FEEDING ECOLOGY AND FREE-LIVING ENERGETICS OF THE LITTLE PENGUIN, *Eudyptula minor*, IN TASMANIA

by

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DECLARATION

This thesis contains no material which has been accepted for the award of any degree or diploma in any university, and to the best of my knowledge and belief, this thesis contains no copy or paraphrase of material previously published or written by any other person, except where due reference is made in the text of the thesis.

Rosemary Gales

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ABSTRACT

Of all birds, penguins are the most specialised in terms of their adaptations to a marine lifestyle. The little penguin, *Eudyptula minor*, is the smallest of all the penguin species and holds an important position in the functioning of the marine ecosystems across its range. To examine the role of penguins in the marine environment it is essential to have information concerning their food and energy requirements, and how these change in space and time. In this study, the feeding ecology and free-living energetics of little penguins in Tasmania was examined by investigating their diet, energy and food consumption rates and their behaviour at sea. Where possible, this information was collected over the annual cycle, and combined with population estimates in order to assess the population requirements of little penguins in Bass Strait, the stronghold of the species distribution in Australia.

Information on the diet was collected by stomach flushing penguins over a two year period at three sites around Tasmania. The stomach flushing technique was first validated by feeding trials and was found to be effective in collecting complete stomach contents provided penguins were flushed repeatedly until only clear water is ejected. The stomach samples collected from the penguins were often highly digested and so extensive use had to be made of diagnostic remains from prey items in order to quantitatively determine the species composition of the diet, as well as the size of prey items consumed. It was evident that fish were important in the diet and the validity of using the otoliths from fish to determine the number and size of fish consumed was tested by feeding trials and subsequent retrieval and examination of the penguin stomach contents. This showed that the rate of digestion of otoliths decreased with meal size but increased with time after ingestion, and only otoliths which are not affected by digestion should be used to assess the original size of the fish consumed. Fish were the most important prey taxon consumed by little penguins in terms of frequency of occurrence, numbers, mass and energy contribution, with cephalopods and crustaceans contributing to the diet to a lesser degree. The diet of the little penguin in Tasmania showed local, seasonal and annual changes which probably reflects the local availability of the prey species. There was no difference in the diet or stomach content mass between male and female penguins, the sex of penguins being determined on the basis of beak morphology, which was shown to be a reliable criterion. The prey of little penguins was characterised as being small, schooling species which occur in relatively shallow water, consistent with the foraging behaviour of the penguins.

The behaviour of little penguins at sea was studied using a new archival electronic activity recorder and the results showed that foraging occurred mainly in the top 15 m, at mean swimming speeds of between 8 to 9 km h^{-1}. Characteristics of

searching and foraging behaviours were hypothesised on the basis of speed and depth profiles. In interpreting the swimming behaviours the effect of carrying the electronic recorder was assessed by simultaneously measuring the water and energy flux rates via isotope turnover techniques. This showed that there were significant effects of carrying instruments while foraging, and these effects were evident even when the devices constituted as little as 0.1 % of penguin mass, or 1.4 % penguin cross-sectional area.

The accuracy of the isotope turnover techniques was assessed by comparing estimates of water, sodium and energy turnovers determined from tritium, sodium-22 and doubly labelled water turnovers in captive little penguins with estimates from simultaneous materials balance trials. This validation allowed the identification of appropriate equilibration times and turnover rate requirements for all three isotopes which have to be met to ensure reliable results from the use of the isotopes in field studies. In conjunction with analyses of the water, sodium and energy status of a variety of little penguin prey items, these trials also provided information on energy assimilation rates which are required for the conversion of water, sodium and energy flux rates into food and seawater consumption rates.

The metabolic rates and food consumption rates of free-living little penguins in Bass Strait were studied over the annual cycle. These estimates of energy turnover during both breeding and non-breeding activities were used to construct time/energy budgets. The period of highest energy demands occurred during chick-rearing which occupies only 16 % of the annual time budget, but requires 31 % of the annual energy budget. Another energetically expensive period also occurs over the winter non-breeding period when adult energy expenditure exceeds the net energy gain acquired from feeding. Over the annual cycle, non-breeding birds require *ca.* 477 500 kJ and breeding birds require *ca.* 533 500 kJ, which when coupled to information on the diet and energy content of the dietary items, translates to 115 and 137 kg of food penguin^{-1} year^{-1}. When combined with the size of the little penguin population in Bass Strait, I estimated that *ca.* 37 000 tonnes of food are consumed by little penguins each year, which comprises 25 000 tonnes of fish, 11 000 tonnes of cephalopods and 1 000 of tonnes crustaceans. All facets of this study, detailing the diet, foraging behaviour and food and energy requirements were synthesised to identify the critical periods with respect to the dynamics of little penguin energy and food acquisition rates and the vulnerability of little penguins to fluctuations in food availability, resulting from either natural perturbations or from commercial fishing activities.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND AND AIMS

Seabirds are defined as birds which feed primarily on marine organisms (Lack, 1967). Although all seabirds are united in their dependence on land for reproduction, it is thought that the availability of food and energy has played an important part in their speciation, and in the determination of their numbers and distribution (Rahn & Whittow, 1984). Seabirds form a heterogeneous group which exhibits a considerable degree of variation in the extent of adaptations to the marine environment and of the 285 species of seabirds, penguins are unique in their degree of specialisation for a marine existence. There are about 16 species of penguins, comprising six genera within the Order Sphenesciformes. All are confined to the southern hemisphere and with their large biomass and high energy demands, penguins are considered to be important consumers of marine resources (Croxall, 1984).

However, despite the potential importance of the role of penguins in marine ecosystems, only recently has this area become a focus of research. The increase in research into the food and energy requirements of penguins over the last decade has been due to the realisation that in many regions penguins consume prey species which are also the target of commercial fishing operations, and also to the development of appropriate field and laboratory techniques which have allowed the pursuit of previously impossible avenues of investigation.

The greatest abundance of penguins occurs in the sub-Antarctic and on the Antarctic continent, and it is the species which occur in these areas that have received greater attention than the more temperate species. Eudyptulid penguins, the smallest of all marine homeotherms, are restricted to Australasia, and have received little attention in comparison with their Antarctic counterparts. There are six subspecies of *Eudyptula minor*, five in New Zealand and one in Australia, and the distribution and characteristics of each subspecies have been described by Kinsky & Falla (1976). The ecology and life history of the little penguin has been well documented by O'Brien (1940), Richdale (1940), Kinsky (1960) and Gales (1985, 1987*a*) in New Zealand; Reilly (1974), Reilly & Cullen (1979, 1981, 1982, 1983) and Dann (1988) in Victoria; and Warham (1958) and Hodgson (1975) in Tasmania. There has also been a suite of laboratory based physiological studies which have concentrated on aspects of metabolic rate, thermoregulation and ventilation (Stahel & Nicol, 1982, 1988; Baudinette & Gill, 1985; Baudinette *et al.*, 1986; Stahel *et al.*, 1984, 1987).

In Victoria, where little penguins are the focus of a large tourist attraction at Phillip Island, there has been a growing concern about recent declines in the number of birds, which it is thought may be associated with changes in the food supply. This concern resulted in a study into the diet of the little penguin in Victoria (Montague & Cullen, 1988), and similar concerns gave rise to a dietary study of the species in Western Australia (Klomp & Wooller, 1988*a*). In these investigations, identification of little penguin prey species provided evidence for both indirect and direct competition with commercial fishing interests, although in the absence of data relating to penguin food consumption rates and foraging behaviour, the precise nature and dynamics of the interactions of little penguins with their prey remained unclear.

In order to more clearly define this issue, a number of questions relating to predators and their interactions with their prey must be addressed. These include:
1) how many are there? 2) What do they eat ? 3) How much energy (i.e. food) do they need ? 4) When, where and how do they take their prey ? (Croxall, 1987).

The aim of the present study was to address these questions in terms of the little penguin in Tasmania, which is the area of greatest abundance of the species in Australia. The population size was assessed from data provided by the Tasmanian Department of Lands, Parks and Wildlife (N. Brothers, unpublished data) and the diet of the little penguin was examined by an extensive field study, during which I obtained stomach contents using the stomach flushing technique which was shown to be both humane and effective. In order to assess how the diet changed in space and time, the diet study was carried out at three sites around Tasmania over a period encompassing two breeding seasons. These facets of the study provided answers to the first two of the questions, but more elaborate studies were required to address the final two, more complicated questions in order to extend our understanding of the little penguin in its marine environment.

Penguins are difficult to observe at sea and remote techniques for assessing energy use and foraging behaviour of free-living penguins have been developed only recently, thus freeing researchers from the constraints of land based experimentation and theoretical extrapolation of results. Recently it has become feasible to apply isotope turnover techniques to free-living penguins, resulting in the first quantitative estimates of energy and food consumption rates, and these data have been reviewed by Green & Gales (in press). Prior to the present study, these techniques had been applied to little penguins (Costa *et al.*, 1986; Green *et al.*, 1988), although a significant feature of all published accounts of free-living penguins are that sample sizes are small, and studies have been restricted to the summer period when breeding and moulting occur.

In order to redress this situation, the free-living energetics of little penguins were measured during both the breeding and non-breeding season in the present study, this being the first study of any free-living seabird for which quantitative measurements of the energy budget have been made over the complete annual cycle. These results were then combined with the dietary and population data, a synthesis which allowed predictions of the annual energy and food requirements for the population of little penguins in Bass Strait.

The behaviour of penguins at sea is central to the last of the four points raised by Croxall (1987), this facet being a fundamental aspect of penguin feeding ecology, influencing their foraging efficiency and their role as predators in the marine ecosystem. In order to examine the foraging behaviour of little penguins, an archival electronic activity recorder was developed which simultaneously measures swimming speed and depth against time. These were successfully deployed and retrieved from little penguins and so resulted in the first integrated data of foraging behaviour of little penguins, from which it was possible to distinguish between several types of foraging and travelling behaviours and estimate foraging efficiency and range.

The results of all facets of this study, when considered together, allowed an assessment of the role of little penguins in the marine environment more accurately than has been possible in the past. These data also contribute to providing the essential information that is required to address the wider questions concerning the management and conservation of marine ecosystems.

1.2 ORGANISATON OF THESIS

Many of the techniques used in this study had previously not been validated in their application to seabird, or more specifically, penguin studies. Therefore, prior to the field studies, all techniques were subjected to validation trials with little penguins in order to quantitatively assess their reliability as estimates of magnitude of error are essential for complete interpretation of the results of the field studies. These methodological and validation studies comprise the first part of this thesis, Section A: Chapters 2 - 6 inclusive.

The results of the application of these techniques to field studies, in terms of the free-living energetics, foraging behaviour and diet, are the subject of the second part of the thesis, Section B: Chapters 7 - 10. Each chapter is self-contained and contains a full description of methodology and a discussion of results and comparison with other studies. The major findings of the study are then synthesised in the General Discussion (Chapter 11), in which further avenues of research are also suggested. Appendix 4 contains reprints of parts of this study which have already been published.

SECTION A

VALIDATION AND METHODLOGICAL STUDIES

CHAPTERS 2 - 6

CHAPTER 2

DETERMINATION OF SEX OF ADULT LITTLE PENGUINS BY EXTERNAL MEASUREMENTS

2.1 INTRODUCTION

In many species of penguins, the sexes differ in size (Croxall, 1985), and in most, the males are about 10% heavier than females. This is apparent in little penguins (*Eudyptula minor*) but the large annual variation in their body mass and the large overlap between sexes make body mass unreliable for sexing.

The beak of *E.minor* is its most dimorphic character and the sexes of little penguins of all the six subspecies can be distinguished by comparing the shapes of the beaks (O'Brien, 1940; Kinsky, 1960; Phillips,1960; Reilly & Balmford, 1972; Kinsky & Falla, 1976). These workers showed that in general the beak of the male is stouter and has a more acutely hooked tip on the upper mandible than that of the female. The female beak is more slender and tapered. However, this difference, although often described, has not been subjected to statistical analyses. In studying the Australian subspecies, the little penguin (*E. minor novaehollandiae*), in Tasmania, I have had to sex adults by their beak measurements and so could quantify the reliability of this sexing technique.

2.2 METHODS

In Tasmania between 1984 and 1986, I determined the sex of 136 adult little penguins either by dissecting freshly dead birds or by examining the cloaca for signs of swelling and distension at the time of egg laying (Serventy, 1956). I measured the beak length (after Baldwin *et al.*, 1931) and beak depth (after Warham, 1975) of each bird to the nearest 0.1 mm. I analysed these data by Discriminant Function Analysis (DFA, Genstat) and calculated a discriminant score for each bird. DFA weights characters by their powers of discriminating between groups of unknown individuals, using data from individuals of known sex (reference, or known group).

With this technique I classified the sex of 107 little penguins (wild-group), including 23 breeding pairs, which I measured in the field on Albatross Island (40°24'S, 144°32'E), Bass Strait, in the 1985/86 breeding season. By comparing the discriminant scores with the known group, I classified each bird as male or female. In addition, to examine the reliability of classifying sex by applying a single DFA, derived from the Australian subspecies, to penguins of a New Zealand subspecies, I calculated discriminant scores from the beak measurements of 40 Southern blue penguins (*E. minor minor*) of known sex. I had determined the sex of these birds

either by dissection or by cloacal examination (see above) in southern New Zealand between 1982 and 1984 (Gales, 1984). The discriminant scores of these birds were then compared with the scores from the known-sex group of *E. minor novaehollandiae* and classified as male or female. The number which was incorrectly sexed by the DFA method was then used to provide an index of reliability of using a single DFA between subspecies.

2.3 RESULTS AND DISCUSSION

The mean beak measurements of the known groups of the two subspecies of *E. minor* are shown in Table 2.1. In both subspecies the differences between male and female beak measurements were significant but nonetheless showed considerable overlap. The difference between the beak lengths of the two known sex groups was not significant for males ($t = 1.50$, $df = 88$, $P > 0.05$) or females ($t = 0.82$, $df = 84$, $P > 0.05$). However, the groups showed highly significant differences in beak depth (males: $t = 4.89$, $df = 88$, $P < 0.05$; females: $t = 3.47$, $df = 84$, $P < 0.05$), with *E. minor minor* having the larger beaks in both sexes.

The classification formula which was derived from the *E. minor novaehollandiae* known sex (reference) group was:

$$D = -83.10 + (10.06 \ln BL) + (17.99 \ln BD)$$

where D is the discriminant score, ln is the natural logarithm, BL is the beak length (mm) and BD is the beak depth (mm).

The sex of a little penguin can be determined by applying the bird's beak measurements to this formula. When D is positive, the penguin is classified as male, and when negative, female. Using this formula, of the 107 wild-group penguins measured in the field, I classified 56 (52 %) as female and 51 (48 %) as male (Table 2.1). As would be expected, there was no significant difference between the *E. minor novaehollandiae* known-sex group and the wild-group (DFA classified sexes) in either beak length (males: $t = 1.45$, $df = 115$, $P > 0.05$; females: $t = 1.58$, $df = 124$, $P > 0.05$) or beak depth (males: $t = 1.49$, $df = 115$, $P > 0.05$; females $t = 0.004$, $df = 124$, $P > 0.05$).

The differences between the discriminant scores of males and females within groups were all significant (Table 2.2) and the distributions of these scores are shown in Figure 2.1. Of the 136 birds in the *E. minor novaehollandiae* known-sex group, 128 were classified as the correct sex, giving a classification reliability of 94 %. The eight penguins which were incorrectly classified by the discriminant formula were four males with relatively small beaks and four females with relatively large beaks.

TABLE 2.1 Beak measurements (mm) of reference and wild specimens of *Eudyptula minor*

Specimens	Character	Sex	n	Mean	S.D.	Range	t-statistic
E. m. novaehollandiae reference group	length	male	66	39.1	1.44	36.0 - 42.3	9.94*
		female	70	36.8	1.17	34.0 - 40.1	
	depth	male	66	14.3	0.67	12.6 - 15.8	17.25*
		female	70	12.4	0.60	11.2 - 13.9	
E. m. novaehollandiae wild group #	length	male	51	38.7	1.07	36.4 - 42.0	9.51*
		female	56	36.5	1.32	34.2 - 40.1	
	depth	male	51	14.5	0.59	13.5 - 16.0	19.54*
		female	56	12.4	0.07	11.2 - 13.4	
E. m. minor reference group	length	male	20	38.8	1.48	36.8 - 41.8	2.54*
		female	20	37.4	1.95	34.2 - 40.9	
	depth	male	20	14.9	0.55	13.5 - 15.8	9.93*
		female	20	13.2	0.54	12.1 - 14.0	

* indicates $P < 0.05$
# indicates sex classified by DFA

TABLE 2.2 Discriminant scores of beak measurements of *E. minor*

Specimens	Sex	n	Mean	S.D.	Range	t-statistic
E. m. novaehollandiae reference group	male	66	1.59	1.016	-1.23 to 3.51	17.82 *
	female	70	-1.49	0.992	-3.68 to 0.68	
E. m. novaehollandiae wild group	male	51	1.70	0.879	0.15 to 3.51	18.94 *
	female	56	-1.57	0.893	-4.00 to -0.03	
E. m. minor reference group	male	20	2.32	0.738	1.13 to 3.88	8.83 *
	female	20	-0.32	1.111	-2.57 to 1.23	

* indicates $P < 0.05$

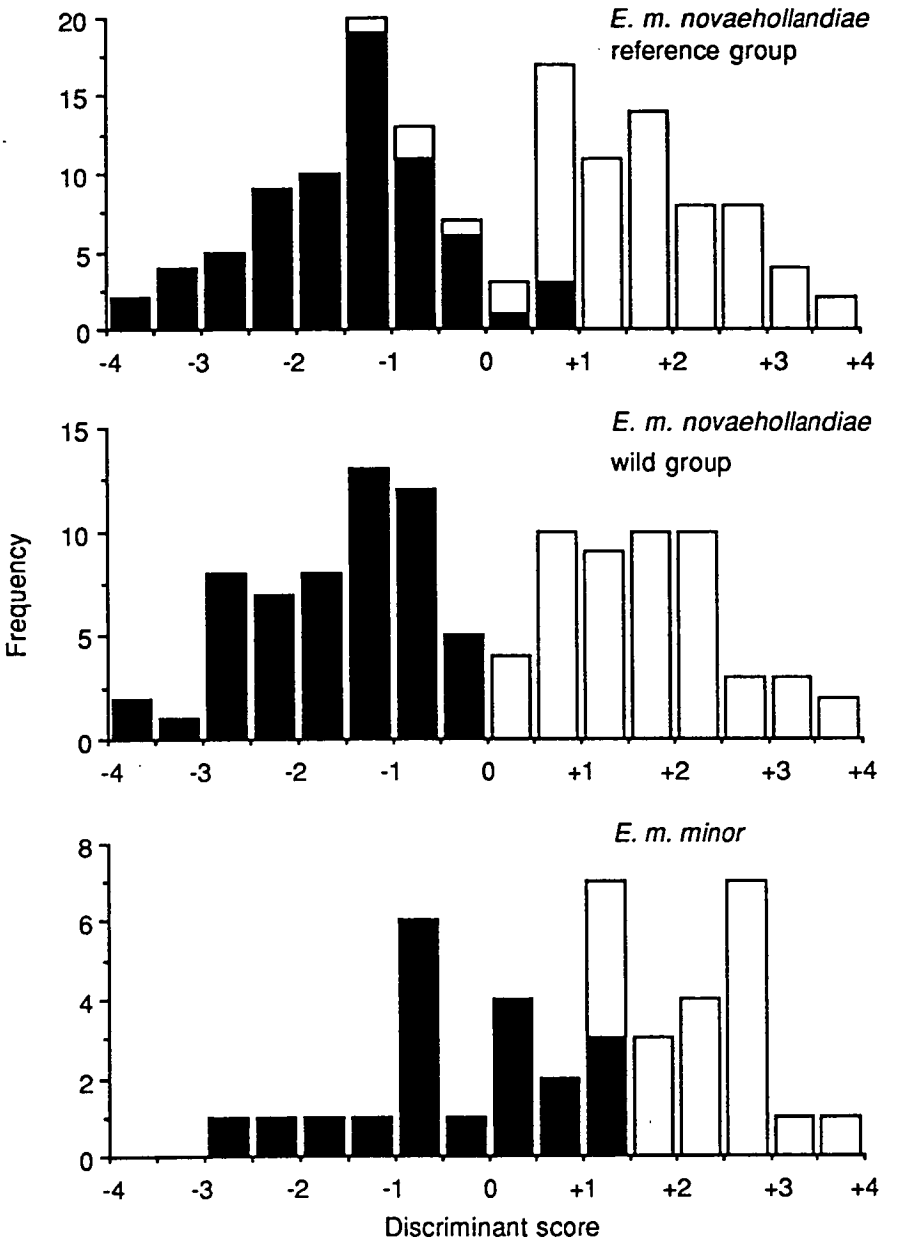

FIGURE 2.1 Discriminant scores of female (■) and male (□) reference and live specimens of *E. m. novaehollandiae* and reference *E. m. minor* classified as male or female.

The numbers of males and females of the 107 wild-group penguins whose sex was classified by the discriminant formula represents a female : male sex ratio on 1 : 0.91, which compares well with that of 1 : 0.86 for the same subspecies found by Hodgson (1975). Of the 23 breeding pairs, every pair was classified as a male-female pair.

When I used the formula derived from the *E. minor novaehollandiae* known-sex group to classify the sex of the *E.minor minor* group, the formula classified only 31 of the 40 New Zealand birds as the correct sex. This represents a classification reliability between subspecies of 78 %. All nine of the misclassified birds were females, which were classified as males. The relatively low level of reliability is a result of the larger *E. minor minor* beaks, as shown in Table 2.1. This is also evident in the differences in the discriminant scores between the two subspecies (males: $t = 2.97$, $df = 84$, $P < 0.05$; females: $t = 4.57$, $df = 88$, $P < 0.05$).

The differences in the beak dimensions between the sexes and between the six subspecies of *E. minor* were illustrated by Kinsky & Falla (1976). From their data and the results of the present study, the conclusion is that a discriminant formula derived from one subspecies cannot be used reliably to sex other subspecies. Juvenile birds may make the difference worse. The beaks of *E. minor* fledglings are on average only 91 % of the adult length and 81% of the adult depth (Gales, 1987*a*) and the age at which they reach adult dimensions is not known. However, the formula presented here for adult little Penguins in Australia gives a high reliability of classifying the correct sex from beak measurements. In practice, one can rapidly sex the adults of *E. minor novaehollandiae* in the field, at any time of year, with 94 % accuracy simply by taking the two beak measurements and calculating the discriminant score.

2.4 SUMMARY

The sex of 136 adult little penguins was determined either by dissection or by evidence of distension of the cloaca after egg laying. Beak measurements of these birds were taken and subjected to Discriminant Function Analysis to assess the reliability of sexing birds in the field. A formula was calculated which gave a high reliability (94 %) of classifying birds of the Australian subspecies to the correct sex from beak measurements.This formula cannot be used to reliably determine the sex of little penguins belonging to other subspecies.

CHAPTER 3

VALIDATION OF THE STOMACH FLUSHING TECHNIQUE FOR OBTAINING STOMACH CONTENTS OF PENGUINS

3.1 INTRODUCTION

Diet studies are essential when addressing the question of the role of penguins in marine ecosystems and there is mounting interest in the impact of penguins on marine resources (e.g., Croxall *et al.*, 1984). Unlike many other seabirds, penguins do not regurgitate when disturbed and so most diet studies to date have required the killing of many penguins in order to obtain stomach samples (e.g., Ealey, 1954; Volkman *et al.*, 1980; Croxall & Furse, 1980; Croxall & Prince, 1980; Lishman, 1985). Several methods have been developed to obtain stomach contents without killing the birds. Emetics are not only unsuccessful in some species but are sometimes fatal (Horne, 1985; Montague & Cullen, 1985). Stomach pumps (Emison, 1968; Dahlgren, 1982) do not provide complete, or indeed representative samples (Croxall & Prince, 1980; Volkman *et al.*, 1980; Horne, 1985). More recently, the technique of stomach flushing has been applied to penguins (Randall & Davidson, 1981; Wilson, 1984; Offredo *et al.*, 1985). Randall & Davidson (1981) developed a flushing device for obtaining food samples from jackass penguins (*Spheniscus demersus*) but the technique was unsuitable for retrieving large food items and the associated trauma led to some nest desertion. An improved and simplified technique has been developed by Wilson (1984) who claimed successful results with no associated nest desertions. The reception of this technique has been varied among seabird researchers, with Ryan & Jackson (1986) claiming that the technique largely eliminates the need to kill birds while Lishman (1985) states that the method is ineffective because it does not always obtain the complete contents, particularly from birds with full stomachs.

The stomach flushing technique now has been used on at least seven species of penguins, and it is surprising that comprehensive validation trials with this group of birds have not been published to date. In the present study, efficiency trials were run on little penguins *Eudyptula minor* to address the following questions: 1) How effective is the stomach flushing technique at obtaining complete stomach contents? 2) How does the state of stomach fullness affect the food recovery rate? 3) How does the time between feeding and stomach flushing affect the recovery rate?

Further validation trials were also run on gentoo penguins (*Pygoscelis papua*) and rockhopper penguins (*Eudyptes chrysocome*) in order to broaden the scope of the trials and to address the final question: 4) How does the size of penguin affect recovery rate?

3.2 METHODS

3.2.1 APPARATUS AND FLUSHING METHOD

The stomach flushing method used was identical in principle to that described by Wilson (1984). To simplify and speed up the process a commercial garden pressure sprayer (Hills 3.5 litre) was adapted to pump water under pressure (2 litre/min) into the stomach of the penguin. After filling the spray tank with seawater at ambient temperature the tank was sealed and pressurized by pumping. A plastic catheter (external diameter: 5 mm; internal: 3.5 mm) attached to the nozzle of the spray gun was inserted into the penguin's mouth and pushed gently down to the base of the stomach (≈30 cm). A switch on the spray gun was then released and seawater was introduced into the stomach under pressure until water flowed out of the corner of the mouth. The catheter was then quickly removed, the bird inverted over a bucket and pressure applied to the base of its stomach with the heel of the operator's hand while its throat was massaged with the fingers to dislodge any large food boluses which may otherwise have blocked the passage. Finally, the mouth of the penguin was sprayed to wash out any food remains which may have caught on the barbed tongue.

For the larger penguin species a multipurpose hand-operated hydraulic pump (Led Multipump) was utilized because it delivered water at a faster rate than the pressure sprayer. The subsequent procedure using this pump followed that described by Wilson (1984).

3.2.2 VALIDATION TRIAL PROTOCOL

The validation trials with little penguins were carried out on Albatross Island (40° 24'S, 144° 32'E), Northwest Bass Strait, Australia in September 1984. During a two-week period 40 penguins were collected and were used in the trials. The penguins used had all been on land for at least 24 hours prior to capture and thus were unlikely to have had food in their stomachs (Wilson *et al.*, 1985). The birds were force-fed pre-weighed thawed atherinids (*Atherinason hepsetoides*) (mean mass 2.8 ± 0.8 g, n = 50), and then held in hessian sacks until stomach flushing. Time intervals between feeding and stomach flushing were 1, 2, 4, 8 and 16 hours and the number of fish per feed was 5, 10, 20 or 50. Thus, for each of the four categories of fish numbers there were five time periods, resulting in a total of 20 different treatments. Each treatment was duplicated and individuals were used only once.

When the prescribed amount of time had elapsed after feeding, the penguins were stomach-flushed as described above. Stomach flushing was repeated until the water ejected from a penguin contained no food material and was totally clear. The condition of food material and number of otoliths ejected with each flush were recorded, and the water was drained off prior to storage in 90% alcohol. After

flushing, penguins were banded and held in sacks for at least one hour to ensure their recovery before release.

In addition to the validation feeding trial, five little penguins were stomach flushed as they came ashore at dusk on Wedge Island (43° 08'S, 147° 40'E), Southeast Tasmania, in August 1984. Stomach flushing was repeated on each bird until the last flush was clean. The penguins were then killed and stored frozen until their stomachs were dissected and examined for food material.

Validation trials on gentoo and rockhopper penguins were carried out on Macquarie Island (54° 30'S, 158° 55'E) in November 1984. I first stomach-flushed 20 gentoo and 10 rockhopper penguins as they came ashore in order to familiarize myself with the technique on larger penguins. Six gentoo penguins and four rockhopper penguins were then fed pre-weighed amounts of school whiting (*Sillago bassensis*) (mean mass 84.7 ± 5.4 g, n = 25), held for a recorded period of time and then stomach-flushed until the last flush was clear. Stomach contents were sieved and stored in alcohol.

In the laboratory all stomach samples were examined for sagittal otoliths which were removed, counted, sorted into left and right, and stored dry. These otoliths were used to calculate the number of fish obtained by stomach flushing. In order to describe fish condition an index of digestion (DI) was devised as follows:

Stage 0 - fish intact without any signs of digestion.
Stage 1 - fish not intact but scales present.
Stage 2 - fish flesh in fillets only, vertebrae articulated, tails present.
Stage 3 - only fragments of fish flesh, vertebrae not articulated, no tails, no scales, otoliths not eroded by digestion.
Stage 4 - no flesh or bones, otoliths partially eroded, sulcus acusticus worn.
Stage 5 - no fish remains, water stained green through bile secretion, empty stomach.

3.3 RESULTS

No food remains were found in the dissected stomachs of the five little penguins which were sacrificed after being stomach-flushed, indicating that stomach flushing yielded 100 % of the stomach contents. The penguins had been feeding on mixed diets of pelagic fish (*Atherinason hepsetoides*, *Engraulis australis*, *Hyporhamphus melanochir*), squid (*Nototodarus gouldi*) and euphausiid crustaceans (*Nyctiphanes australis*).

Results for the validation feeding trial on little penguins are shown in Table 3.1. The rate of retrieval of fish was 90 - 100 % in samples with digestion indices (DI) of 2 or less, i.e. where the fish remained relatively undigested (Table 3.2). In these cases the number of flushes required to empty the stomach ranged from three to ten (Fig. 3.I). As digestion progressed the retrieval rate decreased and no samples in which digestion was well advanced and the otoliths were worn (i.e. DI = 4) scored 100 % retrieval. By definition, those samples with a DI of 5 were empty and thus had 0 % retrieval. The number of flushes required to empty the stomach also decreased as digestion progressed (Fig. 3.1). For up to 2 hrs after feeding there was little difference in the proportion of fish retrieved between the different states of stomach fullness. After 4 hrs the proportion retrieved increased with the stomachs of increasing fullness, and by 16 hrs only penguins fed 50 fish had any food in their stomachs (Fig. 3.2).

Retrieval rates from the gentoo and rockhopper penguins were consistently high: 88 - 100 % (Table 3.3). The rates of retrieval during the early phases of digestion (DI $\leq$ 2) were similar to those of the little penguin despite the differences in body mass between the species (gentoo mean mass = 5.3 $\pm$ 0.70 kg, n = 6; rockhopper mean mass = 3.4 $\pm$ 0.42 kg, n = 4; little penguin mean mass = 1.0 $\pm$ 0.13 kg, n = 40). The mass of the fish meals fed to the gentoo penguins were 14 % of mean body mass, similar to the proportions by body mass of fish fed to rockhopper penguins (12 %) and the 50 fish fed to the little penguins (13 %). The inverse relationships between the number of flushes and the time between feeding and stomach flushing were similar among species (Fig. 3.3). The rates of digestion of the larger penguins, however, were slower than that of the little penguin, and therefore high rates of retrieval were maintained for longer periods.

3.4 DISCUSSION

The stomach-flushing technique has been shown here to be an efficient method of obtaining complete stomach contents. The validation feeding trials on the little penguins showed consistently high rates of stomach content retrieval and the efficiency of this technique was further verified by the subsequent killing of five birds. During a concurrent study of the feeding biology of the little penguin in Tasmania, 761 penguins were stomach-flushed between July 1984 and February 1986 (Chapter 10). As far as I could ascertain, no little penguins died as a result of the stomach flushing and no breeding birds which were stomach flushed deserted their nests. The inclusion of the rockhopper and gentoo penguins in the feeding validation trials further demonstrated the success of the technique across a five-fold variation in body size.

TABLE 3.1 Results of validation trials on little penguins: a and b are results for individual penguins

Fish fed (n)	Time between feeding and flushing (hr)	Retrieval			
		Otoliths (n)		Fish (%)	
		a	b	a	b
5	1	10	9	100	100
	2	10	9	100	100
	4	7	6	80	80
	8	4	4	40	40
	16	0	0	0	0
10	1	19	18	100	90
	2	20	18	100	90
	4	19	17	100	90
	8	15	13	80	70
	16	0	0	0	0
20	1	36	34	95	90
	2	38	37	100	95
	4	39	38	100	100
	8	33	28	85	75
	16	0	0	0	0
50	1	100	100	100	100
	2	98	92	98	98
	4	94	90	96	96
	8	92	79	92	80
	16	63	42	64	42

TABLE 3.2 Digestion indices and fish retrieval (%) from validation trials on little penguins

Digestion index (DI)	Fish retrieval (%)	n
0	90 - 100	5
1	90 - 98	3
2	90 - 100	12
3	75 - 100	8
4	40 - 80	6

TABLE 3.3 Results of validation trials on rockhopper and gentoo penguins

	Fish fed	Time between feeding and flushing	Retrieval		Digestion index	Flushes
	(n)	(hr)	Otoliths (n)	Fish (%)	(DI)	(n)
Rockhopper	5	4	10	100	1	6
		8	10	100	1	5
		16	10	100	3	3
		16	9	100	4	3
Gentoo	9	1	18	100	0	10
		2	17	100	1	8
		4	16	88	1	6
		8	18	100	2	6
		16	18	100	3	3
		16	16	88	4	2

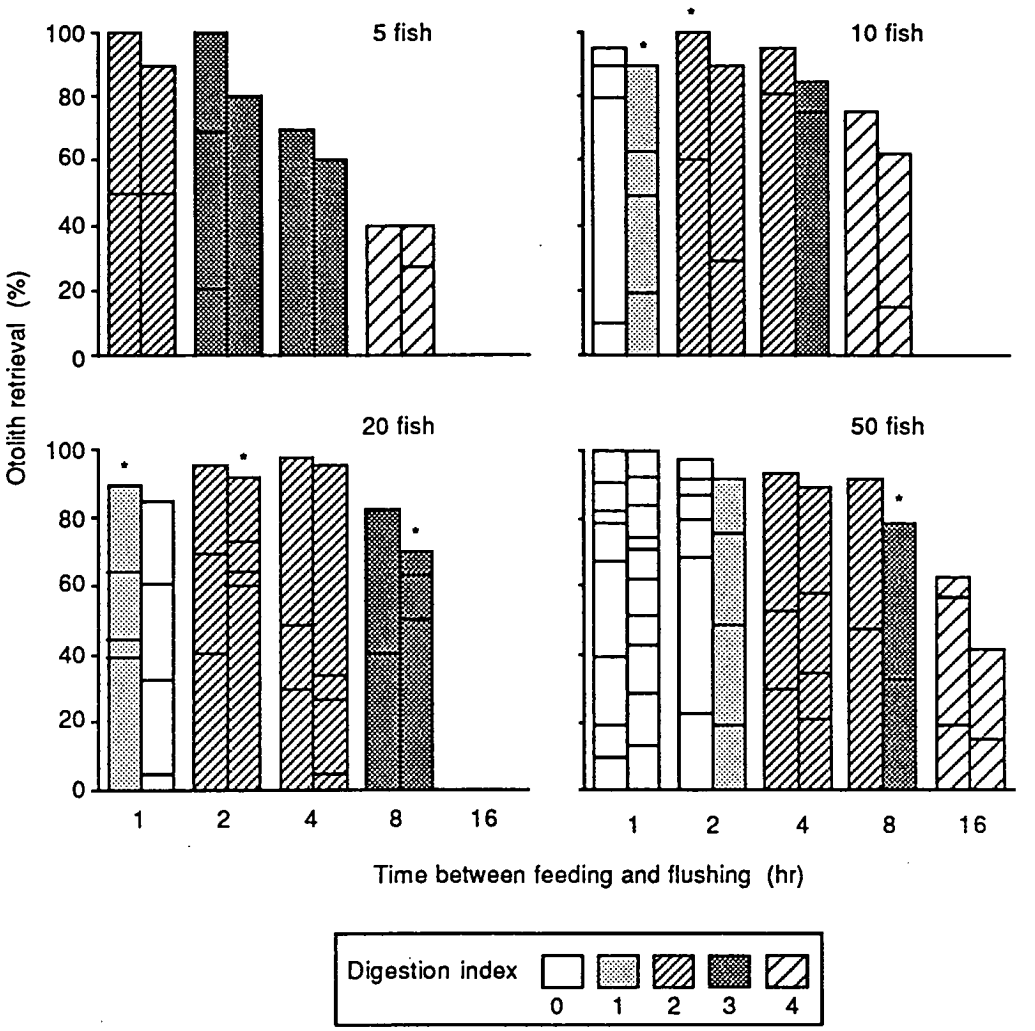

FIGURE 3.1 Results of validation trials on little penguins (replicates n = 2) in relation to meal size. The state of digestion of stomach contents is indicated by shading. The number of flushes required to empty the stomach and the percentage of otoliths retrieved in each flush are indicated by the horizontal bars. Samples in which the first flush retrieved no otoliths are indicated by *.

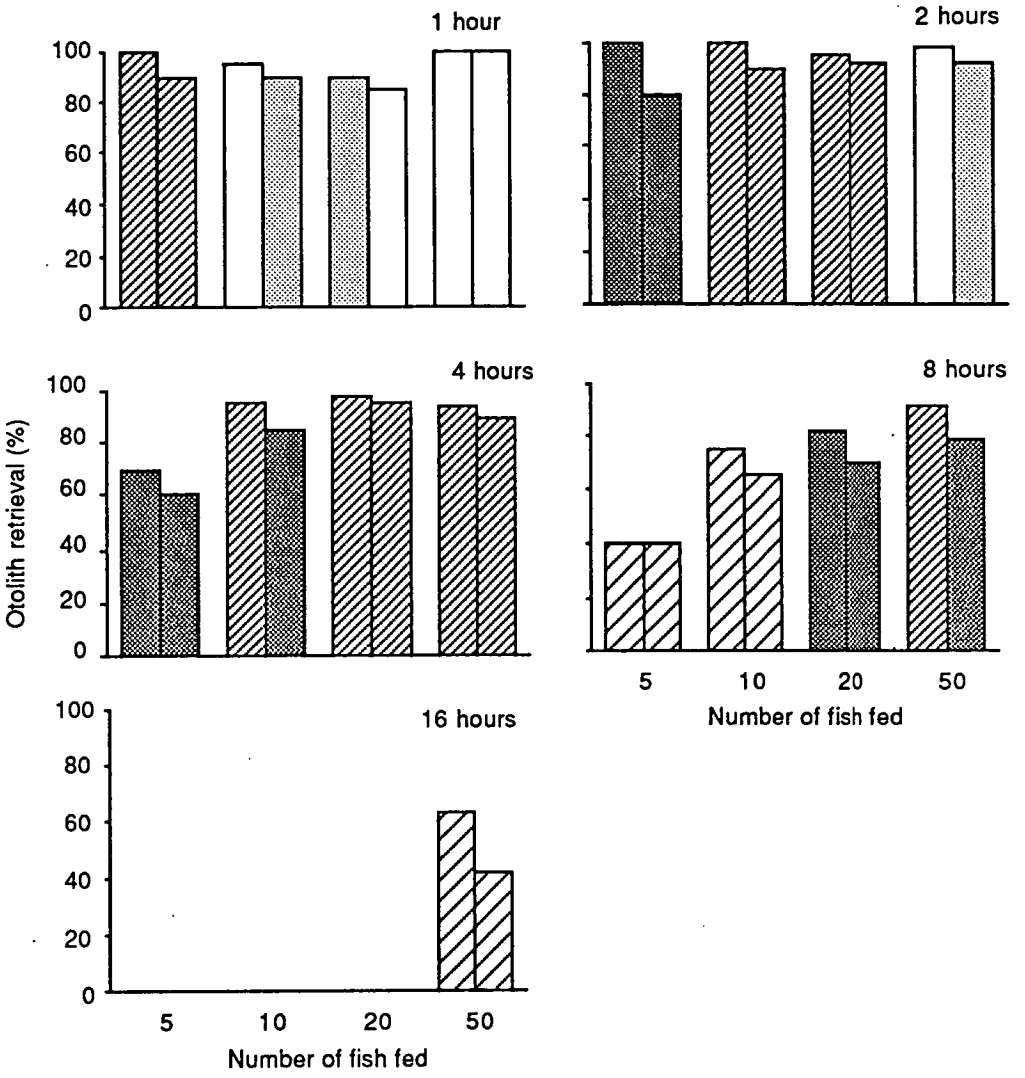

FIGURE 3.2 Results of validation trials on little penguins (replicates n = 2) in relation to time between feeding and flushing. The state of digestion of stomach contents is indicated by shading, the legend being the same as in Figure 3.1.

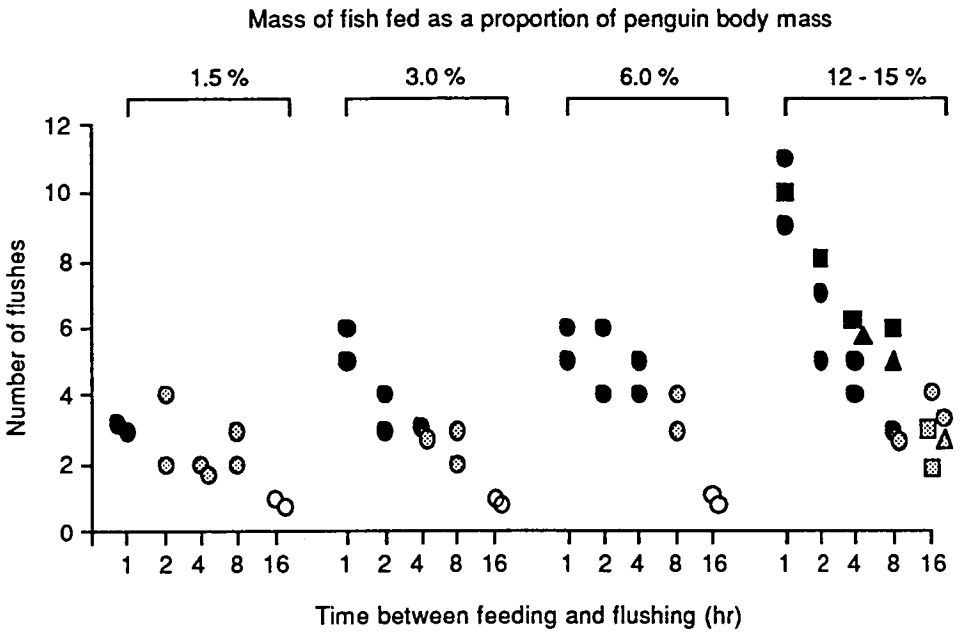

FIGURE 3.3 Relationship between number of flushes, digestion index and time between feeding and flushing. Circles represent little penguins, squares gentoo penguins and triangles rockhopper penguins. Digestion index depicted as: black symbol DI = ≤ 2; shaded symbol DI = 3 - 4; open symbol DI = 5.

Inconsistencies in the methods of different workers using the stomach-flushing technique, together with the lack of precise detail in the published accounts, appear to have led to the conflicting results reported and hence the lack of agreement regarding the efficiency of the technique. This is best exemplified by the question of the number of flushes required to empty the stomach. In the descriptive account by Wilson (1984) the number of flushes required was not stated, nor was it mentioned in many diet studies in which the technique has been used (LaCock *et al.*, 1984; Wilson, 1985*a*; Offredo *et al.*, 1985). In her work on rockhopper and royal penguins (*Eudyptes chrysolophus shlegeli*), Horne (1985) used the technique according to Wilson's specifications but flushed the birds only once. In the present study, it was shown that multiple flushes are essential to empty the stomachs of the three species of penguins tested. Indeed, the greater the fullness of the stomach, the greater the number of flushes required (Fig. 3.3). In cases where the first flush acts to loosen the contents but returns only cloudy water, if only a single flush was used the penguin would be erroneously recorded as having an empty stomach. In birds with full stomachs many consecutive flushes may be required until the complete stomach contents have been retrieved and the last flush is totally clear (Fig. 3.1)

The necessity for multiple flushing was also observed by Randall & Davidson (1981). Birds with empty stomachs are quickly identified by stomach flushing as the colour of the water from the first flush is tainted green by bile secretion (Randall & Davidson,1981; Wilson *et al.*, 1985). In the present study, any bird that did not emit green coloured water in the first flush was subsequently proved to have food in the stomach.

Horne (1985) estimated meal sizes of royal penguins from stomach samples obtained by single stomach flushes and compared results to meal sizes of macaroni penguins (*Eudyptes c. chrysolophus*) derived from the stomach samples from sacrificed birds (Croxall & Prince, 1980; Croxall & Furse, 1980). The royal penguins' average meal size ranged between 7 % and 15 % of those of the macaroni penguins. It is possible that this difference was an artefact of the stomach flushing being restricted to a single flush and thus only retrieving a portion of the stomach contents. Had the royal penguins been stomach flushed until the last flush was clear the meal sizes may have been more comparable to those obtained by other workers who killed the birds.

