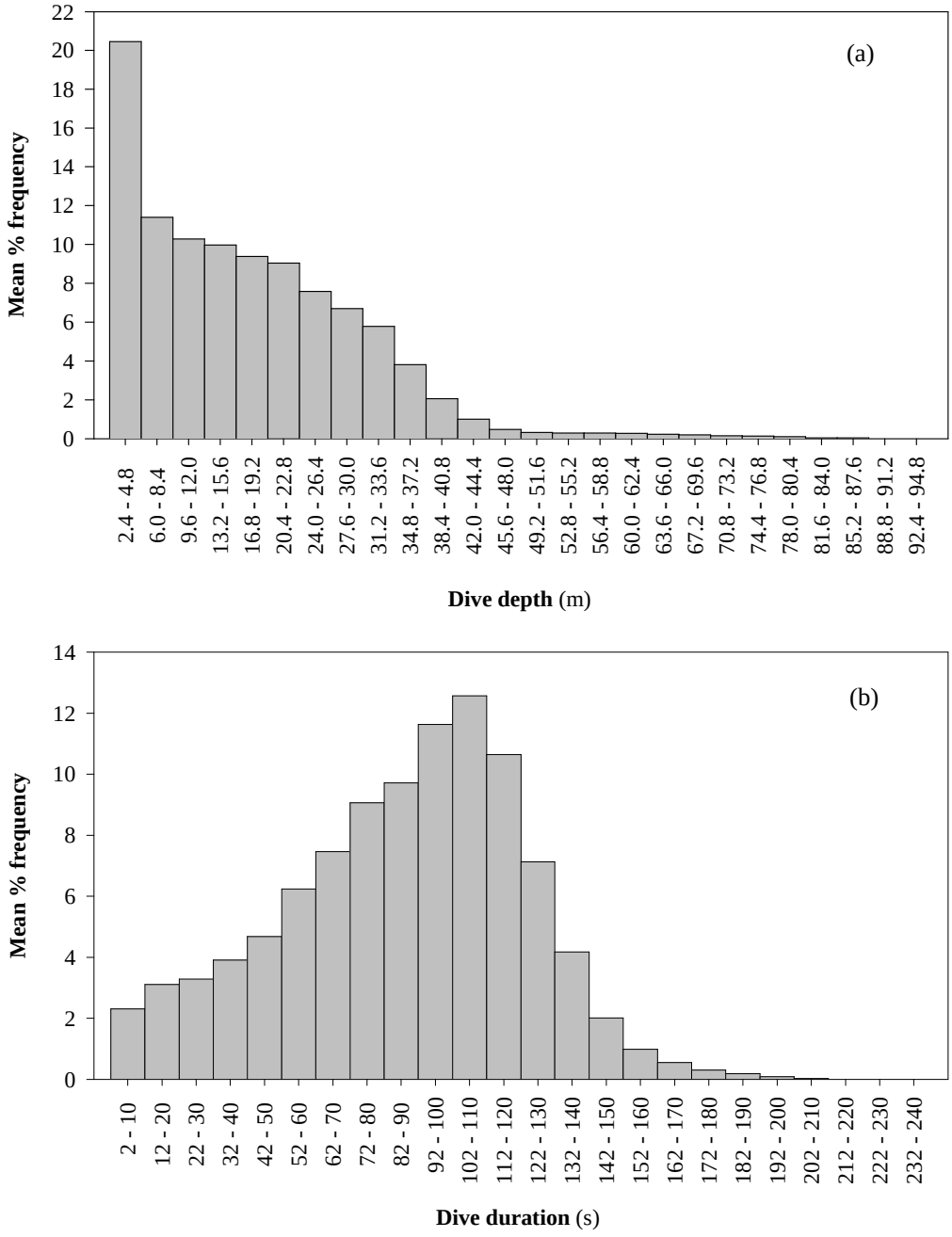

Figure 5.5. Mean frequency distributions of a) dive depth and b) dive duration from dives within diving bouts from 13 breeding female macaroni penguins.

