Available online at www.sciencedirect.com

ScienceDirect

Comparative Biochemistry and Physiology, Part A 150 (2008) 222–230

CBP

www.elsevier.com/locate/cbpa

Predicting rate of oxygen consumption from heart rate while little penguins work, rest and play ☆

J.A. Green [a,b,*], P.B. Frappell [a], T.D. Clark [a,1], P.J. Butler [b]

[a] *Department of Zoology, La Trobe University, Bundoora, Victoria, 3086, Australia*
[b] *Centre for Ornithology, School of Biosciences, University of Birmingham,*
Edgbaston, Birmingham, B15 2TT, UK

Received 12 December 2005; received in revised form 15 June 2006; accepted 16 June 2006
Available online 21 June 2006

Abstract

The relationship between heart rate (f_H) and rate of oxygen consumption ($\dot{V}_{O_2}$) was investigated under changing conditions of ambient temperature, digestive state and exercise state in the little penguin (*Eudyptula minor*). Both f_H and $\dot{V}_{O_2}$ were recorded simultaneously from 12 little penguins while they each (a) rested and exercised within their reported thermo-neutral zone (TNZ), (b) rested and exercised below their reported TNZ and (c) digested a meal of sardines within their reported TNZ. Contrary to our expectations, we found that minimum $\dot{V}_{O_2}$ did not vary between the two temperatures used. Comparison with values from the literature suggests that both minimum $\dot{V}_{O_2}$ and the extent of the TNZ in this species may vary along a latitudinal gradient. Furthermore, while minimum $\dot{V}_{O_2}$ was unchanged at the lower temperature, minimum f_H was significantly higher, suggesting a hitherto undescribed cardiac response to lowered ambient temperature in an avian species. This response was maintained when the penguins exercised within and below their apparent TNZ as f_H was significantly greater in cold conditions for a given level of $\dot{V}_{O_2}$. Furthermore, both f_H and $\dot{V}_{O_2}$ were slightly but significantly elevated for a given walking speed during exercise at the lower temperature. This suggests that the penguins may have been close to their TNZ and that the measures employed to counteract heat loss while at rest may have been compromised during exercise. There was no significant difference in the relationship between f_H and $\dot{V}_{O_2}$ while the penguins were inactive in a post-digestive state or inactive and digesting a meal within their TNZ, though both of these relationships were significantly different from that during exercise. This suggests that while digestion has no effect on the $f_H/\dot{V}_{O_2}$ relationship, for little penguins at least, it is of little value in deriving a predictive relationship for application to active free-ranging animals.

Keywords: Penguin; Energetics; Heart rate; Exercise; Digestion; Thermoregulation; Geographical variation

1. Introduction

Recent studies have shown that heart rate (f_H) can successfully be used as a proxy for the measurement of rate of oxygen consumption ($\dot{V}_{O_2}$) and/or metabolic rate in free-ranging animals (Butler et al., 2004). Application of this method requires a calibration between f_H and $\dot{V}_{O_2}$ under controlled conditions. This is often obtained by subjecting an animal to a variable workload through exercise such as walking (Bevan et al., 1994), swimming (Butler et al., 1992) or flying (Ward et al., 2002). However, exercise is not the only method which has been used. The aim of these 'calibration studies' is not to examine the effects of exercise per se, but to elevate levels of f_H and $\dot{V}_{O_2}$ to those likely to be found under natural conditions in free-ranging animals and to describe a relationship between the two. For example, other studies have examined the relationships between f_H and $\dot{V}_{O_2}$ by varying ambient temperature (Froget, 2002; Morhardt and Morhardt, 1971), digestive state (McPhee et al., 2003) or both (Clark et al., 2005a).

A key finding of these studies is that the $f_H/\dot{V}_{O_2}$ relationship can vary under differing conditions, which underlines the importance of tailoring calibration procedures to the likely

---

☆ Presented as part of the Russell V. Baudinette Memorial Symposium held in Adelaide, Australia, 1–2 October 2005.

* Corresponding author. Department of Zoology, La Trobe University, Bundoora, Victoria, 3086, Australia. Tel.: +61 3 9479 1983; fax: +61 3 9479 1151.

E-mail address: jon.green@latrobe.edu.au (J.A. Green).

[1] Current address: School of Earth and Environmental Sciences, University of Adelaide, South Australia, 5005, Australia.

1095-6433/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2006.06.017

range of activities undertaken and conditions experienced by free-ranging animals. A series of examples illustrate this point. The $f_H/\dot{V}_{O_2}$ relationship has been found to vary if measured on different dates within a season in American kestrels (*Falco sparverius*, Gessaman, 1980). Relationships varied between seasons of the year in white tailed deer (*Odocoileus virginianus*, Holter et al., 1976), perhaps due to a reduction in heart mass and hence stroke volume during periods when the animals were less active. However, there was no significant difference in the relationship in macaroni penguins when inactive/fasting, active/breeding and inactive/moulting (Green et al., 2001, 2005b). Some apparent seasonal changes may be due in part to changes in body mass and/or body composition and more recent models have emphasised the importance of correcting for body mass in studies of this type (Fahlman et al., 2004; Green et al., 2005a). Other studies which have detected differences between individuals and modes of locomotion may have drawn incorrect conclusions through the inappropriate incorporation of these body mass effects (Packard and Boardman, 1999).