It is also likely that a single flush produces biased results as the food items retrieved in the first flush represents that eaten most recently and/or the lighter prey remains. In instances where little penguin stomach contents contained crustaceans and fish, the crustaceans were usually retrieved in flushes before the fish. If a single flush is assumed to obtain the complete stomach contents the bias in the results would have

far reaching implications to interpretations relating to the importance of prey types. Further, restriction to a single flush, may lead to errors in the interpretation of foraging ranges.

Digestion proceeds with time and so recovery rates decrease with time after food intake (Fig. 3.1). The duration of digestion increases with the fullness of the stomach and consequently high retrieval rates are maintained for a longer period (Fig. 3.2). Wilson *et al.* (1985) found that jackass penguins completely digested 50 g of anchovy in 10 hr and 100 g in 14 hr. These evacuation rates are comparable to those of the little penguins which digested meals of 5, 10 and 20 fish in 8 - 16 hr, but meals of 50 fish (140 g) took in excess of 16 hr to be completely digested.

While the results of this study have verified that the stomach-flushing technique is reliable in obtaining complete stomach samples from little penguins, and shows great promise for gentoo and rockhopper penguins, it is essential that the technique is validated in each species to which the technique is applied. This can be achieved by stomach flushing and subsequently killing the penguins and examining the stomachs for food items (Davies, 1956). More profitable are validation feeding trials which, although more labour intensive, also give indications of digestion rate.

In the validation feeding trials on the little penguins, the fish used were atherinids, which are consumed by little penguins in Tasmanian waters (Chapter 10). It was not feasible in the present study to use local prey items in the gentoo and rockhopper penguin feeding trials. The foreign nature of the school whiting used in these trials may have accounted for the slower rate of digestion of these penguins during the trials. It is preferable in validation feeding trials, where possible, to use food species which are important in the diet of the predators. It would also be beneficial to use the different prey taxa (e.g. fish, squid and krill) and ideally, a combination of the prey types in feeding trials with species which prey on animals from different taxa.

In many studies penguins have been killed only to obtain stomach contents. These penguins are usually killed during the breeding season as they return to their nest from the sea. Consequently, not only are the individuals sacrificed but the breeding success of the colony is also decreased by the disruption of pair bonds and the concomitant death of embryos and chicks. The killing of relatively large numbers of penguins may be unacceptable either on ethical grounds or for practical reasons (e.g., of rare species) in concurrent breeding and population dynamics studies, and when working on protected wildlife. Where a satisfactory alternative for obtaining stomachs is available, killing penguins for diet studies is largely unwarranted, The

stomach-flushing technique provides that alternative. This technique offers great potential for humane and long term penguin studies and in particular, makes the use of penguins as environmental monitors and indicators of resources more feasible.

3.5 SUMMARY

The efficiency of the stomach flushing technique in obtaining complete stomach contents was tested on little, gentoo and rockhopper penguins. This technique was validated by feeding the penguins known amounts of fish and subsequently flushing their stomachs after specified time intervals. Examination of the contents showed that the method is very effective in obtaining complete stomach contents and offers an alternative to killing of penguins in order to obtain stomach contents. The effects of different states of stomach fullness on food recovery rates were shown and these highlighted the necessity for multiple flushing. Quantitative information on the effect of time between feeding and stomach flushing on the recovery rates was also obtained. Where stomach contents were relatively undigested the rate of retrieval of fish was 90 - 100 %, but this rate decreased with time and in no cases in which stomach contents were in advanced stage of digestion was the retrieval rate higher than 80 %. Inclusion of the gentoo and rockhopper penguins in the validation trials showed that the size of the penguin does not effect recovery rate.

CHAPTER 4

THE USE OF OTOLITHS AS INDICATORS OF LITTLE PENGUIN DIET

4.1 INTRODUCTION

In many studies of the diets of pelagic seabirds the stomach contents are in advanced stages of digestion with little readily identifiable material (Brown *et al.*, 1981; Croxall *et al.*, 1985; Lishman, 1985). Fish otoliths are increasingly being used in diet studies of piscivorous marine birds and mammals as diagnostic prey remains because otoliths are the most dense structure in teleost fish and are the most resistant to digestion (Treacy & Crawford, 1981). Further, the shapes of the sagittal otoliths are species-specific and otolith dimensions can provide information on the age and size of fish (Fitch & Brownell, 1968; Ross *et al.*, 1979; Frost & Lowry, 1981).

Until recently the influence of digestion on the analyses and interpretation of dietary studies has been largely ignored and most researchers have assumed that digestion rates were similar between prey types. Recently, however, differential digestion has been found to lead to errors in the determination of dietary importance (Furness *et al.*, 1984; Da Silva & Neilson, 1985; Murie & Lavingne, 1985; Wilson *et al.*, 1985). The retention and/or differential digestion of the diagnostic prey remains will also bias the interpretation of results. Common biases are the over-estimation of the importance of squid in the diet due to the accumulation of the keratinous beaks in predator stomachs (e.g., Furness *et al.*, 1984), and the under-estimation of the importance of fish, because otoliths are both relatively small and subject to digestion (Prime, 1979; Duffy & Laurenson, 1983; Murie & Lavigne, 1986).

The primary objective of the present study was to investigate the digestion of otoliths by little penguins *Eudyptula minor* to determine whether (1) otolith numbers can be used to calculate intake (meal or daily consumption) of food; (2) the rate of digestion of ingested otoliths is affected by meal size and/or time of retention; (3) the size of ingested otoliths can be used to calculate the size of fish, and which parameter of the otolith, length or mass, is most appropriate in such calculations.

4.2 METHODS

4.2.1 COLLECTION OF SAMPLES

Forty wild little penguins (mean mass: 1.0 kg, S.E. = 0.02, n = 40) on Albatross Island (40° 24'S, 144° 32'E) Northwestern Bass Strait, were used in feeding and stomach flushing trials during September 1984. The birds had been on land for at least 24 hours prior to the trials and so were unlikely to have had any food in their

stomachs. They were then force-fed thawed atherinids (*Atherinason hepsetoides*), a prey item of the little penguin in Tasmania (Chapter 10). These fish (mean length : 74 mm, S.E. = 0.66, n = 100; mean mass: 2.8 g, S.E. = 0.11, n = 50) were fed to the penguins in meals of 5, 10, 20 or 50 fish per feed. After 1, 2, 4, 8 or 16 hours the birds were then stomach flushed with seawater and released. Thus, for each of the four categories of meal size there were five time periods resulting in a total of 20 treatments and two penguins were used for each treatment. Stomach samples were stored in 90% alcohol until otoliths were removed. For full details of experimental protocol see Gales (1987*b*, also Chapter 3).

4.2.2 ANALYSIS OF REFERENCE MATERIAL

Sagittal otoliths were removed from fresh atherinids, washed in detergent and stored dry. Fish standard length (FSL) was measured from the snout tip to the tip of the last caudal vertebra (± 0.5 mm). To determine whether there were differences in otolith size within pairs, both otoliths were then measured along their maximum length (anterior-posterior) with a micrometer eyepiece (± 0.05 mm), and then weighed on an electronic balance (± 0.005 mg).

Relationships between otolith length (OL) and FSL, and otolith mass (OM) and FSL were investigated by regression analyses. Fish lengths in the reference collection extended from 31 mm (juvenile) to 97 mm (maximum length) (Last *et al.*, 1983). Otoliths were also extracted from a sample of 100 fish of the size range fed to the penguins to provide a reference sample of the sizes of ingested otoliths.

4.2.3 ANALYSIS OF SAMPLES

The otoliths from the stomach samples were removed, washed in detergent, sorted into left and right where possible and stored dry. They were then measured and weighed as described above. The apparent standard lengths of the fish eaten were then calculated from the regression equations. In order to estimate the number of fish eaten, where the otoliths could be categorized into left and right, the maximum number was used, but where categorization was not possible, the total number of otoliths was divided by two.

By comparison with the reference material an index of otolith digestion (DI) was devised in order to describe otolith condition :

DI = 0 - no apparent digestion.

DI = 1 - slight digestion - margin crenulations less distinct but rostrum and sulcus acusticus still well defined.

DI = 2 - moderate digestion - margin crenulations disappeared, rostrum and sulcus acusticus less distinct.

DI = 3 - advanced digestion - no rostrum or sulcus acusticus apparent, otolith lost all diagnostic features.

4.3 RESULTS

There were no significant differences within pairs of otoliths in either length (t = 0.327, d.f. = 22, P > 0.05) or mass (t = 0.825, d.f. = 22, P > 0.05). The relationships between otolith length (OL, mm) and FSL (mm) and otolith mass (OM, mg) and FSL were :

$$FSL = 21.1\ OL^{1.21} \quad (r = 0.99, n = 23, P < 0.001)$$
$$FSL = 50.5\ OM^{0.41} \quad (r = 0.98, n = 23, P < 0.001)$$

The number of recovered otoliths and the number of fish these represented are given in Table 4.1. The percentage of otoliths recovered over time are presented in Figure 4.1 which shows that the initial recovery rates were variable. One hundred per cent of ingested otoliths were recovered from at least one of the penguins which received replicate treatments after the meals of 5, 10 and 50 fish within the first two hours. At no time were 100 % of otoliths recovered after the 20 fish meal. After 8 hr post- feeding the proportion of recovered otoliths ranged from 40 to 79 %, with more from the larger meal sizes. Only after meals of 50 fish were otoliths present in the stomachs after 16 hr. The variability of otolith recovery rates was indicated by treatments in which 20 fish were fed, where the recovery of otoliths was higher after 2 and 4 hr than after 1 hr. The variation was also evident in otoliths recovered from penguins which had received the same treatment, the incidence and magnitude of this variation increasing with time after ingestion (Tables 4.1 & 4.2).

It is unlikely that any otoliths were passed out in faeces. The penguins were held for at least one hour after being stomach flushed and all faeces passed during this time were examined for evidence of otoliths. No otoliths were found in these faeces, nor in the faeces which have been examined from other little penguins (personal observation).

The mean length of otoliths extracted from the reference sample of fish fed to the penguins was 2.8 mm (S.E. = 0.02, range = 2.5 - 3.6) and the mean mass was 2.58 mg (S.E. = 0.6, range = 1.82 - 4.96). Otoliths recovered from the stomach samples decreased in both length and mass with time after ingestion (Tables 4.1 & 4.2). The variation in size of otoliths retrieved from replicate treatments also increased with stomach residence time.

Using both the length and mass of recovered otoliths, apparent fish lengths were calculated using the regression equations from the reference group (Tables 4.1 &

TABLE 4.1 Lengths of retrieved otoliths from little penguin stomachs and calculated fish standard lengths (FSL) with tests for differences in calculated FSL between replicates and between calculated FSL and ingested FSL. a and b are results for individual penguins.

Fish fed n	Time h		Retrieval otoliths n	Retrieval fish %	Otolith length (mm) mean ± S.E. (range)	Calculated FSL (mm) mean ± S.E. (range)	t-statistic between replicates	t-statistic calc. FSL vs ingested FSL
5	1	a	10	100	2.7 ± 0.06 (2.5 - 3.0)	71 ± 1.9 (64 - 80)	0.088	1.227
		b	9	100	2.7 ± 0.02 (2.7 - 2.8)	71 ± 0.6 (70 - 73)		1.050
	2	a	10	100	2.6 ± 0.04 (2.5 - 2.9)	67 ± 1.4 (64 - 77)	2.010	1.254
		b	9	100	2.5 ± 0.02 (2.5 - 2.6)	65 ± 0.7 (64 - 67)		3.101 **
	4	a	7	80	2.5 ± 0.09 (2.3 - 2.6)	63 ± 2.7 (58 - 67)	2.123	2.680 **
		b	6	80	2.2 ± 0.07 (2.1 - 2.5)	53 ± 2.1 (52 - 64)		4.107 ***
	8	a	4	40	1.8 ± 0.12 (1.3 - 2.3)	45 ± 3.4 (29 - 58)	1.823	11.124 ***
		b	4	40	1.4 ± 0.25 (0.9 - 2.1)	32 ± 7.0 (19 - 52)		10.961 ***
10	1	a	19	100	2.7 ± 0.03 (2.6 - 2.9)	70 ± 1.0 (67 - 77)	2.020	2.011
		b	18	90	2.8 ± 0.04 (2.5 - 3.0)	73 ± 1.2 (64 - 80)		0.451
	2	a	20	100	2.7 + 0.05 (2.2 - 3.0)	70 ± 1.7 (55 - 80)	0.150	2.043 *
		b	18	90	2.7 ± 0.05 (2.5 - 3.2)	71 ± 1.5 (64 - 86)		1.835
	4	a	19	100	2.2 ± 0.04 (1.9 - 2.5)	54 ± 1.2 (46 - 64)	4.809 ***	11.154 ***
		b	17	90	2.6 ± 0.08 (1.7 - 3.0)	67 ± 2.4 (40 - 80)		3.496 ***
	8	a	15	80	1.5 ± 0.12 (0.8 - 1.9)	35 ± 3.3 (16 - 46)	2.212 *	14.525 ***
		b	13	70	1.8 ± 0.14 (1.1 - 2.1)	43 ± 4.2 (24 - 52)		12.047 ***
20	1	a	36	95	2.8 ± 0.02 (2.6 - 3.3)	73 ± 0.7 (67 - 89)	1.224	0.879
		b	34	90	2.7 ± 0.03 (2.4 - 3.2)	71 ± 1.0 (61 - 86)		1.859
	2	a	38	100	2.7 ± 0.03 (2.4 - 3.1)	72 ± 1.0 (61 - 83)	0.699	1.740
		b	37	95	2.7 ± 0.02 (2.4 - 2.9)	71 ± 0.7 (61 - 76)		2.170 *
	4	a	39	100	2.7 ± 0.03 (2.1 - 3.2)	72 ± 1.0 (52 - 86)	0.329	1.738
		b	38	100	2.7 ± 0.02 (2.3 - 2.9)	71 ± 0.7 (58 - 76)		2.173 *
	8	a	33	85	2.2 ± 0.05 (1.4 - 2.6)	55 ± 1.4 (32 - 67)	3.115 **	12.466 ***
		b	28	75	2.4 ± 0.04 (1.7 - 2.7)	62 ± 1.3 (40 - 70)		7.917 ***
50	1	a	100	100	2.7 ± 0.02 (2.2 - 3.1)	71 ± 0.6 (55 - 83)	0.196	3.681 ***
		b	100	100	2.7 ± 0.02 (2.2 - 3.1)	72 ± 0.5 (52 - 83)		2.459 *
	2	a	98	98	2.4 ± 0.02 (1.9 - 3.0)	60 ± 0.7 (46 - 80)	0.726	14.201 ***
		b	92	98	2.3 ± 0.03 (1.9 - 2.9)	59 ± 0.8 (46 - 76)		14.110 ***
	4	a	94	96	2.3 ± 0.02 (1.8 - 3.0)	57 ± 0.7 (43 - 80)	0.557	16.500 ***
		b	90	96	2.3 ± 0.02 (1.6 - 2.8)	57 ± 0.7 (37 - 73)		17.529 ***
	8	a	92	92	2.2 ± 0.02 (1.7 - 2.7)	54 ± 0.7 (40 - 70)	7.488 ***	20.333 ***
		b	79	80	1.9 ± 0.03 (1.4 - 2.3)	46 ± 0.8 (32 - 58)		26.404 ***
	16	a	63	64	1.9 ± 0.04 (0.9 - 2.6)	45 ± 1.2 (19 - 67)	2.012 *	22.396 ***
		b	42	42	1.7 ± 0.07 (0.5 - 2.6)	40 ± 1.9 (9 - 67)		19.911 ***

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

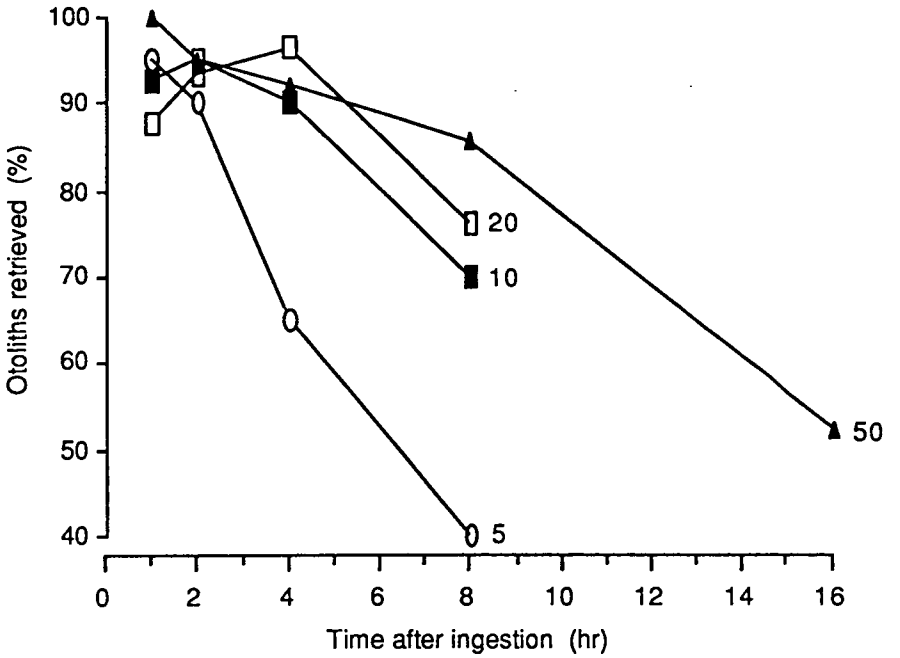

FIGURE 4.1 Per cent of retrieved otoliths from little penguins versus time of flushing after feeding each of the meal sizes. Each point is the mean of the results of the replicate treatment for two penguins.

TABLE 4.2 Mass of retrieved otoliths from little penguin stomachs and calculated fish standard lengths (FSL) with tests for differences in calculated FSL between replicates and between calculated FSL and ingested FSL. a and b are results for individual penguins.

Fish fed n	Time h		Retrieval otoliths n	Otolith mass (mg) mean ± S.E. (range)	Calculated FSL (mm) mean ± S.E. (range)	t-statistic between replicates	t-statistic calc. FSL vs ingested FSL
5	1	a	10	2.36 ± 0.091 (1.99 - 2.81)	72 ± 1.1 (67 - 77)	0.425	0.648
		b	9	2.41 ± 0.073 (2.08 - 2.59)	72 ± 0.9 (68 - 75)		1.001
	2	a	10	2.06 ± 0.099 (1.60 - 2.60)	67 ± 1.3 (61 - 75)	2.087	2.089
		b	9	1.77 ± 0.046 (1.64 - 1.93)	64 ± 0.7 (62 - 66)		3.498 ***
	4	a	7	1.64 ± 0.271 (1.18 - 2.12)	61 ± 4.2 (54 - 69)	1.517	3.009 **
		b	6	1.14 ± 0.095 (1.39 - 2.01)	53 ± 2.0 (58 - 67)		4.126 ***
	8	a	4	0.60 ± 0.117 (0.19 - 1.13)	39 ± 3.4 (26 - 53)	1.144	11.819 ***
		b	4	0.35 ± 0.189 (0.07 - 0.89)	29 ± 7.0 (17 - 48)		13.551 ***
10	1	a	19	2.34 ± 0.081 (1.96 - 2.98)	71 ± 1.0 (66 - 79)	1.690	1.405
		b	18	2.55 ± 0.094 (2.10 - 3.18)	74 ± 1.1 (68 - 81)		0.034
	2	a	20	2.25 + 0.111 (1.21 - 2.96)	70 ± 1.5 (55 - 79)	0.807	2.283 *
		b	18	2.34 ± 0.077 (1.94 - 3.06)	71 ± 0.9 (66 - 80)		1.490
	4	a	19	1.04 ± 0.070 (0.51 - 1.81)	51 ± 1.4 (38 - 64)	5.922 ***	12.988 ***
		b	17	2.09 ± 0.166 (0.62 - 3.13)	67 ± 2.4 (41 - 81)		3.356 **
	8	a	15	0.41 ± 0.180 (0.34 - 0.74)	35 ± 2.3 (32 - 45)	2.427 *	10.833 ***
		b	13	0.64 ± 0.170 (0.41 - 1.50)	42 ± 2.7 (35 - 60)		9.846 ***
20	1	a	36	2.52 ± 0.071 (1.82 - 4.56)	74 ± 0.8 (65 - 94)	1.465	0.332
		b	34	2.39 ± 0.055 (1.78 - 3.05)	72 ± 0.7 (64 - 80)		1.465
	2	a	38	2.47 ± 0.046 (2.00 - 3.23)	73 ± 0.6 (67 - 82)	1.661	0.792
		b	37	2.37 ± 0.034 (2.00 - 2.76)	72 ± 0.4 (67 - 77)		1.871
	4	a	39	2.46 ± 0.102 (1.02 - 4.95)	73 ± 1.2 (51 - 97)	1.296	1.095
		b	38	2.29 ± 0.062 (1.43 - 2.99)	71 ± 0.8 (58 - 79)		2.235 *
	8	a	33	1.21 ± 0.066 (0.34 - 1.88)	54 ± 1.3 (32 - 65)	4.525 ***	13.834 ***
		b	28	1.66 ± 0.062 (0.67 - 2.25)	62 ± 1.0 (43 - 70)		8.422 ***
50	1	a	100	2.42 ± 0.031 (1.57 - 3.24)	72 ± 0.4 (61 - 82)	0.825	2.055 *
		b	100	2.45 ± 0.030 (1.55 - 3.16)	73 ± 0.4 (60 - 81)		1.699
	2	a	98	1.75 ± 0.056 (1.01 - 3.49)	63 ± 0.8 (51 - 84)	0.839	9.845 ***
		b	92	1.74 ± 0.062 (1.05 - 3.63)	63 ± 0.9 (52 - 86)		10.288 ***
	4	a	94	1.58 ± 0.050 (0.65 - 3.63)	60 ± 0.7 (42 - 86)	0.589	13.373 ***
		b	90	1.53 ± 0.039 (0.92 - 2.61)	60 ± 0.6 (49 - 75)		14.962 ***
	8	a	92	1.32 ± 0.045 (0.60 - 2.49)	56 ± 0.8 (41 - 73)	9.150 ***	17.116 ***
		b	79	0.78 ± 0.047 (0.17 - 2.13)	44 ± 1.0 (24 - 68)		24.569 ***
	16	a	63	0.86 ± 0.052 (0.07 - 1.75)	46 ± 1.3 (17 - 63)	3.495 ***	20.898 ***
		b	42	0.60 ± 0.068 (0.01 - 1.57)	38 ± 2.1 (8 - 61)		20.752 ***

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

4.2). In no case were there any significant differences between the apparent fish lengths calculated from either otolith length or mass. There were no significant differences in calculated fish sizes between replicates for up to 2 hr post-feeding for any meal size. Variation between replicates was also insignificant at 4 and 8 hr post-feeding for the meals of 5 fish. This variation, however, tended to increase with both meal size and retention time and was significant after 2 hr when 10 and 50 fish were fed and after 4 hr when 20 fish were fed.

When apparent fish length, calculated from otolith size, is compared to the actual fish length ingested it is clear that the under-estimation of fish size increased with time of retention (Fig. 4.2, Tables 4.1 & 4.2). At 2 hr after ingestion of all meal sizes, in at least one of the replicates, the fish lengths calculated from length of retrieved otoliths were significantly smaller than the lengths of fish actually ingested. With the largest meal size of 50 fish this difference was significant in both replicates after only 1 hr post-feeding. After 16 hr the calculated fish length underestimated the actual fish length by 43 %.

After the initial rapid rate of digestion which occurred during the first hour after being fed 50 fish, the rates of decrease of calculated fish lengths were rapid for the 5 and 10 fish meals but slower for the 20 and 50 fish meals, respectively. This is evident from Figure 4.2 and, for example, for the apparent fish sizes after 8 hr post-ingestion of all meal sizes. After meals of 5 and 10 fish, the calculated fish lengths were approximately 52 % of the actual fish lengths, and were 79 and 68 % for the 20 and 50 fish meals, respectively. Thus, subsequent to the first hour after ingestion, the smaller meals which represented 1.4 and 2.8 % of the penguin body mass, digestion proceeded at a similar rapid rate, but for the larger meal sizes which represented 5.6 and 14 % of penguin body mass, digestion proceeded more slowly.

A sample of otoliths retrieved from stomach samples was described by the digestion index and their lengths and calculated fish lengths are shown in Table 4.3. No otoliths which were retrieved from the stomach samples but were still within skull cases showed any sign of degradation and hence were classified as DI = 0. However, some skull cases retained only one sagittal otolith indicating that there may be differential digestion within a pair, as most loose otoliths in the stomach contents scored 1 to 3 on the digestion index. When the calculated fish sizes are compared with the actual fish sizes, only those estimates derived from otoliths with a DI of 0 do not significantly underestimate the original fish length. The precision of the estimates decreased with increases in DI scores.

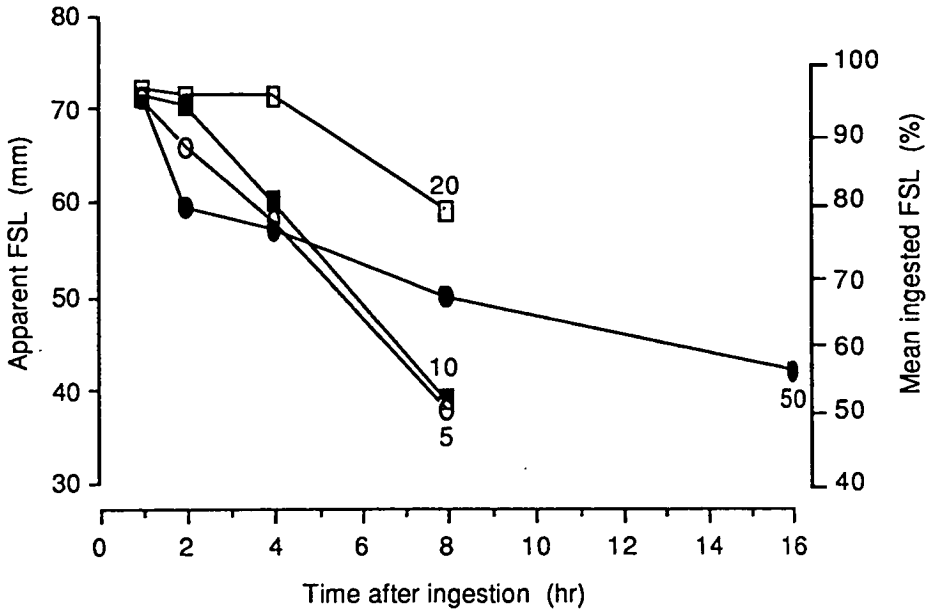

FIGURE 4.2 Apparent fish standard lengths FSL (mm) calculated from otolith lengths from little penguin stomachs versus time after ingestion for each of the meal sizes. Apparent FSL is also expressed as the per cent of the mean FSL actually ingested (74 mm = 100 %)

TABLE 4.3 Comparison of fish standard lengths from otolith lengths from different little penguin digestion indices and ingested fish standard lengths

Digestion index (n = 50)	Otolith length mean ± S.E. (range)	Calculated FSL mean ± S.E. (range)	t-statistic calc. FSL vs. ingested FSL
0	2.8 ± 0.02 (2.6 - 3.3)	74 ± 0.6 (67 - 89)	0.385
1	2.3 ± 0.02 (2.0 - 2.6)	58 ± 0.5 (49 - 67)	14.935 ***
2	1.9 ± 0.03 (1.6 - 2.3)	47 ± 0.9 (37 - 59)	22.753 ***
3	1.5 ± 0.04 (0.8 - 1.9)	34 ± 1.0 (16 - 46)	32.218 ***

*** = $P < 0.001$

4.4 DISCUSSION

Knowledge of the diets of seabirds is essential to an understanding of their role as predators in the marine ecosystem (Croxall *et al.*, 1984) and the primary objective of diet studies is to determine quantitatively the composition of the diet by analyses which are free from bias (Hyslop, 1980). The use of diagnostic prey remains to identify prey species and calculate original prey size is becoming increasingly common, but inherent in this practice is the assumption that digestion leaves hard parts unaffected. Squid beaks have been found to be unaffected by digestion (Bigg & Fawcett, 1985) and may be retained in seabird stomachs for considerable periods of time (e.g., Furness *et al.*, 1984). Thus, while the problem of squid beak accumulation is frequently discussed, little comment is made about the implications, or indeed the presence, of degraded otoliths in seabird stomach contents (e.g., Ainley *et al.*, 1981; Jackson, 1984; LaCock *et al.*, 1984; Wilson, 1985*a*; Wilson *et al.*, 1985). More attention is, however, now being focused on otolith digestion particularly by workers on seals (e.g., Prime, 1979; Bigg & Fawcett, 1985; Da Silva & Neilson, 1985; Murie & Lavigne, 1985, 1986).

In this study it was shown that atherinid otoliths are rapidly digested after ingestion by little penguins. Similarly, in a study of digestion in jackass penguins (*Spheniscus demersus*), meals of 50 g of fish were completely digested, and no otoliths remained, after 10 hr post ingestion, and meals of 100 g had been completely digested after 14 hr (Wilson *et al.*, 1985). These meal sizes represent 1.7 and 3.0 % of the jackass penguin body mass and are equivalent to the 5 and 10 fish meals fed to the little penguins which were completely evacuated between 8 and 16 hr after feeding. Murie & Lavigne (1985) found that in grey seals (*Halichoerus grypus*), no otoliths remained in the stomach after 18 hr. They also found that 30 % or more of the ingested otoliths had been completely digested by 3 to 6 hr post-feeding, a result which was reflected by the little penguins which digested between 20 and 60 % of the otoliths by 4 to 8 hr after ingesting 5 fish.

A consistent element between the feeding studies is that the digestion and passage rates of otoliths are subject to variation. The little penguin data show that otolith digestion rates vary with stomach fullness and time of retention which together confound interpretation of results. Prime (1979), working with harbour seals (*Phoca vitulina*), retrieved only a negligible proportion of ingested otoliths and concluded that no otoliths are retained for more than 48 hr. These observations demonstrate that if digestion of otoliths is not taken into account then any estimate of the number of fish consumed will be too low.

Not only are otolith numbers reduced, but otolith size is also affected by digestion. While otoliths can provide information on size and age of fish, any

calculation of original fish size, when based on otoliths which are in any way affected by digestion, will underestimate the size of fish. In the present study, calculations using retrieved otoliths resulted in significant underestimates of ingested fish length after only 1 to 2 hr post-feeding, and the difference between apparent and actual fish length increased rapidly until errors were in the order of 40 %. In an effort to eliminate this latter bias, Frost & Lowry (1980) calculated the original fish size from only non-degraded otoliths. This also proved to be effective in the present study, as when otoliths with a DI of 0 were used to estimate size of ingested fish, there was no significant difference between apparent and actual fish length. In order to do this, however, a visual inspection under a microscope is necessary for each otolith and it is this requirement which makes otolith length a more time-efficient parameter than otolith mass. Although the two otolith measurements produce the same results in calculations, since otolith condition can be inspected simultaneously to measuring their lengths, considerably less time is required than for the double handling required when otoliths are both inspected and weighed.

Most workers do, in fact, use otolith length (Jackson, 1984; Duffy & Laurenson, 1983; North *et al.*, 1984; Wilson, 1985*a*) and, hence, inspecting otoliths and thereby producing accurate estimates of FSL should be feasible. However, as noted by Murie & Lavigne (1985), this procedure may present sample size problems when used on predators whose prey have small, fragile, and hence readily digested otoliths (e.g., Clupeidae).

The relative size and thickness of otoliths vary considerably between fish species (Hecht, 1978) and there is evidence that small otoliths digest more rapidly than large ones (Prime, 1097; Da Silva & Neilson, 1985; Murie & Lavigne, 1986). Therefore, it would be expected that otoliths from different age fish within a species, and from different species of fish, will digest at different rates. Some species of fish have highly morphologically variable otoliths (Morrow, 1977) and so it is likely that these otoliths may also digest at different rates. Therefore, in partially digested meals, some prey may have been digested completely and therefore not included in calculations of amount of prey consumed, and any differential digestion of diagnostic remains makes quantification increasingly difficult.

The absence of otoliths in the little penguin faeces suggests that unrecovered otoliths are completely digested. Otoliths are, however, occasionally found in emperor penguin (*Aptenodytes forsteri*) faeces (K. Green pers. comm.) and it may be that differences in otolith density and/or penguin digestive physiology account for this difference. The initial rapid rate of digestion during the first hour after being fed 50 fish may be explained by the caloric effect of feeding. After feeding little penguins

meals equivalent to the 50 fish meals in the present experiment, Baudinette *et al.* (1986) found that for an initial period the metabolic rate is almost doubled. Further variability in digestion and passage rates is introduced when mixed diets are consumed (Wilson *et al.*, 1985) and also by the activity of the animal (Bigg & Fawcett, 1985).

If a predator has a catholic diet which includes fish with small otoliths, there is considerable potential for bias in results. Where specific data are not available, interpretation of piscivore diet analyses can result in underestimates of biomass of prey consumed and of prey age and size. The implications of these errors may be particularly important if the information from dietary studies is to be used in the assessment of prey resources.

4.5 SUMMARY

The validity of using otoliths from stomach contents quantitatively to determine the number and size of fish consumed was tested on little penguins. They were fed different meal sizes of known number and size of fish and the stomach contents were recovered after various time intervals. There were no differences in estimates of original fish size when calculated from otolith length or mass. Rate of digestion of otoliths tended to decrease with increased meal size but increased with time after ingestion. Digestion of otoliths proceeds rapidly and, if ignored, estimates of numbers of fish consumed and of original fish size can be significantly underestimated. This problem can be partially solved by inspection of otolith condition and restricting calculations of fish size to otoliths unaffected by digestion. Many factors introduce variations into rate of otolith degradation and further species-specific studies are required before appropriate correction factors can be applied.

CHAPTER 5

VALIDATION OF THE USE OF TRITIATED WATER, DOUBLY LABELLED WATER AND SODIUM-22 FOR ESTIMATING FOOD, ENERGY AND WATER INTAKE IN LITTLE PENGUINS

5.1 INTRODUCTION

Of all the seabirds, penguins show the greatest degree of adaptation to the marine environment, and information on their energetics and feeding rates is essential to the understanding of energy flow in marine ecosystems. There have now been studies on the energetics of at least eight of the 17 penguin species (Kooyman *et al.*, 1982; Davis *et al.*, 1983; Nagy *et al.*, 1984; Green & Gales, in press), including the little penguin, *Eudyptula minor* (Costa *et al.*, 1986; Green *et al.*, 1988; Gales *et al.*, 1988; Chapters 7 & 8), and these studies have recently reviewed by Green & Gales (in press).

The little penguin is the smallest penguin species and is restricted to the coasts of New Zealand and southern Australia. In these waters it is an important consumer of marine resources. Results of preliminary field studies in Tasmania have shown a combination of three isotopes to be appropriate for the investigation of feeding rates and foraging efficiencies of the species (Green *et al.*, 1988). Tritium (HTO) and doubly labelled water (DLW; $HT^{18}O$) were used to determine total body water, water turnover rates, CO_2 production and energy metabolism. Sodium-22 (^{22}Na) was also used to measure the total Na flux and to partition the water and Na influx into intake via food and intake via ingestion of seawater.

Despite the increasing attention being paid to the free-living energetics of the little penguin and other avian species (summarised in Nagy, 1987), there are few validation studies of isotope turnover techniques in birds (e.g., LeFebvre, 1964; Hails, 1979; Degen *et al.*, 1981; Williams & Nagy, 1984*b*; Goldstein & Nagy, 1985; Williams, 1985; Williams & Prints, 1986; Buttemer *et al.*, 1986), and none in seabirds. Not only do seabirds scale differently from other birds in allometric analyses of field metabolic rates (Nagy, 1987), but the use of ^{22}Na has only previously been validated in one bird species (Herd, 1985).

In interpreting and designing studies of free-living energetics it is important to know the magnitude of errors that may occur and also the appropriate time required both for equilibration of isotopes with the body pools and for animal recapture. In this study, I have examined the accuracy of measurements of the three isotopes in captive

little penguins by comparing simultaneous isotopic turnover to metabolizable intake measurements. These methods include a relatively new procedure for ^{18}O determinations with a mass spectrometer. In addition, I examined the effect of two different diets on assimilation efficiencies and isotope turnover.

5.2 METHODS

5.2.1 ANIMALS AND EXPERIMENTAL CONDITIONS

The three adult penguins used in this study had been captured from the east coast of Tasmania and held in outside enclosures for at least four months before experimentation. At the beginning of each experiment the penguins were moved into plastic-lined wire mesh cages measuring 1.0 x 0.8 x 0.8 m. They were housed in a controlled temperature room (15.0 ± 1.0°C), which was within the thermoneutral zone of this species (Stahel & Nicol, 1982), with a 12 h light:dark regime. Before the start of each experiment, the penguins were maintained in these conditions for five days in order to acclimate to the laboratory conditions and the feeding regimes.

Validation experiments of the isotope turnover technique were run in two series. The penguins were fed different diets in each series, one comprising a fish diet of whole specimens of sandy sprat (*Hyperlophus vittatus* , Clupeidae), and the other a squid diet of Gould's squid mantles (*Nototodarus gouldi*, Ommastrephidae), both species occurring frequently in the natural diet of little penguins (Klomp & Wooller, 1988*a*; Chapter 10). The squid mantles and fish were stored frozen. Prior to feeding, the food was thawed in sealed plastic bags, rinsed in freshwater and blot-dried before weighing into 150 g batches. Samples of food were retained for analyses of water, Na, and energy content (see below).

Before injecting birds, blood was sampled for background isotope levels. Each penguin was weighed and given intraperitoneal injections of 1.0 ml tritiated water (HTO:185 MBq), 0.5 ml sodium-22 (^{22}Na:185 kBq) and 0.3 ml 18-oxygen (^{18}O: 95 %+ atoms excess). The accuracy of these injection volumes was established gravimetrically, and none varied by more than ± 0.7 % from the indicated value. Blood samples of ≈ 1 ml were taken from a brachial vein, of each penguin, at 1, 2, 4, 6, 12 and 24 h intervals after injection. Food and water were withheld for 12 h before the injections and during the initial 24 h period. Blood samples were obtained by nicking a brachial vein with a scalpel blade and collecting blood directly into non-heparinised plastic vials. They were then centrifuged, and the serum and red cell fractions separated and stored frozen.

Each penguin was force-fed 150 g of squid or fish each day. They were not given access to drinking water. The isotope turnover trials ran for 6 days (from injection to final blood sample), and blood samples were collected after 2, 4 and 6 days. As the plastic cage liners were installed only after the final equilibration sample (24 h post-injection), the materials balance trial ran for only 5 days. The penguins were weighed with spring balances (± 5 g) whenever blood was collected. The same three birds were used for both experiments, which were identical except for diet, and which were separated by a period of three months.

5.2.2 ANALYTICAL PROCEDURES

At the end of the trials, the cages and plastic liners were thoroughly swabbed with cotton wool and distilled water. The excreta (faeces, urine, and salt-gland excreta) and samples of the food were then dried in a vacuum oven at 55°C until their mass was constant. Subsamples of the food and excreta were then ground in a Wiley mill and stored. Dried samples of food and excreta were compressed into 0.5 g pellets, and combusted in a Gallenkamp ballistic bomb calorimeter to determine energy content (in triplicate). Weighed subsamples (0.5 g) of the food items and excreta were also digested in concentrated nitric acid and then diluted with de-ionised water. The Na concentrations were measured in an atomic absorbtion spectrophotometer (Varian Techtron model 1000) with an air acetylene flame. Serum samples (5 µl) were diluted to 2 ml with de-ionised water for estimation of Na concentration.

The red cell fractions were lyophilized to complete dryness (Vaughn & Boling, 1961). Samples of extracted water (10 µl) were added to 3 ml of PCS cocktail (Phase Combining System, Amersham) and assayed for tritiated water activity in a liquid scintillation spectrometer (Beckman LS 2800). I placed 50 µl aliquots of extracted water in Urey tubes together with a standard charge of CO_2 gas. The Urey tubes were incubated overnight at 80° C, after which the equilibrated CO_2 charge was removed and the ^{16}O : ^{18}O ratio determined with a VG Isogas 903 isotope ratio mass spectrometer. The serum samples were bleached with concentrated hydrogen peroxide and oven-dried overnight. They were then mixed with 3 ml PCS cocktail and assayed for ^{22}Na activity by liquid scintillation spectrometry (Green & Dunsmore, 1978).

5.2.3 COMPUTATIONAL PROCEDURES

The penguins' gross energy intake (GEI), during each feeding trial was calculated by multiplying the amount of food consumed by the mass-specific energy content of the food. Dry matter assimilation (DMA) and energy assimilation efficiency (EAE) were calculated with the following equations (Gessaman, 1972):

$$DMA = \frac{\text{dry mass food (g) - dry mass excreta (g)}}{\text{dry mass food (g)}} \times 100$$

and

$$EAE = \frac{\text{GEI - energy in excreta}}{\text{GEI}} \times 100$$

Based on EAE, the amount of energy assimilated daily (DEA) was evaluated as the product of EAE and the amount of food eaten. The use of these calculations in this study assumes that gut-passage rate is less than 24 h. This is true for little penguins on diets of both squid (Montague, 1982) and fish (Gales, 1987*b*; also Chapter 3). Change in penguin body mass (%) was expressed as the change in mass as a percentage of the mean mass over the duration of the experiment.

Exchangeable Na and body water pool sizes (TBW) were calculated by comparing blood isotope levels at equilibration to standard dilutions of the injected isotopes. With ^{18}O standards, a sample of the standard diluent was also retained for mass spectrometry, as were the ^{18}O background samples. Isotope flux rates were calculated following Lifson & McClintock (1966), Nagy (1980), and Nagy & Costa (1980), assuming that changes in the pool size reflected body mass changes and that these changes were linear. The biological half-lives ($T_{1/2}$) of the isotopes were estimated from the formulae presented by Green & Dunsmore (1978).

For the fish diet, metabolic heat production was estimated from each penguin's rate of CO_2 production assuming 25.4 J ml^{-1} CO_2. This factor is based on the chemical composition of anchovy (19.7 % protein, 5.2 % fat, < 0.5% carbohydrate; South African Fisheries Industrial Research Institute, 1980; cited in Nagy *et al.*, 1984). Anchovy is of similar composition to sandy sprat [anchovy: 72.0 % water, energy content 23.9 kJ g^{-1} dry mass (Chapter 6), and see Table 5.1] and belong to the same order, Clupeiformes. For the squid diet, the conversion factor was 24.9 J ml^{-1} CO_2 which is based on the composition of another Ommastrephid squid, Japanese flying squid (76.6 % water, 4.19 kJ g^{-1} wet mass, Croxall & Prince, 1982*a*; and see Table 5.1). The conversion factors used in estimating oxidation water were 0.5 ml water per g of metabolised protein and 1.07 ml water per g metabolised fat (Schmidt-Nielsen, 1975). I assumed that fat and protein had the same fractional assimilations and, as carbohydrates formed < 0.5 % of both diets, they were considered negligible. Unless stated otherwise, all mean values are presented ± S.D., all Student's *t*-tests are paired, and the 0.05 level of probability was accepted as indicating statistical significance.

TABLE 5.1 Water, sodium and energy contents of the fish and squid used in the validation trials

		Water content %	Sodium content mmol/kg	Energy content kJ/g wet mass	Energy content kJ/g dry mass
Fish (sandy sprat)	mean	72.85	75.9	5.47	20.27
	S.D.	1.19	*	0.25	0.73
	n	49		10	10
Squid (Gould's squid)	mean	77.13	96.20	5.46	23.89
	S.D.	0.87	4.83	0.32	1.17
	n	6	6	6	6

* pooled sample

5.3 RESULTS

5.3.1 MATERIALS BALANCE

The water, Na and energy contents of the two diets are shown in Table 5.1. The penguins showed no significant changes in body mass during the experiments (fish diet $t_{(2)} = 1.75$, ns; squid diet $t_{(2)} = 0.95$, ns; Table 5.2), which indicates that they maintained water, Na and energy balance during the trials. The energy content in the penguins' faeces after being fed the fish diet was 10.82 ± 0.01 kJ g^{-1} dry mass ($n = 3$) and after the squid diet was 13.63 ± 0.77 kJ g^{-1} dry mass ($n = 3$). The food consumption, excretory output, apparent dry matter assimilation (DMA), and energy assimilation efficiency (EAE) are shown for each penguin in Table 5.2. When being fed the squid diet the penguins showed higher % DMA ($t_{(2)} = 9.56$, $P < 0.05$) and % EAE ($t_{(2)} = 3.65$, $P < 0.05$) than when on the fish diet.

The mean daily energy intake for the fish and squid diets were 728.0 ± 29.5 kJ kg^{-1} ($n = 3$) and 699.7 ± 13.1 kJ kg^{-1} ($n = 3$), respectively, and did not differ significantly from one another ($t_{(2)} = 1.43$, ns). There was also no significant difference between the two diets in terms of DEA ($t_{(2)} = 0.15$, ns).