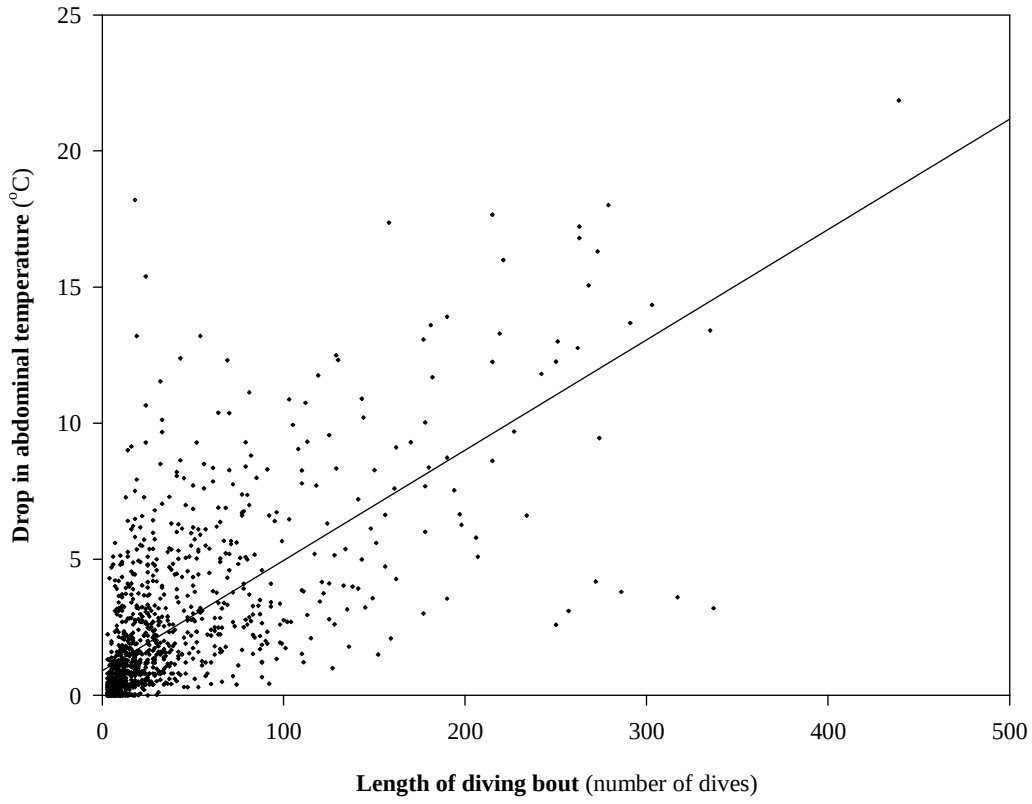

Figure 5.6. Drop in abdominal temperature during diving bouts of different durations, recorded from 13 breeding female macaroni penguins.