Humans show different relationships between leg and arm exercise (Vokac et al., 1975) and during static or dynamic exercise (Maas et al., 1989). Juvenile steller sea lions (*Eumetopias jubatus*) appear to have different relationships while swimming and while digesting food (McPhee et al., 2003). There was no difference in the relationship while barnacle geese walked or swam (Nolet et al., 1992), but Ward et al. (2002) found differences between walking and flying in the same species. Conversely, both Bevan et al. (1995) and Green et al. (2005b) found no difference in the relationships when gentoo (*Pygoscelis papua*) and macaroni (*Eudyptes chrysolophus*) penguins walked or swam using their flippers (wings). In king penguins (*Aptenodytes patagonicus*), the relationship determined for penguins exposed to cold temperatures was different from that determined while the birds exercised (Froget et al., 2002). In addition to this, the nutritional state and body composition in king penguins has a profound and complex effect on these relationships (Fahlman et al., 2005, 2004; Froget et al., 2001). In lower vertebrates it is essential to include the effects of varying temperature in predictive models (Butler et al., 2002; Clark et al., 2006, 2005b).

In the present study we investigate the relationship between f_H and $\dot{V}_{O_2}$ in the little penguin (*Eudyptula minor*). With their large and varied geographical range (Williams, 1995) little penguins must adapt to challenging environments ranging from discontinuous bouts of foraging in cold water, fasting, walking long distances to nest sites, and incubating and brooding in nests and burrows at temperatures up to 43 °C (Ropert-Coudert et al., 2004). Our aim was to determine how the relationship between f_H and $\dot{V}_{O_2}$ in little penguins varies in response to changing levels of exercise, temperature and digestive status, with the ultimate objective of utilising this relationship to predict the $\dot{V}_{O_2}$ of free-ranging animals.

2. Materials and methods

2.1. Animals

All experiments were carried out with La Trobe University Ethics Committee approval (AEC 04/37(L)) and relevant wildlife permits. Twelve little penguins in six breeding pairs were obtained from Phillip Island Nature Park, Victoria, Australia. Penguins were transported by car to La Trobe University, Melbourne, Australia, where all experiments took place. The experiments were performed between September 9 and 21, 2004, which was during the pre-breeding/courtship phase in this year (Peter Dann, personal communication). The penguins were kept in a large sand-based outside enclosure (7.5 m × 5 m) which was half covered to provide protection from sun and rain. They were provided with artificial nesting burrows and a large rectangular swimming tank (2 m × 1.5 m) filled with fresh water. The penguins were hand-fed 150–200 g of sardines twice daily, supplemented daily with a multi-vitamin tablet. The penguins each spent 6 days at La Trobe and during this time they remained in their breeding pairs and each penguin increased in body mass, by an average of approximately 5%.

2.2. Apparatus

Rate of oxygen consumption was measured using an open-circuit respirometry system. A 45 × 45 × 55 cm perspex respirometer was suspended above a variable speed treadmill and brush-type draught excluders ensured a good seal between the respirometer and the treadmill belt. The treadmill and respirometer were placed within a constant temperature room in which ambient temperature could be set between 2 °C and 40 °C. Air was drawn from the respirometer using a vacuum compressor at approximately 16–17 L min^{-1}. Flow rate was monitored using an electronic mass-flow meter (Sierra Instruments Inc.). A subsample of air was drawn from this main flow using a small air pump (AMETEK R-1, Applied Electrochemistry) and passed through a drying column (Drierite, Hammond) and analysed for the fractional content of O_2 and CO_2 by two gas analysers (AMETEK S-3A/I and AMETEK CD-3A). Heart rate was monitored using a customised heart rate transmitter system (POLAR a3, Polar Electro Oy, Finland). The heart rate transmitter was attached dorsally to the feathers using lightweight paper tape and custom-made brass electrodes were inserted under the skin. The transmitter unit had a functional range of approximately 1 m and the receiver unit was placed on top of the respirometer to ensure a strong signal. The outputs from the heart rate receiver, gas analysers, flow meter, treadmill speed and a thermocouple located inside the respirometer were collected at 200 Hz (Powerlab 8SP, ADInstruments) and displayed on a computer using Chart software (ADInstruments). Rate of oxygen consumption was determined from the rate of airflow from the respirometer and the difference in the fractional oxygen concentration between ambient and outflowing air. Instantaneous corrections of the gas concentrations were calculated dry at standard temperature (273 K) and pressure (101.3 kPa) using the method of Frappell et al. (1989) assuming a first order linear system (chamber volume = 101l; flow = 17 L min^{-1}; tau = 4.71 min, determined from a semi-logarithmic plot of concentration against time following a perturbation, r^2 = 0.99). $\dot{V}_{O_2}$ was calculated with consideration of RQ related errors (Frappell et al., 1992). Whole system accuracy was determined to be 6% by bleeding CO_2 into