5.3.2 RATES OF ISOTOPE EQUILIBRATION

The HTO had equilibrated in the penguin body pools by 2 h, and the levels in the samples taken between 2 and 12 h after injection remained relatively unchanged. This is reflected in the estimates of total body water (TBW), calculated from HTO dilution (Fig. 5.1A). Samples taken only 1 h post-injection resulted in very high estimates of TBW. When TBW, determined at each of the equilibration sampling times, is expressed as a percentage of body mass at the time of injection versus at time of sample collection, the differences are minimal (i.e., < 1 %) until 6 h post-injection. Owing to loss of body mass these differences increased after 6 h, reaching 2.3 % at 12 h, and 4.3 % after 24 h, and these differences are significant (12 h: $t_{(2)} = 5.71$, $P < 0.05$; 24 h: $t_{(2)} = 12.65$, $P < 0.05$). A period of between 2 and 6 h is, therefore, considered to be both adequate and convenient for equilibration of injected HTO with the body water pool.

As with HTO, ^{18}O was equilibrated with body water at 2 h and remained relatively constant, until 12 h post-injection. After 12 h, the concentration started to decline and was significantly lower than equilibration values at 24 h post-injection ($t_{(2)} = 4.34$, $P < 0.05$; Fig. 5.1B).

TABLE 5.2 Digestibility of food and energy intake of little penguins.

Penguin No.	Initial body mass kg	Mass change %	Total food consumption			Faecal production		DMA * %	EAE ** %	DEA *** kJ/kg.day
			Fresh mass kg	Dry mass kg	Energy kJ	Dry mass g	Energy kJ			
					Fish diet					
1	1.085	-0.9	0.748	0.203	4115	113.6	1246	43.8	69.7	531.3
2	1.165	-0.9	0.747	0.203	4115	108.9	1176	46.3	71.4	506.7
3	1.180	-4.2	0.748	0.203	4115	113.9	1213	43.8	70.5	502.5
mean	1.143	-2.0	0.748	0.203	4115	112.1	1212	44.6	70.5	513.5
S.D.	0.051	1.9	-	-	-	2.8	35	1.4	0.8	15.6
					Squid diet					
1	1.170	+0.7	0.750	0.171	4085	81.1	1173	52.6	71.3	496.1
2	1.190	-0.7	0.750	0.171	4085	80.7	1087	52.6	73.4	505.6
3	1.165	-3.7	0.750	0.171	4085	80.6	1051	52.6	74.3	530.4
mean	1.175	-1.2	0.750	0.171	4085	80.8	1104	52.6	73.0	510.7
S.D.	0.130	2.5	-	-	-	0.3	63	-	1.5	17.7

* Dry matter assimilation
** Energy assimilation efficiency
*** Daily energy assimilation

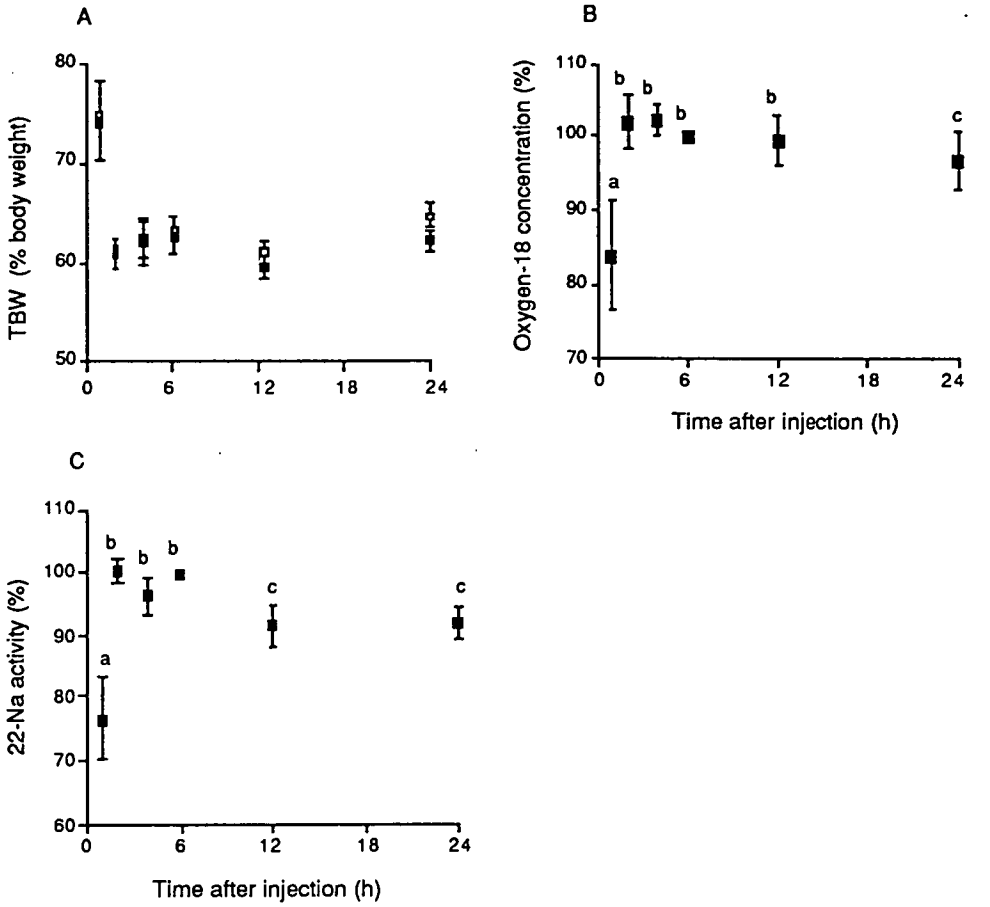

FIGURE 5.1

A Total body water, calculated from HTO dilution, expressed as a per cent (±SE) of body mass at time of injection (closed box) and mass at bleeding (open box) for intervals up to 24 h post-injection.

B Mean 18-O concentration (±SE) expressed as a per cent of concentration 6 h after injection. Means with different superscripts are significantly different ($P < 0.05$). Sample size for each point is six, being the three penguins on the two diets.

C Mean serum 22-Na specific activity expressed as a per cent of activity 6 h after injection. Means with different superscripts are significantly different ($P < 0.05$). Sample sizes as in B.

The ^{22}Na concentration in the serum increased rapidly until 2 h post-injection (Fig. 5.1C). Between 2 and 6 h ^{22}Na remained relatively constant but started to decline after 6 h. Therefore, a period of between 2 and 6 h is appropriate for equilibration of injected ^{22}Na with the exchangeable Na in the little penguin. When equilibrated, the exchangeable Na was 43.6 ± 4.5 mmol (n = 6) or 37.6 ± 3.4 mmol kg^{-1} body mass. These levels are equivalent to 55 mmol Na l^{-1} body water.

5.3.3 VALIDATION OF WATER TURNOVER RATES

TBW, determined from HTO equilibration, ranged between 57 % and 71 % of body mass (Table 5.3). When TBWs were determined from both HTO and ^{18}O equilibration samples at 6 h after injection, the estimate derived from ^{18}O was only -1.6 ± 2.2 % (n = 6) less than that determined from HTO, and this difference was not significant ($t_{(2)}$= 0.30, ns). The greatest discrepancy between the two methods was -5.0%, with ^{18}O giving the lower estimate. The biological half-life of HTO in the penguins was 4.3 ± 0.26 days (n = 6), and there was no significant difference between diets ($t_{(2)}$ = 0.15, ns). There were also no significant differences between the water influx and efflux rates (Table 5.3), and it was assumed that the birds were in water balance (fish diet: $t_{(2)}$ = 1.318, ns; squid diet: $t_{(2)}$ = 0.948, ns).

The validation of the water turnover rates was assessed by comparing the estimated water intake rates of the two diets to the water influx rates estimated from the HTO turnover over the 6 days, at the completion of the trials. The preformed water provided by the fish diet was 0.728 ml g^{-1} fresh mass. The metabolic water obtained via oxidation of the metabolizable fat and protein was estimated as 0.154 ml g^{-1} fresh mass. Thus, the total water yield from the fish diet was 0.882 ml g^{-1} fresh mass. For the squid diet, the preformed water represented 0.771 ml g^{-1} fresh mass. The metabolic water provided by the squid diet was estimated as 0.09 ml g^{-1} fresh mass, the total water yield being 0.861 ml g^{-1} fresh food.

For little penguins on the fish diet, total water intake, as estimated by HTO over the 6 day period, was between +7.4 % and +13.1 % of the calculated value, while on the squid diet the range was between -8.6 % and +12.3 % (Table 5.3). On average, HTO overestimated water intake by 6.5 ± 0.08 % (n = 6). At only 2 days after injection, the HTO activity had decreased by 20.1 ± 3.3 % from equilibration values (Fig. 5.2A). The rate of water influx calculated from the 2 day HTO turnover was 26 % lower than the estimated dietary water intake and differed significantly from evaluation of samples taken at 4 and 6 days post-injection ($P < 0.05$; Fig. 5.2A). The

TABLE 5.3 Components of the water metabolism and dietary water intake of the little penguins

Penguin No.	Mean body mass kg	TBW ml/kg	HTO T 1/2 days	Water influx (I) ml/kg.day	Water efflux ml/kg.day	Dietary water intake (II)* ml/kg.day	Ratio I/II
				Fish diet			
1	1.080	650	4.15	109.3	110.2	101.8	1.074
2	1.160	608	4.17	101.8	102	94.8	1.074
3	1.155	709	4.60	107.7	112.7	95.2	1.131
mean	1.132	656	4.31	106.3	108.3	97.3	1.093
S.D.	0.045	51	0.25	4.0	5.6	3.9	0.033
				Squid diet			
1	1.174	567	4.69	83.8	83.0	91.7	0.914
2	1.186	604	4.30	97.4	98.1	90.7	1.074
3	1.144	620	4.07	105.7	109.3	94.1	1.123
mean	1.168	597	4.35	95.6	96.8	92.2	1.037
S.D.	0.022	27	0.31	11.1	13.2	1.7	0.11

* Calculated as preformed and metabolic water

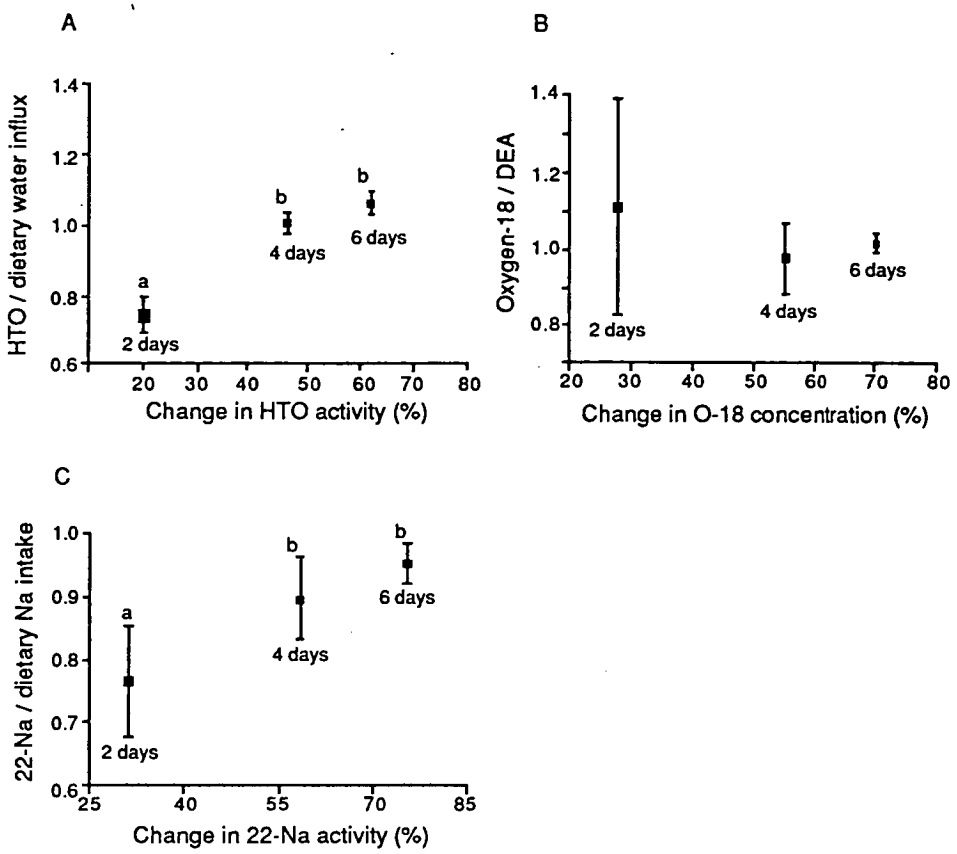

FIGURE 5.2

A Water influx (ml/kg.day) derived from HTO turnover expressed as a ratio of dietary water intake (± SE) to the per cent change in HTO activity from equilibration (6 h) to samples after 2, 4 and 6 days. Means with different superscripts are significantly different ($P < 0.05$). Sample sizes are six for 2 and 6 days, being the three penguins on both diets, and five for day 4 as one sample was lost.

B Metabolic rate (kJ/kg.day) derived from DLW turnover as a ratio of DEA (± SE) in relation to per cent change in 18-O concentration from equilibration (6 h) to samples after 2, 4 and 6 days. Sample sizes as in A.

C Na influx (mmol/kg.day) derived from 22-Na turnover as a ratio of dietary sodium influx (± SE) in relation to the per cent change in 22-Na activity from equilibration (6 h) to samples after 2, 4 and 6 days. Means with different superscripts are significantly different ($P < 0.05$), sample sizes as in A.

HTO turnovers after 4 days overestimated water influx by 1.0 ± 0.1 % and this was not significantly different from the estimate derived over the 6 days.

5.3.4 VALIDATION OF METABOLIC RATES

The biological half-life of ^{18}O during the two trials was 3.43 ± 0.12 days (n = 6), and the different diets had no effect on the half-life ($t_{(2)} = 0.116$, ns).

Estimates of CO_2 production from DLW turnover in the validation trial using the fish diet averaged 0.878 ml g^{-1} h^{-1}, and the average was 0.847 ml g^{-1} h^{-1} during the squid feeding trial (Table 5.4). Thermal equivalents for CO_2 production were assumed to be 25.4 and 24.9 kJ l^{-1} CO_2 for the fish and squid diets, respectively (see Section 5.2). Mean metabolic rates were thus 535.2 kJ $kg^{-1}day^{-1}$ and 506.0 kJ $kg^{-1}day^{-1}$ for the fish and squid diets, respectively, and these values were not significantly different ($t_{(2)}$ = 1.675, ns). When compared to mean DEA estimated from materials balance (Table 5.2), DLW estimates of energy metabolism were 4.3 % higher for the fish diet, which is not significantly different from the former value ($t_{(2)}$ = 1.053, ns). For the squid diet the discrepancy between the average metabolic rate predicted by the DLW method and the material balance method was 0.8 % and the difference was not significant ($t_{(2)} = 0.346$, ns). On average, DLW overestimated metabolic rate by 1.8 ± 0.06 %, and this is again not significant ($t_{(2)} = 0.677$, ns).

After two days, the ^{18}O concentration had decreased from initial equilibration levels by 28.1 ± 4.6 %, by 55.0 ± 0.81 % after 4 days, and by 70.3 ± 1.4 % after 6 days (Fig. 5.2B). Pooling data from both diets, metabolic rates estimated from DLW turnover were 10.9 % higher than that estimated from materials balance after 2 days, 2.4 % lower than DEA after 4 days, and 1.7 % higher after 6 days. The degree of variation in values within the three time periods were very different and this would account for the lack of statistical difference between samples (Fig. 5.2B).

5.3.5 VALIDATION OF SODIUM TURNOVER RATES

The biological half-life of ^{22}Na did not differ significantly between diets ($t_{(2)}$ = 1.941, ns) and averaged 2.95 ± 0.16 days (n = 6). There were no significant differences between Na influx and efflux rates associated with either the fish or the squid diets, suggesting that the penguins were in Na balance (fish diet: $t_{(2)}$ = 1.94, ns; squid diet: $t_{(2)}$ = 1.17, ns) (Table 5.5).

The exchangeable Na pools of the penguins were consistently lower on the fish diet than during the squid diet. Whilst on the fish diet, the penguins had an Na pool of

TABLE 5.4 Validation of the DLW method as an estimate of metabolic rate and food consumption

Penguin No.	Metabolic rate		Ratio (I)/DEA
	ml CO_2/g.h	kJ/kg.day (I)	
	Fish diet		
1	0.882	537.7	1.012
2	0.825	502.9	0.993
3	0.927	565.1	1.125
mean	0.878	535.2	1.043
S.D.	0.05	31.2	0.071
	Squid diet		
1	0.863	515.7	1.039
2	0.836	499.6	0.988
3	0.841	502.6	0.948
mean	0.847	506.0	0.992
S.D.	0.014	8.6	0.046

TABLE 5.5 Components of the sodium metabolism and dietary sodium intake of the little penguins

Penguin No.	Exchangeable sodium pool mmol/kg	22-sodium influx mmol/kg.day	22-sodium efflux mmol/kg.day	Total 22-sodium turnover (I) mmol	Total sodium intake (II) mmol	Ratio I/II
			Fish diet			
1	34.80	8.84	8.90	57.3	56.8	1.009
2	32.67	8.10	8.15	56.4	56.7	0.995
3	37.38	8.63	8.86	59.2	56.8	1.042
mean	34.95	8.52	8.64	57.6	56.8	1.015
S.D.	2.36	0.38	0.42	1.4	-	1.024
			Squid diet			
1	39.38	8.73	8.72	61.5	72.1	0.853
2	40.06	9.06	9.10	64.4	72.1	0.893
3	41.51	9.56	9.82	65.6	72.1	0.910
mean	40.32	9.12	9.21	63.8	72.1	0.885
S.D.	1.09	0.42	0.56	2.1	-	0.029

53.8 ± 0.21 mmol l^{-1} TBW, and during the squid trial, 67.6 ± 1.6 mmol l^{-1} TBW. The final estimates of Na influx by ^{22}Na turnover and estimated dietary Na intake were in closer agreement with the fish than the squid diet. On average, ^{22}Na turnover overestimated Na intake from the fish diet by 1.5 %, but it underestimated calculated Na intake of the squid diet by 11.5 % (Table 5.5). When the data are pooled, there was no significant difference between estimates of ^{22}Na turnover and and Na intake ($t_{(2)}$ =1.726, ns).

When sampled after 2 days, the ^{22}Na activity had decreased by 31.1 ± 7.4 % from the initial equilibration value, decreased to 58.4 ± 5.9 % after 4 days, and to 75.5 ± 1.7 % by the final sample (6 days; Fig. 5.2C). Na influx calculated from ^{22}Na turnover was 39.8 % lower than the estimated dietary Na intake for the 2 day sample and was also significantly lower than the dietary values estimated for 4 or 6 days (Fig. 5.2C). The ^{22}Na-derived estimate of Na influx at day 4 was not significantly different from that obtained after 6 days, although the variation in values was greater at 4 days.

5.4 DISCUSSION

Energy assimilation efficiencies are essential for accurately modelling the food requirements of natural populations. Data for assimilation efficiencies of seabirds (summarised by Adams, 1984) are limited, and many bioenergetic studies rely instead on values from other species, and usually from chicks. Assimilation efficiencies can, however, vary with species, diet type, and temperature (Wiens, 1984).

In the present study, the squid had a higher energy content than the fish on a dry mass basis and the little penguins showed higher DMA and EAE when being fed the squid diet (Tables 5.1 and 5.2). The EAE values for both diet types, however, were at the lower limit of the range for seabirds for which data are available (Adams, 1984). The values of assimilation efficiency obtained in this study are the lowest recorded for penguins, and such species-specific variation in this parameter may importantly affect estimates of their food requirements. In their study of energetic requirements of little penguins, Costa *et al.*. (1986) assumed an assimilation efficiency of 77.9 % for an exclusively fish diet, a value obtained from jackass penguins (*Spheniscus demersus*) by Nagy *et al.* (1984). Substituting the EAE value determined in the present study for that used by Costa *et al.* (1986) produces a value of 4.64 kJ g^{-1} fresh matter available to the penguins, which is 10 % lower than the 5.13 kJ g^{-1} based on jackass penguins. This discrepancy would compound in further calculations of estimated food intake and demonstrates the need, where possible, to use values appropriate to the species and diet type.

It is also important to know the period of time required for isotopes to equilibrate with the body pool as sampling before complete equilibration leads to errors in determination of pool size and subsequent turnover rates of the isotopes. Route of injection has been found to affect rate of isotope equilibration in mammals (Smith & Sykes, 1974), but there was no difference in the rate of HTO equilibration in chukar partridges (*Alectoris chukar*) after either intramuscular (IM) or intravenous (IV) injections (Degen *et al.*, 1981). For relatively small birds, where route of injection was either IM or IV, 1 h has been determined as sufficient for species ranging in size from 20 to 600 g (LeFebvre, 1964; Degen *et al.*, 1981; Williams & Nagy, 1984*a*, *b*; Williams, 1985). In larger birds, however, equilibration periods have rarely been measured.

Equilibration time for HTO was determined in the grey-headed albatross (*Diomedea chrysostoma*, *ca.* 3.5 kg) and was complete in all cases by 2 h after IM injections (Costa & Prince, 1987). With emus (*Dromaius novaehollandiae*, *ca.* 35 kg), plasma ^{22}Na concentration was stable from 6 h to 24 h after IM injections (Herd, 1985). None of the isotopes used in the present study had equilibrated by 1 h post-injection, the route of injection being intraperitoneal (IP). By 2 h, however, all isotopes had equilibrated with the penguins' body pools, with HTO and ^{18}O remaining unchanged through 12 h and ^{22}Na through 6 h post-injection. An appropriate equilibration period for all three isotopes then, after IP injection, is 2-6 h for the little penguin.

In isotope studies, appropriate sampling intervals are important. If insufficient isotope turnover has occurred, or if isotope levels are too close to background levels, large errors in estimating their turnover rates may result (Nagy, 1983). Suggested recapture intervals are often presented as multiples of the biological half-life of an isotope, and this varies with taxon, size, and activity level of a given animal. Biological half-lives of HTO in birds have been summarised by Streit (1982), who presents a predictive formula that predicts a theoretical half-life of 8.6 ± 0.1 days for the little penguins of this study, double the value found in the present study. Using the formula presented for the biological half-life of ^{18}O in free-living birds (Nagy, 1983), one finds that the theoretical half-life in little penguins is 2.1 ± 0.03 days, shorter than the 3.4 days determined in these experiments on captive animals. HTO is accurate for more than five half-lives, so calculated water fluxes are generally reliable for a longer period than are calculated CO_2 production rates, where reliable results are obtained between one and two half-lives of the ^{18}O isotope (Nagy, 1983).

Published values of the biological half-life of ^{22}Na in birds range from 8.1 days for glaucous-winged gulls, *Larus glaucescens* (Roberts & Hughes, 1984), to 18.9 days for emus (Herd, 1985). These values are well in excess of the 2.9 days calculated for little penguins. These differences reflect the variety which may exist, not only between taxa, but also within species depending on body mass, metabolic rate, environment, diet, stage of breeding cycle and experimental procedure (Streit, 1982).

Mean TBW of little penguins in this study was 62.6 % body mass, a figure which is typical of normally hydrated adult birds (Skadhauge, 1981; Mahoney & Jehl, 1984). This value agrees with TBWs determined via isotope dilution of adult little penguins (63.2 %, Costa *et al.*, 1986; 64 %, Green *et al.*, 1988; 63 %, Gales *et al.*, 1988, also Chapter 7) and the value of 61 ± 2.3 % determined by desiccation of five adult little penguins (B. Green, unpubl. data). Errors in estimation of TBW by HTO in mammals have been summarised by Nagy & Costa (1980), and these range from -5.7 to +12.0 %. Degen *et al.* (1981) found that in chukar partridges and sand partridges (*Ammoperdix heyi*) there was no significant difference between desiccated and HTO-determined water space, although small differences were found in chukar partridges by Crum *et al.* (1985), depending on methods of analyses.

In the present study TBW determined from HTO dilution was 1.6 % higher than TBW determined from ^{18}O dilution, but the difference was not significant. TBW estimated from ^{18}O dilution in three species of sparrows (*Melospiza melodia, Zonotrichia albicolis* and *Passer domesticus*) averaged 3.1 % higher than values obtained by desiccation (Williams, 1985) after correcting for the feather water pool. If no allowance is made for the water contained in the feathers, which is not penetrated by isotope, then the discrepancy between TBWs determined by desiccation and isotope dilution decreases (Williams, 1985).

A further source of discrepancy between desiccated and isotopically determined TBW is the time of weighing, as loss of body fluids having an isotope concentration lower than that at equilibration will result in an under-estimation of TBW (Degen *et al.*, 1981). This was reflected in the present study (Fig. 5.1A) when, owing to loss of body mass, the discrepancy between TBWs calculated from mass at injection and bleeding increased after 6 h post-injection. Animals should therefore be weighed as close as possible to the time at which the isotopes equilibrate.

In this study, HTO overestimated actual water intake by 6.5 % with a range of -8.6 % to +13.1 %. This is similar to the range of differences between measured and isotopically determined water intake in sand partridges (-9.3 % to +13.3 %) and chukar partridges (-8.0 % to +6.4%) (Degen *et al.*, 1981), and in a variety of mammals (-7 % to +4 %) (summarised in Nagy & Costa, 1980). In most cases the water flux rates measured with HTO are expected to be within ± 10 % of the actual flux rates Nagy & Costa (1980).

From this study, however, it is clear that timing of sampling importantly affects the reliability of results. When water intake was estimated from HTO turnover after only 2 days, or a 20 % decrease in HTO activity, the discrepancy between estimated and actual water intake was significantly larger (26 %) than at the end of the trial. In this study at least a 40 % change in HTO activity had to occur before reliable estimates of water turnover could be estimated from HTO turnover. This is due to the fact that, where insufficient turnover has occurred, any analytical limitations and/or errors are greatly magnified. The reasons, however, for the consistent underestimates from HTO and ^{22}Na after 2 days (Figs. 5.2A, 5.2C) are unclear. It could be associated with the deprivation of food during the 24 h equilibration sampling, resulting in the birds' not being in water and Na balance at the beginning of the trial. The balance may then have been regained after the 2 day sample, hence the closer agreement between estimates after this time.

There have now been a substantial number of studies of field energetics of mammals and birds using DLW (summarised in Nagy, 1987), including studies on free-living penguins (Kooyman *et al.*, 1982; Davis *et al.*, 1983; Nagy *et al.*, 1984; Costa *et al.*, 1986; Davis *et al.*, 1989; Green & Gales, in press). Using both respirometric measurement and energy balance techniques, the DLW method has been validated in several species of mammals and birds (Hails, 1979; Nagy, 1980; Williams & Nagy, 1984*a,b*, Weathers *et al.*, 1984; Goldstein & Nagy, 1985; Williams, 1985; Williams & Prints, 1986), and generally discrepancies are less than 10 %. However, no validation studies have included seabirds, despite the increasing use of DLW with this group (e.g. Flint & Nagy, 1984; Nagy *et al.*, 1984; Costa *et al.*, 1986; Roby & Ricklefs, 1986; Costa & Prince, 1987; Gabrielsen *et al.*, 1987; Obst *et al.*, 1987).

On average in this study, the difference between DEA and the DLW estimates was small, with DLW resulting in only a slightly higher (1.75 %) value. The difference in values determined by the two methods ranged between -5.2 % and +12.5 %, and this range is typical in validation studies of birds (summarised in Williams &

Prints, 1986). The sources of error are probably due to a combination of operator errors, small violations of underlying assumptions, and inherent analytical uncertainties in both methods being compared (Nagy, 1980; Williams & Nagy, 1984*b*; Goldstein & Nagy, 1985).

While on average the agreement between energy balance and DLW values for metabolic rate was good at each sampling interval, the range of errors was greatest when sampled after only 2 days, or after only a 28 % decrease in ^{18}O concentration. From this study, it appears that reliable results cannot be obtained until at least a 50 % decrease in ^{18}O concentration has occurred, or until at least one biological half-life has elapsed.

Estimates of food intake from DLW averaged 1.4 % above the measured amounts. An alternative method for determining food intake is the use of ^{22}Na, an isotope which is much less expensive than DLW and the analysis of which is relatively simple. This method has been examined in several species of captive mammals (Green, 1978; Green & Dunsmore, 1978; Green & Eberhard, 1979; Williams & Green, 1982) and has also been used to estimate food intake in free-living mammals (Green *et al.*, 1978; Williams & Dudzinski, 1982; Williams & Ridpath, 1982; Green & Eberhard, 1983; Tedman & Green, 1987). This technique has been validated in mammals and lizards and has also been shown to be a useful technique for determining food intake of emus (Table 5.6). The value of using this isotope in seabirds, and in other marine animals, is that it allows the partitioning of water and Na flux into intake via food and intake via ingestion of seawater. Studies on seabird feeding rates have previously been based on the assumption that no seawater is ingested (Kooyman *et al.*, 1982; Costa *et al.*, 1986, Adams *et al.*, 1986; Costa & Prince, 1987). However, through the use of ^{22}Na in free-living seabirds, it has been found that seawater ingestion does occur (Green *et al.*, 1988, Green & Brothers, 1989) and can be of a sufficiently high magnitude that, if it is not taken into account, estimates of feeding rates may be significantly overestimated.

The turnover of ^{22}Na in little penguins agreed closely with dietary Na intake and supports the method as being useful for assessing rates of food intake. In most validation studies using ^{22}Na, there is only slight discrepancy between ^{22}Na turnover and dietary Na intake, but the differences in some cases are relatively high (Table 5.6). From this study, it was important that the turnover of ^{22}Na between samples was at least 50 % before food intake rates were reliably estimated. This is consistent with

TABLE 5.6 Validation studies of the sodium-22 method

Species	Mass kg	Mean error % (n)	Error range %	Source
Skink *Lampropholis guichenoti*	0.001	-7.6 (12)	-21.1 to +7.8	Gallagher et al. (1983)
Eastern Quoll *Dasyurus viverrinus*	1.3	-15.7 (4)	-21.9 to -6.8	Green & Eberhard (1979)
Rabbit *Oryctolagus cuniculus*	1.8	-22.6 (11)	-28.9 to -12.4	Green & Dunsmore (1978)
Tasmanian devil *Sarcophilus harrisii*	3.8	-6.6 (6)	-18.8 to +2.4	Green & Eberhard (1979)
Dingo *Canis familiaris dingo*	16	+13.7 (10)	-25.4 to +5.8	Green (1978)
Buffalo *Bubalus bubalis*	293	+1.0 (5)	-	Williams & Green (1982)
Little penguin *Eudyptula minor*	1.15	-5.0 (6)	-14.7 to +4.2	This study
Emu *Dromaius novaehollandiae*	35	-2.6 (12)	-39 to +37	Herd (1985)

results from emus, where samples should be taken between one and five half-lives to yield reliable results (Herd, 1985).

Large discrepancies between measured versus ^{22}Na-estimated rates of food intake have been attributed to lack of absorbence of Na in the undigested fraction of the diet. In little penguins, when fed fish, the discrepancy between the two methods was small (+1.5 %), but increased when the diet was changed to squid (-11.5 %). Because dry matter assimilation was lower with the fish diet than with the squid, decreased digestibility of prey cannot be responsible for the difference. Herd (1985) takes the reflux of urine into the colon and the possible exchange of endogenous and dietary Na to account, at least in part, for the success of this technique with the emu. Little penguins also reflux urine into the lower intestine but this cannot explain the difference in results with the two diets. Squid, however, have an appreciably higher concentration of Na than fish (Chapter 6) and it may be some difference in the way in which Na is compartmentalised in the two prey types that affects its uptake during digestion. No other studies have investigated the effect of prey type on ^{22}Na turnover, and further work is required before the source of this difference can be understood.

5.5 SUMMARY

The water, sodium and energy turnovers of little penguins were examined by comparing estimates determined from tritium (HTO), sodium-22 (^{22}Na), and doubly labelled water (DLW) turnovers with estimates from simultaneous materials balance trials. Two diets were used to assess the effect of food type on assimilation efficiencies and on turnover rates. Energy assimilation efficiencies were higher with the squid diet than with the fish diet but were still the lowest recorded for any penguin species. All three isotopes had equilibrated with body pools between 2 and 6 hours after injection and biological half-lives were 4.3, 3.4 and 2.9 days for HTO, ^{18}O and ^{22}Na, respectively, with no difference between diets. On average, HTO-derived estimates were significantly higher than measured intake (6.5 %), but DLW-derived estimates of metabolic rate were not significantly different from materials balance estimates. On average, ^{22}Na underestimated dietary sodium intake by 5.0 %, and, although this difference was not significant, discrepancies were greater with the squid diet than with the fish diet. Changes of approximately 50 % in isotope levels in the blood are required between injection and sampling to ensure reliable results from turnovers of all three isotopes.

CHAPTER 6

WATER, ENERGY AND SODIUM CONTENT OF PELAGIC FISH, SQUID AND KRILL FROM SOUTHERN AUSTRALIAN WATERS: THEIR USE IN STUDIES OF MARINE PREDATORS

6.1 INTRODUCTION

When energy flow through populations or communities is assessed in terms of amount of food consumed, seabirds assume an important position in the functioning of marine ecosystems. To project these patterns in any detail requires knowledge of the composition of predator diets and the energy content of the various prey items (Wiens, 1984). In the course of research into the feeding ecology and energetics of seabirds in southern Australia, a problem has been the scarcity of information on the composition of known prey species.

My study has concentrated on little penguins (*Eudyptula minor*) while other local seabird studies have concentrated on a variety of procellariiform species (Green & Brothers, 1989; N. Brothers and B. Green, unpublished data). These seabirds together consume a wide variety of schooling pelagic species of fish, squid and krill; the contribution of each varying with seabird species, location and season (N. Brothers and R. Gales, unpublished data). In studies of the free-living energetics of these seabird species, determined via isotope turnover techniques (e.g., Gales, 1989; Gales *et al.*, 1988; Green *et al.*, 1988; Green & Brothers, 1989; also Chapters 5, 7 & 8), it is required that the composition of the diet is known, with respect to the available water and sodium content of the prey, in order to determine rates of food and seawater consumption from isotopically determined water and sodium turnover rates. It is also essential to know the energy content of the prey consumed.

To facilitate these calculations, I collected samples of 19 species of common southern Australian fish, representing 14 families, together with representatives of two cephalopod families and samples of one species of a euphausiid crustacean. These samples were then analysed in terms of their water, energy and sodium contents. A limited number of samples were also analysed for lipid content. The species examined are important in the diets of many seabirds, as well as being preyed upon by marine mammals and fish, and in some cases are commercially important to man.

Models of energy flow through seabird communities in the North Atlantic, North Pacific and South Africa have been used to estimate that seabirds may consume between 20 and 30 % of the available fisheries stocks in the vicinity of the seabird colonies (Wiens & Scott, 1975; Furness, 1978, 1982; Furness & Cooper, 1982).

These estimates, which rely on accurate determinations of the composition and energy contents of seabird prey, imply the significant role of seabirds in the trophic and production dynamics of most near-shore marine ecosystems (Wiens, 1984). Also, if food consumption rates and assimilation efficiencies are known, then with the knowledge of the chemical composition of the prey, the nutrient returns to the marine ecosystem can also be estimated.

This Chapter presents results on the composition of a variety of common pelagic species which have been applied to dietary information and data relating to the food consumption and energy flux rates in little penguins (Chapters 5, 8 & 10). They are also required for use in other studies of marine predators and subsequent modelling of trophic interactions, and also to increase fundamental knowledge on the composition of marine organisms.

6.2 METHODS

All samples were collected from southern Australian waters between 1985 and 1987. The specimens were rinsed in fresh water, blot dried and weighed before freezing. Frozen samples were then thawed and rinsed before blot drying and weighing. All samples were then dried to constant mass either in an oven at 55 - 60° C, or by freeze drying, to provide solid and free-water contents of samples. Samples were then ground and weighed sub-samples combusted in a ballistic bomb calorimeter (Gallenkamp), while similar samples were digested in 25 % nitric acid and diluted with de-ionised water before the sodium content was determined by atomic absorbtion spectrophotometry (Varian model 1000). Lipid was extracted using a Soxhlet apparatus with CCl_4 as the solvent. Analytical procedures were performed at the CSIRO Division of Wildlife and Ecology, Canberra.

All data are presented as mean ± 1 S.D., and data are compared using unpaired, two-tailed Student's t-tests.

6.3 RESULTS

The water, sodium and energy contents of the prey species are summarized in Table 6.1. The water content was the most uniform compositional parameter measured in fish, the mean for the 19 species being 76.0 ± 4.3 %. The average value for the energy content of fish was 5.34 ± 1.01 kJ g^{-1} wet, with a wide range from 3.24 kJ g^{-1} (Tasmanian whitebait, *Lovettia sealii*) to 7.53 kJ g^{-1} (jack mackerel, *Trachurus declivis*). The sodium content of fish showed an even larger range, this being due to the inclusion of three species of salmoniform fish, which are either anadromous or diadromous, while all the other species are marine (Last *et al.*, 1983). Excluding the salmoniform fish, the mean sodium content of the 16 species of marine fish was

TABLE 6.1 Size and composition of prey species.

		Sample **		Length	Mass	Water content	Energy content		Sodium	Lipid
		Location	Date	mean ± SD (n) mm	mean ± SD g	mean ± SD %	mean ± SD (n) kJ/g dry	mean ± SD kJ/g wet	mean ± SD (n) mmol/kg wet	mean ± SD (n) %
FISH										
CLUPEIFORMES										
DUSSUMIERIIDAE										
Spratelloides robustus	Blue sprat	Vic	May-86	51.6 ± 8.1 (12)	1.33 ± 0.62	73.7 ± 2.1	24.30 ± 0.97 (4)	6.07 ± 0.22	57.7 ± 5.0 (5)	-
CLUPEIDAE										
Sardinops neopilchardus	Pilchard	Vic	May-86	138.0 ± 7.2 (6)	26.10 ± 3.00	74.3 ±1.7	20.45 ± 0.91 (6)	5.25 ± 0.14	123.5 ± 18.6 (6)	-
Hyperlophus vittatus	Sandy sprat	SA	Jan-85	62.7 ± 7.9 (50)	2.81 ± 72.8	72.8 ± 1.2	20.27 ± 0.73 (10)	5.47 ± 0.25	75.9 (*)	-
ENGRAULIDAE										
Engraulis australis	Anchovy	Tas	Sep-85	123.2 ± 2.5 (5)	19.67 ± 1.10	72.0 ± 1.7	23.96 ± 0.96 (5)	6.63 ± 0.47	46.8 ± 2.8 (5)	-
SALMONIFORMES										
APLOCHITONIDAE										
Lovettia sealii	Tasmanian whitebait	Tas	Sep-85	46.8 ± 2.9 (15)	0.59 ± 0.08	86.4 ± 0.6	23.82 ± 2.14 (*)	3.24	11.1 (*)	-
GALAXIIDAE										
Galaxias maculatus	Common jollytail	Tas	Sep-85	39.6 ± 1.1 (15)	0.28 ± 0.04	85.0 ± 0.3	28.63 ± 1.10 (*)	4.29	14.0 (*)	-
Galaxias truttaceus	Spotted galaxias	Tas	Sep-85	44.1 ± 1.6 (15)	0.44 ± 0.06	79.0 ± 1.1	24.59 ± 1.07 (*)	5.16	25.2 (*)	-
GADIFORMES										
MORIDAE										
Macruronus novaezelandiae	Blue grenadier	Tas	May-86	381.5 ± 6.2 (4)	308.9 ± 250.0	74.9 ± 2.6	25.68 ± 2.81 (4)	6.33 ± 1.42	52.5 ± 5.7 (4)	6.43 ± 3.93 (4)
ATHERINIFORMES										
ATHERINIDAE										
Atherinason esox	Pike-headed hardyhead	Tas	Aug-85	68.1 ± 3.0 (7)	2.74 ± 0.40	77.7 ± 1.1	20.79 ± 1.10 (*)	4.64	55.3 ± 2.0 (2)	-
Atherinason sp.	Short-headed hardyhead	Tas	Aug-85	63.5 ± 5.6 (6)	2.14 ± 0.54	75.1 ± 0.9	22.50 ± 0.36 (*)	5.60	57.1 ± 11.4 (2)	-
Atherinasoma microstoma	Small mouthed hardyhead	Tas	Aug-85	73.5 ± 11.7 (9)	4.43 ± 1.94	73.9 ± 0.7	20.22 ± 0.82 (*)	5.28	49.2 (*)	-
Atherinasoma presbytoides	Silverfish	Vic	May-86	51.9 ±10.2 (10)	1.44 ±0.73	76.5 ± 1.8	22.99 ± 0.74 (3)	5.68 ± 0.59	52.6 (*)	-
PERCIFORMES										
SILLAGINIDAE										
Sillago bassensis	School whiting	Tas	Sep-85	150.2 ± 1.1 (5)	41.73 ± 3.74	73.5 ± 1.4	21.11 ± 0.53 (5)	5.59 ± 0.32	59.8 ± 5.1 (5)	-
		Vic	May-86	175.2 ± 10.6 (6)	70.10 ± 10.41	73.9 ± 0.6	22.02 ± 0.75 (6)	5.78 ± 0.30	-	-
CARANGIDAE										
Trachurus declivis	Jack mackeral	Tas	Jan-86	287.5 ± 8.9 (6)	318.7 ± 19.5	69.0 ± 3.6	24.08 ± 2.05 (6)	7.53 ± 1.37	65.0 ± 4.2 (6)	8.56 ± 3.09 (6)
ARRIPIDAE										
Aripis trutta	Australian salmon	Tas	Oct-85	103.2 ± 47.8 (5)	30.21 ± 32.99	74.5 ± 1.0	21.32 ± 1.23 (5)	5.45 ± 0.50	50.2 ± 7.6 (5)	-

TABLE 6.1 continued

		Sample **		Length	Mass	Water content	Energy content		Sodium	Lipid
		Location	Date	mean ± SD (n) mm	mean ± SD g	mean ± SD %	mean ± SD (n) kJ/g dry	mean ± SD kJ/g wet	mean ± SD (n) mmol/kg wet	mean ± SD (n) %
EMMELICHTHYIDAE										
Emmelichthys nitidus	Redbait	Tas	Jul-87	255.0 ± 18.0 (3)	236.3 ± 50.8	72.6 ± 0.2	20.35 ± 0.72 (3)	5.57 ± 0.16	70.7 ± 3.11 (3)	3.27 ± 0.84 (3)
MUGLIDAE										
Aldrechetta forsteri	Yellow-eyed mullet	Tas	Jul-85	55.2 ± 11.5 (6)	1.3 ± 0.9	71.2 ± 1.2	21.8*	6.28 (*)	-	-
		Tas	Apr-86	318.2 ± 31.7 (5)	494.9 ±152.1	75.0 ± 2.4	21.6 ±1.0 (7)	5.42 ± 0.72	45.6 ± 6.3 (5)	3.75 ± 1.70 (5)
PLEURONECTIDAE										
Rhombosolea tapirina	Greenback flounder	Tas	Oct-85	78.3 ± 35.9 (6)	16.17 ± 15.31	80.0 ± 0.4	20.67 ± 2.43 (6)	4.13 ± 0.51	62.3 ± 2.8 (4)	-
TETRADONTIFORMES										
MONACANTHIDAE										
Acanthaluteres spiromelanurus	Bridled leatherjacket	Tas	Jun-86	82.3 ± 3.1 (6)	9.62 ± 1.20	79.6 ± 0.9	19.07 ± 0.26 (*)	3.89	64.7 ± 8.5 (3)	-
SQUID										
TEUTHOIDAE										
Sepioteuthis australis	Calamari	Vic	May-86	126.6 ± 21.6 (8)	104.9 ± 44.1	80.3 ± 1.1	24.76 ± 1.51 (8)	4.87 ± 0.19	115.14 ± 8.0 (5)	-
OMMASTREPHIDAE										
Nototodarus gouldi	Gould's squid	Vic	May-86	148.6 ± 12.7 (8)	60.0 ± 18.2	77.4 ± 1.6	24.25 ± 1.31 (8)	5.48 ± 0.61	91.06 ± 9.35 (8)	-
		Tas	Apr-86	230.3 ± 41.7 (10)	338.3 ± 197.7	77.0 ± 1.1	23.48 ± 0.75 (10)	5.39 ± 0.29	129.85 ± 11.70 (10)	2.12 ± 0.75 (6)
KRILL										
EUPHAUSIDAE										
Nyctiphanes australis	Australian krill	Tas	Sep-86	adults (n = 200)	-	87.2	20.22 (*)	2.58	62.5 (*)	-
		Tas	Jul-85	adults (n = 200)	-	82.2	19.61 (*)	3.49	160.0 (*)	-

* standard length for fish and dorsal mantle length for squid.
* indicates pooled sample.
- indicates data not collected.
** Location: Vic = Victoria; Tas = Tasmania; SA = South Australia.