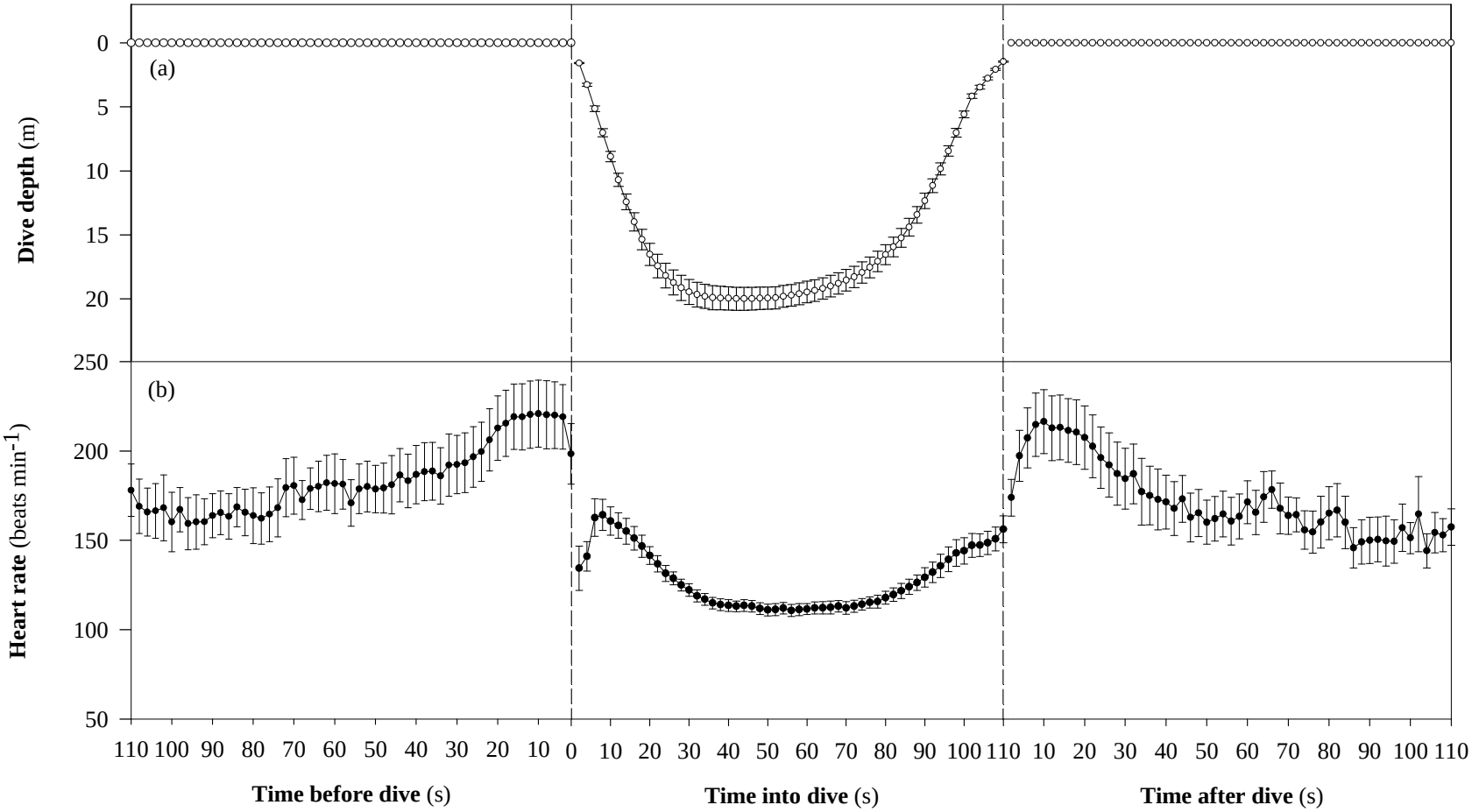

Figure 5.7. Changes in (a) mean depth (± S.E.M.) during dives of 102 – 110 seconds and associated changes in (b) mean heart rate (± S.E.M.), recorded from 13 breeding female macaroni penguins

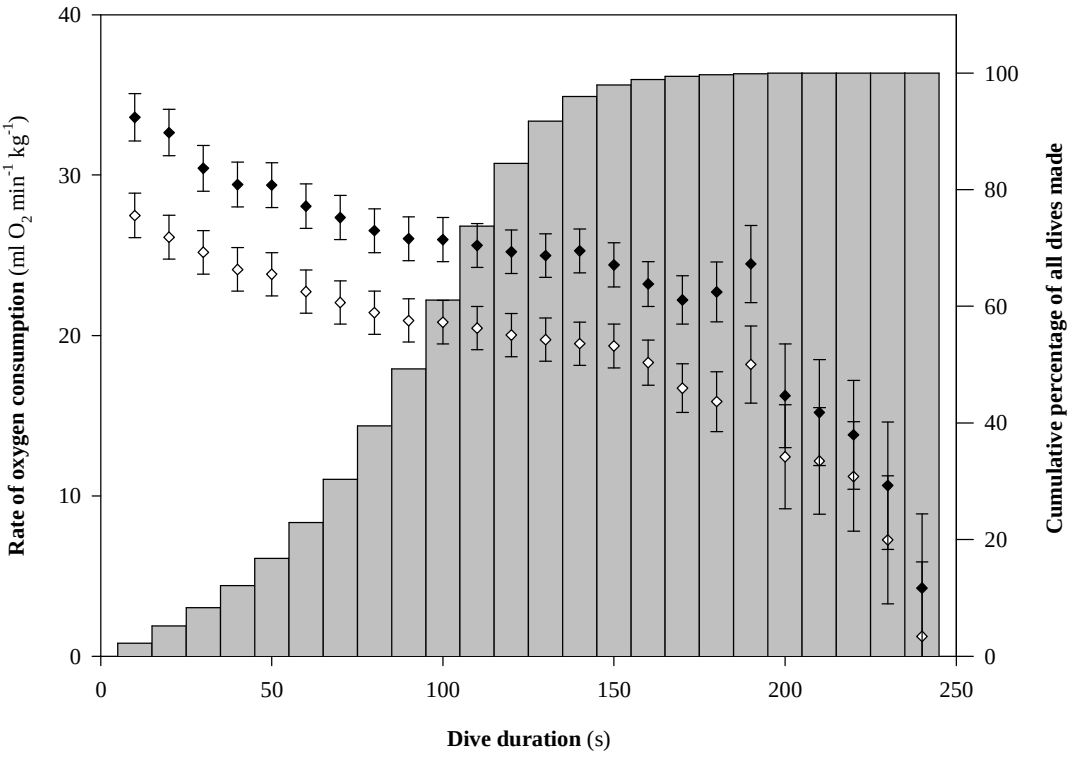

Figure 5.8. Rate of oxygen consumption calculated from heart rate while diving (open symbols) and mean heart rate while diving and surfacing (closed symbols) at different dive durations. Also shown is the cumulative frequency of observed dive durations (grey bars).

References

Bengston J.L., Croll D.A., and Goebel M.E. (1993). Diving behaviour of chinstrap penguins at Seal Island. Antarctic Science **5**: 9-15

Bethge P., Nicol S., Culik B.M., and Wilson R.P. (1997). Diving behaviour and energetics in breeding little penguins (*Eudyptula minor*). Journal of Zoology **242**: 483-502

Bevan R.M., and Butler P.J. (1992). Cardiac output and blood flow distribution during swimming and voluntary diving of the tufted duck (*Athya Fuligula*). Journal of Experimental Biology **168**: 199-217

Bevan R.M., Keijer E., and Butler P.J. (1992). A method for controlling the feeding behaviour of aquatic birds: Heart rate and oxygen consumption during dives of different duration. Journal of Experimental Biology **162**: 91-106

Bevan R.M., Butler P.J., Woakes A.J., and Prince P.A. (1995). The energy expenditure of free-ranging black-browed albatrosses. Philosophical Transactions of the Royal Society of London B **350**: 119-131

Bevan R.M., Boyd I.L., Butler P.J., Reid K., Woakes A.J., and Croxall J.P. (1997). Heart rates and abdominal temperatures of free-ranging South Georgian shags, *Phalocrocorax georgianus*. Journal of Experimental Biology **200**: 661-675

Bevan R.M., Bishop C.M., and Butler P.J. (1998). The physiology of polar birds. In: Portner H.O. and Playle R.C. (eds) Cold Ocean Physiology. Society of Experimental Biology seminar series, 66. Cambridge University Press, Cambridge

Boyd I.L., and Croxall J.P. (1996). Dive durations in pinnipeds and seabirds. Canadian Journal of Zoology **74**: 1696-1705

Boyd I.L., Arnould J.P.Y., Barton T., and Croxall J.P. (1994). Foraging behaviour of Antarctic fur seals during periods of contrasting prey abundance. Journal of Animal Ecology **63**: 703-713

Butler P.J. (1988). The exercise response and the "classical" diving response during natural submersion in birds and mammals. Canadian Journal of Zoology **66**: 29-39

Butler P.J. (1993). To what extent can heart rate be used as an indicator of metabolic rate in free-living marine mammals. Symposium of the Zoological Society of London **66**: 317-332

Butler P.J. (2000). Energetic costs of surface swimming and diving of birds. Physiological and Biochemical Zoology **73**: 699-705

Butler P.J., and Jones D.R. (1997). Physiology of diving of birds and mammals. Physiological Reviews **77**: 837-899

Butler P.J., and Woakes A.J. (1979). Changes in heart rate and respiratory frequency during natural behaviour of ducks, with particular reference to diving. Journal of Experimental Biology **79**: 283-300

Butler P.J., and Woakes A.J. (1984). Heart rate and aerobic metabolism in humboldt penguins, *Spheniscus Humboldti*, during voluntary diving. Journal of Experimental Biology **108**: 419-428

Carbone C., and Houston A.I. (1996). The Optimal allocation of time over a dive cycle: An approach based on aerobic and anaerobic respiration. Animal Behaviour **51**: 1247-1255

Castellini M.A., Davis R.W., and Kooyman G.L. (1988). Blood chemistry regulation during repetitive diving in Weddell seals. Physiological Zoology **61**: 379-386