60.3 ± 13.6 mmol kg^{-1} wet, while that of the three not wholly marine salmoniform species was 16.8 ± 7.4 mmol kg^{-1} wet.

The two squid species were similar to fish in terms of water content and also energy content on a wet mass basis (t-tests = 0.25 and 0.36 respectively, $P > 0.05$). The sodium content of the squid showed a wide range, both the highest and lowest values being for the two samples of Gould's squid (*Nototodarus gouldi*). The intra-specific range of sodium content was even higher in the krill, with a 2.5 fold difference being measured for the two samples of *Nyctiphanes*, the maximum being 160 mmol kg^{-1} wet. The two samples of krill both comprised adult animals (D. O'Brien, personal communication), and both were collected in Storm Bay (S.E. Tasmania), collection dates being separated by a period of 14 months (July 1985, Sept 1986).

6.4 DISCUSSION

A validation study comparing measured food intake and that estimated from isotope turnover techniques, showed close agreement in little penguins when being fed on fish and squid diets of known species and proximate composition (Gales, 1989; also Chapter 5). When extrapolated into the field, such close agreement depends largely upon detailed information about the proximate composition of prey species and their relative proportions in the diet. In studies which assume an 'average' diet for the species, results may be misleading if the study animals took disproportionate amounts of high or low energy content prey. Misleading results could also occur in calculations of food intake when estimated from sodium turnover rates and the sodium content of prey items, as this can vary markedly both within and between taxa and species (Table 6.1).

On a wet mass basis, fish were the group containing highest energy and lowest sodium levels, although there was a large degree of variation between species. There was a two-fold difference in the energy content between species of fish, the highest being the lipid rich jack mackerel. Following this was the anchovy (*Engraulis australis*) which had an energy content of 6.63 kJ g^{-1}. This is similar to the values reported for the Cape anchovy (*E. capensis*): 6.37 kJ g^{-1} (Cooper, 1978) and 6.58 kJ g^{-1} (Nagy *et al.*, 1984). Much lower values were measured for other species including Tasmanian whitebait and bridled leatherjackets (*Acanthaluteres spiromelanurus*). This inter-specific variation is important in energy flow studies of predator-prey relationships as little penguins, for instance, consume fish species which contain a wide range of energy values (Table 6.1 and Chapter 10). In terms of sodium content of fish, marked variation was also evident between species, marine species being considerably higher in sodium content than either anadromous or diadromous species.

In cephalopods, there were not marked differences in the water, sodium and energy values between the two species examined. However, in the two samples of Gould's squid, intra-specific variation was evident, where energy levels were similar but marked differences in sodium content occurred. On average, squid were similar in energy and higher in sodium than the fish species. It has been recognised that marine invertebrates are higher in sodium than marine vertebrates (Schmidt-Nielsen, 1960), and that there can also be appreciable variation in the energy content between squid species (Croxall & Prince, 1982*a*). Of the 17 species of squid examined by Croxall & Prince (1982*a*), 16 of which were Northern hemisphere species, all were lower in energy content than the two species measured in the present study. Two species of common South African squid (*Loligo reynaudi* and *Todaropsis eblanae*) (Cooper, 1979) were also lower in energy content than the species measured in the present study.

Major variations in the energy contents of both fish and squid in other studies have been attributed to differences in reproductive status, condition and age (e.g., Crawford, 1979; Croxall & Prince, 1982*a*; Montevecchi & Piatt, 1984). In the present study, from the differences in the size of the two groups of Gould's squid, it is likely that the smaller individuals (mean DML 14.9 cm) had not yet reached sexual maturity as juveniles of the species grow to 15 cm in the second month after spawning, whereas the larger group (mean DML 23.0 cm) probably comprised predominantly sexually mature individuals, maturity being reached between 22 and 30 cm DML (Smith, 1983, O'Sullivan & Cullen, 1983). Although these two groups were similar in their energy content, it may be this size, and possibly maturity difference, which accounts for the difference in sodium levels between the two groups, but the physiological rationale behind this is not yet clear.

In krill, lipid and energy contents are highly correlated with the state of sexual maturity and different classes of animals may predominate in different swarms (Clarke, 1980, 1984). An average value for antarctic krill (*Euphausia superba*) has been given as 4.2 - 4.5 kJ g^{-1}, as gravid females contain energy levels of 5.45 kJ g^{-1}, and mature males contain 3.84 kJ g^{-1} (Clarke & Prince, 1980). While such correlation with maturity levels and energy content probably exists for other krill species, a slightly lower average value has been given for *E. chrystallorophius* (3.85 kJ g^{-1} ; B. Green personal, communication) and this is similar to one of my samples of the Australian euphausiid krill (*Nyctiphanes australis*) in which I recorded an energy level of 3.49 kJ g^{-1}, and a sodium level of 160 mmol kg^{-1}. This level of sodium is also similar to that which has been recorded for *E. pacifica* (149 mmol kg^{-1}; Hirano *et al.*, 1964). However, in another sample of Australian krill which I collected 14 months later, I measured considerably lower levels of both energy and sodium (2.58 kJ g^{-1} and 62.5

mmol kg^{-1}, respectively). Although I have no data on the sexual status of the individuals within these two groups of krill, all individuals were adults, but they may have differed in their age composition and stage of sexual maturity, information which may have assisted in the explanation of this intra-specific variation.

Knowledge of the composition of marine prey species, and the variation which exists within taxa and species, is important in bioenergetic studies of seabirds and other marine predators. In estimating the food consumption rates of seals, the problems associated with the high levels of variation which exist in the caloric content of fish, both between and within species, have been addressed by Harwood & Croxall (1988). In some of the marine species measured in the present study, both the energy and sodium levels varied significantly, both between species and within species at different times of year, and so the application of average values of diet and compositional data of prey species could potentially be misleading. In studies of trophic relationships between predator and prey species in marine ecosystems, when the energy contents of prey are considered, dietary differences between seasons or predator species may have enhanced significance in terms of contribution of energy input from different prey species. There is evidence, for example, that seabirds breed more successfully when able to select a diet with a high energy value (Furness & Cooper, 1982).

When applying prey composition data to predator food consumption, or metabolic rate studies, ideally one should consider not only the appropriate species being consumed, but also, the size class, sex and reproductive status of the prey. This, however, is rarely possible, and a variety of compromise solutions are usually suggested (Harwood & Croxall, 1988). The full extent of intra-specific variation could not be addressed in the present study, but the results at least indicate the level of variation which exists between species and so provides a more sound data base for use in the predator energy requirement calculations than existed previously. These results, in association with precise seasonal dietary information, were employed in a study of the seasonal energy and food consumption rates of little penguins (Chapters 8 & 10). This study also served to illustrate the magnitude of intra-specific variation in energy and sodium levels which occur in some marine species. The levels of variation in energy and sodium levels which exist within fish, cephalopod and krill species, both between sites and seasons, and the physiological basis of such variation, are the topic of a current investigation (R. Gales, K. Newgrain & B. Green, unpublished data).

6.5 SUMMARY

The water, energy and sodium contents of 19 fish species, two squid species and one species of krill occurring in southern Australian waters are presented, together with some data on lipid content. These data showed considerable variation both within and between species, as well as between taxa. The use of these data, and the source and implications of the variation of the data are briefly discussed in reference to studies of the bioenergetics and food consumption rates of marine predators, particularly seabirds.

SECTION B

APPLIED AND FIELD STUDIES

CHAPTERS 7 - 10

CHAPTER 7

THE ENERGETICS OF FREE-LIVING LITTLE PENGUINS DURING MOULT

7.1 INTRODUCTION

All adult penguins undergo an annual moult during which the entire plumage is renewed. All species spend the duration of the moult ashore and hence cannot feed but subsist on fat and protein reserves accumulated during an intensive pre-moult foraging period. The moult period is one of starvation and rapid mass loss during which energy expenditure increases markedly from basal levels due to intense protein mobilization for feather synthesis and decreased insulation (Groscolas, 1982). Changes in plasma lipid levels during the moult of the emperor penguin, *Aptenodytes forsteri*, have been measured by Groscolas (1978, 1982) in order to assist in characterising energy metabolism during moult.

From studies of body composition of macaroni, *Eudyptes chrysolophus*, and rockhopper penguins, *Eudyptes chrysocome*, Williams *et al.* (1977) have shown the changes in body composition during moult. Energy consumption rates of moulting penguins have been summarised for 13 of the 17 species by Croxall (1982). These energy consumption data were calculated indirectly from rates of body mass loss, much of which was collected from captive birds, and changes in body composition (Williams *et al.*, 1977). These data show the high energy costs of replacing the complete feather coat in a relatively short time. Laboratory studies using metabolic chambers have also shown that the energetic costs are elevated during moult in macaroni and rockhopper penguins (Brown, 1985) and also in the little penguin, *Eudyptula minor*, (Stahel, 1984; Baudinette *et al.*, 1986).

To date, however, there are no published studies on the energetics of moult of free-living penguins in their natural environments. The feasibility of estimating metabolic rates of free-living penguins by use of isotope turnover techniques has been demonstrated in: king penguins, *Aptenodytes patagonicus* (Kooyman *et al.*, 1982); gentoo penguins, *Pygoscelis papua*, and macaroni penguins, (Davis *et al.*, 1983, 1989); and jackass penguins, *Spheniscus demersus* (Nagy *et al.*, 1984). Recently, the energy requirements of four free-ranging little penguins, two fasting on shore and two foraging at sea, have also been reported by Costa *et al.* (1986). A preliminary study of the water, sodium and energy turnover rates in free-living little penguins in both adults and chicks has also recently been completed (Green *et al.*, 1988), as well as a validation study of the use of isotopes in studies of little penguin energetics (Gales, 1989, also Chapter 5).

The little penguin is the smallest of the 17 penguin species and its distribution is restricted to Australia and New Zealand. Descriptions of the changes in mass loss and plumage during the moult of the little penguin are presented by Richdale (1940) and Kinsky (1960). The seasonal timing of moult and location of the moulting site in relation to the breeding burrow has been reported for the species by Reilly & Cullen (1983). In the present study, I report on the changes in plasma lipids and the energetic costs of moult in free-living little penguins determined with tritiated and doubly labelled water.

7.2 METHODS

The field study was carried out during March and April 1985 at Marion Bay, south-east Tasmania (42°50'S., 147°52'E.) and Albatross Island, north-west Bass Strait (40°24'S., 144°32'E.). The colony of little penguins at Marion Bay is amongst sand dunes whereas that at Albatross Island is on rocky slopes and in caves. Birds were marked with flipper bands when they were first sighted ashore at the beginning of moult, within 1 day of their arrival. The sex of the birds was determined from beak measurements (Gales, 1988*a*; also Chapter 2). At Marion Bay, approximately 2 ml of blood was extracted from the brachial vein of moulting penguins for analyses of total triglyceride and total cholesterol levels. These blood samples were separated by centrifugation into red cell and serum fractions and stored frozen. Lipid assays were run on an Abbott Biochromatic Analyzer (ABA-100). For this aspect of the study, the birds from which blood samples were collected were weighed, and categorised into one of four moult stages, as follows:

Pre-moult :	initiation of moult, old feathers 'plumped' out.
Moult 1 :	beginning of loss of old feathers, new feathers just visible
Moult 2 :	half of new feathers replaced, old feathers falling in sheaves
End moult :	new feather coat complete, birds about to return to sea.

Blood samples were also taken in the previous breeding season (December 1984) and levels of triglycerides and cholesterol were analysed. Water turnover rates and field metabolic rates (CO_2 production) were measured by means of tritiated water (HTO) and doubly labelled water (DLW; $HT^{18}O$) (Lifson & McClintock, 1960; Nagy, 1980; Nagy & Costa, 1980; Degen *et al.*, 1981).

Birds were caught at their moult roost sites and weighed (± 10 g). Fourteen birds were then given an intraperitoneal injection of 1ml of HTO (185 MBq) only, and twelve others were given 1 ml of HTO (185 MBq) and 0.3 ml of 95 % atoms excess ^{18}O. Birds were then returned to their roost sites. After 5 - 6 h they were recaptured and a blood sample (≈ 2 ml) was taken from the brachial vein. They were again returned to their roost site and were checked daily to determine any movement away

from the original roost. After 3 - 14 days the birds were recaptured, weighed, and a blood sample was taken. They were then re-injected with 0.5 ml HTO (185 MBq) and a blood sample obtained 5 - 6 h later. Six birds, which were injected with HTO only at the completion of moult, returned to sea for 1 - 9 days. Blood samples were taken from these birds on their return from the sea in order to measure water fluxes of birds subsequent to termination of moult.

Blood samples were separated by centrifugation and frozen until later analyses. Water was vacuum distilled from the red-cell fraction and the HTO levels determined by liquid scintillation spectrometry using PCS (Phase Combining System, Amersham) cocktail and a Beckman liquid scintillation counter (Model LS2800). Subsamples of extracted water samples were also prepared for mass spectrometry by means of Urey exchange with standard carbon dioxide charges at 80°C overnight. The ^{18}O levels of the resulting equilibrated carbon dioxide samples were measured in an isotope ratio mass spectrometer (V.G. Isogas, Model 903).

Rates of water flux and CO_2 production were calculated from the changes in isotope levels in the blood during the experimental periods (Lifson & McClintock, 1966; Nagy, 1980); it was assumed that mass specific body water pools remained constant and that any changes in body mass were linear. Field metabolic rates (FMR, in kJ kg^{-1} day^{-1}) were converted from units of CO_2 production (ml $g^{-1}h^{-1}$) using the constant of 28.0 kJ per litre carbon dioxide (Kleiber, 1961), as fasting penguins primarily catabolise body fat reserves (Groscolas & Clement, 1976; Williams *et al.*, 1977).

Fresh, undigested samples of stomach contents were obtained by stomach flushing (Gales, 1987*b*; also Chapter 3) from seven little penguins arriving from sea at Albatross Island at the initiation of moult. These food samples were oven dried to constant mass at 55°C. Dried samples were then compressed into pellets of 0.5 g and combusted in a Gallenkamp ballistic bomb calorimeter to determine energy content. The food consumption required to sustain penguins through the moult process were calculated from values of energy content of the food samples brought ashore by penguins prior to moult, and the mean energy assimilation efficiency for the dietary components.

These seven food samples obtained from the penguins comprised mixed species of fish and squid. The mean water content was 74.5 ± 2.4 % and the dry matter energy content 22.1 ± 1.1 kJ g^{-1}. The mean energy assimilation efficiency of little penguins feeding on fish and squid diets is 72 % (Gales, 1989; also Chapter 5).

The food samples therefore contained a metabolizable energy content of 4.06 kJ g^{-1} fresh mass.

Data are presented as mean values ± SD. Student's *t* - tests (two-tailed) were used to test for significant differences between means, and the 5 % level of probability was accepted as denoting statistical significance.

7.3 RESULTS

The pattern of mass loss during moult in little penguins exhibited a significant linear relationship ($r = -0.94$; $n = 46$; $P < 0.001$) (Fig. 7.1). For those birds in which the entire period of moult was monitored, the moult process lasted 16 - 18 days (mean = 16.7 ± 0.79; $n = 8$). The mean rate of mass loss was 55 g day^{-1} and total mass loss during moult represented a total of 46 % of the initial mass.

The levels of triglycerides and cholesterols at the beginning of moult did not differ significantly from the levels obtained from breeding birds (triglycerides: $t = 1.24$; $n = 29$; $P > 0.05$; cholesterols: $t = 0.187$; $n = 29$; $P > 0.05$). During the process of moult, however, triglyceride and cholesterol levels showed increases of 2.5 times and 1.8 times, respectively, from pre-moult to end moult (Fig. 7.2). In moulting birds total body water (TBW) ranged between 54 and 70 % (mean = 63 ± 4.3 %; $n = 46$) body mass. TBW expressed as a per cent of body mass increased during moult ($r = 0.62$; $n = 46$; $P < 0.001$) and showed the relationship: $y = 0.5x + 58.8$, where y is TBW (%) and x is days after the beginning of moult.

Moulting birds showed a mean water influx rate of 16.9 ± 3.6 ml $kg^{-1}day^{-1}$ compared with a mean water efflux rate of 40.6 + 5.9 ml kg^{-1} day^{-1}. The rates of mass change and the water influx rates (Table 7.1) were significantly correlated ($r = -0.72$; $n = 21$; $P < 0.001$) and showed the relationship: $y = -4.0x + 0.32$, where y is water influx (ml kg^{-1} day^{-1}) and x is mass change (% day^{-1}). The mean FMRs of little penguins moulting at Marion Bay and at Albatross Island were not significantly different ($t = 0.506$; $n = 12$; $P > 0.05$). The difference between the FMRs of female and male little penguins was also not significant ($t = 0.216$; $n = 12$; $P > 0.05$). The mean energy cost for a penguin during moult was 657 ± 105 kJ kg^{-1} day^{-1} ($n = 12$) (Table 7.1). Changes in activity of injected isotopes over time were not sufficient to allow subdivision of energy costs within the moult process.

The difference between the water influx rates of penguins that restricted movement to a minimum during moult (i.e. birds which were repeatedly located in the same roost sites) and those birds which were located in different roost sites, and were

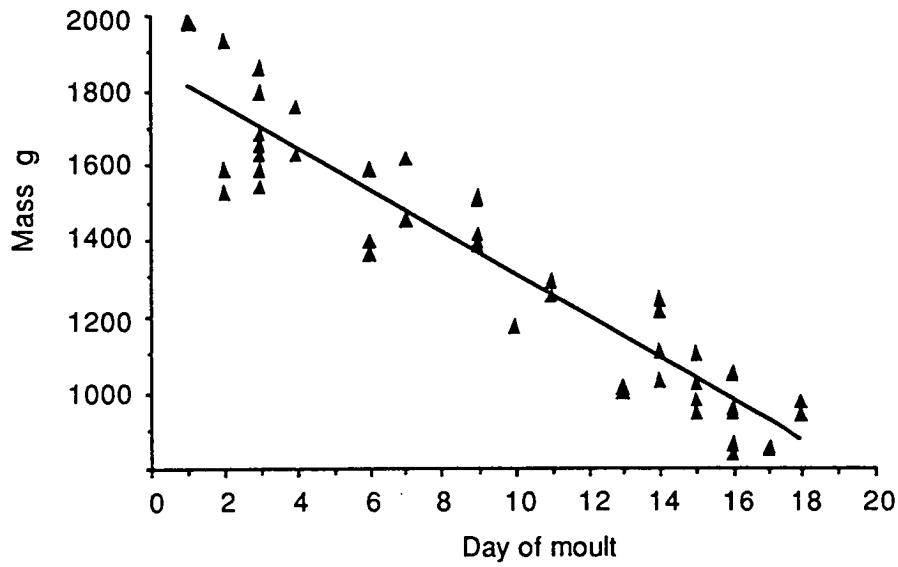

FIGURE 7.1 Decrease in mass of little penguins during moult

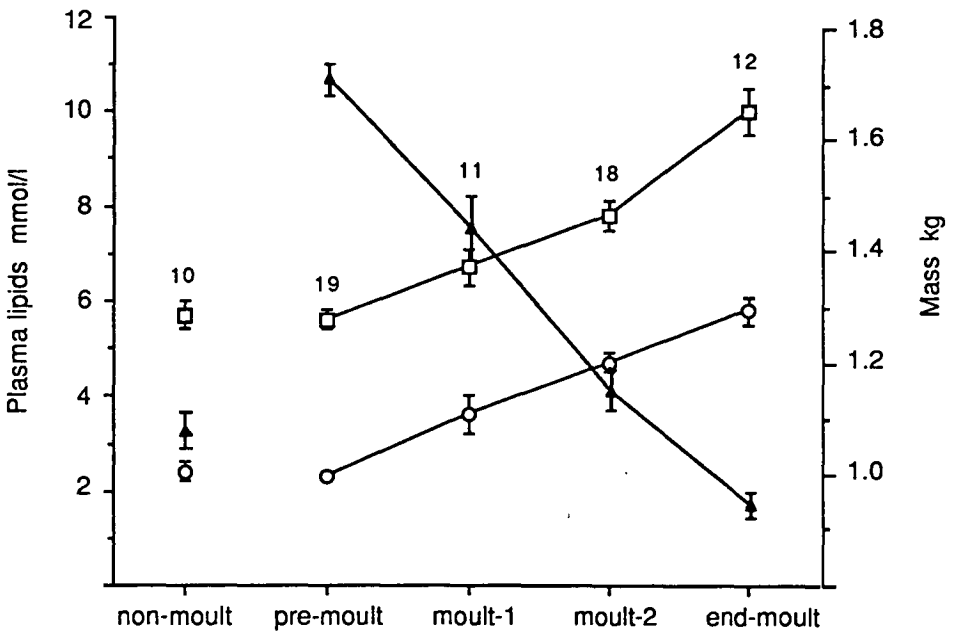

FIGURE 7.2 Changes in levels of plasma lipids and body mass during moult (mean ± SE; sample sizes in superscript are identical for all three categories). Triangles denote mass, circles triglycerides and squares cholesterol.

TABLE 7.1 Mass changes, and water and energy turnover in adult little penguins during moult

		Mean	S.D.	n
Mass (g)	initial	1580	213	21
	final	1109	194	21
Mass change (% d^{-1})		-4.2	0.7	21
Water influx (ml $kg^{-1}day^{-1}$)		16.9	3.6	21
Water efflux (ml $kg^{-1}day^{-1}$)		40.6	5.9	21
CO_2 production (ml $g^{-1}h^{-1}$)		0.99	0.16	12
Field metabolic rate (kJ $kg^{-1}day^{-1}$)		657	105	12

often seen traversing between these sites, was not significant ($t = 0.311$; $n = 21$; $P > 0.05$).

The six birds which went to sea after completion of moult foraged for an average of 3 ± 3 days before returning to land and were in relatively stable energy balance (mass change = -0.26 ± 1.9%). The water influx and efflux rates of these birds were 183 ± 70 ml kg^{-1} day^{-1} and 183 ± 64 ml kg^{-1} day^{-1}, respectively. These levels are considerably higher, 11 times and 4.5 times, than the influx and efflux rates of moulting birds (Table 7.1).

7.4 DISCUSSION

The little penguin, like most other species of penguins, moults after breeding, in late summer. The timing appears critical because the initiation of the moulting season, which ranges over 2 weeks, is less variable than is the onset of breeding, which ranges over about 10 weeks (Reilly & Cullen, 1981). The pattern and rate of mass loss reported in this study is similar to those recorded elsewhere for the species (Richdale, 1940; Kinsky, 1960; Hodgson, 1975) with approximately a 46 % decrease from the initial mass during the 15 - 18 day moult period.

Changes in circulating lipids are often examined in order to characterise the physiological basis of phases of the annual cycle in birds (Berry *et al.*, 1979; de Graw *et al.*, 1979; Groscolas, 1982). Triglycerides are the most important form in which dietary fat is transported in the Cape cormorant, *Phalacrocorax capensis* (Berry *et al.*, 1979), a species which has a diet of predominantly small pelagic fish, broadly similar to the diet of the little penguin (Chapter 10). In the emperor penguin, Groscolas (1982) reported that plasma lipids were depressed during the period of feather formation, as lipid levels were lower than those of non-moulting, breeding birds. In the little penguin, the levels of tryglycerides and cholesterols in birds which were breeding did not differ from the levels in birds which were initiating moult.

During moult of the emperor penguin all plasma lipids showed marked and identical increases in levels and reached maxima at the end of the moult fast (Groscolas, 1982). The magnitude of these increases was 1.85 times, a similar elevation to the 1.7 times increase recorded for plasma lipids during moult in eudyptid penguins (Tollu, 1978; cited in Groscolas, 1982). In the little penguin the magnitude of the elevation of cholesterol levels during moult was identical (1.8 times) but that of triglycerides was even higher (2.5 times). Groscolas (1982) concluded that the changes in plasma lipids in breeding and moulting emperor penguins are related to endocrine influences, rather than to starvation, provided fat stores are not depleted. In the Cape cormorant, Berry *et al.*, (1979) showed positive correlations between the

levels of plasma triglycerides and the total mass of fish ingested. During the moult fast in the little penguin, comparison with the linear decrease in body mass implies that there is an inverse relationship of plasma lipid levels and fat mobilization, at least during this phase of the annual cycle (Fig. 7.2).

The mean TBW of moulting little penguins was 63 ± 4.3 % with a range of 54 - 70%. These are similar to the TBWs of gentoo and macaroni penguins reported by Davis *et al.*, (1983) and for moulting eudyptid penguins (Williams *et al.*, 1977). The mean water influx rate of little penguins whilst moulting was 16.9 ml kg^{-1} day^{-1} which is only slightly lower than the 18.1 ml kg^{-1} day^{-1} reported by Costa *et al.* (1986) for two fasting little penguins. Both these values are within the range of 13 - 25 ml kg^{-1} day^{-1} reported by Green *et al.* (1988) for 3 incubating and one moulting little penguin. It is assumed that this water is entirely metabolic water because the influx rates are low and show relatively little variation (Table 7.1). Although eudyptid penguins drink readily during the moult fast (Williams *et al.*, 1977), little penguins, unlike some other penguin species, have never been observed to drink during moult (personal observation).

Many of the data concerning metabolic rates of moulting birds are derived indirectly from mass loss data and a fundamental difficulty in this technique is the lack of information on the exact composition of material lost (Croxall, 1982). This problem, however, is alleviated when determining precursor-product relationships of FMRs from the isotope turnover technique (Kleiber, 1961; Davis *et al.*, 1983).

The composition of mass lost during moult has been determined for at least two species of penguins and ranges from 40.2 % fat and 7.2 % non-feather protein for rockhopper penguins to 36.4 % fat and 4.9 % non-feather protein for macaroni penguins (Williams *et al.*, 1977). Using the energy equivalent of 28.0 kJ l^{-1} CO_2 in the conversion of CO_2 production to FMR assumes that all metabolic water is derived from oxidation of fat. From this assumption it can be calculated that a mean of 17.9 ml H_2O kg^{-1} day^{-1} would be produced, only slightly higher than the 16.9 ml H_2O kg^{-1} day^{-1} measured by HTO turnover. It is likely this difference is due to the oxidation of a small proportion of protein which liberates less water per millilitre of CO_2 than does the oxidation of fat (Schmidt-Nielson, 1979).

It is common practice to describe costs of various activities or energy budgets as multiples of the basal metabolic rate, BMR (King, 1974; Ellis, 1984). The BMR (postabsorbtive resting metabolic rate at thermoneutrality) of little penguins in the laboratory has been reported as 426 kJ kg^{-1} day^{-1} (Stahel & Nicol, 1982). Slightly lower values were subsequently presented by Stahel *et al.* (1984), and these values

were not substantially different from the predicted BMR of 389 kJ kg^{-1} day^{-1} for a bird of similar size from the equation of Aschoff & Pohl (1970). Baudinette *et al.* (1986) have reported a substantially lower BMR of 270 kJ kg^{-1} day^{-1} for little penguins. For consistency, the FMRs in the present study have been expressed as multiples of the BMR reported by Stahel & Nicol (1982) and as used by Brown (1984), Ellis (1984) and Costa *et al.* (1986).

The average FMR of moulting little penguins in the present study was 657 kJ kg^{-1} day^{-1}, or 1.5 times BMR. Baudinette *et al.* (1986), using metabolic chambers, reported that little penguins during moult showed an increase in oxygen consumption of approximately 40 % . These moult-induced increases in energy turnover appear similar to those reported for other penguins. Brown (1985), using metabolic chambers, has reported elevations of 1.36 and 1.32 times resting metabolic rate for macaroni and rockhopper penguins respectively (levels which can only be compared indirectly), but were slightly lower than those measured by Williams *et al.* (1977) for the same species (1.6 and 2.1 times BMR respectively). Croxall (1982) summarised information on ratios of metabolic costs of moult (calculated from mass loss) to predicted metabolic rates for 13 penguin species. He concluded that energy costs during moult are approximately 2.0 times BMR, and increase of 0.7 times BMR above the value for fasting, non-moulting penguins (1.3 times BMR). Brown (1985) states that estimates of energy expenditure based on mass loss were approximately 30 % greater than $V(O_2)$ measurements in macaroni and rockhopper penguins.

To date, all these measurements of metabolic rates of penguins are derived indirectly either from mass loss data or from determination of $V(O_2)$ in metabolic chambers. These methods are useful in that they can demonstrate patterns of energy utilization during moult, rather than an integrated value. Groscolas (1978) has shown that moult in the emperor penguin is divisible into a number of phases, and these are associated with different biochemical events and rates of mass loss. The restrictive conditions of metabolic cages, however, may affect results, although Croxall (1982) presumes that moulting penguins tend to minimise movement. Indeed, Groscolas (1982) states that moulting emperor penguins are lethargic and that confinement has no effect on their behaviour. Brown (1985) concurs that moulting eudyptid penguins are invariably sedentary and concludes that it is only by minimising activity and movement that penguins can survive the rigours of moult.

This is not the case with little penguins. While some of the little penguins in this study were relatively sedentary during the sampling period, others were seen to range between sites, some changing moult roosts every night. From observations of other birds, this was not a result of the disturbance involved in the isotope study, but is

rather a characteristic of some individuals. Recent observations also show that Adelie penguins, *Pygoscelis adeliae*, and king penguins also move considerable distances during the moult fast (personal observation). Sample sizes were not sufficient to assess the effect of movement on the FMR of little penguins. Therefore, the water influx rates of sedentary and mobile individuals were compared, following the assumption that all water influx is metabolic water. The lack of significant differences between water influx rates suggest that, while the cost of moult is high, at least a certain amount of movement during this time does not add a significant cost to the energetics of moult.

Given the range in BMRs presented for the species, and the artificial conditions inherent in laboratory measurements, it is logical to assess the cost of moult in relation to other studies of free-living, non-moulting, little penguins. The costs of incubation have been measured in three little penguins by Green *et al.* (1988) who report an FMR of 570 kJ kg^{-1} day^{-1}, similar to the 560 kJ kg^{-1} day^{-1} reported by Costa *et al.* (1986) for two fasting little penguins. The cost of moult in the present study is approximately 1.2 times higher than these values. An FMR of 1170 kJ kg^{-1} day^{-1} was measured by Green *et al.* (1988) for four penguins undertaking incubation shifts interspersed with foraging bouts. Thus, energy expended during moult is approximately half that expended by these breeding birds. Further, in terms of water influx, for two penguins undertaking extended foraging trips, Green *et al.* (1988) reported an average of 567 ml kg^{-1} day^{-1} which is 34 times that of the water influx of moulting penguins. Given this information it is clear that the cost of moulting, whilst elevated above resting, fasting levels, is considerably less than the energy expended during foraging trips. Probably the most energetically expensive stage is incurred during the pre-moult foraging period when penguins almost double their mass to ensure sufficient reserves for the period when restricted to land. Penguins arriving ashore for the moult period are obese, but depart very lean and almost emaciated. Given that moult lasts on average 16.7 days and little penguins expend about 657 kJ kg^{-1} day^{-1} during moult, the total cost is 10 972 kJ kg^{-1}. Incorporating the water content and metabolizable energy of food samples the penguins would need to take in approximately 2 700 g fresh food kg^{-1} extra during the pre-moult foraging period to supply adequate energy reserves for the moult.

If the penguins were simply resting on land for the same period of time, using the expenditure of 560 kJ kg^{-1} day^{-1} presented by Costa *et al.* (1986) for two fasting penguins, these penguins would require approximately 2 300 g fresh food kg^{-1}. This figure is 15 % less than that required by moulting penguins. It should be noted that little penguins spend extended times ashore only during the moult.

When little penguins have completed moult, their energy reserves are depleted and the initial post-moult foraging trips are generally short. In the present study, the six birds monitored with HTO during these trips stayed at sea an average of 3 days, retained a stable mass and their water influx (183 ml kg^{-1} day^{-1}) mirrored water efflux (183 ml kg^{-1} day^{-1}). These influx rates are 20 % higher than that measured by Costa *et al.* (1986) on two foraging birds, both of which were in negative energy balance. The post-moult influx rates are similar to the influx rates of birds foraging between incubation shifts, but lower than birds which are foraging and feeding young (Green *et al.*, 1988). It appears then that little penguins, whilst recovering from moult, forage conservatively in energetic terms, compared with the pre-moult or chick rearing foraging demands, at least until energy stores and normal mass are attained.

7.5 SUMMARY

Levels of circulating triglycerides and cholesterol in moulting little penguins in Tasmania were measured before, and throughout the moult. Levels at the initiation of moult were similar to those in breeding birds but increased by 2.5 times (triglycerides) and 1.8 times (cholesterol) during the moult. Water flux rates and field metabolic rate (FMR) were measured throughout moult using tritiated and doubly labelled water. TBW ranged from 54 to 70 % body mass and increased during moult. Water influx rates were significantly correlated with rate of mass change. Mean FMR of moulting little penguins was 657 kJ kg^{-1} day^{-1}, or 1.5 times basal metabolic rate (BMR), and there was no difference between sites or sexes. The water influx rates of birds foraging immediately after moult were 11 times higher than in moulting birds. The energy required to sustain a moulting little penguin is 15 % higher than that required for a resting, non-moulting penguin. Although the cost of moult is elevated above BMR the main energetic expense is met during the pre-moult foraging period when birds must consume enough food to ensure that they lay down sufficient fat reserves to sustain the moult.

CHAPTER 8

THE ANNUAL ENERGETICS CYCLE OF ADULT LITTLE PENGUINS

8.1 INTRODUCTION

Penguins are receiving increasing attention in terms of their role as important consumers of marine resources, and studies of their energy and food requirements in their natural environments include Antarctic, sub-Antarctic and temperate penguin species. Published accounts of the species examined also encompass almost the entire size range of penguins, from the king penguin, *Aptenodytes patagonicus*, weighing approximately 13 kg (Kooyman *et al.*, 1982), to the smallest penguin species, the little penguin, *Eudyptula minor*, weighing approximately 1 kg (Costa *et al.*, 1986; Gales *et al.*, 1988; Green *et al.*, 1988). The allometric relationships of water and energy flux of free-living seabirds have been assessed by Nagy (1987) and Green & Brothers (1989), and of penguins in particular by Green & Gales (in press).

Significant features of the published accounts of free-living penguin energetics, are that the sample sizes are generally small, and that the studies have been restricted to the summer period, when breeding and moulting occur. Due to the pelagic nature of many southern penguin species in the non-breeding season, it is not feasible to quantitatively assess the entire energy budget, including both breeding and non-breeding periods. As a result, the role of penguins in the Southern Ocean has been examined from models of seabird energetics (Furness, 1978; Croxall & Prince, 1982*b*). However, ideally, analyses of ecological energetics should consider the entire annual cycle, the major iterative component of avian life histories (Walsberg, 1983). The present study of the little penguin, is the first for any free-living seabird for which quantitative measurements have been made of the energy budget over the complete annual cycle.

The little penguin is restricted to New Zealand and southern Australia, and the adults of this species do not desert the breeding colonies during the non-breeding, winter period. This feature, together with the frequency and predictability with which they attend the colonies during the breeding season, allowed a study of the water and energy flux rates over the annual cycle. These data are necessary to quantitatively determine food and energy consumption rates of individuals, with extrapolation to population requirements. This was considered important as little penguins are regarded as significant predators in the marine communities of which they are a part, and in some areas there is concern regarding competition between little penguins and commercial fisheries. This study was undertaken in Bass Strait which is the stronghold of the species in Australia with respect to population numbers.

The water, sodium and energy fluxes of free-living, adult little penguins were measured by means of isotope turnover techniques (Lifson & McClintock, 1966; Green, 1978; Nagy, 1980; Nagy & Costa, 1980; Nagy, 1983). The combined use of three isotopes, tritium (H_3O), sodium-22 (^{22}Na) and oxygen-18 ($H_2{}^{18}O$), allows the assessment of field metabolic rates, and the partitioning, via water and sodium flux rates, of food and seawater consumption rates (Green *et al.*, 1988; Green & Brothers, 1989).

In order to estimate food consumption rates via isotope turnover, it is important that the contributions of the different prey taxa to the diet are also known. Consequently, a diet study was conducted simultaneously with the energetics study at the same location and during each sampling period. (Chapter 10). The major objectives of this study were to construct a complete energy budget for adult little penguins and to compare the roles of the two sexes in terms of energy use. Also, in conjunction with the dietary information and estimates of the population size, an attempt was made to predict the annual energy and food requirements for the population of little penguins in Bass Strait.

8.2 METHODS

8.2.1 STUDY SPECIES AND SITE

Little penguins are the only species of penguin breeding in Tasmania and are found in many locations around the coast and on offshore islands. The adults are monomorphic, with the exception that the males are usually slightly heavier, and have stouter bills (Gales, 1988*a*; also Chapter 2). The timing of their breeding seasons is variable (Reilly & Cullen, 1981) but in Tasmania they usually breed during summer, and moult after breeding (Fig. 8.1). Nests are always under cover; either in burrows, between boulders, under vegetation, or in caves. Two eggs are laid, which are incubated for about 36 days. The chicks are continuously brooded until they are about 3 weeks old and fledge at approximately 8 weeks of age. Both parents share equally in the incubation and care of chicks, and nest relief periods are short, usually every one to two days. During the breeding season, most foraging adults leave the island in the hour preceding dawn and return in the first hour after sunset i.e., they forage during the daylight hours and are restricted to inshore areas.

After the chicks fledge, the adults forage for a period of several weeks in order to attain the fat stores required to sustain them during the moult period which usually occurs between February and March, and lasts about two and a half weeks. Following moult, adult little penguins return to their colonies periodically during the winter, non-breeding season, the frequency of visits increasing as the breeding season approaches.

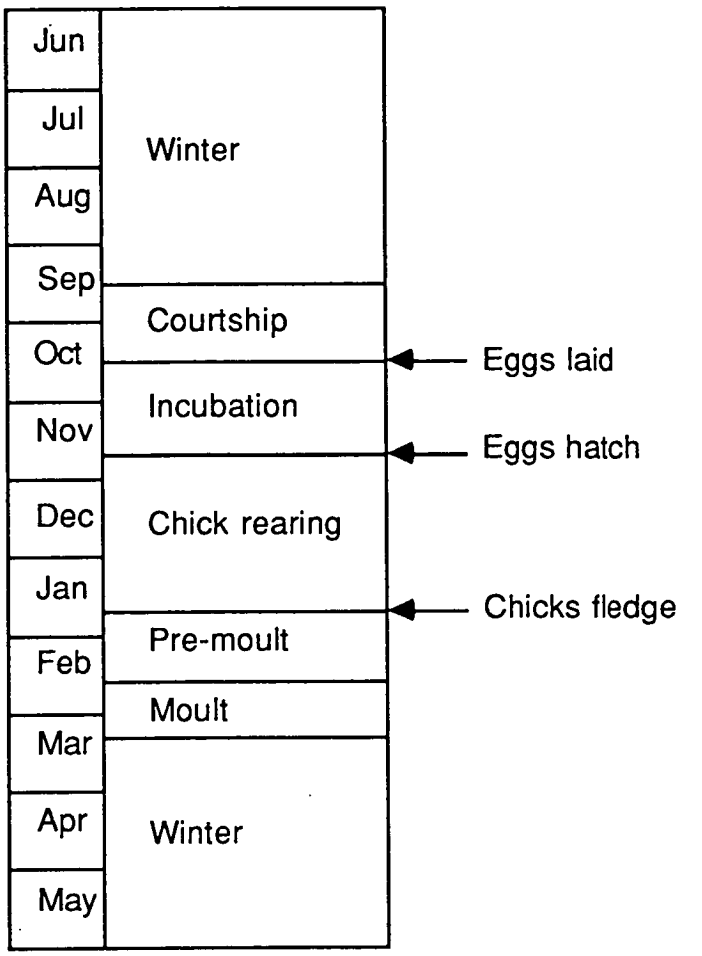

FIGURE 8.1 Typical pattern of the annual cycle of little penguins in Tasmania

This study was carried out in Tasmania on Albatross Island, north-west Bass Strait (40° 24' S., 144° 32' E). Approximately 500 pairs of little penguins breed on the Island, many of them nesting in caves. The field work was carried out during a series of visits, each of approximately two weeks, covering a period of 17 months, and the timing of these visits encompassed all the major facets of the annual cycle (Table 8.1). The penguins on Albatross Island are highly specific in their departure and arrival sites and this enabled relatively easy recapture of the penguins as well as partitioning of their time at sea and ashore.

The penguins sampled in this study nested on the surface within caves, rather than in confined burrows, and this situation reduced the possibility of inhalation of unlabelled CO_2, which would increase isotopically measured CO_2 production rates (Nagy, 1980). Also, checking occupants of surface nests is less disruptive than checking burrow occupants. When feasible, both members of a breeding pair were studied at the same time. When the hatching date was not known, the ages of chicks being fed by adults were estimated from the chick growth data in Hodgson (1975) and Gales (1987*a*).

8.2.2 WATER, SODIUM AND ENERGY FLUX RATES

Water and sodium turnover rates and field metabolic rates were measured via isotope turnover techniques. The use of tritium (HTO; ^3H_2O) provides a measure of water flux rates, and when used together with $H_2{}^{18}O$ allows determination of metabolic rates (CO_2 production rates). The added use of ^{22}Na provides a simultaneous measure of sodium flux rates, and when used in conjunction with water influx rates, allows the partitioning of food and seawater derived water and sodium intake. The underlying principles, assumptions, errors and methodology of isotope turnover techniques have been discussed by Lifson & M^{c}Clintock (1966), Green (1978), Nagy (1980, 1983), and Nagy & Costa (1980). Isotope turnover studies of penguins have been recently reviewed by Green & Gales (in press) and the use of the three isotopes has been validated in little penguins by Gales (1989, also Chapter 5).

A similar sampling protocol was used during each of the trips, only the number of penguins being sampled and the dose and combinations of the isotopes used, varying between trips. Penguins were caught by hand and banded with individually numbered stainless steel flipper bands, before being weighed in a bag to the nearest 10 g using a spring balance. At the beginning of each period a blood sample of ≈ 1 ml was taken from a brachial vein of some penguins to determine background levels of $H_2{}^{18}O$. Birds were then given intra-peritoneal injections of ^3H_2O (185 MBq), $H_2{}^{18}O$ (95 %+ atoms excess) and $^{22}NaCl$ (185 kBq) in various

TABLE 8.1 Number of penguins injected with one or a combination of isotopes during the study period (number of penguins injected with tritium indicates total number of penguins injected).

Date	Activity	Tritium	Sodium-22	Oxygen-18
Sep-84	Courtship	5	4	0
Dec-84	Chick rearing	16	0	0
Mar-85	Moult/post moult *	13	3	6
Jul-85	Over-winter	10	6	6
Sep-85	Courtship	14	12	6
Nov-85	Incubation + chick rearing	16	13	13
Jan-86	Chick rearing	12	12	12

* Results of energetics of moult presented in Gales et al. (1988; also Chapter 7).

combinations, and in no case did the total injected volume exceed 2 ml. The numbers of injections given to penguins, and the combinations of isotopes used during each period, are given in Table 8.1.

Following injections, penguins were returned to their nest sites for a period of 5 to 6 hours during which time the isotopes equilibrated with the body pools (Gales, 1989; also Chapter 5). After this time a blood sample of 1 - 2 ml was collected from a brachial vein into a non-heparinised vial in order to assay for determination of body pool sizes. Following collection of these equilibration blood samples, the penguins were again weighed, a coloured spot painted on their chests, and then were returned to their nests. The activity of the penguins was then monitored for the remainder of the sampling period (up to 14 days) by thorough searches of the colony during the day and night, and by observing the arrival and departure of penguins at their landing sites around dawn and dusk. The percentage of time at sea or on land was recorded for each penguin during each sampling period. During this period, the penguins were caught and blood samples taken in order to determine the flux rates, via the rates of decline of isotope levels in the body pools. Most penguins were sampled more than once during each period, and so the number of isotope turnovers exceeds the number of penguins injected.

Blood samples were allowed to clot, after which they were centrifuged in the field, the serum drawn off and stored in a separate vial. Both red cell and serum fractions were then frozen for later analyses. In the laboratory, samples of pure water were extracted from the red cell fraction by lyophilization (Vaughn & Boling, 1961). Aliquots of 10 µl extracted water were added to 3 ml of PCS cocktail and the HTO activity levels were measured in a liquid scintillation counter (Beckman LS 2800). Larger aliquots of extracted water (50 µl) were assayed for $H_2{}^{18}O$ levels by isotope ratio mass spectrometry (VG Isogas 903) after Urey exchange and equilibration with a standard charge of CO_2 overnight. The ^{18}O background samples taken from animals prior to injection were retained, along with a sample of the standard diluent, for mass spectrometry. The serum samples were bleached with concentrated hydrogen peroxide and then oven dried. They were then mixed with 3 ml of PCS cocktail and assayed for ^{22}Na activity determined by liquid scintillation spectrometry. Sodium concentrations of sera were determined by atomic absorption spectrophotometry (Varian Model 1000) after diluting sera with deionised water (1: 400).

Total body water (TBW) and exchangeable sodium (ES) pools were determined by comparing the specific activities of diluted standards with blood samples taken after isotope equilibration. Isotope flux rates were determined from the decline of isotope levels in the serial blood samples, in conjunction with the pool sizes

(Lifson & McClintock, 1966; Nagy, 1980; Nagy & Costa, 1980). It was assumed that any changes in mass, when less than 10 % were accompanied by similar relative changes in pool size. When changes in body mass greater than 10 % were recorded over the experimental period, pool sizes were re-estimated at the completion of the turnover period by re-injection and re-equilibration of isotopes. It was also assumed that changes in body mass and pool sizes were linear during the experimental periods.

To convert CO_2 production rates into units of energy expenditure (kJ) it is necessary to know which substrates are being catabolised. For fasting birds the predominant metabolite is fat (Groscolas & Clement, 1976; Williams *et al.*, 1977) and so a thermal equivalent of 28.0 kJ per litre CO_2 (Kleiber, 1961) was assumed. As protein rather than fat is the major metabolic substrate in active animals, the thermal equivalents that apply to foraging penguins depend primarily on the composition of the diet. The diet was determined for between 20 and 30 adult penguins on each trip by means of the multiple stomach flushing technique (Wilson, 1984; Ryan & Jackson, 1986; Gales, 1987*b*; also Chapter 3). The relative contribution of the prey to the diet in terms of mass of stomach contents, and the water, energy and sodium status of the different prey types were determined in the laboratory (Chapters 6 and 10), and summarized results of these analyses are shown in Tables 8.2 and 8.3.

Metabolic rates of feeding penguins were converted from units of CO_2 production using the appropriate combinations of the thermal equivalents for fish (25.4 J ml^{-1} CO_2), squid (24.9 J ml^{-1} CO_2) and krill (26.0 J ml^{-1} CO_2). These were calculated from the chemical composition of a clupeid fish (Wilson *et al.*, 1984), an ommastrephid squid (Croxall & Prince, 1982*a*) and a euphausiid species of krill (Clarke, 1980; Davis *et al.*, 1989).

8.2.3 FOOD AND SEAWATER CONSUMPTION RATES

Estimates of food consumption rates by adult little penguins were determined from water and sodium flux rates, in conjunction with data of the water and sodium status of the diet during each period of study. Recently, it has been shown that penguins ingest variable amounts of seawater, and so estimates of food consumption rates based on water influx alone result in over-estimates (Green *et al.*, 1988; Robertson *et al.*, 1988; Green & Gales, in press). If the available water and sodium contents of the diet are known, and if it is assumed that seawater has a sodium concentration of 470 mmol l^{-1}, the volume of ingested seawater can be calculated. Hence, water influx rates can be partitioned between water derived from food and that derived from seawater ingestion, and estimates of food ingestion can then be refined. Examples of the iterative process by which this is achieved are shown in Green *et al.* (1988), Green & Brothers (1989) and Appendix 1. In these calculations it is necessary

TABLE 8.2 Water, sodium and energy status of the different prey taxa used in the energetics calculations, n refers to number of species examined.

		Water	Sodium	Energy		
		%	mmol kg fresh	total kJ/g dry	total kJ/g fresh	metabolizable kJ/g fresh
Fish	mean	74.3	65.7	21.89	5.64	3.98
	SD	2.4	20.3	1.92	0.84	
	n	15	7	15	15	
Squid	mean	78.8	110.4	24.31	5.16	3.77
	SD	2.2	6.7	0.63	0.40	
	n	2	2	2	2	
Krill	mean	84.7	111.3	19.9	3.03	2.24
	SD	-	-	-	-	
	n	1	1	1	1	

TABLE 8.3 The seasonal composition of little penguin diet at Albatross Island *

Date	Diet % mass			Water % **	Sodium mmol/kg	Metabolizable energy	
	Fish	Cephalopod	Crustacean			kJ/g fresh	kJ/l CO_2
Sep-84	77.1	18.1	4.8	90.0	76.0	3.86	25.3
Dec-84	69.6	23.5	6.9	90.1	79.3	3.81	25.3
Mar-85	25.1	74.5	0.4	89.9	99.2	3.82	25.0
Jul-85	73.8	24.6	1.6	89.8	77.4	3.97	25.3
Sep-85	96.7	3.1	0.2	89.7	67.2	3.97	25.4
Nov-85	79.8	10.6	9.6	90.2	74.8	3.79	25.4
Jan-86	61.8	37.8	0.4	89.8	82.8	3.89	25.2
Mean	69.1	27.5	3.4	89.9	79.5	3.87	25.3
SD	22.2	23.5	3.7	0.2	9.9	0.07	0.1

* Summarized from information presented in Chapter 10
** Free + metabolic water

to know the water available to the penguins from food, both the free and the metabolic water. The rates of metabolic water production in little penguins on a fish diet is 0.154 ml g^{-1} fresh and on a squid diet is 0.110 ml g^{-1} fresh (Gales, 1989; also Chapter 5). The values for the krill component of the diet was assumed to be 0.10 ml g^{-1} fresh, based on the composition of *Euphausia superba* (male/female mean) (Clarke, 1980) and the experiments of Davis *et al.* (1989) with gentoo penguins (*Pygoscelis papua*). The water (free + metabolic) and sodium status of the diet during each period of study are shown in Table 8.3.

8.2.4 ENERGY BUDGET AND POPULATION REQUIREMENTS

Energy budgets were calculated from time/activity profiles and measured rates of energy expenditure over the annual cycle. Males and females were compared to see if any sexual differences in energy commitment were evident. For breeding adults, the annual cycle was divided into the functional units of the breeding season: courtship, incubation, chick rearing; as well as pre-moult, moult and the non-breeding period. The time commitment of each of these activities were drawn from personal observations as well as from a number of different sources summarized in Stahel & Gales (1987). The life history parameters of age at breeding, life expectancy and proportion of non-breeding individuals are from Reilly & Cullen (1982). The population estimates of little penguins in Bass Strait are from Harris & Norman (1981) for the Victorian sector, and from unpublished data (N. Brothers, personal communication) for the Tasmanian sector.

In determining the food consumption rates necessary to satisfy the annual population energy requirements mean values of the diet energy content were used (Table 8.2). Energy assimilation efficiencies used in these calculations were 70.5 % for the fish component of the diet, 73 % for the cephalopod component (Gales, 1989; also Chapter 5), and 74 % for krill (Davis *et al.*, 1989)).

8.2.5 STATISTICS

All values are given as means ± SD. Differences between means were tested by two-tailed t-tests or ANOVA. Following a significant result from ANOVA, comparisons between means were tested using Fisher's protected least significant difference test (PLSD) as discussed by Chew (1977). In all tests the 5 % level of probability was accepted as indicating statistical significance. Regression lines were calculated using the least squares linear regression method.

8.3 RESULTS

8.3.1 BODY MASS, AND WATER AND SODIUM POOL SIZES

There were no significant differences (t-tests, $P > 0.05$) between males and females in terms of mass, total body water (TBW) or exchangeable sodium (ES) pool sizes measured at the same times of year and so these data were pooled to provide mean seasonal data for both sexes (Fig. 8.2A). The mean body mass of penguins during the study ranged between 1.0 and 1.4 kg, and the seasonal difference in mass was significant ($F_{6, 79} = 8.60$, $P < 0.001$). This significant variation was due to the wide range of masses recorded in March 1985 from moulting birds (mean mass: 1358 ± 357 g, n = 13). When these data are excluded from analyses, there are no significant differences in mass between months ($F_{5, 67} = 1.73$, ns), and the mean mass for penguins outside the moulting season was 1063 ± 99 g (n = 73). Mass specific TBW showed a significant variation over the study ($F_{6, 79} = 21.07$, $P < 0.001$) and generally was inverse to body mass, lighter birds having higher mass specific TBW (Fig. 8.2B). Similarly, lighter birds had higher exchangeable sodium pool sizes (Fig. 8.2C) and these were variable between seasons ($F_{5, 44} = 5.64$, $P < 0.001$).

The mean changes in mass registered by the penguins between release and recapture for each study period was always less than ± 2.5 %/day, and was usually less than ± 1.0 %/day. The period of greatest mass loss was in July 1985 (mean: -2.1 ± 2.2 %/day, n = 17), and of greatest mass gain was in January 1986 (mean: +1.6 ± 3.7 %/day, n = 40). This variation probably reflects whether the penguins had fed recently prior to recapture, and also the extra food bought ashore when feeding chicks.

8.3.2 DATA GROUPING AND TIME PARTITIONING

The water and sodium influx rates, together with field metabolic rates (FMR), i.e. CO_2 production and energy expenditure rates, recorded over the 17 month study period are shown in Table 8.4. These, together with the calculated food and seawater consumption rates are shown graphically in Figures 8.3 to 8.6. At no time of year was there a difference between males and females in any of the parameters measured, and so these data have been pooled for each month. Also, there were no significant differences in results between parameters measured in different years but at corresponding times (i.e. courtship period: September 1984 and 1985) and so these data have been pooled also.

Following this, data were also pooled for the chick rearing period which encompassed December 1984, November 1985 and January 1986, as any differences evident were functionally based, i.e. due to the sizes of the chicks being raised, rather than due to differences between months or years. This was tested by comparing influx

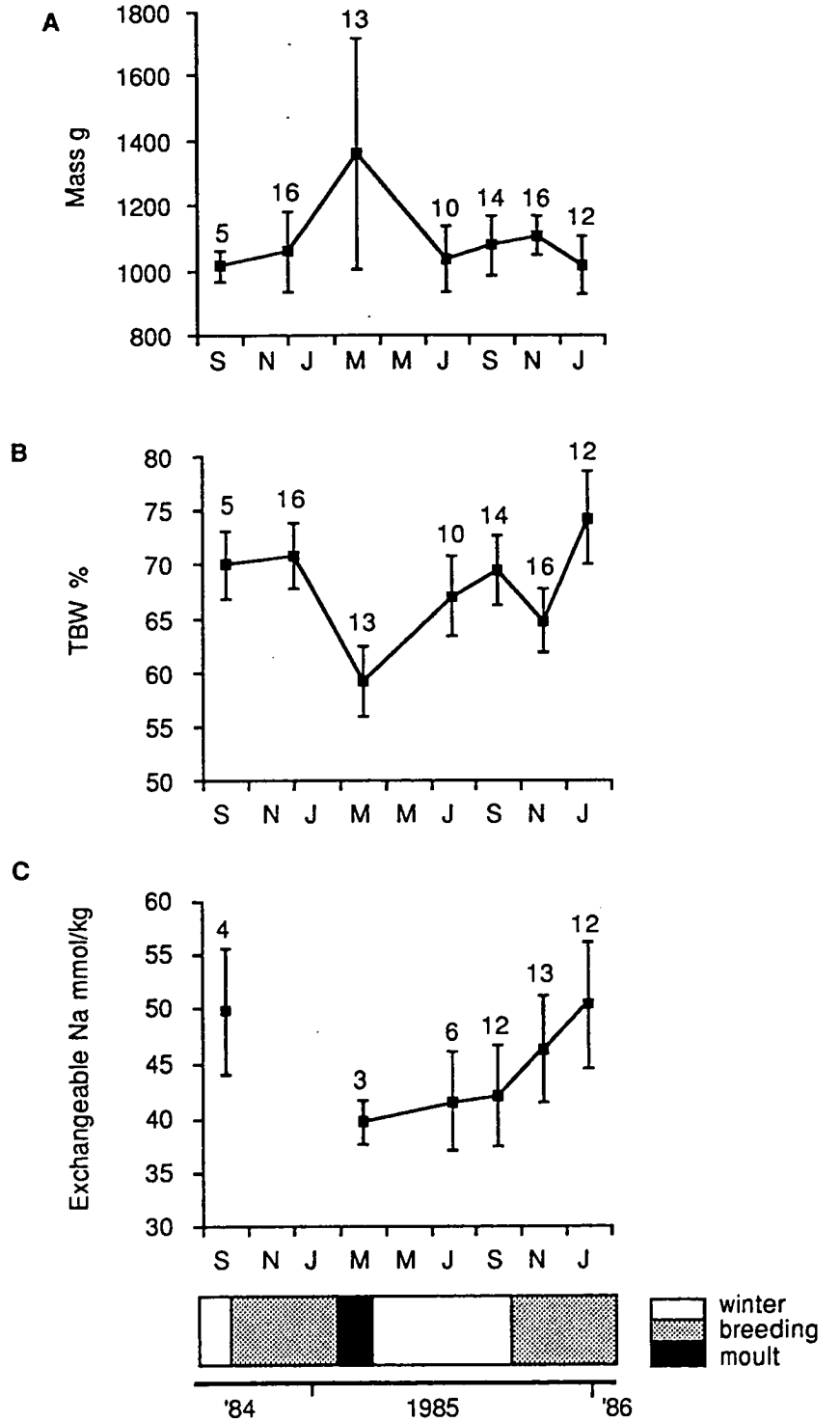

FIGURE 8.2 Seasonal changes in mean body mass (A), total body water (B), and exchangeable sodium pool size (C) in little penguins. Sample sizes (n) shown above SD bars.

TABLE 8.4 Water and sodium influx rates and field metabolic rates (mean ± SD) of adult little penguins over the annual cycle

Month	Stage	Activity**	Water influx ml/kg.day	Sodium influx mmol/kg.day	Field metabolic rate (FMR) ml/g.h	Field metabolic rate (FMR) kJ/kg.day
March	moult	fast	16.9 ± 3.6	-	0.99 ± 0.16	657 ± 105
	post-moult	forage+	181 ± 74.4	33.4 ± 13.2	no data	no data
July	winter	fast	25.6 ± 11.3	-	no data	no data
		forage+	102.3 ± 86.2	6.0 ± 6.0	2.01 ± 0.40	1207 ± 242
September	courtship	fast	18.0 ± 6.4	-	0.86 ± 0.12	577 ± 79
		forage+	261.5 ± 124.6	21.0 ± 9.5	2.39 ± 0.50	1457 ± 305
November	incubation	fast	19.5 ± 7.4	-	0.98 ± 0.15	661 ± 103
		forage+	347.3 ± 2..1	no data	no data	no data
November to January	chick rearing -early	forage+	252.6 ± 81.9	34.8 ± 11.6	2.08 ± 0.49	1261 ± 295
	-mid	forage+	520.0 ± 167.3	55.0 ± 19.6	2.95 ± 0.60	1788 ± 362
	-late	forage+	628.4 ± 63.4	76.6 ± 8.0	4.19 ± 0.40	2532 ± 257

- denotes no detectable turnover. Samples sizes shown in Figs. 8.3 to 8.6.

** fast indicates all time on land, forage+ indicates foraging at sea plus some time on land

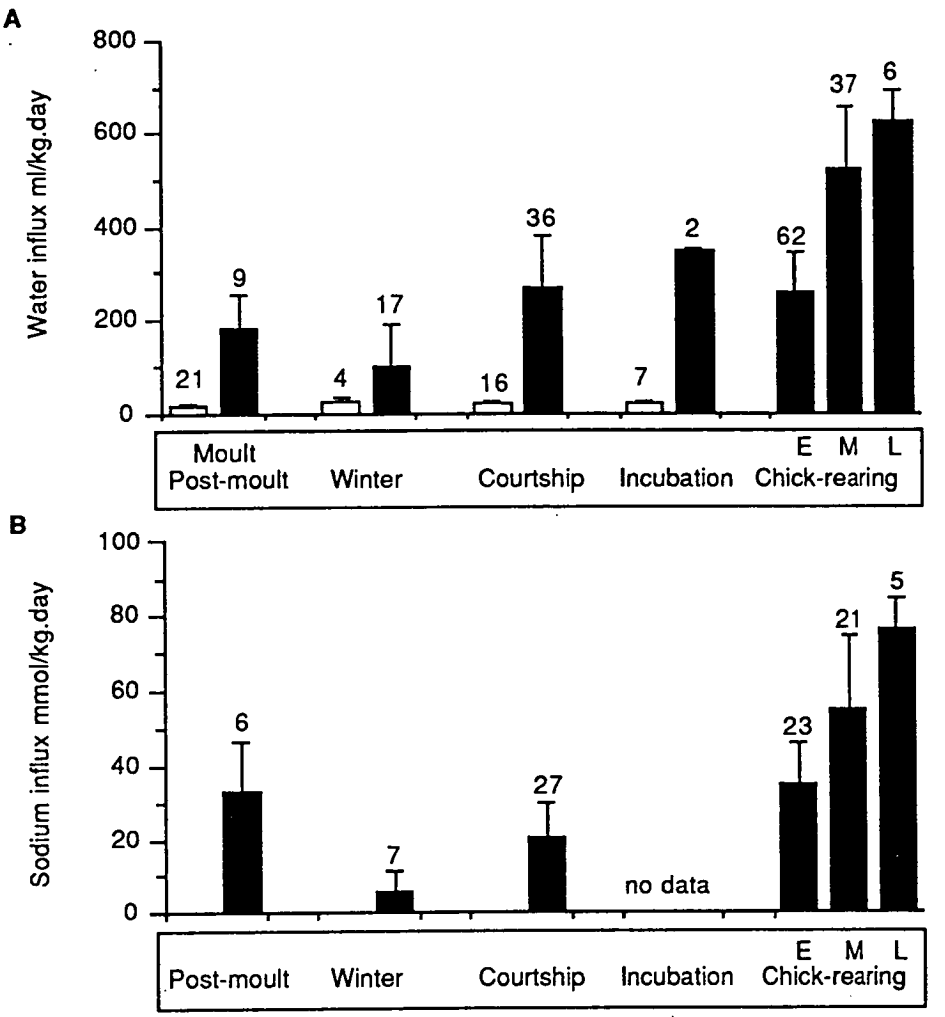

FIGURE 8.3 Mean rates of water (A) and sodium (B) influx in adult little penguins over the annual cycle. Open bars indicate fasting birds, closed bars indicate foraging birds, and E, M, L indicate early, mid and late stage chick rearing. Sample sizes shown above SD bars.

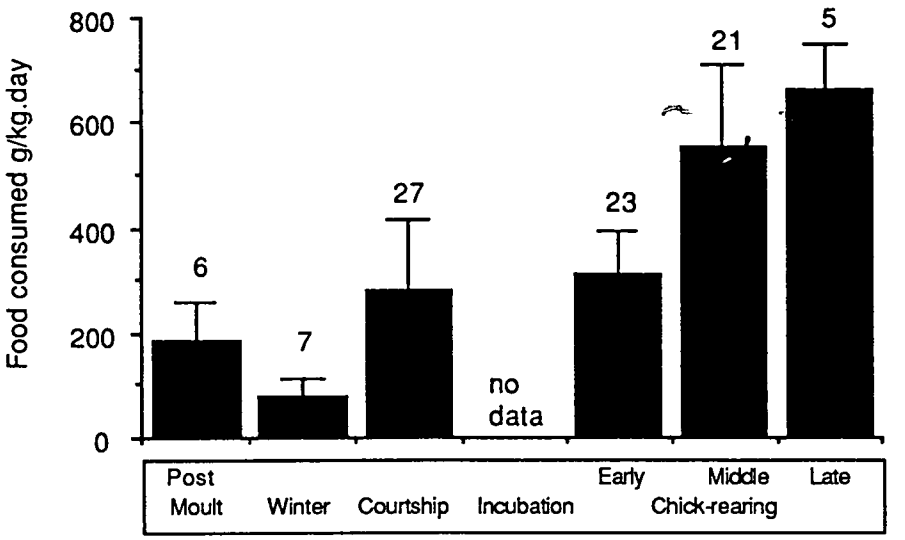

FIGURE 8.4 Mean rates of food consumption by little penguins over the annual cycle. Sample sizes shown over SD bars.

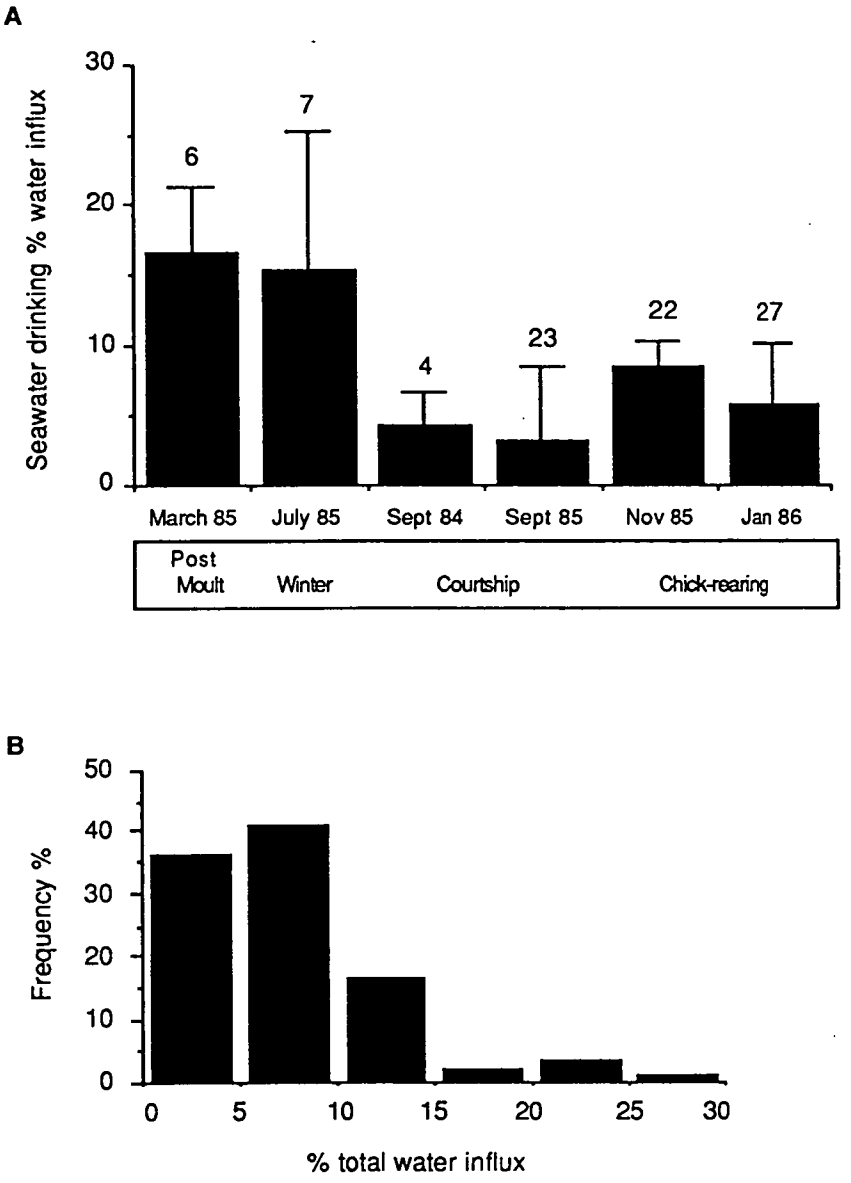

FIGURE 8.5 Seawater ingestion rates of foraging little penguins,
(A) Distribution over the annual cycle, mean ± SD (n).
(B) Frequency of ingestion rates.

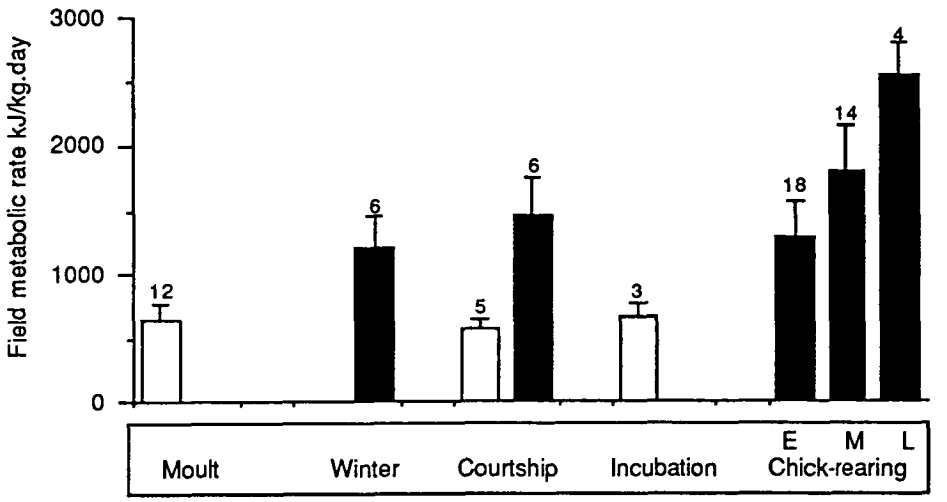

FIGURE 8.6 Field metabolic rates of adult little penguins, mean ± SD (n). Open bars indicate fasting birds, closed bars foraging bars.

rates and metabolic rates of adults raising similar sized chicks, but measured in different months, and there were no significant differences (ANOVAs, $P > 0.05$). During the chick rearing period, (Nov - Jan) the data have been divided into three groups based on the age and mass of chicks being reared: adults feeding early (age: 0 - 7 days; mass 73 ± 86 g; brood size: 1.8 ± 0.4), mid (age: 8 - 35 days; mass 360 ± 269 g; brood size: 1.6 ± 0.5) or late-stage chicks (age: 36 - 60 days; mass: 1150 ± 151 g; brood size 1.3 ± 0.5). Essentially, early chicks represent very young chicks soon after hatching which were being continuously brooded, mid-stage chicks incorporate chicks on the linear part of the logarithmic growth curve, and so include the age at the point of inflection of the growth curve and the end of the guard stage, and late-stage chicks are those which approached or had attained asymptotic weight prior to fledging. The mean brood size decreased as the chicks got older. This was due to a greater proportion of one-chick broods vs two-chick broods in broods containing late stage chicks relative to the number of chicks in broods containing early stage chicks, which is a reflection of the death of one chick within some two-chick broods.

Most penguins departed and returned to the colony within 24 hours. The average durations of foraging trips to sea were approximately 12.5 h (52 % of the day) in March, 11.5 h (48 %) in July, 13.5 h (56 %) in September, 16.5 h (69 %) in November, and about 17 h (71 %) in January; foraging trips increasing in time with increasing day length during summer. However, some birds stayed at sea for periods in excess of 24 h during every phase of the annual cycle, and these extended trips more common immediately after moult and in the winter period than at any other time of year.

8.3.3 WATER AND SODIUM FLUX RATES

The water influx rates of both fasting and foraging birds and the sodium influx rates of foraging birds over the annual cycle are shown in Table 8.4 and Figure 8.2. The penguins which are in the fasting category remained on land for the entire turnover period, whereas the birds in the foraging category were at sea for all, or part of the turnover period. Sodium turnover was undetectable in fasting birds . The water influx rates of fasting birds represents metabolic water formation derived from the catabolism of body reserves and were similar at all stages of the year ($F_{3, 44} = 2.50$, ns; Fig. 8.3A). For the foraging birds, changes in mass were highly correlated with water influx during each period (Table 8.5). Rates of water and sodium influx of foraging birds were also highly correlated (Table 8.5) and a pattern was shown by these influx rates over the study period which reflected the activity of the penguins.

Water influx rates of foraging penguins varied significantly over the annual cycle ($F_{6, 162} = 42.88$, $P < 0.001$; Table 8.4, Fig. 8.3A). After moult the mean water

TABLE 8.5 The significance levels of correlations between parameters measured in foraging little penguins

	Mass change and Water influx	Water influx and Sodium influx	Water influx and FMR
Moult	**	**	no data
Winter	*	*	ns
Courtship	**	***	*
Chick rearing	***	***	***

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$; ns not significant

TABLE 8.6 Water and sodium influx rates, food consmption rates and FMRs of adults raising either broods of either 1 or 2 early, mid or late stage chicks. {Mean ± SD (n)}

Chick stage	Brood size	Water influx ml/kg.day	Sodium influx mmol/kg.day	Food consumed g/kg.day	FMR kJ/kg.day
Early	1	234.5 ± 127.8 (11)	61.7 (1)	624.8 (1)	1882 (1)
	2	256.7 ± 68.9 (49)	33.6 ± 10.2 (22)	285.4 ± 59.6 (22)	1225 ± 259 (17)
Mid	1	573.3 ± 152.8 (16)	57.8 ± 17.0 (11)	600.6 ± 171.9 (11)	1935 ± 329 (6)
	2	473.5 ± 166.4 (21)	52.0 ± 22.7 (10)	494.3 ± 185.2 (10)	1678 ± 365 (8)
Late	1	629.3 ± 64.3 (4)	74.1 ± 6.5 (4)	653.5 ± 96.7 (4)	2532 ± 257 (4)
	2	626.6 ± 94.6 (2)	86.8 (1)	704.9 (1)	no data

influx rate in foraging penguins was 181 ml H_2O kg^{-1} day^{-1}, compared to 102 ml H_2O kg^{-1} day^{-1} during the non-breeding, winter period, but this difference was not significant (PLSD = 93.09, ns). These values correspond to sodium influx rates of 33.4 and 6.0 mmol Na kg^{-1} day^{-1} respectively, and this decline in sodium influx was significant (PLSD = 14.56, $P < 0.05$). Influx rates then increased to levels recorded during the courtship period when birds were feeding intensively and increasing in mass in preparation for raising chicks, and the increase was significant both in terms of water influx (PLSD = 66.45, $P < 0.01$) and sodium influx (PLSD = 11.10, $P < 0.01$). There were no significant differences in water influx rates between courtship, incubation and when raising early stage chicks ($F_{2, 97} = 0.94$, ns) but sodium influx rates did show a significant increase between courtship and early chick rearing (PLSD = 7.43, $P < 0.05$). Influx rates of both sodium and water then increased rapidly and significantly during the chick rearing stage (water influx: $F_{2, 102} = 74.08$, $P < 0.001$; sodium influx: $F_{2, 46} = 19.26$, $P < 0.0001$). Maximum levels achieved were 628 ml H_2O kg^{-1} day^{-1} and 76.7 mmol Na kg^{-1} day^{-1} when rearing late-stage chicks.

8.3.4 FOOD AND SEAWATER CONSUMPTION RATES

Food and seawater consumption rates of foraging penguins were calculated assuming that the sodium content of water is 470 mmol l^{-1} and using the appropriate values of sodium and available water content of the diet during each period. Rates of food consumption varied significantly over the study ($F_{5, 83} = 28.29$, $P < 0.001$). Food consumption rates (Fig. 8.4) declined significantly from 183 ± 75 g kg^{-1} day^{-1} recorded during the post-moult period to a low of 74 ± 37 g kg^{-1} day^{-1} recorded during the winter period (PLSD = 188.85, $P < 0.05$). Feeding rates then increased significantly to those achieved during the courtship period (275 ± 135 g kg^{-1} day^{-1} ; PLSD = 140.14, $P < 0.05$), and this feeding rate was not significantly different from the mean of 300 ± 92 g kg^{-1} day^{-1} measured during early chick rearing (PLSD = 74.20, ns). The feeding rate then increased significantly from early-chick stage to mid-chick stage (550 ± 182 g kg^{-1} day^{-1}) (PLSD = 78.95, $P < 0.05$). The rate of food consumption of adults feeding late-stage chicks was 664 ± 87 g kg^{-1} day^{-1}, not significantly higher than when rearing mid-stage chicks (PLSD = 130.12, ns). These food consumption rates include the food fed to the chicks as well as that assimilated by the adults as isotopes equilibrate rapidly with the stomach contents in little penguins (R. Gales & B. Green, unpublished data) and so the stomach contents are effectively part of the body pool.

The mean rate of seawater consumption as a per cent of the total water influx over the entire study period was seasonally variable ($F_{5, 83} = 11.67$, $P < 0.01$). Seawater ingestion was highest during the non-breeding season, the mean seawater ingestion rate during the post-moult and winter period accounting for 16 % of water

influx. Seawater ingestion was also most variable between individuals in the winter period and values ranged between 6 % to 30 % of total water influx between individuals (Fig. 8.5A). The distribution of the seawater consumption rates over the study period and their frequency distribution are shown in Figure 8.5 and in most cases (76 %) seawater ingestion contributed less than 10 % of the total water influx.

8.3.5 FIELD METABOLIC RATES

From the doubly labelled water turnovers, and the dietary energy equivalents, the field metabolic rates (FMR) of penguins were calculated over the annual cycle and are shown in Table 8.4 and Figure 8.6. Throughout the courtship and chick rearing periods FMR was highly correlated with water influx rates (Table 8.5). There were no significant differences between FMRs of fasting birds during the study ($F_{2, 17} = 1.35$, ns); 0.86 ml CO_2 g^{-1} h^{-1} (577 kJ kg^{-1} day^{-1}) during courtship; 0.99 ml CO_2 g^{-1} h^{-1} (675 kJ kg^{-1} day^{-1}) during moult; and 0.98 ml CO_2 g^{-1} h^{-1} (611 kJ kg^{-1} day^{-1}) during incubation.

The FMRs of foraging little penguins showed significant seasonal variation over the annual cycle ($F_{4, 43} = 17.95$, $P < 0.001$). The mean FMR of birds foraging during winter was 2.01 ml CO_2 g^{-1} h^{-1} (1207 kJ kg^{-1} day^{-1}), and this was the only period when there was no correlation between FMR and water influx rates (Table 8.5). The rates of CO_2 production of foraging birds remained relatively constant during winter, courtship and early chick rearing, and there were no significant differences in the FMRs of foraging penguins during these periods ($F_{2, 27} = 1.35$, ns). During chick rearing the FMR increased rapidly and significantly ($F_{2, 33} = 29.48$, $P < 0.001$), reaching a maximum of 4.19 ml CO_2 g^{-1} h^{-1} (2 532 kJ kg^{-1} day^{-1}), when feeding late-stage chicks.

The adults sampled during the chick rearing stage were classified as either successful or unsuccessful depending on whether their chick(s) survived or died during the experimental period. In only one adult, from which two water turnover samples were collected, did the chicks die during the experimentation. The successful adults were raising either one, or two chick broods, and the turnover rates and food consumption rates of these adults are shown in Table 8.6. Where sample sizes were sufficient for statistical comparisons, the turnover rates of adults raising either one or two chick broods within each group were compared, and in no instance were there any significant differences.

8.4 DISCUSSION

8.4.1 TOTAL BODY WATER AND SODIUM POOLS, AND SEX COMPARISONS

Mass specific total body water pools (TBW) are inversely correlated with body fat content (Panaretto, 1963; Green & Eberhard, 1983) and these parameters changed over the annual cycle reflecting the condition of the penguins engaged in various activities. The lowest TBW was recorded during the moult period, before which the penguins double in mass and accumulate body fat reserves to sustain themselves during the 17 day fast that accompanies moult. TBW was highest when the adults were raising late stage chicks, corresponding with the the time at which the adults were lowest in mass. The mean TBW values are similar to those recorded in other studies of little penguins (Costa *et al.*, 1986; Green *et al.*, 1988; Gales, 1989; also Chapter 5) and also in four other penguin species (Green & Gales, in press; and references therein). Generally, the exchangeable sodium pools followed a similar pattern to TBW over the study period and are also similar to those recorded for other penguin species (Green & Gales, in press)

There were no significant differences between the sexes of adult penguins with respect to mass specific body pool sizes, as well as in any of the other parameters measured: water influx, sodium influx, food consumption rates and FMR. This is consistent with the equal division of time devoted to caring for eggs and chicks between the two sexes, and is similar to the results obtained by Davis *et al.* (1989) for gentoo penguins, another monomorphic species, which also showed no significant difference between the sexes in their rates of energy expenditure.

8.4.2 SEAWATER CONSUMPTION RATES

The topic of seawater drinking and ingestion by foraging seabirds has been addressed by a number of authors in terms of the effect on estimates of food consumption rates based on water influx rates (Kooyman *et al.*, 1982; Adams *et al.*, 1986; Costa *et al.*, 1986; Costa & Prince, 1987; Green *et al.*, 1988; Green & Brothers, 1989; Green & Gales, in press; Gales, 1989; also Chapter 5). In many studies it has been assumed that seawater ingestion is negligible, but results from water and sodium turnover in a number of penguin and other seabirds indicate that some species do ingest appreciable amounts of seawater, either deliberately or inadvertently while feeding, although the rates are variable both within and between species. Green *et al.* (1988) have found that mean rate of seawater ingestion was about 10 % of total water influx in little penguins, 5 % in gentoo penguins (Robertson *et al.*, 1988) and about 13 % in Adelie penguins *Pygoscelis adeliae* (B. Green, unpublished data).

In the present study, the rates of water influx of fasting birds were similar during all periods, and this uniformity confirms that little penguins do not drink whilst on land. This is contrary to some Antarctic and sub-Antarctic species which have been observed to drink on land (Davis *et al.*, 1989; personal observation). The mean rate of seawater consumption by foraging little penguins was 7 % of the total water influx, but the rate was variable during the study. The rates of seawater consumption were highest during the post-moult period and winter, in some animals accounting for over 30 % of the total water influx. Rates then decreased to rates of generally less than 10 % during the breeding season. In 77 % of all cases, seawater ingestion represented less than 10 % of total water influx, with higher rates becoming less frequent (Fig. 8.5B).

The rates of seawater ingestion were not correlated with the composition of the diet, suggesting that in little penguins, which are catholic predators, there may not be major differences in the rates resulting from concomitant ingestion of seawater with capture of different prey types. However, the diet samples were not collected from the birds used in the isotope turnover study and so this possibility cannot be excluded. It is difficult to explain the variation in the rates of seawater ingestion; however, it is possible that the high rates during the non-breeding season could perhaps reflect the ingestion of free water, that is active drinking, in an effort to maintain water balance during periods of low rates of food consumption. Drinking is therefore a process which must be addressed when discussing water influx rates in penguins (see Green & Gales, in press) as food consumption rates may be overestimated, and by variable degrees, if food consumption rates are based on water influx rates alone.

8.4.3 ENERGETICS AND FOOD CONSUMPTION RATES DURING THE NON-BREEDING PERIOD

The non-breeding activities of penguins include those associated with the moulting cycle and the time spent subsequent to moult until the onset of the next breeding season. The free-living energetics of moulting little penguins have been discussed by Gales *et al.* (1988, also Chapter 7) and those results were similar to those recorded by Green *et al.* (1988). While water influx rates during the post-moult period were not significantly different from those recorded in winter, there was a significant difference in the sodium influx rates between the two periods. This was reflected in the rates of food consumption during these periods which declined to the lowest rate which was recorded during winter (74 g kg^{-1} day^{-1}). During this period the birds sampled were also declining in mass. Little penguins in negative energy balance were also recorded by Costa *et al.* (1986) for two birds just prior to the breeding season and these birds had water influx rates within the range of those measured during winter in the present study. FMRs were relatively high during winter and this was the only period during the study when FMRs were not significantly correlated with rates of

water influx, or food consumption rates. This situation indicated that the birds were working hard for little gain in food, i.e., in negative energy balance.

There are no other data regarding free-living energetics of penguins during the winter period with which these data may be compared as many penguin species remain at sea during the non-breeding season. Little penguins also may remain at sea for long periods during the winter period, presumably to cut the costs of travelling between the colony and the feeding grounds. The data collected in this study are from birds which were coming ashore regularly and hence commuting between the colony and feeding areas, and these may reflect a harsher picture in energetic terms than would be shown by those birds which remain at sea.

8.4.4 ENERGETICS AND FOOD CONSUMPTION RATES DURING THE BREEDING PERIOD

During the breeding season little penguins are either fasting on land while courting, nest building, incubating or brooding, or are foraging at sea. While penguins were restricted to land, there were no differences in the FMRs during the courtship and incubation periods, which again were not different from the penguins when moulting. These metabolic rates, however, are integrated values including the costs incurred in all land-based activities such as walking, preening, mating and territorial defence, and therefore reflect the total FMR of all activities in which the penguins are engaged over the turnover period. Differences in the actual costs, of the processes of incubation *vs* courtship, if they existed for instance, could therefore be masked as there is a greater component of active behaviours such as aggression, and mating activities in the courtship period. The results of this study, however, are consistent with other studies of FMR in little penguins while fasting on land (Costa *et al.*, 1986; Green *et al.*, 1988). The mass specific FMRs and water influx rates of fasting little penguins are higher than have been recorded for seven other penguin species which are all larger species. Allometric lines relating penguin body mass to rates of daily energy expenditure (0.67) and water influx (0.86) have been described for fasting penguins by Green & Gales (in press).

In foraging birds, rates of water influx were positively correlated with rates of mass change, sodium influx and FMR throughout the breeding season. Water influx rates were similar in foraging birds during the courtship period and when raising small chicks, and this was also reflected in a uniformity in rates of food consumption and FMR during these periods. In a previous study, Green *et al.* (1988) found a significant increase in water influx rates between little penguin adults which were foraging and feeding small chicks and those which were foraging between incubation shifts, but this was not reflected in FMRs which were similar between the two periods. In the present

study I was unable to measure FMR during the foraging period of the incubation phase, but the water influx rates and FMRs while foraging and rearing small chicks were similar between the two studies. This uniformity in water influx and FMR during incubation and early chick rearing is consistent with results for gentoo penguins (Davis *et al.*, 1989) and Adelie penguins (B. Green, unpublished data), although in the latter species there was a difference between the two sexes in water influx rates. In situations where FMRs remain uniform but food consumption rates increase, it has been suggested that this reflects an increase in availability of prey, meaning that the birds can increase food consumption rates without an increase in energy cost (Green *et al.*, 1988; Davis *et al.*, 1989). However, in this study, both food consumption rates and field metabolic rates remained uniform from courtship to early chick stage, indicating that there was little change in food supply and demand, and that the additional food consumed to feed small chicks is not a significant burden on the adults.

As chicks increased in size, the rates of water and sodium influx, food consumption and FMR of the foraging adults increased rapidly, reflecting the increasing demands of the chicks during growth. When little penguins were feeding late stage chicks just prior to fledging, food consumption rates increased to 664 g kg^{-1} day^{-1}. The cost of raising late stage chicks was previously unknown and the rates of water, sodium and energy flux at this time are higher than have previously been reported for little penguins. They are also much higher on a mass specific basis than have been reported for any other penguin species. Allometric analyses of water influx and FMR in six species of foraging penguins provide slopes of 0.79 and 0.72, respectively (Green & Gales, in press).

As only one adult sampled in this study was raising broods in which a chick died during the experimental period, it was not possible to compare energetics of adults which were either successful or unsuccessful in their chick rearing abilities. However, adults were raising broods containing either one or two chicks, so it was possible to compare the energetics of adults within each group of either early, mid or late stage chicks. In none of the parameters measured in which it was possible to compare adults of one and two chick broods, were there any significant differences reflected in adult turnover rates of water, sodium or energy, as well as food consumption rates (Table 8.6). Differences between flux rates of adults raising chicks were due to the stage of development of their chicks, rather than the number of chicks within the brood. This indicates that if the adults were capable of raising chicks through each stage, then the amount of food they consumed was enough to sustain two chicks as well as maintaining themselves, and was independent of the number of chicks in their brood.

This study, however, was carried out in seasons when fledging success was high, reflecting adequate food availability, as little penguins have been shown to exhibit brood reduction in which both chicks are fed in "good" years, but in "bad" years the stronger chick resumes feeding before the requirements of the smaller chick have been met (Gales, 1987*a*). I did not look at the rates of provisioning between chicks within broods, or the growth rates of chicks within and between broods, but it is clear that in this study the food consumption rates of adults was sufficient to sustain two chicks.

After experimental manipulation of clutch size, little penguins were shown to be able to successfully fledge three chicks per pair, and clutches of three eggs would produce more surviving offspring than the usual clutch of two eggs (Dann, 1988). Therefore, if there is a sustainable energetic limit in terms of parental energy expenditure, as suggested by Drent & Daan (1980), it seems that in years of adequate food availability, this limit may accommodate the rearing of more than two chicks per pair. Comparisons of the energetics of adults raising chicks in years of both high and low food availability, comparison of the growth of chicks within and between broods, and experiments of manipulation of brood size with simultaneous monitoring of parental energy investment is needed to understand how the brood reduction strategy is manifest in terms of cost to the adult and subsequent chick provisioning.

8.4.5 EXPRESSION OF RATES OF ENERGY EXPENDITURE

The most common comparative unit used for energy expenditure is basal metabolic rate (BMR) and the energy expenditures of individuals are often expressed as multiples of BMR in order to facilitate comparisons between species or communities (e.g., Ellis, 1984; Laugksch & Duffy, 1984). There have been five studies in which little penguin BMR has been measured in metabolic chambers (Stahel & Nicol, 1982; Stahel & Nicol, 1988; Stahel *et al.*, 1984, 1987; Baudinette *et al.*, 1986). The results from these studies show a large range in values of BMR (max: 426 kJ kg^{-1} day^{-1}, min: 268 kJ kg^{-1} day^{-1}) and thus the interpretation of energy expenditure of little penguins may vary with the BMR selected to serve as a baseline, particularly in comparative analyses. Where BMRs have not been measured, many energy use data are expressed as multiples of predicted BMR from the equations of Lasiewski & Dawson (1967) and Aschoff & Pohl (1970). In little penguins, these predicted values are within the range of the measured values (Table 8.7). Alternatively, existence metabolic rate (EMR) is sometimes used as an expression of metabolic rate as this value integrates BMR together with the added costs of temperature regulation, calorigenic effect of feeding and the energy expended whilst in a cage. A measure of EMR was estimated from a materials balance study of little penguins (512 kJ kg^{-1} day^{-1}, Gales, 1989; also Chapter 5).