Chappell M.A., Shoemaker V.H., Janes D.N., Bucher T.L., and Maloney S.K. (1993). Diving behaviour during foraging in breeding Adelie penguins. Ecology **74**: 1204-1215

Costa D.P. (1988). Methods for studying the energetics of freely diving animals. Canadian Journal of Zoology **66**: 45-52

Croxall J.P., Davis R.W., and O'Connell M.J. (1988). Diving patterns in relation to diet of gentoo and macaroni penguins at South Georgia. The Condor **90**: 157-167

Croxall J.P., Briggs D.R., Kato A., Naito Y., Watanuki Y., and Williams T.D. (1993). Diving pattern and performance in the macaroni penguin *Eudyptes chrysolophus*. Journal of Zoology **230**: 31-47

Croxall J.P., Prince P.A., and Reid K. (1997). Dietary segregation of krill-eating South Georgian seabirds. Journal of Zoology **242**: 531-536

Croxall J.P., Reid K., and Prince P.A. (1999). Diet, Provisioning and productivity responses of marine predators to differences in availability of Antarctic krill. Marine Ecology Progress Series **177**: 115-131

Culik B.M., and Wilson R.P. (1991). Energetics of under-water swimming in Adélie penguins (*Pygoscelis adeliae*). Journal of Comparative Physiology B **161**: 285-291

Culik B.M., Wilson R.P., and Bannasch R. (1994). Underwater swimming at low energetic costs by pygoscelid penguins. Journal of Experimental Biology **197**: 65-78

Culik B.M., Pütz K., Wilson R.P., Allers D., Lage J., Bost C.-A., and Le Maho Y. (1996a). Diving energetics in king penguins (*Aptenodytes patagonicus*). Journal of Experimental Biology **199**: 973-983

Culik B.M., Pütz K., Wilson R.P., Bost C.-A., Le Maho Y., and Verselin J.-L. (1996b). Core temperature variability in diving king penguins (*Aptenoydytes patagonicus*) a preliminary analysis. Polar Biology **16**: 371-378

Davis R.W., and Kanatous S.B. (1999). Convective oxygen transport and tissue oxygen consumption in weddell seals during aerobic dives. Journal of Experimental Biology **202**: 1091-1113

Davis R.W., Kooyman G.L., and Croxall J.P. (1983). Water Flux and estimated metabolism of free-ranging gentoo and macaroni penguins at South Georgia. Polar Biology **2**: 41-46

Davis R.W., Croxall J.P., and O'Connell M.J. (1989). The reproductive energetics of gentoo (*Pygoscelis papua*) and macaroni (*Eudyptes chrysolophus*) penguins at South Georgia. Journal of Animal Ecology **58**: 59-74

Everson I. (ed) (2000). Krill: Biology, Ecology and Fisheries. Blackwell Science, Oxford

Fedak M.A. (1986). Diving and exercise in seals: a benthic perspective. In: Brubakk A.D., Kanwisher J.W. and Sundess G. (eds) Diving in animals and man. Tapir, Trondheim

Fedak M.A., and Thompson D. (1993). Behavioural and physiology options in diving seals. Symposium of the Zoological Society of London **66**: 333-348

Fedak M.A., Pullen M.R., and Kanwisher J. (1988). Circulatory responses of seals to periodic breathing: heart rate and breathing during exercise and diving in the laboratory and open sea. Canadian Journal of Zoology **66**: 53-60

Green J.A., Butler P.J., Woakes A.J., Boyd I.L., and Holder R.L. (2001). Heart rate and rate of oxygen consumption of exercising macaroni penguins. Journal of Experimental Biology **204**: 673-684

Green K., Williams R., and Green M.G. (1998). Foraging ecology and diving behaviour of macaroni penguins *Eudyptes chrysolophus* at Heard Island. Marine Ornithology **26**: 27-34

Handrich Y., Bevan R.M., Charrassin J.-B., Butler P.J., Pütz K., Woakes A.J., Lage J., and Le Maho Y. (1997). Hypothermia in foraging king penguins. Nature **388**: 64-67

Heldmaier G., and Ruf T. (1992). Body Temperature and metabolic rate during natural hypothermia in endotherms. Journal of Comparative Physiology B **162**: 696-706

Hill R.D., Schneider R.C., Liggins G.C., Schuette A.H., Elliott R.L., Guppy M., Hochachka P.W., Qvist J.E., Falke K.J., and Zapol W.M. (1987). Heart rate and body temperature during free diving of Weddell seals. American Journal of Physiology **253**: R344-R351

Houston A.I., and Carbone C. (1992). The optimal allocation of time during the diving cycle. Behavioral Ecology **3**: 255-265

Hull C.L. (1997). The effect of carrying devices on breeding royal penguins. The Condor **99**: 530-534

Kato A., Naito Y., Watanuki Y., and Shaughnessy P.D. (1996). Diving pattern and stomach temperature of foraging king cormorants at subantarctic Macquarie Island. The Condor **98**: 844-848

Kooyman G.L. (1989). Diverse Divers. Springer-Verlag, Berlin

Kooyman G.L., and Kooyman T.G. (1995). Diving behavior of emperor penguins nurturing chicks at Coulman Island, Antarctica. The Condor **97**: 536-549

Kooyman G.L., and Ponganis P.J. (1990). Behavior and physiology of diving in emperor and king penguins. In: Davis L.S. and Darby J.T. (eds) Penguin Biology. Academic Press, New York

Kooyman G.L., and Ponganis P.J. (1998). The physiological basis of diving to depth: Birds and mammals. Annual Review of Physiology **60**: 19-32

Kooyman G.L., Wahrenbrock E.A., Castellini M.A., Davis R.W., and Sinnett E.E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. Journal of Comparative Physiology B **138**: 335-346

Kooyman G.L., Castellini M.A., Davis R.W., and Maue R.A. (1983). Aerobic dive limits in immature Weddell seals. Journal of Comparative Physiology b **138**: 335-346

Kooyman G.L., Cherel Y., Le Maho Y., Croxall J.P., Thorson P.H., Ridoux V., and Kooyman C.A. (1992a). Diving behavior and energetics during foraging cycles in king penguins. Ecological Monographs **62**: 143-163

Kooyman G.L., Ponganis P.J., Castellini M.A., Ponganis E.P., Ponganis K.V., Thorson P.H., Eckert S.A., and Le Maho Y. (1992b). Heart rate and swim speeds of emperor penguins diving under sea ice. Journal of Experimental Biology **165**: 161-180

Kramer D.L. (1988). The behavioral ecology of air breathing by aquatic animals. Canadian Journal of Zoology **66**: 89-94

Maughan R., Gleeson M., and Greenhaff P.L. (1997). Biochemistry of Exercise & Training. Oxford University Press, Oxford

Millard R.W., Johansen K., and Milsom W.K. (1973). Radiotelemetry of cardiovascular responses to exercise and diving in penguins. Comparative Biochemistry and Physiology A **46**: 227-240

Mori Y. (1998). The optimal patch use in divers: optimal time budget and the number of dive cycles during bout. Journal of Theoretical Biology **190**: 187-199

Ponganis P.J., and Kooyman G.L. (2000). Diving physiology of birds: a history of studies on polar species. Comparative Biochemistry and Physiology a **126**: 143-151

Ponganis P.J., Kooyman G.L., Baranov E.A., Thorson P.H., and Stewart B.S. (1997a). The aerobic submersion limit of Baikal seals, *Phoca sibirica*. Canadian Journal of Zoology **75**: 1323-1327

Ponganis P.J., Kooyman G.L., Stark L.N., Kooyman C.A., and Kooyman T.G. (1997b). Post-dive blood lactate concentrations in emperor penguins, *Aptenodytes forsteri*. Journal of Experimental Biology **200**: 1623-1626

Ponganis P.J., Kooyman G.L., Starke L.N., Kooyman C.A., and Kooyman T.G. (1997c). Post-dive blood lactate concentrations in emperor penguins, *Aptenodytes forsteri*. Journal of Experimental Biology **200**: 1623-1626

Ponganis P.J., Kooyman G.L., Winter L.M., and Starke L.N. (1997d). Heart rate and Plasma lactate response during submerged swimming and trained diving in California sea lions, *Zapholus californianus*. Journal of Comparative Physiology B **197**: 9-16

Pütz K., Wilson R.P., Kierspel M.A.M., Culik B.M., Adelung D., Charrassin J.-B., Raclot T., Lage J., and Le Maho Y. (1998). Foraging strategy of king penguins (*Aptenodytes patagonicus*) during summer at the Crozet Islands. Ecology **79**: 1905-1921