TABLE 8.7 Expression of field metabolic rate (FMR) compared to various standard rates

Stage	Activity *	FMR kJ/kg.day	Multiples of standard rates #					
			FMR/BMR 1	FMR/BMR 2	FMR/L & D	FMR/A & P	FMR/EMR	FMR/IMR
Moult	fast	657	1.54	2.45	1.94	1.68	1.28	1.14
Winter	forage +	1207	2.83	4.50	3.57	3.09	2.36	2.09
Courtship	fast	577	1.35	2.15	1.71	1.48	1.13	1
	forage +	1457	3.42	5.44	4.31	3.73	2.84	2.52
Incubation	fast	661	1.55	2.47	1.96	1.69	1.29	1.15
Chick rearing								
early	forage +	1261	2.96	4.71	3.73	3.22	2.46	2.18
mid	forage +	1788	4.20	6.67	5.29	4.57	3.49	3.10
late	forage +	2532	5.94	9.45	7.49	6.48	4.95	4.39

* forage + indicates FMR of foraging plus some time on land

\# BMR 1 (426 kJ/kg.day; Stahel & Nicol,1982); BMR 2 (268 kJ/kg.day; Baudinette et al.,1986), L & D (338 kJ/kg.day; Lasiewski & Dawson, 1967); A & P (391 kJ/kg.day; Aschoff & Pohl, 1970) EMR (512 kJ/kg.day; Gales,1989; also Chapter 5); IMR (577 kJ/kg.day, this study).

Due to the range in the values of BMR, and the highly controlled conditions under which BMR is measured, it has been suggested that it may be more instructive to express the FMRs of foraging birds by comparing metabolic rates of foraging ("active" metabolic rate, AMR) and non-foraging birds ("inactive" metabolic rate, IMR) under field conditions, and comparing measurements derived by the same technique (Green & Gales, in press). This has been done for 6 penguin species and values of AMR/IMR ranged between 1.7 and 3.3, with most values about 2 times IMR (Green & Gales, in press). The FMRs measured in the present study are similar to those for little penguins summarized in Green & Gales (in press). However, the maximum rate of energy expenditure that was recorded when little penguins were foraging for late stage chicks, (AMR/IMR ratio = 4.4) (Table 8.7) exceeds other calculated AMR/IMR ratios. The only other data on the costs of raising late stage chicks, is that for Adelie penguins and this was 3.3 times IMR (B. Green, unpublished data). Drent & Daan (1980) suggest that the "energetic ceiling" for birds is about 4 times BMR. Even if the highest measured rate of little penguin BMR (Stahel & Nicol, 1982) is selected, the cost of raising late stage chicks would be 5.9 times BMR, which again is higher than has been reported for other seabirds.

The rates of energy expenditure which have been measured for little penguins in this study, however, by virtue of the isotope turnover technique and the sampling protocol, represent an integrated value of the cost of foraging plus some degree of cost of time on land. Using appropriate time budgets, these integrated costs have been partitioned so as to derive a refined estimate of the cost of foraging (Nagy *et al.*, 1984; Costa *et al.*, 1988; Davis *et al.*, 1989). I have also partitioned the time spent on land and at sea, as described in Davis *et al.* (1989), and in this process I have assumed that land based FMR in winter was the same as land based FMR measured during the courtship period, and that FMR on land during chick rearing was the same as FMR measured during incubation (Table 8.8). These results show that although the lengths of foraging trips vary over the year with increasing daylight in summer the energy expended while foraging at sea is lowest in winter, and increases with the breeding season, parallel to the increasing rates of food consumption.

The duration of foraging trips of adults feeding chicks are similar throughout the chick rearing period but the cost of foraging increases rapidly, presumably reflecting the more intensive foraging required to procure the increasing amounts of food consumed which is subsequently fed to the growing chicks. Costa *et al.* (1988) estimated that while foraging between incubation shifts, little penguins expend 1 800 kJ kg^{-1} day^{-1} which is similar to the rates estimated here while foraging in winter (Table 8.8). My data are also broadly similar to those of Davis *et al.* (1989) for gentoo

TABLE 8.8 Estimated field metabolic rates (FMR) at sea of little penguins *

Stage	FMR on land kJ/kg.day	FMR on land & sea kJ/kg.day	Mean % turnover at sea	FMR at sea only kJ/kg.day	FMR at sea as multiples of standard rates #			
					FMR/BMR 1	FMR/BMR 2	FMR/EMR	FMR/IMR
Winter	577	1207	51	1812	4.2	6.8	3.5	3.1
Courtship	577	1457	67	1890	4.4	7.0	3.7	3.3
Chick rearing								
early	661	1261	40	2161	5.1	8.1	4.2	3.7
mid	661	1788	54	2748	6.5	10.2	5.4	4.8
late	661	2532	79	3029	7.1	11.3	5.9	5.2

* FMR on land in winter assumed to be 577 kJ/kg.day as measured during courtship.
FMR on land during chick rearing assumed to be 611 kJ/kg.day as measured during incubation
FMR on land and at sea (forage +) as shown in Table 8.6

\# Standard metabolic rates as shown in Table 8.6

and macaroni (*Eudyptes chrysolophus*) penguins, and also of Nagy *et al.* (1984) who estimated that jackass penguins, *Spheniscus demersus*, foraging to feed small chicks expended 6.6 times SMR while at sea. These again are integrated values of FMR at sea, incorporating the costs of swimming, diving, preening and resting on the surface, and Nagy *et al.* (1984) estimated that when actually swimming jackass penguins would have metabolic rates close to 9.8 times SMR.

In another study, it was found that little penguins spent 95 % of their time at sea actively swimming (Gales *et al.*, in press; also Chapter 9), and so the foraging FMRs in Table 8.8 would probably be close to that for swimming and diving. It has been assumed that underwater swimming is less expensive than flying (Schmidt Nielsen, 1972) but the results of this study, and those of Nagy *et al.* (1984), show that the metabolic cost of penguin swimming can approach those of flapping flight, and are considerably greater than for gliding or soaring flight (Berger *et al.*, 1970; Tucker, 1972; Ellis, 1984; Flint & Nagy, 1984; Adams *et al.*, 1986; Costa & Prince, 1987).

8.4.6 ANNUAL ENERGY BUDGETS

Recently, through the combined use of isotope turnover methods and time budgets it has been possible to draw up proximate estimates of the overall rates of energy expenditure for penguins (Nagy *et al.*, 1984; Davis *et al.*, 1989; Green & Gales, in press). These budgets are valuable, but must still be considered rudimentary as the data upon which they are based are confined to certain phases during the breeding season, and the energy expenditures of the rest of the year are estimates. In this study, however, it was possible to construct an energy budget based on a more complete suite of information than exists for any other seabird species. From the results of the isotope turnover rates and the proportion of time dedicated to various activities, total energy requirements were calculated for both breeding and non-breeding birds (Table 8.9). There remain, inherent in these calculations, several assumptions. It is impossible to measure the FMR of free-living penguins in the pre-moult period and the FMR of non-breeding penguins, as these birds remain at sea for prolonged periods making the isotope turnover technique impossible due to isotope washout. Therefore, the "maintenance" level of energy expenditure measured during the winter (1243 kJ kg^{-1} day^{-1}) was assumed to apply to penguins at these times. I considered this appropriate in these analyses, but I do recognise that non-breeders, and failed breeders may show rates of energy expenditure which are different from the "maintenance " levels shown by breeding birds. It could be that this rate is even higher than the maintenance levels, as the foraging efficiency of non-breeders and failed breeders may be lower than successful breeding birds.

TABLE 8.9. Energy budget for an adult little penguin.

A. BREEDING BIRD

Stage	FMR kJ/day	Time * days	Total energy kJ
Courtship	644	10 fasting	6 440
	1565	20 foraging +	31 300
Eggs (female only)			620
Incubation	732	19 fasting	13 910
	1430	17 foraging +	24 310
Chick rearing-early	1366	8 foraging +	10 930
-mid	1888	27 foraging +	50 980
-late	2876	22 foraging +	63 270
Chick stored energy **			7 900
Chick expenditure **			29 430
Pre-moult	1243	24 foraging +	29 830
Moult	880	17 fasting	14 960
Winter	1243	201 foraging +	249 840
TOTAL - male			533 100
- female			533 720

B. NON-BREEDING BIRD

Stage	FMR kJ/day	Time days	Total energy kJ
Moult	880	17 fasting	14 960
Remainder	1243	348 foraging +	432 564
TOTAL - male/female			477 520

* "fasting" indicates complete fast, "foraging+" indicates an integrated FMR including foraging with some period of time on land.

** Cost per adult = rate per chick x 1.7/2

In the construction of a complete annual energy budget, the additional costs of egg production and that invested in chick rearing, must also be considered. In another study, Gales & Green (unpublished data) found that the stored energy in a little penguin egg was 308 ± 53 kJ egg^{-1} (n = 14). As little penguins invariably lay a clutch of two eggs, the stored energy in a clutch would be 616 kJ $clutch^{-1}$, and this energy cost would be incurred by the female only. The energy which is both stored in, and expended by, chicks must also be included, as this is also not accounted for in the energy turnovers of adults as measured by doubly labelled water. In order to determine these values I have assumed that the average non-mineral composition of a little penguin fledgling would constitute approximately 60 % water, 20 % protein and 10 % fat (B. Green, unpublished data). The average mass of little penguins fledging from Albatross Island during this study was 1.1 kg and so the stored energy in a little penguin fledgling, using the energy equivalents of Kleiber (1961), would be (220 x 24.0) + (110 x 39.3) = 9 600 kJ. Subtracting the stored energy of the egg from this value, the energy stored in the chick after hatching would be 9 290 kJ.

In order to evaluate the energy expended by a chick during growth, I have used the data acquired from a separate study of the energetics of little penguin chick growth using doubly labelled water (R. Gales & B. Green, unpublished data). The chick period was determined as 57 days and the data were grouped into two stages of growth: 0 - 16 days and 17 - 57 days of age. For the first 17 days, the mean mass of chicks was 243 g and the mean FMR was 1 445 kJ kg^{-1} day^{-1}. Therefore, the total chick expenditure for this period of growth would be 5 970 kJ. In the following 40 days, the mean mass of chicks was 826 g, and mean FMR was 867 kJ kg^{-1} day^{-1}, resulting in an energy expenditure for this period of 28 650 kJ. Therefore, the total energy expenditure of a chick over the 57 day growth period would be 34 620 kJ $chick^{-1}$.

In the calculation of the energy budget for breeding little penguins (Table 8.9A, Fig. 8.7) the energy requirement data are summarized from Table 8.4 and these have been multiplied by the appropriate time budget data. The energy involved in laying eggs and raising chicks is calculated for clutches of two eggs and the successful raising of 1.7 chicks per pair, as these were the observed breeding parameters during this study.

Without the burden of breeding activities, the annual energy cost for a non-breeding bird would be 448 MJ $year^{-1}$. For a breeding bird, however, the annual energy cost would be approximately 533 MJ $year^{-1}$, and for a pair to raise 1.7 chicks the total energy cost would be approximately 1 067 MJ $year^{-1}$ (Tables 8.9 and 8.10). The annual time and energy profiles for a pair of little penguins to raise 1.7 chicks are

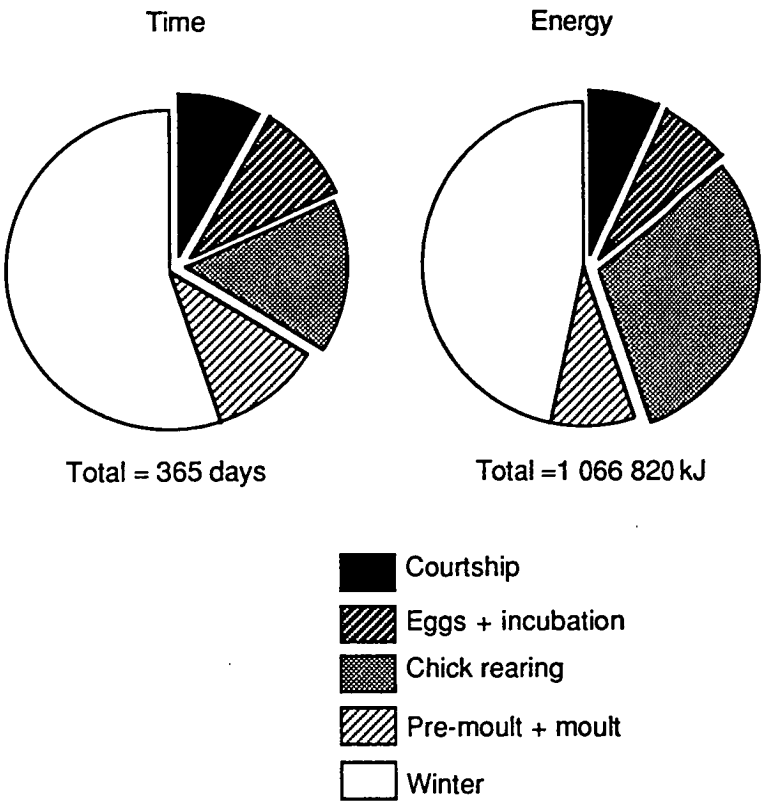

FIGURE 8.7 Annual time and energy budget of a pair of little penguins to raise a brood of 1.7 chicks.

TABLE 8.10. Time and energy budget for a breeding pair of little penguins raising 1.7 chicks to fledging.

Stage	Time		Energy	
	days	%	kJ	%
Courtship	30	8.2	75 480	7.1
Eggs + incubation	36	9.9	77 060	7.2
Chick rearing	57	15.6	325 020	30.5
Pre-moult + moult	41	11.2	89 580	8.4
Winter	201	55.1	499 680	46.8
TOTAL	365	100	1 066 820	100
FOOD REQUIRED			274 kg	

depicted in Figure 8.7. The breeding season is the most energetically expensive period for little penguins, with the chick rearing period in particular accounting for 31 % of the total yearly energy expenditure, during 16 % of the time. This commitment is higher than estimated for other penguin species (Green & Gales, in press) but the data for the other species are based on less complete data sets, and any errors in assumptions may result in significant errors in the overall energy budgets. However, notwithstanding the potential sources of variation, with the construction of energy budgets based on quantitative data, the dynamics of the cost of survival and reproduction can at least now be confidently addressed.

8.4.7 POPULATION REQUIREMENTS

Using values for the composition of the diet and metabolizable energy yields averaged over the annual cycle, and applying these figures to the energy budget data, it can be calculated that a breeding little penguin raising 0.85 chick/year (1.7 chicks/pair), would consume 137 kg of food each year. Using a similar process, a non-breeding little penguin would require 115 kg food/year for maintenance (Table 8.11). Little penguins usually start breeding at three years of age, and live for 6.5 years (Reilly & Cullen, 1982). Therefore, during its lifetime, an "average" little penguin would consume approximately 830 kg food.

If the food requirement data are coupled to the diet data, then the food consumed would comprise approximately 67 % fish, 30 % cephalopod and 3 % crustacea. These data can then be applied to population data for little penguins feeding in the Bass Strait region. In total there are approximately 190 000 breeding birds, based on the estimates of Harris & Norman (1982) and N. Brothers (unpublished data). It has been suggested that a stable population of little penguins, would comprise 50 % non-breeding juveniles (Reilly & Cullen, 1982) and so approximately 95 000 juvenile little penguins would be feeding in the Bass Strait area. The number of penguins feeding in Bass Strait, however, may vary, particularly in response to seasons of low breeding success, which would result in a lower proportion of non-breeders, and a higher proportion of failed breeders. However, if the energy and diet data are extended to the food requirements of the population, as assessed by current estimates, and assuming a stable population, it is estimated that a total of 37 000 tonnes of food are consumed by little penguins annually (Table 8.11).

There are no data regarding the prey biomass for Bass Strait, but even if there were data available, given the concentrated nature of little penguin foraging it would be of limited application in addressing the question of consumption rates of little penguins in relation to prey stocks. There are also no comparative data of rates of prey consumption by other top-level consumers in Bass Strait, but given the population

TABLE 8.11 Food requirements for the little penguin population in Bass Strait

	Population size	Food kg/penguin.yr	Total Food tonne/yr	Fish tonne/yr	Cephalopod tonne/yr	Crustacean tonne/yr
Breeding population	190 000	137	26 030	17 544	7 861	625
Non-breeding population	95 000	115	10 925	7 364	3 299	262
Total	285 000		36 955	24 908	11 160	887

energy requirements of little penguins, these penguins must be considered as important members of the Bass Strait ecosystem. It is clear that populations of little penguins are most vulnerable to energetic stress during chick-rearing and also in winter. Reduction in prey stocks, either by natural means or by competition from commercial fisheries could be expected to seriously affect the survival of chicks and adults, respectively, at these times.

8.6 SUMMARY

Isotope turnover techniques were used to measure the metabolic rates and food consumption rates of free-living little penguins throughout the annual cycle in Bass Strait. The estimates of energy turnover were used to construct time/energy budgets for comparison with time/activity budgets. Although chick-rearing occupies only 16 % of the annual time budget, this period accounts for 31 % of the annual energy budget. The high energy costs of chick-rearing are particularly extreme towards the end of chick growth. At this time the daily rates of food consumption by attending adults exceed 60 % of adult body mass. During the non-breeding season (winter) adult energy expenditure exceeds that acquired from feeding and the birds lose body mass. The total annual food requirements of little penguins in Bass Strait are assessed in terms of biomass of fish, squid and krill. The critical periods are identified with respect to penguin energy acquisition and their vulnerability to fluctuations in food availability, either as a result of natural perturbations or from commercial fishing activities.

CHAPTER 9

FORAGING BEHAVIOUR OF THE LITTLE PENGUIN: INITIAL RESULTS AND ASSESSMENT OF INSTRUMENT EFFECT

9.1 INTRODUCTION

The behaviour of penguins at sea is a fundamental aspect of their feeding ecology, influencing their foraging efficiency and their role as predators in the marine ecosystem. Penguins are notoriously difficult to observe at sea, and so the recent development of appropriate recording instruments has led to increased knowledge of penguin foraging behaviour.

Of all the parameters of penguin foraging behaviour, diving depth has received most attention with maximum diving depths being recorded for emperor (*Aptenodytes forsteri*; Kooyman *et al.*, 1971), jackass (*Spheniscus demersus*; Wilson & Bain, 1984*a*), gentoo (*Pygoscelis papua*; Adams & Brown, 1983) and little penguins (*Eudyptula minor*; Montague, 1985). Multiple depth recorders, which log the number of dives to defined depths, have been used on king (*A. patagonicus*; Kooyman *et al.*, 1982) and chinstrap penguins (*P. antarctica*; Lishman & Croxall, 1983). Autoradiographic depth and speed gauges (Wilson & Bain, 1984*a,b*) have been used on jackass penguins where the cumulative times at certain depths and speeds are recorded (Wilson, 1985*b*), and other data from these speed gauges have also been used to estimate the foraging ranges of macaroni (*Eudyptes chrysolophus*) and rockhopper (*E. chrysocome*) penguins (Brown, 1987). Foraging data, relating feeding ecology and diving performance in penguins, have recently been reviewed by Croxall & Lishman (1987), Kooyman & Davis (1987), Croxall & Davis (in press) and Wilson & Wilson (in press).

Recently, studies of the feeding ecology of the pygoscelid penguins have included direct measurements of the foraging range and travelling speeds, incorporating radiotelemetry techniques, which allow differentiation of behaviour types during foraging trips (Trivelpiece *et al.*, 1986; Adams & Wilson, 1987; Davis *et al.*, 1988). However, in none of these studies was it possible to measure both swimming speed and depth against time and thus produce an integrated pattern of swimming performance whilst foraging. To achieve this, for this study, Mr. C. Williams (University of Tasmania) designed and constructed an archival electronic activity recorder. These recorders were then successfully deployed and retrieved from little penguins in Bass Strait, southern Australia.

Central to any study involving the attachment of instruments to free-living animals, is the effect of the instrument on the overall energy balance and behaviour of the animal, increases in energy demand probably influencing behaviour (e.g. Caccamise & Hedin, 1985; Costa & Gentry, 1986; Kenward, 1987; Gessaman & Nagy, 1988; Obrecht *et al.*, 1988). Wilson *et al.* (1986) have shown that, by comparing the swimming speeds of jackass penguins fitted with devices of varying cross sectional areas, increasing the size of the device decreased the penguin's swimming speed. In a recent study of the diving patterns and diets of gentoo and macaroni penguins, Croxall *et al.* (1988) showed that when using depth histogram recorders, the mass of the prey bought ashore was not significantly different between instrumented and control birds, and there was little difference between the durations of foraging trips for the two groups of animals. They concluded however, that they cannot exclude the possibility that instrumented birds used more energy, although Wilson *et al.* (1986) found that jackass penguins fitted with devices did not use significantly more energy per foraging trip than did non-instrumented penguins.

The significance of "instrument effect" is crucial when interpreting the data recorded by instruments fitted to free-living penguins. To examine this, I used a combination of tritium and 18-oxygen to measure the carbon dioxide production (and hence energy expenditure) and water influx of penguins, foraging either with or without the activity recorder. Similar trials were also run on penguins fitted with "mock" recorders of two different sizes to determine any size effect. I report here on the first integrated data on the foraging behaviour of the little penguin, the smallest of all penguin species, and the effect of carrying instruments ranging between 0.1 and 6 % penguin mass, or 1.4 and 11.8 % penguin cross sectional area.

9.2 METHODS

9.2.1 STUDY AREA AND THE BIRDS

The field study was carried out on Albatross Island, north-west Bass Strait (40°25'S., 144°32'E.) during visits to the Island in March, September and December 1987. In March, the penguins are either moulting or undertaking post-moult foraging trips when recovering from the confinement to land during the moult process. During the breeding season, September to February, little penguins forage during daylight hours and return to the nest on most nights. Most penguins leave the Island in the hour preceding sunrise, and return in the first hour after sunset. The diet consists of fish, squid and krill, and the relative proportions of these dietary items vary according to location and season (Chapter 10). During the September sampling period, the penguins were engaged in courtship and nest building activities, the nests being built in crevices in a large, open-ended cave. During the December sampling period, the study birds were raising either one or two chicks, aged between one and four weeks.

9.2.2 WATER FLUX AND METABOLIC RATES

Water turnover rates and field metabolic rates (CO_2 production) were measured by means of tritiated water (HTO) and doubly labeled water (DLW; $HT^{18}O$) (Lifson & McClintock, 1966; Nagy, 1980; Nagy & Costa, 1980), the use of these isotopes having recently been validated in little penguins (Gales, 1989; also Chapter 5).

No penguins were injected with isotopes in March. In September, six adults were caught at their nest sites, banded and weighed (± 10 g). They were then given intraperitoneal injections of 1 ml HTO (185 MBq) and 0.5 ml 95 %+ atoms excess ^{18}O. Birds were then returned to their nest sites. After 5 - 6 hours, the birds were recaptured and a 2 ml blood sample was collected from a brachial vein into a non-heparinised vial. Nests were then checked several times during the day and night to determine whether the birds were ashore or at sea. Birds were recaptured between two and 10 days after injection when a further blood sample was taken, and the birds were weighed and released. Some birds were sampled several times during the study period.

Blood samples were centrifuged and stored frozen until later analyses at CSIRO, Dept. of Wildlife and Ecology, Canberra. The red cell fractions were vacuum-distilled to extract water from which HTO levels were measured using liquid spectrophotometry (Beckman LS 2800) and $^{18}0$ levels determined by mass spectrometry (V.G. Isogas 903). Rates of water flux and CO_2 production were calculated from the changes in isotope levels in the blood (Lifson & McClintock, 1966; Nagy, 1980; Nagy & Costa, 1980), assuming that changes in pool size reflected body mass changes, and that these changes were linear (see Chapters 5 & 8).

In December, the field protocol was repeated, with 11 penguins being injected with 1 ml HTO (185 MBq), and three of these penguins were also injected with 0.5 ml 95 %+ atoms excess ^{18}O. During both the September and December sampling periods, 'mock' recorders were fitted and removed from the study birds providing measurements of water influx and CO_2 production rates whilst birds were foraging either with, or without instruments attached (see below).

Field metabolic rates were converted from units of CO_2 production (ml g^{-1} h^{-1}) to kilojoules by using the chemical composition of prey species which are similar to those found in the diet of little penguins in Bass Strait {anchovy *Engraulis capensis*: 19.7 % protein, 5.2 % fat, Nagy *et al.* (1984); and an ommastrephid squid: 20.6 % protein, 0.96 % fat, Croxall & Prince (1982*a*)}. Penguins from Albatross Island commonly consume anchovies, and another species of an ommastrephid squid, *Nototodarus gouldi*. I assumed that the fish and squid contained negligible amounts of carbohydrates (energy equivalents from Schmidt-Nielsen, 1975). The diet was not

studied simultaneously during 1987 sampling periods, but had been examined, using stomach flushing (Gales, 1987*b*), at approximately the same times of year, two years previously at the same location. During those times the diet by % mass, comprised 96.7 % fish, 3.1 % cephalopod and 0.1 % crustacean in September 1985, and 61.8 % fish, 37.8 % cephalopod and O.4 % crustacean in January 1986 (Chapter 10). These compositional data were used in the present study and the small crustacean contribution was considered to have the same protein and fat contents as the fish component.

Therefore, to convert amounts of CO_2 production to units of energy expenditure, I used the factors of 1 l CO_2 = 25.4 kJ for the September samples, and 25.2 kJ for the December samples. For any penguin which fasted during either of the experimental periods, the conversion factor of 28 kJ l^{-1} CO_2 was used as fasting penguins metabolise predominantly fat (Groscolas & Clement , 1976; Williams *et al.*, 1977), whereas protein from food is the major metabolic substrate in active animals.

9.2.3 Instruments and attachments

The electronic activity recorder is a miniaturised device designed for logging components of penguin activity at sea. Functions recorded are depth and swimming speed against a time base. Each unit weighs approximately 60 g, is neutrally buoyant in seawater and is constructed in two linked modules. One module containing the speed transducer, is 3 x 4 x 1.5 cm; the other module containing the depth transducer, memory and batteries, and is 11 x 3.5 x 1 cm. As surface swimming may be a normal component of penguin activity, the speed transducer was mounted on the belly, because speed is recorded as revolutions of a paddle wheel which needs to be constantly immersed. The other module was mounted on the back of the penguin. The design of the recorder was such that it was as flat as possible and contoured to the form of the penguin so as to minimise drag.

Each function is recorded into one of eight pre-set speed and depth categories (depth: 0 < 2, 2 < 5, 5 < 7, 7 < 10, 10 < 15, 15 < 20, 20 < 50, 50+ m; speed: 0 < 0.5, 0.5 < 1.0, 1.0 < 1.4, 1.4 < 1.8, 1.8 < 2.4, 2.4 < 3.2, 3.2 < 4.4, 4.4+ m s^{-1}). Sampling frequency was set at approximately every 9 s, and the data are stored in internal RAM. Capacity of the meter was set at *ca.* 8,000 readings which gives a total logging time of about 20 h for each foraging trip. Battery life is about 2 months with 50 % usage, after which new batteries are installed. The meters were calibrated in the sea by lowering to known depths and also towing the meters along side a boat travelling at known speeds. Data were retrieved from the recorder and the instrument reset using a dedicated, portable interface. Data are recorded onto discs using a portable computer (*Epson PX 8*) in the field and then transferred onto an *Apple Macintosh* computer for subsequent data analyses.

The mock packs were moulded resin, designed to simulate the shape, mass and cross sectional area of the electronic activity recorder with the exception that there was no lead connecting the dorsally and ventrally mounted units. The combined mass of the mock units was 60 g, and the cross sectional area constituted 11.8 % of the penguin cross sectional area, the same dimensions as the electronic activity recorder.

In March, the first electronic activity recorder was fitted to a penguin, and was successfully retrieved. This penguin was not injected with isotopes, and so the data for March are restricted to foraging behavioural information recorded by the meter. During September and December, all study birds were injected as described above, and five penguins in each month were fitted with 'mock' recorders of the same dimensions as the electronic activity recorder. In December, three penguins were also fitted with electronic activity recorders. Also in December, four penguins were fitted with plastic 2 ml syringe barrels from which the ends had been removed, resulting in a hollow, open-ended cylinder. These barrels were mounted on the penguins' backs, weighed 1.3 g, and constituted 1.4 % of the penguin's cross sectional area (Fig. 9.1).

All devices were attached to the penguins using *Loctite* 422, a cyanoacrylate adhesive. When the birds returned to the Island after a foraging trip, after a blood sample had been collected, the attachments were removed using a round ended knife, which resulted in no, or minimal, feather loss.

During the experimental period, all three types of attachments were sequentially fitted and removed from the penguins, resulting in isotope turnover samples from birds foraging either with, or without, the electronic activity recorder, the mock recorders, or the syringe barrels.

9.2.4 FORAGING BEHAVIOUR ANALYSES

As the activity recorder collects speed and depth data in range categories, the data were analysed using the value at the lower end of the range, thus treating the data conservatively. Types of foraging behaviour were identified by graphing and visually scanning the data from each trip. To estimate the duration of dives, the data were scanned and any sequential runs of data points where the depth was consistently below 2 m were counted. Dive times were then calculated as the sum of the time spent at depths below 2 m before rising to above 2 m. The possibility exists however that the penguins may have surfaced and re-submerged in between data readings, i.e. within 9 s. Therefore, in sequences where depths below 10 m were recorded, if the penguin ascended to the 2 - 5 m depth, and subsequently descended again, this was treated as two dives.

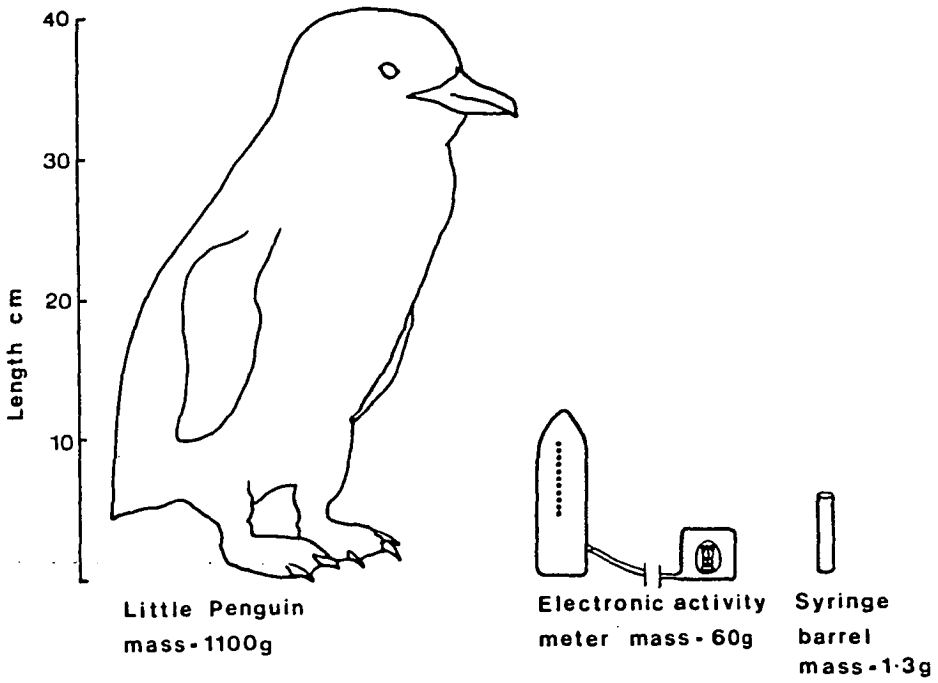

FIGURE 9.1

Size of electronic activity recorder and syringe barrel relative to size of a little penguin

Distance travelled by the penguins was estimated by two methods. First, I simply multiplied the time spent swimming at each speed category by the speed. However, this estimate integrates both the horizontal and vertical components of swimming associated with diving and travelling. To minimise inclusion of the vertical component I scanned the data and included only that which was confined to the top 2 m for periods of at least 3 consecutive data points, i.e. 3 x 9 s. This assumes that little penguins mainly travel in the top 2 m, consistent with the results of other studies (Adams & Brown, 1983; Trivelpiece *et al.*, 1986; Wilson *et al.*, 1986).

9.2.5 STATISTICS

Results are presented as mean ± standard deviation and two-tailed t-tests were used to determine the significance of differences between means. Unless indicated, all t-tests are unpaired. The 5 % level was accepted as denoting statistical significance.

9.3 RESULTS

9.3.1 ASSESSMENT OF INSTRUMENT EFFECT

Six birds were injected with the isotopes in September. These birds had a mean mass of 994 ± 45 g, and a total body water content (TBW) of 67.2 ± 5.6 %, values not significantly different from those of the 10 birds injected in December whose mass was 964 ± 79 g and TBW was 67.1 ± 3.4 % ($t = 0.85$, and $t = 0.03$, d.f. = 14, for mass and TBW, respectively).

The penguin which carried the electronic activity recorder in March weighed 890 g, maintained mass during the sampling period and was not injected with isotopes. Therefore, the data collected to assess the effects of carrying instruments are from the September and December trips, when the duration of foraging trips, changes in mass, water and energy flux rates are compared between instrumented and non-instrumented birds. Sample sizes do not refer to the number of individuals sampled, but rather to the number of turnover samples obtained, some of which may have been taken from the same individual, whilst either carrying or not carrying instruments.

9.3.1.1 Duration of foraging trips

In September, foraging trips by all birds ranged between 12 - 14.5 h, and 16 - 18.5 h in December, increased day length accounting for the longer foraging periods. I detected no significant differences in the duration of the times at sea between birds with or without attachments. Observations on land of birds fitted with attachments showed that these birds behaved normally, and that these birds departed and arrived back at the Island at similar times to penguins which had not been handled at all. The 3.1 h foraging trip by the instrumented penguin in March, was not unusual. When penguins

have just completed moult, they are emaciated and duration of foraging trips is highly variable. These birds may stay at sea for a few hours before returning to the Island, remaining under the cover of rocks before proceeding to roost sites after sunset (personal observation).

9.3.1.2 Mass loss

The birds which were sampled whilst not carrying instruments lost mass at a rate of 0.3 ± 4.0 % day^{-1} (n = 6) in September, and 0.96 ± 3.12 % day^{-1} (n = 7) in December, and these are not significantly different (t = 0.33, d.f. = 11). In September, the birds while carrying packs lost 1.7 ± 6.8 % day^{-1} which is not different to the rate of mass loss of birds not carrying packs (t = 0.44, d.f. = 11), and also not different when using paired comparisons of instrumented and control trips for the same individuals (t = 0.25, d.f. = 5). Similarly, there was no difference in December between the rates of mass loss of birds on trips either with or without packs (mass loss = 3.6 ± 4.9 % day^{-1}, n = 7; t = 1.32, d.f. = 8, paired t = 0.61, d.f. = 2), or syringe barrels (mass loss = 0.2 ± 1.9 % day^{-1}, n = 6; t = 1.18, d.f. = 8, paired t = 1.03, d.f. = 3). The rates of mass change for the penguins are shown in Figure 9.2.

9.3.1.3 Water influx rates

Water influx rates of birds on control and instrumented trips are shown in Figure 9.3. Penguins on control trips had a mean water influx rate of 306.6 ± 47 ml kg^{-1} day^{-1} (n = 6) in September, and 473.6 ± 79.3 ml kg^{-1} day^{-1} (n = 7) in December, and these rates are significantly different (t = 4.51, d.f. = 11, $P < 0.05$). In September, while birds were on trips while carrying mock packs, their mean water influx rate decreased to 159.9 ± 36.8 ml kg^{-1} day^{-1} (n = 7), and this is significantly lower than the rates during the control trips in the same month (t = 6.31, d.f. = 11; paired t = 7.51, d.f. = 5, $P < 0.05$).

In December, birds carrying packs showed water influx rates of 295.0 ± 86.8 ml kg^{-1} day^{-1} (n = 7), and this was significantly higher than birds carrying the same packs in September (t = 3.79, d.f. = 12, $P < 0.05$). The water influx rates of birds while on trips carrying syringes in December was 335.5 ± 84.2 ml kg^{-1} day^{-1} (n = 6), which is not different from the influx rates of birds carrying packs (t = 0.85, d.f. = 11). Birds without attachments in December showed significantly higher rates of water influx when compared to birds either foraging with packs (t = 4.02, d.f. = 12; paired t = 3.90, d.f. = 2), or syringes (t = 3.04, d.f. = 11; paired t = 3.44, d.f. = 3).

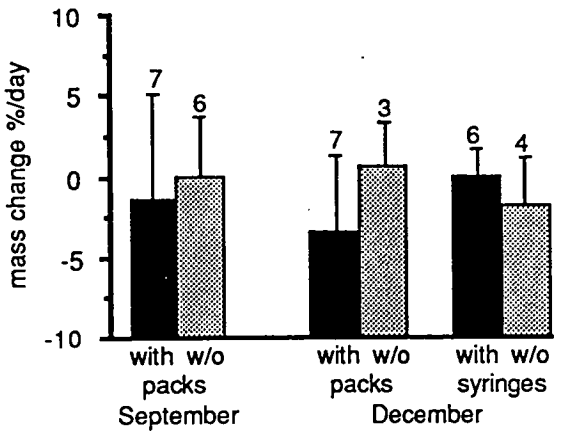

FIGURE 9.2 Mean rates of changes in mass (± SD) of little penguins foraging either with (solid bars) or without (hatched bars) devices attached. Packs constitute 11.8 %, and syringes 1.4 % penguin cross sectional area. Sample size indicated for each group.

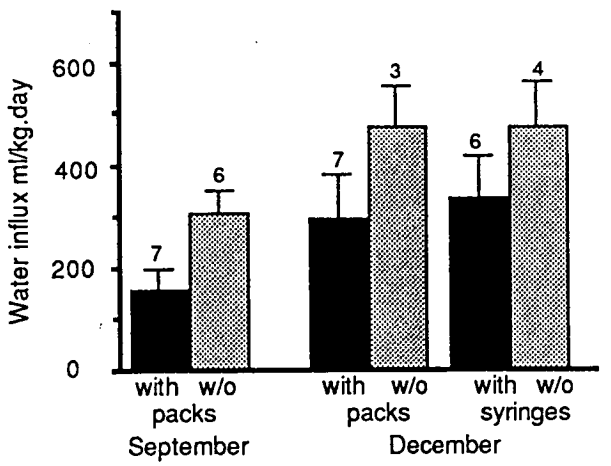

FIGURE 9.3 Mean rates of water influx (± SD) of little penguins foraging either with (solid bars) or without (hatched bars) devices attached. Packs and syringes as in Fig. 9.2. Sample size indicated for each group.

9.3.1.4 Metabolic rates

In September, the mean rate of CO_2 production of penguins carrying packs was 2.21 ± 0.18 ml g^{-1} h^{-1} (n = 7), compared to the higher rate of 2.74 ± 0.21 ml g^{-1} h^{-1} (n = 4) of control birds, and these rates are significantly different (t = 4.34, d.f. = 9; paired t = 3.65, d.f. = 3), (Fig. 9.4). Consequently, the field metabolic rates (FMR) of birds on control trips was also significantly higher (control trips: 1670.5 ± 130.1 kJ kg^{-1} day^{-1}, n = 4; with packs: 1348.8 ± 111.6 kJ kg^{-1} day^{-1}, n = 6). The CO_2 production rates of the birds which carried the electronic activity recorders in December were 2.50 and 3.01 ml g^{-1} h^{-1}, the former value being for the bird carrying the data logger from which data was retrieved. In neither of these birds could I measure CO_2 production rates when they were not carrying the recorders.

9.3.2 FORAGING BEHAVIOUR

The single electronic activity recorder deployed in March was retrieved and the data recovered. In December, of the three meters deployed, one penguin failed to return before my departure from the Island, two meters were retrieved, but only one provided data. The penguin from the March sample was engaged in post-moult foraging, and the penguin in December was raising two chicks, two to three weeks of age. The basic foraging parameters; duration of time at sea, number of records, mean and maximum speed and depths are shown in Table 9.1.

9.3.2.1 Swimming speed and depth

Analyses of the frequency of swimming speeds shows that in both samples, less than 10 % of time was spent swimming at less than 1.0 m s^{-1} (3.6 km h^{-1}), and that most of the time (> 60 %) was spent swimming at speeds greater than 2.4 m s^{-1} (8.6 km h^{-1}) (Fig. 9.5). However, neither penguin reached the maximum speed category of 4.4+ m s^{-1} (15.8+ km h^{-1}). In terms of depth of swimming, over 75 % of the time was spent in the top 5 m, with < 2 % of the time spent at depths deeper than 15 m. The deepest depth category (50+ m) was not reached.

In the March sample, it appeared that as depth increased, swimming speed decreased (Fig. 9.6). The variances around the means however, are high (Table 9.2) and given that the data are recorded in range categories, any biological significance would be speculative. A linear relationship between speed and depth was not evident in the December sample, the two functions appearing independent (Fig. 9.6, Table 9.2). Using the procedure outlined in Section 9.2.4, the duration of dives recorded were 21.7 ± 11.4 s (n = 208 dives) in March, and 20.6 ± 13.2 s (n = 827) in December.

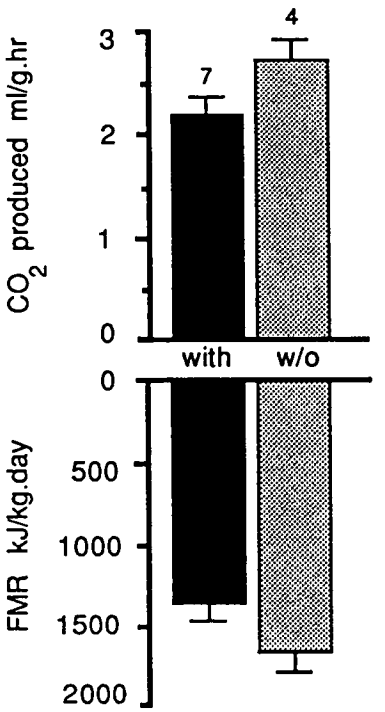

FIGURE 9.4 Mean (± SD) CO_2 production rates and field metabolic rates (FMR) of little penguins foraging in September either with packs (solid bars) or not carrying attachments (hatched bars). Sample sizes indicated for each group.

TABLE 9.1 Foraging parameters of two little penguins

Foraging parameters			March	December
Foraging trip duration			3 hr 5 min	18 hr 2 min
Speed and depth records n			1237	7013
Speed	mean ± SD	m/s	2.4 ± 0.83	2.3 ± 0.54
	mean	km/h	8.6	8.3
	maximum	m/s	3.2	3.2
		km/h	11.5	11.5
Depth	mean ± SD	m	2.1 ± 3.14	1.3 ± 2.94
	maximum	m	15 - 20	20 - 50

TABLE 9.2 Differences between the mean swimming speeds (± 95 % confidence limits) at consecutive depth ranges

Depth ranges (m) compared	Difference between mean speeds (m/s) ± 95% confidence limits	
	March	December
0-2 and 2-5	0.2 ± 0.14	-0.3 ± 0.02
2-5 and 5-7	0.1 ± 0.20	0.1 ± 0.03
5-7 and 7-10	0.5 ± 0.22	-0.1 ± 0.04
7 -10 and 10-15	0.3 ± 0.19	0.1 ± 0.07
10-15 and 15-20	-	0.1 ± 0.10
15-20 and 20-50	-	-0.2 ± 0.19

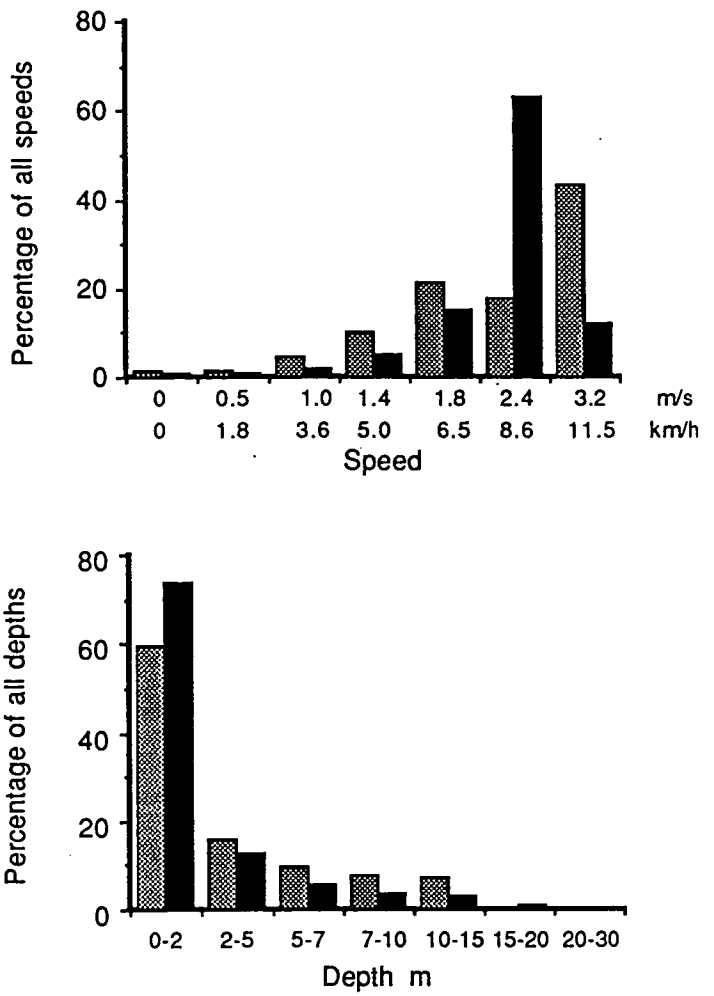

FIGURE 9.5 Frequency histograms of swimming speeds and depths for little penguins, hatched bars for March sample (n = 1237), solid bars for December sample (n = 7013).