Schmidt-Nielsen K. (1997). Animal Physiology, 5th edn. Cambridge University Press, Cambridge

Scholander P.F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. Hvalrådets Skrifter **22**: 1-131

Speakman J.R. (1997). Doubly Labelled Water. Chapman & Hall, London

Stephenson R., Butler P.J., and Woakes A.J. (1986). Diving behaviour and heart rate in tufted ducks *Aythya fuligula*. Journal of Experimental Biology **126**: 341-359

Williams T.D. (1989). Agression, incubation behaviour and egg-loss in macaroni penguins, *Eudyptes chrysolophus*, at South Georgia. Oikos **55**: 19-22

Williams T.D., and Croxall J.P. (1991). Annual variation in breeding biology of macaroni penguins, *Eudyptes chrysolophus*, at Bird Island, South Georgia. Journal of Zoology **223**: 189-202

Williams T.D., Briggs D.R., Croxall J.P., Naito Y., and Kato A. (1992). Diving pattern and performance in relation to foraging ecology in the gentoo penguin, *Pygoscelis papua*. Journal of Zoology **227**: 211-230

Williams T.M., Davis R.W., Fuiman L.A., Francis J., Le Bouef B.J., Horning M., Calambokidis J., and Croll D.A. (2000). Sink or swim: strategies for cost-efficient diving by marine mammals. Science **288**: 133-136

Wilson R.P., Pütz K., Bost C.A., Culik B.M., Bannasch R., Reins T., and Adelung D. (1993). Diet dive depth in penguins in relation to diel vertical migration of prey: whose dinner by candlelight? Marine Ecology Progress Series **94**: 101-104

Woakes A.J., Butler P.J., and Bevan R.M. (1995). Implantable data logging system for heart rate and body temperature: Its application to the estimation of field metabolic rates in Antarctic predators. Medical & Biological Engineering & Computing **33**: 145-151

Ydenberg R.C., and Clark C.W. (1989). Aerobiosis and anaerobiosis during diving by western grebes: an optimal foraging approach. Journal of Theoretical Biology **139**: 437-449

Zar J.H. (1999). Biostatistical Analysis, 4th edn. Prentice-Hall International Inc., New Jersey

Chapter 6

General Discussion

The overall aim of the present study was to measure the heart rate and abdominal temperature of macaroni penguins and use these data to answer questions about their ecology, behaviour and physiology. All of the principle objectives stated in Chapter 1 have been addressed and though not exhaustive and comprehensive, this study has proved the utility of the f_H technique and improved our understanding of this important species.

The heart rate method has now been established as a valid and valuable method for the investigation of metabolic rate in the field (FMR). Chapter 2 addresses objectives 1 and 2 and describes the relationship between heart rate (f_H) and rate of oxygen consumption $\dot{V}_{O_2}$ for this species and develops the statistical techniques necessary to investigate the errors associated with the estimation of $\dot{V}_{O_2}$. One of the many problems associated with the use of doubly labelled water to estimate FMR is the inability to quantify the amount of error associated with this technique and how this should influence experimental design. The work presented in Chapter 2 improves upon this position by modelling the error of estimates made using the f_H technique and how the nature of this error affects the number of experimental animals used and amount of data collected from them both in the laboratory calibration process and in the field.

Chapter 2 also shows that the relationship between f_H and $\dot{V}_{O_2}$ does not change as physiological status changes but that it can vary between the sexes. Further work in this area should look further into these sex differences, which are also suggested by the results from Chapter 4. Other work might investigate whether the $f_H/\dot{V}_{O_2}$ varies when different types of locomotion or muscle groups are used. A previous study of gentoo penguins (Bevan et al. 1995) showed no difference in the relationship while swimming in a static water canal or walking on a treadmill. However, more recent work with barnacle geese suggests a different relationship while flying in a wind tunnel and exercising on a treadmill (S. Ward, C.M.

Bishop, P.J. Butler & A.J. Woakes, unpublished data). Certainly this subject stands further investigation.