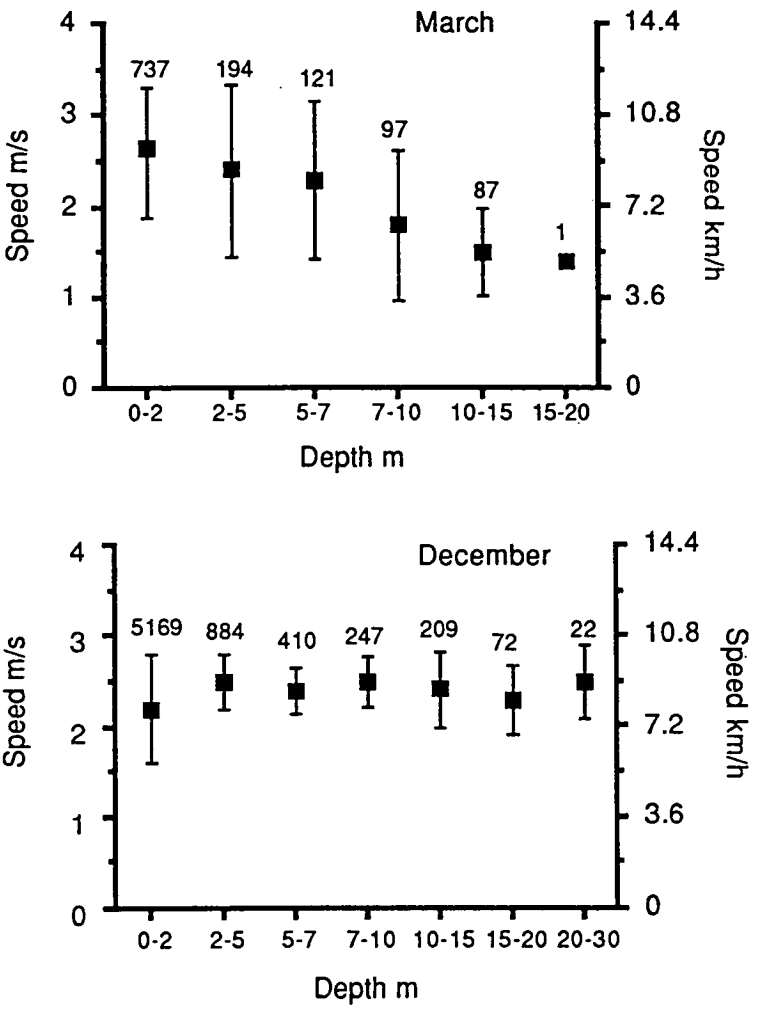

FIGURE 9.6 Relationships between swimming speed and depth for two little penguins, means ± SD. Sample size indicated for each group.

9.3.2.2 Temporal patterns

Frequency profiles of swimming speed and diving depth against time are shown in Figure 9.7. This reflects the predominance of shallow (< 5 m) swimming at reasonably high speeds throughout the foraging period. This pattern is similar in both the March and December samples, but the brevity of the March sample precludes detailed time analyses. In the December record, the first half of the foraging trip was spent almost entirely swimming at speeds between 1.8 and 3.2 m s^{-1} in the top 2 m. In the second half, the speeds were more concentrated in the 2.4 to 3.2 m s^{-1} range, with a more scattered pattern in the depth profile. The use of deeper depths was most prevalent in the middle of the trip.

The distribution of the variances around speed and depth for the December sample is shown in Figure 9.8. The variance around the speed data is high during the first 8 h of foraging, after which it reduces, particularly during the last 5 h. Diving depth data show greater variances during the middle, rather than at either end of the trip. During the first hour the penguin remained entirely in the top 2 m, and only after it had been at sea for more than 3 h, were less than 90 % of the dives in this zone.

9.3.2.3 Foraging behaviour patterns

In the March data, three types of foraging behaviour were identified (Fig. 9.9A). The frequencies of the speed and depths within each type were assessed. Type 1 consisted of reasonably rapid, shallow swimming, with only 5 % of dives exceeding 2 m depth. The second category, type 2, consisted of rapid changes in both swimming speeds and depths which are reflected in the high variations around the the mean speed and depth (Table 9.3). It was during this behaviour mode that the penguin made most dives below 7 m. The final category, type 3, consisted of faster swimming, 70 % of the time at speeds $\geq$ 2.4 m s^{-1}, most of which was within 5 m of the surface. Samples of the data, and the distribution of the types during the foraging trip, are shown in Figure 9.9A.

Examination of the December data identified four types of behaviour (Fig. 9.9B, Table 9.3). During sub-surface travel (type A), 99 % of time is spent in the top 2 m at relatively low speeds. This was uninterrupted for the first 1.5 h of the trip and followed by extended periods of faster, but still shallow travel (type B). After 4.5 h of sub-surface swimming (types A and B), the penguin started diving intensively, type C. This consisted of relatively short bouts of high speed, deep dives, some reaching 20+ m, probably indicating intensive feeding bouts. Each bout was followed immediately by periods in the upper 2 m zone at low speeds (types A and B). Following this the penguin undertook two long periods (2.4 and 1.8 h) of a different foraging behaviour,

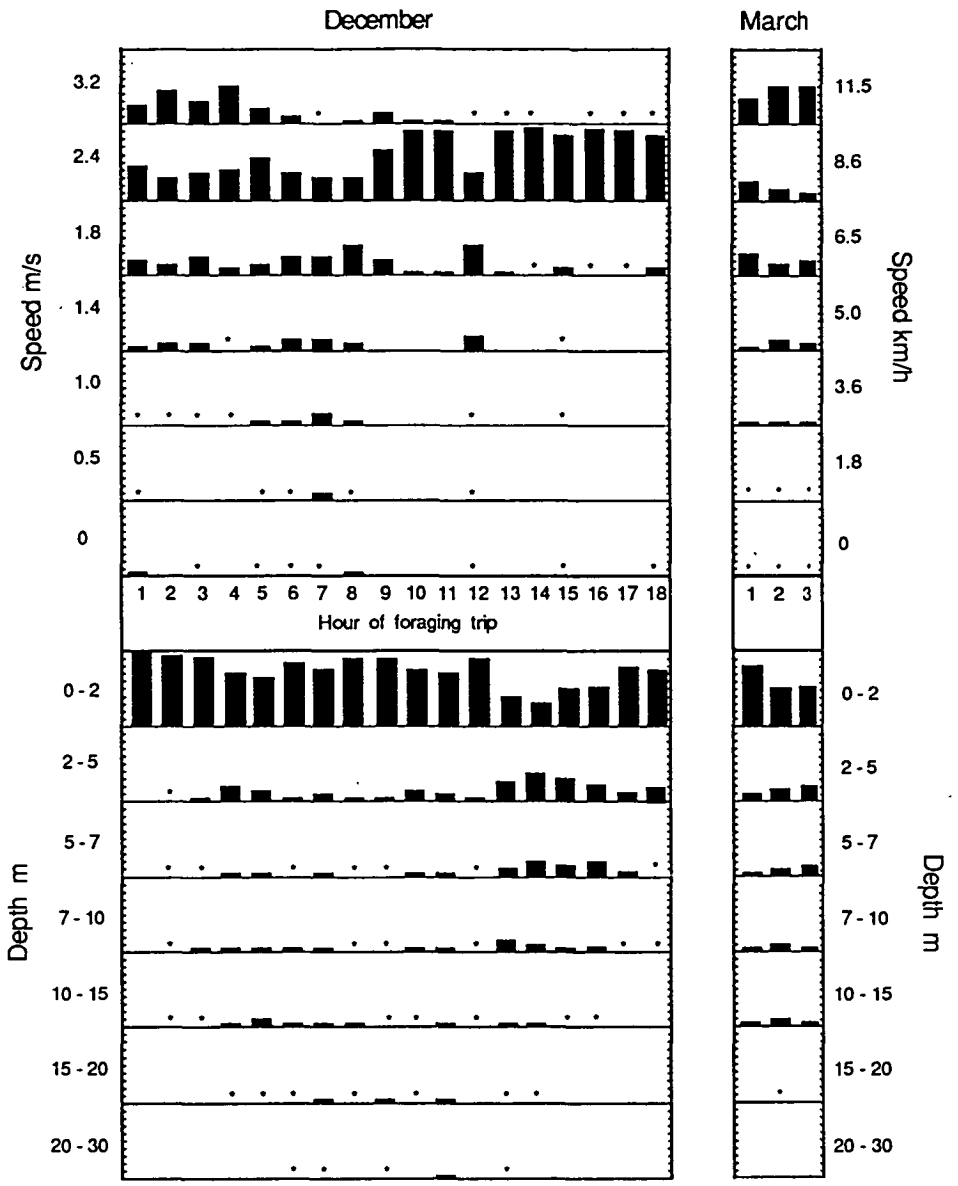

FIGURE 9.7 Frequency profiles of swimming speed and depth during each foraging trip where the % of time spent at each speed and depth category is calculated separately for each hour of the foraging trip. * indicates that in that hour, less than 5 % of time spent in relevant category.

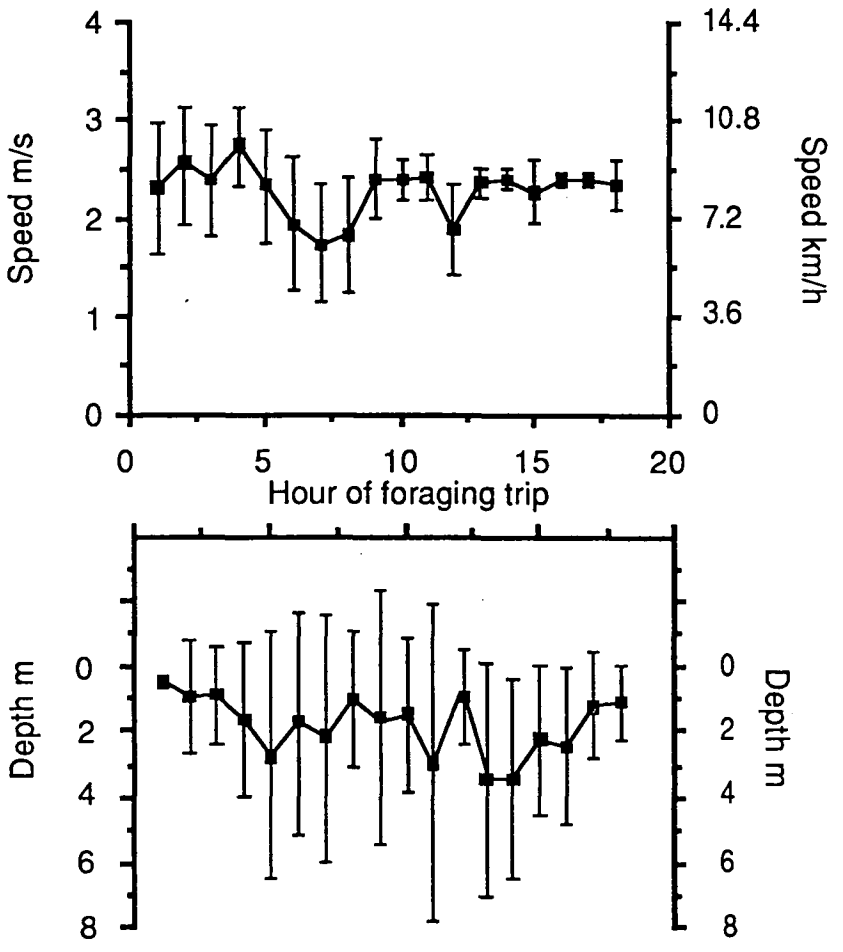

FIGURE 9.8 Mean ± SD swimming speeds and depths during the December foraging trip (total trip time: 18 h 2 min)

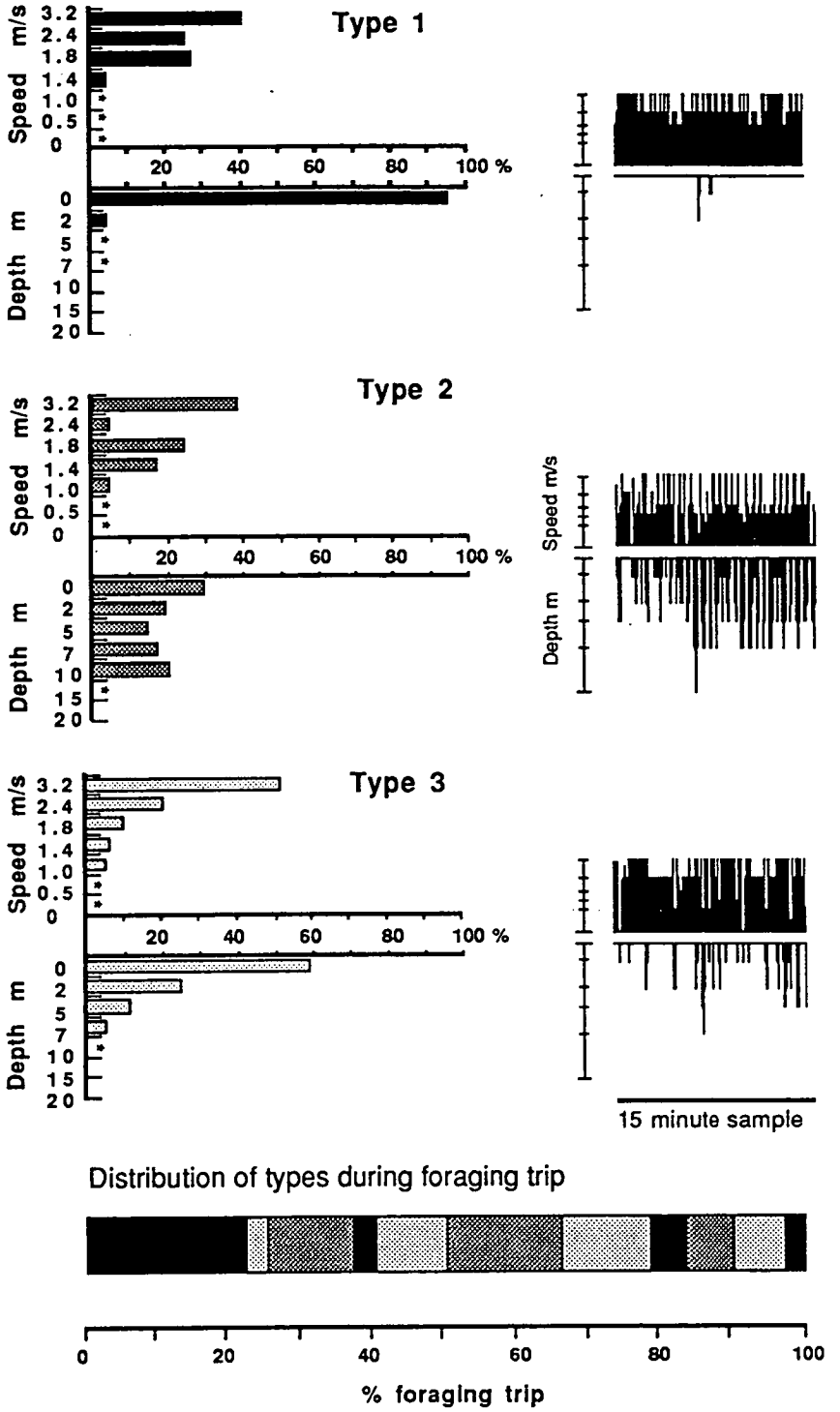

FIGURE 9.9A Characteristics of the three foraging behaviour types identified for the penguin foraging in March, description in Table 9.3 and text. For each type, frequency histograms of swimming speeds and depths, and representative samples (15 min) of the raw data are presented. * signifies that less than 5 % of time in relevant category. The distribution of the three types during the trip are also shown, the shading corresponding to the frequency histograms of each type.

TABLE 9.3 Characteristics and descriptions of the types of foraging behaviours identified for little penguins

Behaviour type	Speed m/s mean ± SD range	Depth m mean ± SD range	n	Behaviour % total	Behaviour description
March					
type 1	2.5 ± 0.7 0 - 3.2	0.1 ± 0.7 0 - 7	388	31.4	underwater travel
type 2	2.2 ± 0.9 0 - 3.2	4.2 ± 3.8 0 - 15	436	35.2	intensive foraging
type 3	2.5 ± 0.8 0 - 3.2	1.6 ± 2.3 0 - 10	413	33.4	foraging + searching plus travel
December					
type A	1.9 ± 0.7 0 - 3.2	0.02 ± 0.3 0 - 7	1997	28.4	slow sub-surface travel
type B	2.5 ± 0.4 0 - 3.2	0.7 ± 1.9 0 - 15	2200	31.4	fast sub-surface travel plus searching
type C	2.4 ± 0.4 0.5 - 3.2	3.5 ± 5.2 0 - 20	996	14.2	intensive foraging
type D	2.4 ± 0.1 0 - 3.2	2.3 ± 2.8 0 - 20	1820	26.0	foraging + travel

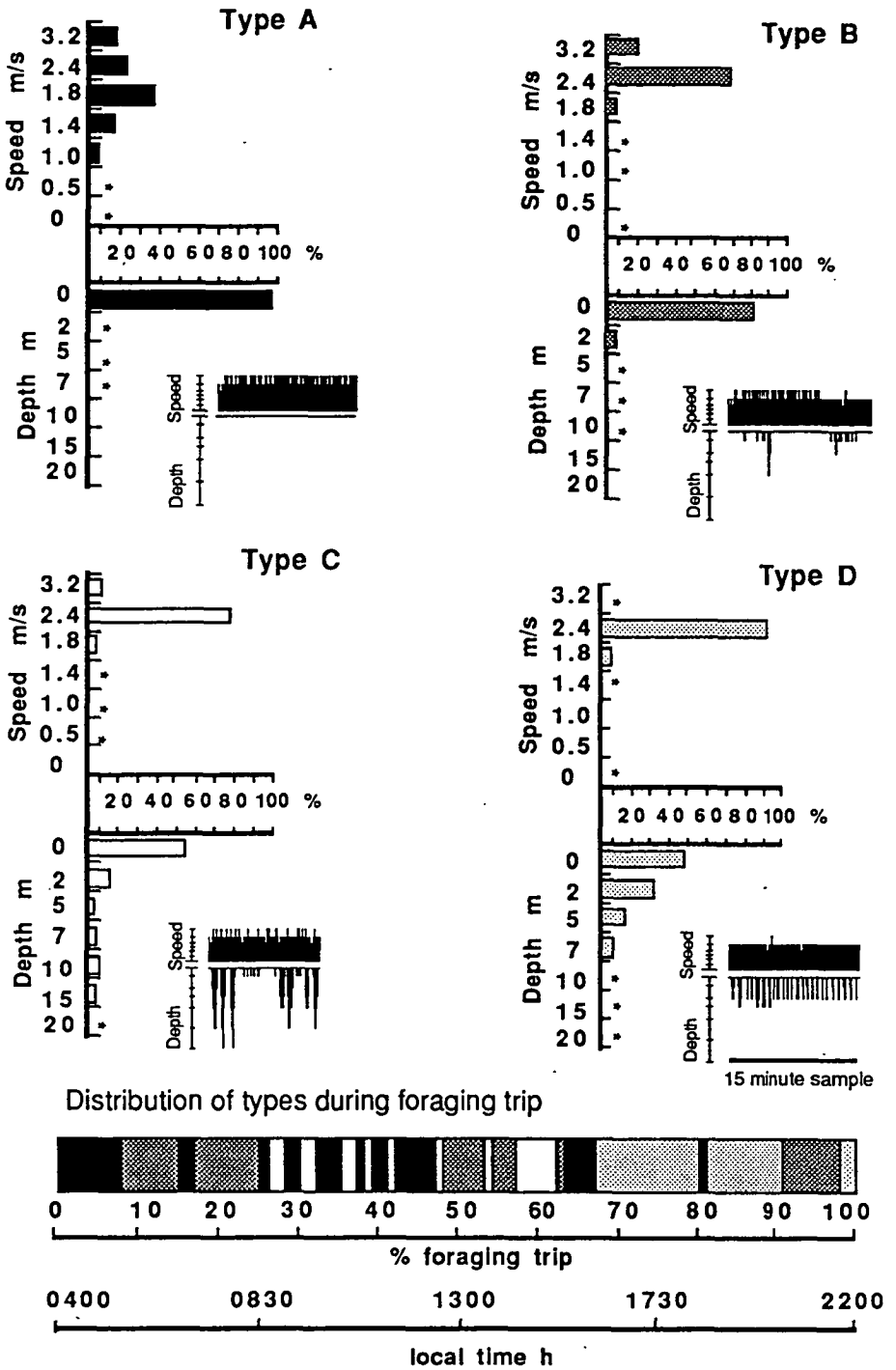

FIGURE 9.9B Characteristics of the four foraging behaviour types identified in the December trip. Format as in Fig. 9.9A. Distribution of the four types are shown in relation to their occurrence during the foraging trip and in relation to local time.

type D. This was characterised by a predominance (90 %) of swimming speeds in the 2.4 to 3.2 m s^{-1} range, over 90 % of which was in the top 7 m.

9.3.2.4 Distance travelled

The first method of calculation of distance travelled resulted in estimates of 26.5 km for the penguin in March and 148.6 km for the December trip. After the vertical components of swimming were extracted (see Section 9.2.4) the revised estimates were 3.6 km in March and 25.9 km in December. Assuming a straight line bearing, these figures represent a maximum foraging distance away from the Island of 1.8 and 13 km respectively.

9.4 DISCUSSION

9.4.1 ASSESSMENT OF INSTRUMENT EFFECT

The development of devices to study the foraging behaviour of aquatic animals is gaining momentum and there are a variety of recorders now available, as well as others still in production stages. Consequently, there is increasing use of recorders in field studies, and widespread acknowledgment of the need to consider the method of attachment, size, shape and mass of the recorder with respect to potential effects on the animals' foraging performance (Wilson *et al.*, 1986; Kenward, 1987; Costa, 1988).

With a new design of recorder, regardless of size, it is important to undertake field testing, although this is difficult to do in the wild. Apart from laboratory experiments (e.g. Obrecht *et al.*, 1988), efforts have been made through comparisons of tagged and untagged animals using such parameters as mass changes, reproductive success (Kenward, 1987), duration of foraging trips (Wilson *et al.*, 1986; Heath, 1987; Croxall *et al.*, 1988), mortality rates (Davis *et al.*, 1988), mass of food brought ashore (Wilson *et al.*, 1986; Croxall *et al.*, 1988) and metabolic rates (Costa & Gentry, 1986; Wilson *et al.*, 1986; Gessaman & Nagy, 1988).

When comparing the duration of foraging trips of jackass penguins fitted with devices by either harnesses or hose clips, Wilson *et al.* (1986) found no significant differences. Heath (1987) reports that, for the same species, use of the same harnesses resulted in feather wear, and reduced manoeuverability and swimming ability, with the result of decreased prey consumption. Both Wilson *et al.* (1986) and Croxall *et al.* (1988) found that durations of foraging trips did not differ significantly between penguins carrying devices or not carrying devices, and this result is consistent with the little penguins in this study. Also, in this study, there were no differences in the changes in mass of the birds while on trips whether or not they were carrying devices (Fig. 9.2).

On the basis of these two parameters alone, it could be concluded that carrying devices did not significantly affect the foraging performance of little penguins. Duration of foraging trips, however, is not a reliable parameter on which to base assessment tests as it does not reflect foraging performance and efficiency, and changes may be difficult to detect in some penguin species (Croxall *et al.*, 1988; Davis *et al.*, 1988). Rates of change in mass may also be an unreliable parameter on which to base such conclusions as there is evidence that in some animals at least, mass trends are generally determined by internal factors, on a seasonal basis, rather than by foraging difficulties (Kenward, 1987).

The mass of food bought ashore by instrumented and non-instrumented gentoo and macaroni penguins did not show any differences (Croxall *et al.*, 1988). Similarly, Wilson *et al.* (1986), found that jackass penguins fitted with small recorders (2.3 % of penguin cross sectional area) returned to the colony with amounts of food similar to that of control birds which were not carrying devices. In the same study, two penguins carrying larger devices (14.6 % penguin cross sectional area) returned in an emaciated condition after extended foraging trips.

One major disadvantage of monitoring the amount of food brought ashore by birds with and without devices is that it does not necessarily reflect the amount of food consumed as digestion proceeds soon after food consumption. (Wilson *et al.*, 1985; Jackson & Ryan, 1986; Gales, 1988*b*, also Chapter 3). Further, the mass of food brought ashore is not indicative of the amount of energy expended to obtain the food. On the basis of lack of detectable differences in food mass brought ashore, and the durations of foraging trips, Croxall *et al.* (1988) concluded that the effect of the meter was probably not great, but noted that there was the possibility that the instrumented birds used more energy than control birds.

Therefore, to assess any recorder affect, I compared the rates of water and energy flux of instrumented and control penguins whilst foraging. Little penguins carrying devices consistently showed significantly lower rates of water influx than when foraging with no devices, and these differences were significant for both small (1.4 % penguin cross sectional area) and large (11.8 % penguin cross sectional area) devices (Fig. 9.3). Although little penguins are known to consume seawater, water intake is predominantly derived from food intake (Green *et al.*, 1988). The consistently lower water influx rates of instrumented penguins therefore most likely represents decreased food intake. In September, the water influx rates of birds on foraging trips while carrying mock recorders was 48 % less than when on control trips. In December, the water influx rates were 38 % less than control trips when birds carried

mock packs or recorders, and 29 % less when carrying syringes. Carrying devices, therefore, significantly affected their performance at sea in terms of food intake.

The penguins carrying packs in December had significantly higher water influx rates than birds in September which were carrying the same packs (Fig. 9.3). The control birds in December also had significantly higher water intakes than in September and this may reflect a temporal difference in prey type, abundance and/or availability, together with the increased demands of chick rearing in December. Conceivably, high food availability could reduce, or offset, the effect of carrying a device, i.e., in periods of low food availability it is likely that the effects of carrying recorders would be greater than in periods of high food availability. Hence the effect of recorder attachment is probably not of a fixed magnitude, but may vary between seasons and locations.

Wilson *et al.* (1986) re-analysed data in Nagy *et al.* (1984) and showed that in jackass penguins, attachment of devices did not result in changes in metabolic rates while foraging. Costa & Gentry (1986) also compared metabolic rates during foraging trips of Northern fur seals (*Callorhinus ursinus*) and found that, overall, there was no significant difference between instrumented and control trips, but when the two types of trips were compared for the same individual (paired comparisons), the metabolic rates were, on average, 19 % higher on trips with recorders.

I found, however, that in terms of energy use, little penguins when carrying packs used 20 % less energy than when not carrying packs (Fig. 9.4). This was less than half the magnitude of the decrease in water influx (48 %) of the same birds. Therefore, when foraging with packs attached, the birds consumed only half the amount food compared to control trips, but showed 80 % of the energy expenditure of control trips. A ratio of water intake and energy expenditure can be used as a measure of foraging efficiency, a low ratio indicating low efficiency. The ratio of water intake to energy use without packs was 0.18 ml kJ^{-1}, and 0.12 ml kJ^{-1} when carrying a pack, a decrease in efficiency of 33 %.

This ratio is likely to change with prey type, abundance or availability. It is conceivable that if only metabolic rates are compared between instrumented and control birds, the results could be similar, but the efficiency ratio could still be lower in the instrumented birds. Unless rates of water and food intake are also examined at the same time, conclusions could be misleading if the energy expenditure is similar between the two groups, given that the instrumented birds may have consumed significantly less prey for the same energy expended.

Wilson *et al.* (1986) found that there was a relationship between the size of instruments and the effect on jackass penguin foraging performance, small devices (2.3 % penguin cross sectional area) having virtually no effect. This is contrary to the results of my study as attachment of open ended syringe barrels (1.4 % penguin cross sectional area) resulted in reduced water influx rates (29 %), compared to control trips. Further, there was no significant difference between birds with either syringes or the larger packs (11.8 % cross sectional area) attached in terms of water intake, despite the large difference in size of devices. I do not have any data on the rate of energy use of these two groups of birds but it is possible that the birds with the smaller syringes expended less energy to obtain their food than the birds with the larger packs.

Wilson *et al.* (1986) also found that travelling speed was related to device size, speed decreasing as device size increased. If I apply this relationship to little penguins, attachment of the syringe barrels would result in a 4 % reduction of speed, and the packs would result in a 35 % reduction in swimming speed. If this reduction is applied to the mean speed of 2.35 m s^{-1} measured with the recorders in this study, the actual speed would be 3.17 m s^{-1}, or 11.4 km h^{-1}, and the maximum speed would have been 17.0 km h^{-1}. However, due to the lack of detectable differences in water influx rates between the penguins with either syringes or packs, and the significantly lower rates of water influx of these birds compared to control birds, I do not consider that Wilson's equation is applicable to little penguins. In flying birds, homing pigeons (*Columba livia*) worked longer and harder when encumbered with transmitters (Gessaman & Nagy, 1988). Transmitters of 2.5 and 5.0 % body mass had the same slowing down effect on flight performance but mean flight metabolism was 53 % and 85 % higher, repectively, than control flights when carrying transmitters (Gessaman & Nagy, 1988).

9.4.2 LITTLE PENGUIN FORAGING BEHAVIOUR

9.4.2.1 Diving depth and duration

The diving depths recorded in this study are comparable with other accounts of little penguin diving and feeding behaviour. The diet of the little penguin in Bass Strait consists of species of small, schooling fish, squid and krill which occur near the surface (Chapter 10). The only previous study of little penguin diving is one in Victoria where capillary pressure tubes were used to record maximum diving depth (Montague, 1985). In that study, the maximum depths achieved ranged from 6 to 69 m, with most records being in the 10 to 20 m depth range. These values are consistent with the two maximum depths achieved by the two penguins in this study, which were in the ranges of 15 - 20 m and 20 - 50 m. The biological significance of maximum depth records, however, is limited as they only show extreme values.

The two continuous records of depths in this study, showed mean depths of 2.1 m and 1.3 m (Table 9.1), indicating the predominance of the use of the upper layers. Three swimming levels for penguins have been discussed by Adams & Brown (1983); swimming on the surface; travelling at depths of 2 m or less; and foraging at depths, greater than 2 m. In general, surface swimming accounts for only a small component of travelling in penguins (Wilson *et al.*, 1986; Trivelpiece *et al.*, 1986; Adams & Wilson, 1987) and in the little penguin it has been shown that submerged swimming is more efficient than surface swimming as, when submerged, they avoid the major component of drag associated with wave formation (Baudinette & Gill, 1985). Thus, time spent at less than 2 m depth was interpreted as travelling, rather than diving. When the 0 - 2 m depth range is excluded, the mean depths were 5.1 ± 3.0 m (n = 500 records, March) and 5.0 ± 3.8 m (n = 1844 records, December).

Dive durations of free-living little penguins timed with stop-watches are short, 24.3 ± 8.8 s, n = 462 (Lalas, 1983) and 23 ± 9 s, n = 31 (Stahel & Gales, 1987), and are similar to the mean dive durations estimated in this study (21.7 s and 20.6 s in March and December respectively). Little penguins dived 208 times in the March trip (69 ± 32 dives hr^{-1}, range = 33 - 93, n = 3) and 827 dives in the December trip (46 ± 32 dives hr^{-1}, range = 0 - 99, n = 18). As little penguin dives are short in duration, time for ascent and descent is limited as some time must be devoted to the pursuit and capture of prey. Little penguin diving therefore is characterised by short, shallow dives, almost exclusively in the top 20 m.

9.4.2.2 Swimming speeds

There are few estimates of swimming speeds in little penguins, and these are summarised in Table 9.4. Recently, Dann & Cullen (1989) concluded that the estimate of 16.2 km h^{-1} made by Norris (1965) was too high and probably erroneous. The swimming speeds measured in the present study, however, are also higher than most previously recorded speeds for the species (Table 9.4). The methods of measurement, however, vary between the studies, and the observations of Dann & Cullen (1989) are restricted to penguins swimming close to shore, and these may not be typical of speeds undertaken when foraging out at sea.

9.4.2.3 Foraging behaviour patterns

From the data I distinguished several types of behavioural patterns, including what I interpreted as travelling and foraging patterns (Table 9.3). In the March record I categorised three types of behaviour (Fig. 9.9A), type 1 - rapid, shallow swimming, type 2 - swimming at variable speeds and depths, and type 3 - faster swimming, at less variable depths. I hypothesize that the type 1 behaviour represents primarily underwater travel, and the type 2 and 3 represent foraging behaviours. It is possible

TABLE 9.4 Comparison of swimming speeds recorded for little penguins

Speed (km/h)				Distance (m) or duration (h) of measurement	Activity	Method of measurement	Source
mean	SD	n	max				
-	-	1	16.2	-	underwater travel	timed from submarine	Norris (1965)
-	-	1	5.7	c.100 m	underwater travel	timed from boat	Barton (1979)
-	-	3	6.2	short	underwater	timed in tank	Clark & Bemis (1979)
6.4	-	6	8.5	19 m	underwater travel	timed from shore	Dann & Cullen (1989)
5.3	-	2	6.5	19 m	surface paddling	timed from shore	Dann & Cullen (1989)
8.6	3.0	1	11.5	3.1 h	entire foraging trip	electronic recorder	this study
8.3	1.9	1	11.5	18.0 h	entire foraging trip	electronic recorder	this study

that type 2 is a more intensive foraging behaviour than 3 which involves a greater degree of travelling and searching, as well as some foraging. Alternatively, the differences between 2 and 3 may be attributed to exploitation of different prey types. The three types occur with relatively equal frequency (Table 9.2) but are not evenly distributed during the trip. Rapid, shallow swimming (type 1), occurs mainly at the beginning of the trip and this may reflect commuting to the foraging ground. It is then interspersed during the trip, between diving and foraging bouts (types 2 and 3), when the bird was probably travelling between foraging locations and returning back to the Island.

In the December sample, four categories were identified. Two of these, I suggest, are primarily associated with underwater travel, and two with foraging (Fig. 9.9B). The two types associated mainly with travel, A and B, differ in that A is the slower of the two and is restricted to the top 2 m. This category probably incorporates some bathing and resting behaviours and occurs at the beginning, as well as during the trip. The mean speed of this type, 6.8 km h^{-1}, is similar to the mean speed of 6.4 km h^{-1} recorded by Dann & Cullen (1989) who timed little penguins as they left the shore.

The second category associated with travel, B, was characterised by extended periods of fast swimming, with intermittent dives below 2 m in depth, and this probably reflects rapid travel and some degree of searching for prey. The most intensive of the two foraging categories, type C, was characterised by rapid bouts of deep diving, and during the first half of the trip, each bout was immediately followed by a period of resting and travelling (type A). This pattern then changed, the duration of bouts of deep diving (type C) increasing, followed by more extended periods in the upper layers (types A and B). Following this, the penguin undertook two long periods (2.4 and 1.8 h) of medium speed travel with regular dives, mainly in the top 7 m (type D), each of which was followed by period of sub-surface travel (A and B). I interpret D as some travelling plus foraging, the foraging pattern being distinct from that of C. The different foraging types again, may represent exploitation of different prey sources. Of the two types, D was used more extensively, and was restricted to the latter half of the trip. This could be interpreted as intensive foraging prior to return to the Island, obtaining food to be fed to the chicks.

Comparing the types of behaviour identified during both trips shows that the patterns of types 1 and B are broadly similar, rapid submerged travel with limited diving to depths greater than 7 m, both types contributing 31 % to the total foraging trips (Table 9.3). The foraging types, however, show differences. These may be a result of inter-individual variation, the difference in the length of the trips, or alternatively, may reflect seasonal differences in prey resources and the demands upon

the two birds, i.e. post-moult recovery in March, and chick raising in December. Substantially more samples, from throughout the year, are required to resolve this question.

Despite the differences in detail, however, the overall pattern which emerges is similar between the two birds, and is supported by the available literature. Little penguin foraging is characterised by relatively shallow diving at high speeds. Thus, despite the fact that they are limited to the depths to which they can dive by their anaerobic capacity and small size, they efficiently exploit prey in the upper layers by rapid and sustained swimming. This strategy is consistent with their diet, which comprises mainly schooling species of fish, squid and krill, all of which are found in relatively shallow water (Chapter 10). The diet of the little penguin is broadly similar to the jackass penguin, and Wilson (1985*b*) presents swimming speeds of fish which are preyed upon by jackass penguins. These speeds, and those of other clupeoids (Blaxter & Hunter, 1982), are less than those which can be achieved, and sustained, by little penguins.

Information about the hunting techniques of the little penguin is largely anecdotal (Dove, 1910; Roberts, 1951; Schultz, 1987) but from all accounts they are pursuit hunters. This strategy is consistent with the swimming speeds and depth ranges from this study, little penguins employing frequent, shallow dives, at speeds which are faster than their prey. Given the limited anaerobic capacity of the little penguins (Mill & Baldwin, 1983), this interpretation of swimming behaviour and hunting strategy is consistent with Woakes (1988) who characterised a diving strategy of some birds, as consisting of using stored oxygen to allow the active tissues to prepare aerobically during relatively short dives, and then to replace oxygen quickly at the surface between dives. This strategy maximizes the time spent foraging, particularly in shallow water, when the time taken to reach the foraging site is a small proportion of the maximum aerobic dive duration.

9.4.2.4 Foraging range

During the breeding season, little penguins generally attend eggs and chicks on a daily basis and so, during that time, their foraging range is restricted to areas relatively close to the breeding colonies. In this study, a simple multiplication of the time spent at each speed category by the speed, produces total distances travelled of 26.5 km (March) and 148.6 km (December). This difference between the two birds is merely a reflection of the difference in the duration of the two foraging trips, as swimming speeds were similar between the two birds, but time spent travelling different (Table 9.1). The modified calculations of distances travelled, which includes movement only in the horizontal plane, in the upper 2 m, are 3.6 km (March) and 25.9

km (December). These figures result in maximum potential foraging ranges away from Albatross Island of 1.8 and 13 km respectively. These are inevitably overestimates as they assume no deviation from a straight line bearing. Recently, little penguins have been radio tracked during foraging trips from Phillip Island, Victoria, and there, during the summer, most birds foraged within a radius of 15 km from their burrows, and most birds' activity was concentrated within about 5 -10 km of the coastline (Weavers, 1987). These data, although from a different area, are similar to the estimates of distance travelled in my study, and are consistent with aspects of their breeding and feeding ecology.

9.4.2.5 Feeding efficiency

Costa (1988) has introduced the approach of incorporating metabolic rate and foraging behaviour data as a diving efficiency index. This approach does not require the size of the prey to be known (see Croxall *et al.*, 1988), and reflects the overall availability of prey energy. My data of simultaneous measurements of foraging behaviour and metabolic rates are restricted to the penguin in December. This bird had a FMR of 1016 kJ/kg.day, and using the chemical composition of fish and squid, and the assimilation efficiency rates for little penguins from Gales (1989; also Chapter 5), I calculated that this bird consumed 221 g if fish was consumed, or 227 g if squid was consumed. If the assessment of the number of dives (827) is correct, then this penguin consumed an average of 0.3 g food per dive. Prey items of little penguins are usually much larger than 0.3 g (with the exception of krill), and so it is unlikely that every dive results in prey capture. For example, if the average prey consumed weighed 2 g, then only a maximum of 13 % of the dives would need to be successful in prey capture, Further analyses of this approach, when comparative data are available may be extremely useful in analyses of foraging efficiencies, both within and between species.

9.4.2.6 Comparison with other penguin species

The diving depths of little penguins recorded in this study are similar to those of jackass penguins which usually do not dive to depths in excess of 30 m (Wilson, 1985*b*). Assuming that time spent in the top 2 to 3 m constitutes travelling rather than foraging, Wilson (1985*b*) found that the mean time spent at potential foraging depths was 12 % of the foraging trip. The two little penguins in this study spent 40.6 % and 24.6 % of time at sea at depths greater than 2 m, and 26.3 % and 13.7 % of the time deeper than 5 m (March and December records respectively). Other species of penguins are capable of, and consistently dive to depths much greater, and for considerably longer periods, than little penguins (summarised in Kooyman & Davis, 1987; Croxall & Lishman, 1987), and these differences have been attributed to differences in body size, anaerobic capabilities and diet (Baldwin *et al.*, 1984; Croxall & Lishman, 1987).

The little penguin is the smallest penguin species and has the most limited anaerobic capacity (Mill & Baldwin, 1983). Consistent with this, their dive durations are generally less than for other penguins for which data are available, with the exception of jackass penguins. In this latter species the average dive duration was measured as 23 ± 20.2 s, n = 138 (Broni, 1985), although this figure is much shorter than the 2.5 min recorded for the same species by R. Wilson (in Adams & Brown, 1983). The number of dives per hour is much greater in little penguins than in gentoo penguins (Trivelpiece *et al.*, 1986; Croxall *et al.*, 1988) and this difference is probably attributable mainly to the fact that gentoo penguins dive considerably deeper and for longer periods.

Diving ability is essentially a function of diving time and swimming speed (Piatt & Nettleship, 1985). Body size is a major factor influencing diving ability (Stonehouse, 1967; Croxall & Lishman, 1987; Davis *et al.*, 1988), but Clark & Bemis (1979), using diving tanks, found no relationship between size of penguin and maximum swimming speed. Swimming speeds of other penguin species have been summarised by Wilson (1985*b*) and Croxall & Lishman (1987). Despite the variety of conditions and methods of measurement, mean speeds show a wide range, and are seemingly unrelated to body size. The highest mean and maximum speeds recorded are 12.4 km h^{-1} and 18.8 km h^{-1} measured by Wilson (1985*b*) for jackass penguins during underwater sprints and these results are higher than for the little penguin in this study (Table 9.4). Many of the comparative data, however, are timed over short distances and hence may not reflect the usual activity of the birds.

Using autoradiographic speedmeters, Wilson & Wilson (in press) report the mean swimming speed of spheniscid penguins as 4.5 km h^{-1}, although the "normal" travelling speeds are higher (magellanic penguin *Spheniscus magellanicus*, 7.6 km h^{-1}; Humboldt penguin *S. humboldti*, 6.8 km h^{-1}; jackass penguin 7.3 km h^{-1}). More recently, using the same method, Adams & Wilson (1987) reported mean speeds for gentoo penguins (≈ 6 kg) as 7.9 km h^{-1}, and 8.7 km h^{-1} for king penguins (≈ 15 kg) (Adams, 1987), and Brown (1987) measured swimming speeds of between 6.9 and 8.2 km h^{-1} for mararoni (*c* 4.5 kg) and rockhopper (≈ 2.5 kg) penguins. This range of values incorporates the mean swimming speeds recorded here for little penguins (≈ 1 kg) (Table 9.4), despite the 15-fold variation in body mass.

Consistent between all studies is the observation that penguins do not swim at low speeds for sustained periods. There is also the contention of an energetically optimum travelling speed (Adams, 1987; Adams & Wilson, 1987), and this is generally a high speed. In my study, both little penguins spent less than 3 % of the time at sea swimming at speeds less than 3.6 km/h and the swimming speeds were

concentrated at the higher speed categories (Fig. 9.5). The energetically optimum speed for the species probably lies within this range of speeds, although this may change both with location and season.

9.5 SUMMARY

I investigated the foraging behaviour of little penguins using a new archival electronic activity recorder which simultaneously measures speed and depth against time. I present the first integrated data of foraging behaviour of two little penguins, from which I was able to distinguish between several types of travelling and foraging behaviours. From these two samples, little penguins forage mainly within the top 15 m, at mean speeds of about 8 - 9 km h^{-1}, and probably within 10 km of their breeding colony during the breeding season. These data, although limited, have important implications for 1) understanding the role of diving ability in foraging efficiency and energy use, 2) the inter-individual and seasonal variation in foraging behaviour and, 3) competition between commercial fishery operations and little penguins. The results of more extensive use of activity recorders will at least be a start in addressing these important questions. In the interpretation of these data I believe, however, that there is no simple or fixed relationship between the size of a meter and the effect on the bearer. Using attachments ranging between 1.4 and 11.8 % penguin cross sectional area (0.1 and 6 % penguin mass), and isotopic water, I also assessed the effects of carrying devices while foraging. Both water influx and metabolic rates were significantly lower in penguins carrying devices, compared to penguins foraging without devices attached. Even the relatively small attachments resulted in a decreased foraging efficiency. Further studies examining the rates of water influx and energy use on birds at different times of year, and consideration of other parameters such as reproductive success, will aid in solving the question, but substantially more work is required in order to fully interpret results gained with recording devices.