Chapters 3 and 4 concentrate on objectives 3 and 4 and describe the application of the relationships and techniques from Chapter 2 and the conversion of heart rates collected from the field to estimates of FMR. These data can now be combined with data collected simultaneously on diet and foraging locations of macaroni penguins (Barlow et al. 2001), converted to estimates of food consumption and added to models of the Scotia Sea. In Chapter 3, FMR was estimated during two different phases of the breeding season and the results from this compared to energetic data from other studies of penguins. The estimates from the present study were very similar to those from the only previous study of penguin energy expenditure made using the f_H technique. (Bevan et al. 2001). However, both of these estimates were lower than estimates using DLW and time energy budgets combined with respirometry, especially when animals were at-sea and foraging. Possible explanations for this difference are outlined in Chapter 3, but it seems likely that DLW does tend to overestimate FMR in aquatic animals, especially since in studies using DLW, the metabolic scope is greater than is normally sustainable (Peterson et al. 1990, Hammond and Diamond 1997). Another explanation might be differences in the $f_H/\dot{V}_{O_2}$ relationship associated with modes of locomotion (as mentioned above), further underlining the importance of investigation in this area.

Because of logistical constraints, it was not possible to collect FMR data from many individuals throughout the whole of the breeding season. The data presented in Chapter 3 do though allow comparison of FMR during the different phases of the season and for different activities. Furthermore, using the moult fast as an example, Chapter 4 shows how it is possible to track changes in FMR in individuals and groups from day to day over an extended period of time. These data have never been collected from a free-ranging animal before and future work should concentrate on extending such deployments to the whole of the breeding season for these animals as well as the winter period for which we know nothing of their behaviour and location. Further and continuing developments in technology should allow heart rate data loggers to be deployed in experimental animals for over a year. These data will allow us to look in detail at the energetic costs of all the activities associated with foraging,

reproduction and maintenance. This will allow us to further test theories about the temporal demands of reproduction on parents (Ricklefs 1983) and give an insight into how decisions and strategies are made. It was also not possible to collect FMR data from male penguins in the current study and deployments on males should be a priority in any further work to complete the picture of parental energy expenditure.

Chapter 5 explores objectives 5 and 6 and the application of the f_H method to the questions surrounding the physiology and behaviour of diving. The physiological mechanisms which facilitate the diving behaviour observed in many birds and mammals are still not fully understood (Butler and Jones 1997). However, the present study does suggest that most dives by macaroni penguins observed in the field may indeed be sustained by aerobic metabolism following adjustments in circulation and heart rate. A consequence of these adjustments in macaroni penguins and in other species is a lowering of temperature in the abdomen. The extent of this cooling around the body and on what time scale it occurs is unknown and further work should concentrate on investigating this. Lowering of body temperatures is likely to reduce metabolism during diving thus extending diving duration. Understanding how and if this process is facilitated is essential in understanding how diving animals achieve the behaviour we observe. The data gathered on abdominal temperature in the present study are extremely interesting and indicate significant changes in circulation while diving. However, without further investigation it is unfortunately not possible to draw many conclusions from them. The present study suggests that the diving of foraging macaroni penguins is not limited by their physiology, with few dives at the limits of aerobic duration or anaerobic in nature. Further work should focus on the depth, distribution and availability of prey species and how these factors determine diving behaviour.

References

Barlow K.E., Boyd I.L., Croxall J.P., Staniland I.J., Reid K., and Brierley A.S. (2001). Competition for Antarctic resources: are seals supplanting penguins at South Georgia? Journal of Animal Ecology **Submitted**

Bevan R.M., Woakes A.J., Butler P.J., and Croxall J.P. (1995). Heart rate and oxygen consumption of exercising gentoo penguins. Physiological Zoology **68**: 855-877

Bevan R.M., Butler P.J., Woakes A.J., and Boyd I.L. (2001). The energetics of gentoo penguins, *Pygoscelis papua*, during the breeding season. Functional Ecology **Submitted**

Butler P.J., and Jones D.R. (1997). Physiology of diving of birds and mammals. Physiological Reviews **77**: 837-899

Hammond K.A., and Diamond J. (1997). Maximal sustained energy budgets in humans and animals. Nature **386**: 457-462

Peterson C.C., Nagy K.A., and Diamond J. (1990). Sustained metabolic scope. Proceedings of the National Academy of Science **87**: 2324-2328

Ricklefs R.E. (1983). Some considerations on the reproductive energetics of pelagic seabirds. Studies in Avian Biology **8**: 84-94