CHAPTER 10

SEASONAL AND LOCAL VARIATION IN THE DIET OF THE LITTLE PENGUIN IN TASMANIA

10.1 INTRODUCTION

The little penguin, *Eudyptula minor*, the smallest of all penguins, is restricted to the Australasian region. Within its Australian range, the area of highest concentration of little penguins is in Bass Strait. In this region, between the island of Tasmania and mainland Victoria, the breeding population is estimated at between 150 000 to 200 000 pairs (N. Brothers, unpublished data), and as the penguins remain in the region throughout the year, they are considered important members of the local marine ecosystem.

Earlier work on the diet of little penguins was restricted to anecdotal accounts by enthusiastic naturalists, who reported that small fish were the principal prey item (Dove, 1910; O'Brien, 1940; Roberts, 1951). Since then later workers over the last decade have quantified the diet composition of little penguins in Victoria (Montague, 1982; Cullen & Montague, 1987; Montague & Cullen, 1988), Western Australia (Klomp & Wooller, 1988*a*) and Codfish Island, New Zealand (van Heezik, 1988). The results of these studies show that the prey species vary significantly between seasons, years and locations, but consist predominantly of small, schooling animals.

In the present study I investigated the diet of little penguins at three sites around Tasmania, over a period of twenty months, encompassing two successive breeding seasons. Prior to this study, there was no information concerning the diet of the species in Tasmanian waters. This information was considered necessary in order to examine the apparent annual, regional and seasonal differences which occur in the diet of little penguins. The study was carried out simultaneously with a study of the free-living energetics (Chapter 8) and foraging behaviour of little penguins (Chapter 9) in Bass Strait. The results of all three facets of the study are inter-dependent and simultaneous investigation of these aspects is essential to extend our understanding of the role of the little penguin in the marine ecosystem.

This study also differed from the other investigations of little penguin diet in that stomach samples were obtained using the multiple stomach-flushing technique and extensive use was made of the diagnostic prey remains which persist in the stomachs of seabirds. The material from Bass Strait was treated in greatest detail, concentrating on prey species and sizes of prey individuals consumed. Sexual differences in diet were also examined to check whether the dimorphism in little

penguin body mass and beak size (Gales, 1988*a*; also Chapter 2) was reflected in the diet and the amount of food brought ashore. Biases inherent in the analyses of diets of seabirds which consume a catholic diet are discussed, particularly those aspects relevant to the results of this study. The diet of the little penguin is then discussed with reference to both intra- and inter-specific comparisons, as well as an analysis of the potential competition between little penguins and commercial fishing operations.

10.2 METHODS

10.2.1 STUDY SPECIES AND SITES

The breeding season of little penguins varies considerably in timing and duration (Gales, 1985), and in Tasmania they usually breed during summer and moult after breeding. Both parents share equally in the care of eggs and feeding of chicks, and nest relief periods are short, usually between one and two days. Little penguins are diurnal foragers, leaving the colony in the hour preceding dawn and generally returning in the first hour after sunset.

In the present study, stomach samples were collected as soon as penguins arrived back from sea at the three sites around Tasmania: Fort Direction, Marion Bay and Albatross Island (Fig. 10.1). The little penguin colony at Fort Direction is small (< 100 pairs) and is situated on a headland protruding into Storm Bay, adjacent to the mouth of the Derwent River. The Marion Bay site, on the exposed south-east coast of Tasmania, has a breeding colony of 400 - 600 pairs. Albatross Island has an estimated breeding population of 500 pairs of little penguins and is situated north of Tasmania in north-west Bass Strait, a relatively broad area of continental shelf between Tasmania and Victoria.

10.2.2 COLLECTION OF FOOD SAMPLES

A total of 761 little penguins were stomach flushed as they arrived ashore in order to obtain stomach contents using the water off-loading technique (Wilson, 1984). The penguins were flushed several times to ensure collection of complete stomach contents (Gales, 1987*b*, also Chapter 3). The frequency of sampling and the number of samples collected at the three sites are shown in Table 10.1. Sample sizes were low in some months, particularly at Fort Direction, as fewer birds come ashore during winter months and so sampling at this time, particularly at small colonies, becomes increasingly difficult. Captured birds were also weighed to the nearest 10 g using a spring balance, sexed from beak measurements (Gales, 1988*a*, also Chapter 2) and banded with stainless steel flipper bands. After being stomach flushed, birds were held for about one hour to ensure complete recovery before being released at their point of capture. No individuals were sampled more than once during a four month period.

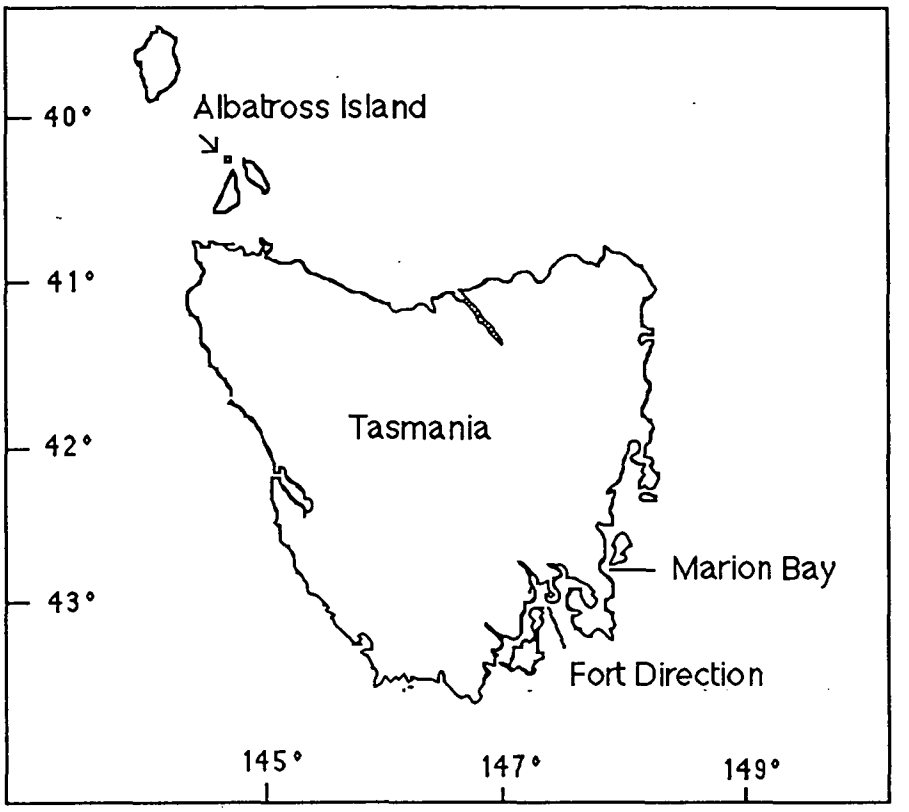

FIGURE 10.1
Map of Tasmania showing location of the three sampling sites

TABLE 10.1 Sampling protocol and numbers of penguins stomach flushed at the three sites around Tasmania.

	Marion Bay	Fort Direction	Albatross Island
Sampling period	Jul 1984 - Feb 1986	Aug 1984 - Feb 1986	Sep 1984 - Jan 1986
Sampling frequency	monthly	monthly	2 - 4 month intervals
No. sampled/month or visit mean ± SD	22 ± 10/month	7 ± 3/month	25 ± 4/visit
Total number sampled	447	138	176
No. penguins with food n (%)	399 (89 %)	125 (91 %)	153 (87 %)

TABLE 10.2 Frequency of occurrence (%) of prey in the stomach contents of little penguins in Tasmania. n equals total number of samples with food

	Marion Bay	Fort Direction	Albatross Island
n	399	125	153
Fish	81	98	85
Cephalopods	80	14	96
Crustaceans	53	11	55
Gastropods	0	0	3

Stomach samples were allowed to settle and excess water decanted off through a 0.5 mm sieve. In this procedure some very fine, highly digested material in suspension was drained off, but before being discarded this suspension was examined for the presence of any diagnostic prey remains. Stomach contents were then stored in alcohol and returned to the laboratory for analyses.

10.2.3 ANALYSIS OF FOOD SAMPLES

In the laboratory the samples were drained, blotted dry and weighed to the nearest 1 g. The whole sample was transferred to a large tray and sorted into fish, cephalopod and crustacean components which were weighed separately to the nearest 0.5 g. Any highly digested, un-identifiable material was assumed to be distributed in proportion to the composition of the identifiable material (Croxall *et al.*, 1985). Intact prey was kept to aid in identification of more digested, diagnostic prey remains. The diet composition from all three sites was assessed as frequency of occurrence (FOO); number of individuals and wet mass.

10.2.3.1 Fish

Loose otoliths were collected from the stomach samples and those still enclosed in fish crania were removed. All otoliths were cleaned, dried, counted and examined to assess the degree of digestion (Gales, 1988*b*; also Chapter 4).

Fish and otoliths were identified by direct comparison with a reference collection compiled for this purpose (R. Gales, unpublished data), and then verified with fish taxonomists at CSIRO Division of Fisheries and Oceanography, Hobart. Otoliths were paired by species and size to estimate the number of fish consumed, together with the number of intact heads enclosing paired otoliths. When large numbers of otoliths were present, they were counted, identified and then the total number divided by two to obtain the total number of fish, odd otoliths being assumed to represent an additional fish.

The lengths of all identified otoliths from Albatross Island samples that showed no signs of digestion were measured using a binocular microscope fitted with a graticule. Regressions relating otolith length (OL) to fish length (standard length, SL; total length, TL; or fork length, FL) and mass (W) were calculated to allow estimation of the original fish size (see Appendix 2). Regressions were obtained from the biometric data derived from the otolith reference collection with the exception of the relationship between length and mass of blue grenadier *Macruronus novaezelandiae* from Lalas (1983). Insufficient reference data precluded length and mass estimates of some fish species. Fish nomenclature followed Last *et al.* (1983).

10.2.3.2 Cephalopods

All loose cephalopod beaks were removed from the stomach contents, sorted into upper and lower beaks, counted and stored in alcohol. Intact buccal masses were counted separately before extraction of lower beaks. The total number of cephalopods was estimated from the combined number of loose lower beaks and intact buccal masses. Intact specimens and beaks were identified, where possible, by comparison with reference material obtained from Dr. C. C. Lu (National Museum of Victoria, Melbourne) and from the literature (Clarke, 1986). Regressions relating lower rostral length (LRL: Clarke, 1962) to dorsal mantle length (DML) and body mass (W) (see Appendix 2) were applied to all squid beaks from Albatross Island samples that did not show signs of digestion in order to estimate the size of squid.

10.2.3.3 Crustaceans

All crustaceans were counted and identified to the lowest taxonomic level possible. The identification of brachyuran megalopa and stomatopod larvae to species or genus level was not possible due to the lack of information on the composition of the crustacean fauna in Tasmanian waters. All krill from Albatross Island samples were examined and intact specimens were measured (Standard length 6: carapace length (CL) from the anterior edge of the carapace to the posterior lateral edge of the carapace; Kirkwood, 1982) using an ocular micrometer in a binocular microscope. CL was then converted to total length (Standard length 1- total length (TL) from the tip of the rostrum to the tip of the uropod; Kirkwood, 1982) using the appropriate relationship (see Appendix 2). Carapace length (standard length 6) was selected for measurement as this is not affected by damage to the rostrum or preservation in alcohol (Mauchline, 1980).

10.2.4 Statistics

All values are given as means ± S.D. Differences between means were tested by two-tailed t-tests or ANOVA. Following a significant result from ANOVA, comparisons between means were tested using Fisher's protected least significant difference test (PLSD). In all tests, the 5 % level of probability was accepted as indicating statistical significance.

10.3 RESULTS

10.3.1 Sample size and sex ratio

Of the 761 penguins which were stomach flushed at the three locations, 677 (89 %) of the birds produced food samples (Table 10.1) and the remaining 84 (11 %) penguins regurgitated bile stained water indicating that their stomachs were empty (Gales, 1987*b*; Wilson *et al.*, 1985; also Chapter 3). Birds with empty stomachs occurred more frequently during the non-breeding season (Fig. 10.2).

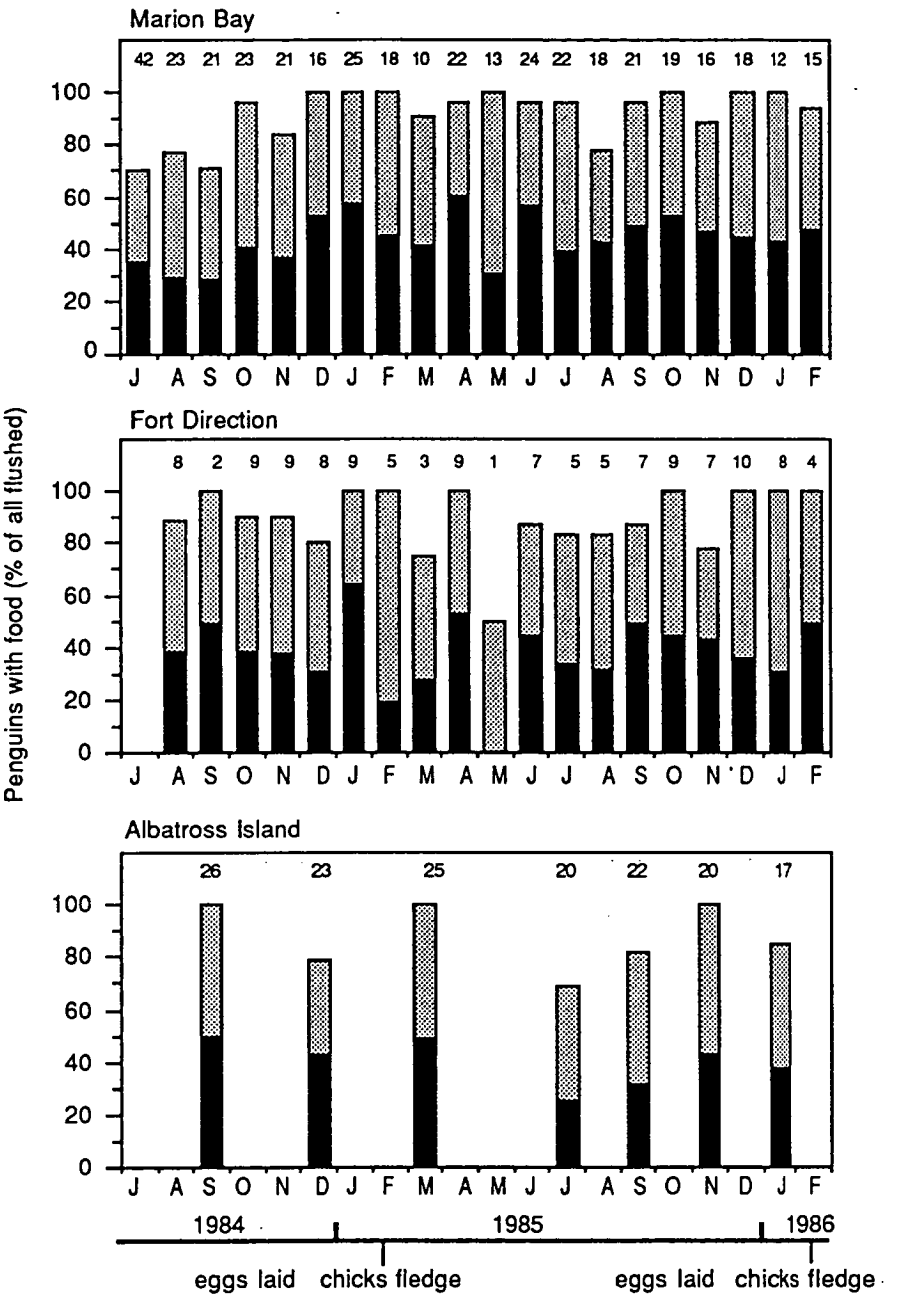

FIGURE 10.2 Per cent of all penguins flushed which produced food. Number of birds producing food shown above bars, proportion of males (solid bars) and females (stippled bars) indicated.

The sex ratio of all the penguins which had food in their stomachs was close to parity; 49 : 51 % (female : male). At Marion Bay 47 % (n = 189) were females , 47 % (n = 59) at Fort Direction, and 53 % (n = 81) at Albatross Island (Fig. 10.2).

10.3.2 PENGUIN BODY MASS

Penguin body masses were compared between sites for the common seven months in which sampling took place at all three sites and there was no significant difference ($F_{2, 308} = 2.09$, ns; Fig. 10.3). Penguin body mass varied seasonally and this was significant ($F_{6, 304} = 4.32$, $P < 0.001$). Maximum body masses were recorded subsequent to breeding when penguins were preparing for moult. Males were significantly heavier (mean mass: 1207 ± 169 g, n = 151) than females (mean mass: 1120 ± 138 g, n = 160) ($F_{1, 309} = 24.67$, $P < 0.001$; all data pooled).

10.3.3 STOMACH CONTENT MASS

The mean wet mass of stomach contents collected for each month at each site are shown in Figure 10.3. The mean wet mass of the 399 stomach contents collected over the 20 months at Marion Bay was 30.9 ± 22.6 g (2.5 ± 1.8 % adult body mass), 47.0 ± 29.2 g (3.7 ± 2.2 % adult body mass) for the 125 samples from Fort Direction over 19 months, and 40.3 ± 31.9 g (3.3 ± 2.5 % adult body mass) for the 153 samples from Albatross Island over seven months. When data from each site are compared for the common seven months for which data are available from all three sites, there was a significant difference in sample mass between sites ($F_{2, 306} = 3.92$, $P < 0.05$), with the heaviest stomach samples collected at Fort Direction (44.9 ± 52.8 g, n = 40), followed by Albatross Island (38.6 ± 49.9 g, n = 153) and Marion Bay samples (25.2 ± 39.6 g, n = 118). When these data are compared within each of the seven months between sites, there were significant local differences in sample mass in only two of the months (September 1984: $F_{2,46} = 5.64$, $P < 0.01$; November 1985: $F_{2,40} = 14.11$, $P < 0.001$).

When wet masses of stomach contents are compared between the seven months for each site, there were significant seasonal differences for Marion Bay ($F_{6,111} = 8.59$, $P < 0.001$) and Albatross Island ($F_{6,146} = 12.30$, $P < 0.001$) but there was no significant seasonal variation in the mass of samples from Fort Direction ($F_{6,33} = 1.35$, ns). However, when the sample mass data from Fort Direction for all 19 months of sampling are compared, the seasonal variation was significant ($F_{18,106} = 2.11$, $P < 0.01$). At all sites the mass of stomach samples was highest when adults were feeding chicks. Variation within months at each site was also highest during the summer months during chick feeding and prior to moult. Mass of stomach contents did not differ between sexes.

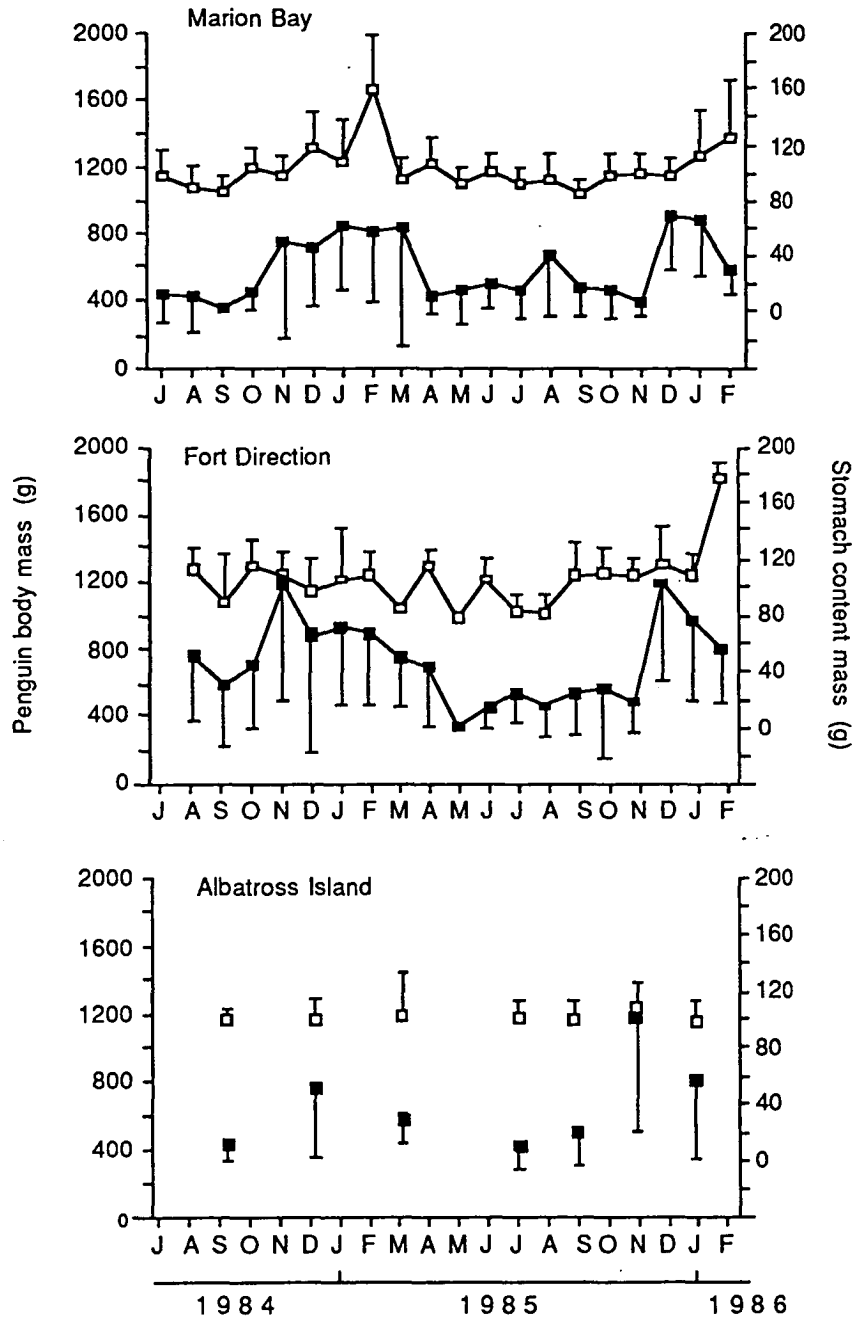

FIGURE 10.3 Penguin body mass (open box) and mass of stomach contents (closed box) collected from penguins at each site (mean ± SD).

10.3.4 General composition of the diet

10.3.4.1 Frequency of occurrence

At all sites, fish was an important prey item in terms of frequency of occurrence (Table 10.2) and appeared in the diet in every month of sampling (Figs. 10.4 & 10.5). Cephalopods were also an important prey group for penguins at Marion Bay and Albatross Island and occurred in the diet every month, but were infrequently eaten by penguins at Fort Direction. Penguins at Fort Direction also rarely consumed crustaceans, while this prey taxon occurred in approximately 54 % of penguin stomach samples from Albatross Island and Marion Bay. Gastropod larvae were only consumed by penguins at Albatross Island, but only occurred in five of the 153 samples.

10.3.4.2 Composition by numbers

Fish was the most important prey taxon in terms of numbers consumed at all sites (Table 10.3). The diet expressed as per cent composition by numbers of the three taxa was similar at Marion Bay and Albatross Island, and at these sites fish accounted for between 50 and 55 % of all items eaten, followed by crustaceans (30 - 35 %) and cephalopods (14 - 16 %), respectively. At Fort Direction, fish accounted for almost 95 % of all items consumed. At Marion Bay, more fish were consumed than items in any other taxon between November and April, with cephalopods and crustaceans being eaten more frequently during the non-breeding season (Fig. 10.4). At Fort Direction, fish constituted over 60 % of the items eaten in all but two of the 19 months, and over 90 % in all but four months (Fig. 10.4). At Albatross Island, the proportion of fish consumed was highest during September of both years, with crustaceans being most numerous in December 1984 and November 1985. Cephalopods were the most numerous in the diet of penguins at Albatross Island during March and July 1985 (Fig. 10. 5).

The mean number of fish per stomach varied through the sampling period at each site (Fig. 10.6). At all sites fewest fish were present in stomach contents collected during winter, with peaks generally occurring between September and January. These peaks were similar in magnitude in the two summer sampling periods recorded at Albatross Island, with between 150 and 180 fish/stomach being recorded in September 1984 and 1985. At Marion Bay and Fort Direction the peaks were higher in magnitude (400 - 600 fish/stomach) but these occurred only in the latter part of 1985. At the same period in the previous year the numbers of fish/stomach were much lower. In total, most fish (66 - 75 %) were represented from counts of heads in stomach contents from all sites (Table 10.3). The numbers of fish/stomach assessed by either paired otoliths or loose diagnostic remains, however, varied during the sampling period at each site (Fig. 10.6). At Albatross Island the two major peaks comprised fish assessed

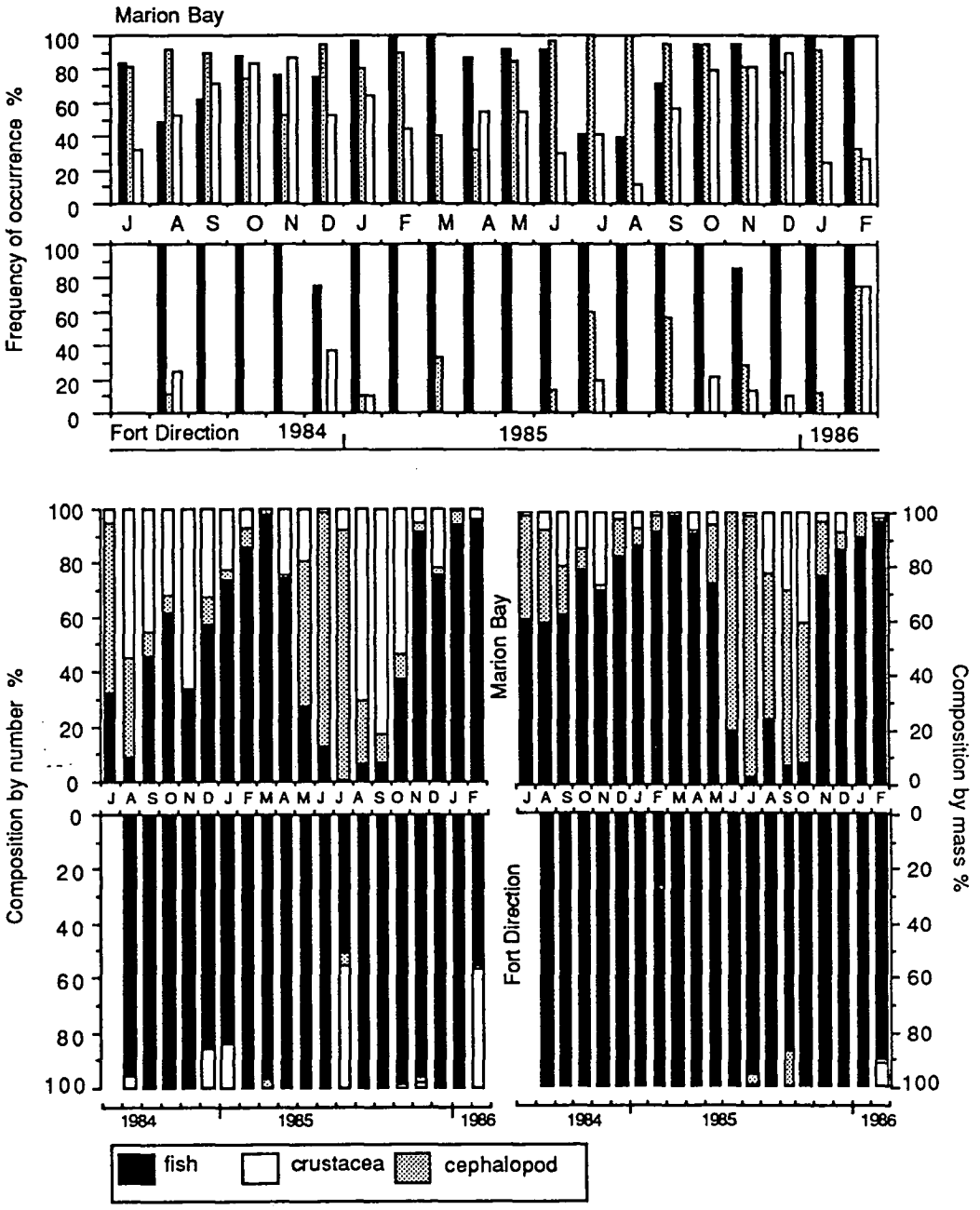

FIGURE 10.4 General composition of little penguin diet from Marion Bay and Fort Direction expressed as frequency of occurrence (%) , composition by number (%) and by wet mass of the three prey taxa.

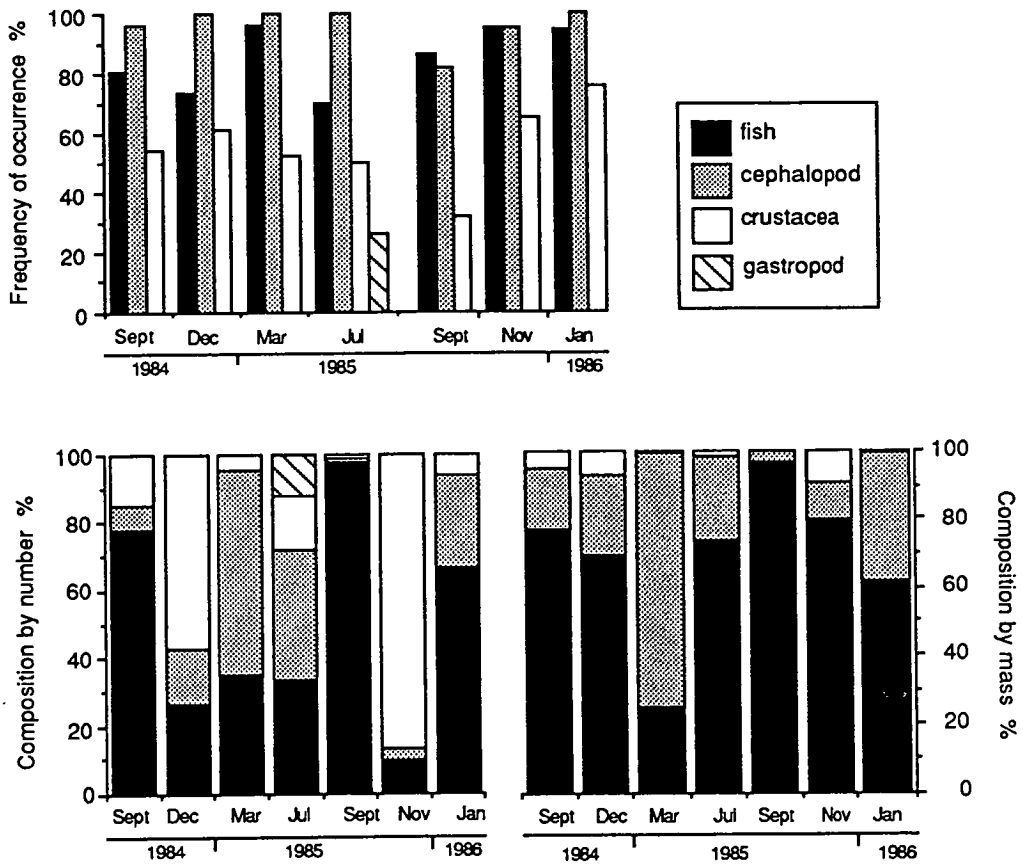

FIGURE 10.5 General composition of little penguin diet from Albatross Island expressed as frequency of occurrence (%), composition by number (%) and by wet mass of the three prey taxa.

TABLE 10.3 Composition by numbers (n) of fish, cephalopods and crustaceans in the stomach contents of little penguins. Contribution within fish and cephalopod taxa indicated by n and (%) of either paired or loose otoliths and beaks, and for crustaceans indicated by n and % of the three identified groups. Contribution of each taxa for penguins at each site indicated by n and (%) of total numbers.

Site		Fish			Cephalopods			Crustaceans			
		paired otoliths	loose otoliths #	total	paired beaks	loose beaks	total	krill	brachyuran megalopa	stomatopod	total
Marion Bay	n	30 128	10 865	40 993	5 000	50 706	10 706	8 246	6 370	8 540	23 156
	%	(73.5)	(26.5)	**(54.8)**	(46.7)	(53.3)	**(14.3)**	(35.6)	(27.5)	(36.9)	**(30.9)**
Fort Direction	n	10 841	3 760	14 601	16	37	53	85	646	21	782
	%	(74.2)	(25.8)	**(94.6)**	(30.2)	(69.8)	**(0.3)**	(10.9)	(86.4)	(2.7)	**(5.1)**
Albatross Island *	n	7 866	3 899	11 765	1 830	1 807	3 637	6 059	191	1 832	8 082
	%	(66.9)	(33.1)	**(50.0)**	(50.3)	(49.7)	**(15.5)**	(75.0)	(2.3)	(22.7)	**(34.3)**

* Samples from this site also include Gastropod larvae (n = 43) which constitute 0.2 % of n for this site.
\# Also included are other diagnostic remains e.g. mandibles and dorsal spines

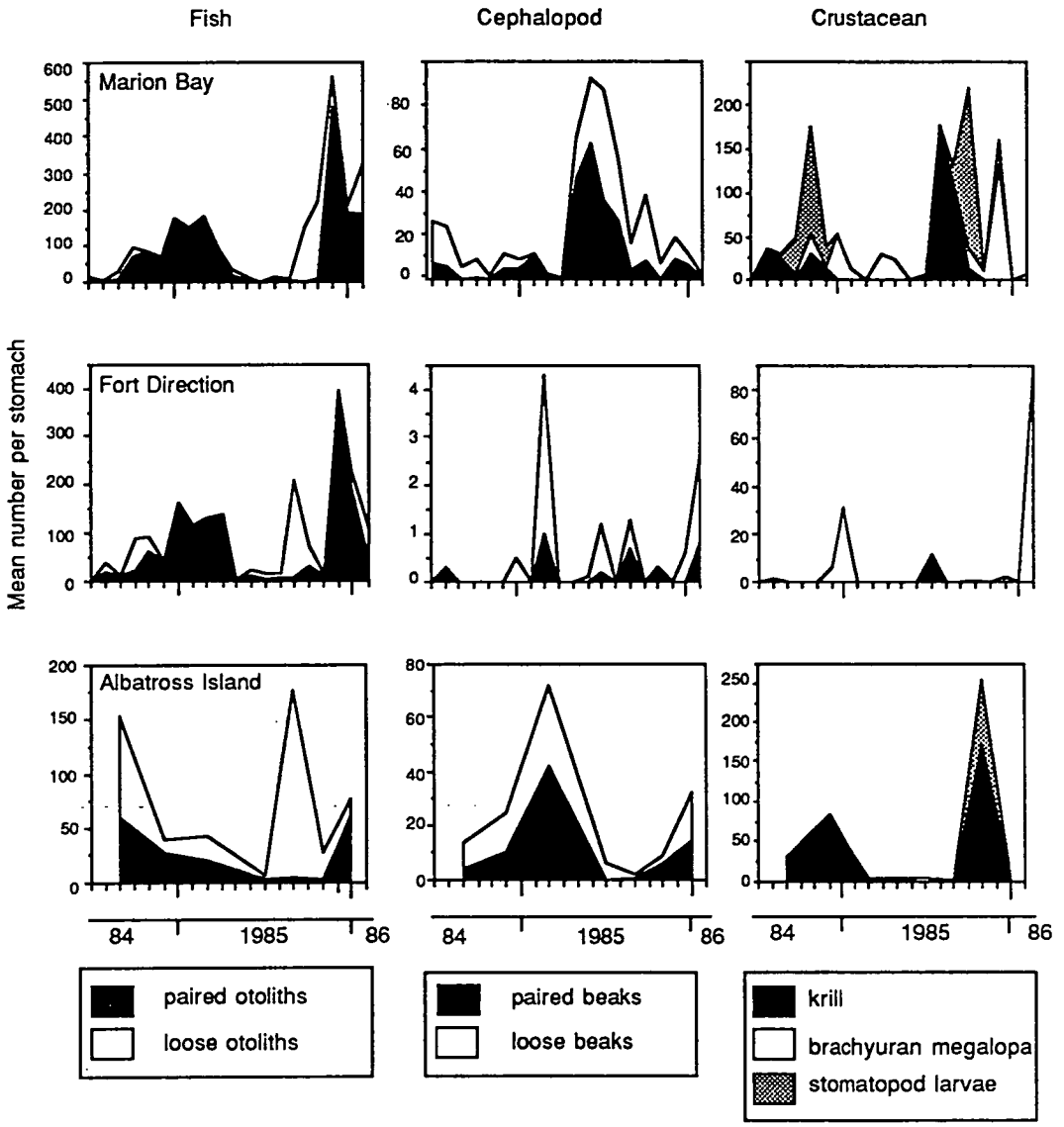

FIGURE 10.6 Mean number of fish, cephalopods and crustaceans per stomach from little penguins in Tasmania. Contribution within each taxon identified by paired otoliths or beaks and loose diagnostic remains are shown for fish and cephalopods, and the contribution of the three identified groups shown for crustaceans.

by large numbers of loose otoliths, but at Fort Direction and Marion Bay, most fish were accounted for by counts of intact heads, the largest numbers of fish diagnostic remains occurring in stomachs during September and October 1985, respectively.

The contribution of counts of paired beaks enclosed in the buccal mass, and the number of loose lower beaks, used to assess the number of cephalopods per stomach, were almost equal at both Marion Bay and Albatross Island. At Fort Direction, however, loose beaks accounted for almost 70 % of instances of occurrence (Table 10.3). The numbers of cephalopods/stomach from penguins at Marion Bay showed one major peak (95/stomach) over the sampling period, during the non-breeding period between May - August 1985 and an earlier peak (March 1985: 72/stomach) was evident at Albatross Island (Fig. 10.6). At Fort Direction, the highest number of cephalopods recorded per stomach occurred in March 1985 (4.2/stomach), at this site relatively few cephalopods were consumed at any time of year. The ratios of cephalopods in stomachs represented by paired or loose beaks were generally consistent, with both being evident in stomach contents at all times of year.

The contribution of the different crustaceans; krill, brachyuran megalopa, stomatopod larvae, varied considerably both between sites and seasonally (Table 10.3, Fig. 10. 6). At Marion Bay all three types were fairly evenly represented in total numbers, brachyuran megalopa were most numerous at Fort Direction (86 %), and krill were most numerous at Albatross Island (75 %). At Marion Bay, stomatopod larvae occurred in large numbers in November 1984 and October 1985, with krill generally occurring earlier in the season, particularly in August and September 1985. Brachyuran megalopa were eaten at several times during the year at this site, with highest numbers occurring in December 1985.

At Fort Direction, crustaceans only constituted 5 % of numbers of all prey items consumed, and these were primarily the brachyuran megalopa eaten between December and February in both years. At Albatross Island, there were two peaks in krill consumption, both occurring over summer, a period when stomatopod larvae were also consumed. Crustacean consumption by penguins was consistently low over the winter months.

10.3.4.3 Composition by wet mass

The diet of little penguins, expressed in terms of wet mass in stomach contents, is shown in Table 10.4. At all sites, fish predominanted in the diet followed by cephalopods and crustaceans, respectively. The diet at the different sites differed in the per cent contribution by each taxon and the seasonal pattern also varied between sites with respect to the relative contribution (% wet mass) by taxon (Fig. 10.7). At

TABLE 10.4 Composition by wet mass (g) of food in the stomach contents of little penguins.

Site	Fish	Cephalopods	Crustaceans
Marion Bay	7 948.5 (71.0 %)	2 253.5 (20.2 %)	986.5 (8.8 %)
Fort Direction	6 530.0 (99.1 %)	34.0 (0.5 %)	27.5 (0.4 %)
Albatross Island	4 054.0 (68.3 %)	1 540.0 (25.9 %)	346.0 (5.8 %)

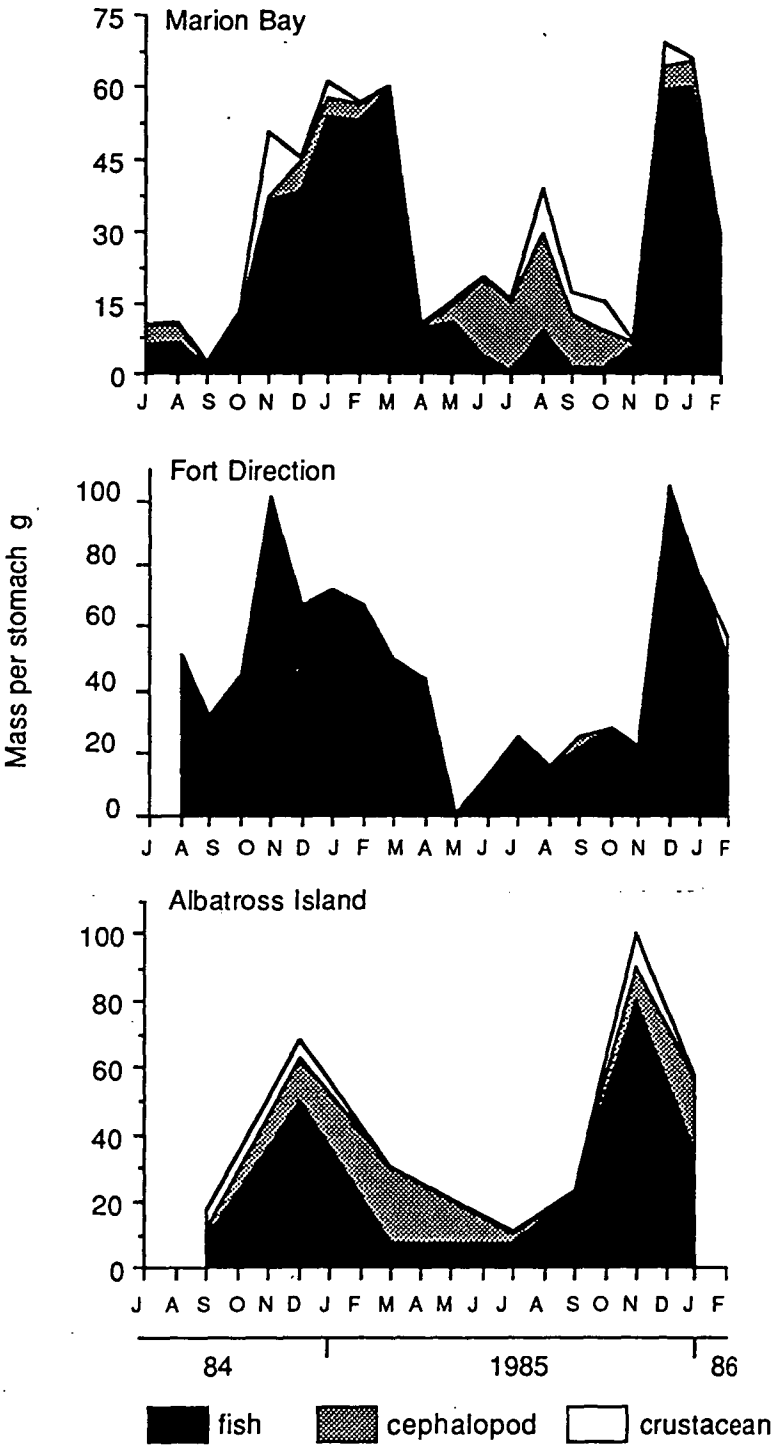

FIGURE 10.7 Composition of little penguin diet shown as wet mass of prey taxa in stomach contents.

Fort Direction, the diet was almost exclusively fish, with cephalopods and crustaceans combined accounting for less than 1 % mass. At Marion Bay fish contributed most mass during the breeding season, with cephalopods being more important than fish in the months from June to October. At Albatross Island, cephalopods were eaten in larger quantities than fish only in March 1985, but consistently consumed in addition to fish during the breeding season. Contributions to the diet by crustaceans was greatest during summer at Albatross Island, but at all sites crustaceans contributed only to a small degree on a mass/stomach basis.

10.3.5 DETAILED COMPOSITION OF THE DIET

Detailed analyses of the diet of little penguins was limited to the stomach samples collected from penguins from Albatross Island. The numbers and frequency of occurrence of identified species are shown in Table 10.5. There were no significant differences in the prey species composition of the diet between male and female little penguins at Albatross Island.

10.3.5.1 Fish

From the total of the 11 765 fish counted from the Albatross Island stomach samples, 17 species were identified and a further two types could not be identified below genus level (Table 10.5). In addition, a species complex could be identified only as post-larval fish; the degree of digestion combined with the small size and undifferentiated nature of the otoliths of fish within this group preventing further identification. Approximately half (50.1 %) of the fish identified were juvenile blue grenadier and these occurred in 23.5 % of all stomach samples. The post-larval group were the next most abundant group of fish and accounted for 23.9 % of the fish diet, occurring in 18.3 % of all stomachs. The remaining 18 species together constituted the remaining 26 % of the fish diet with no other single species accounting for more than 6 %.

The seasonal variation in the fish species recorded in samples is shown in Table 10.6 and the per cent composition by numbers of the eight fish species/groups which each constituted at least 25 % of the fish diet in terms of numbers in any one month is shown in Figure 10.8. While blue grenadier was the most numerous fish species, their presence was restricted essentially to two months, September 1984 and 1985, with two individuals also being recorded in November 1985. Post-larval fish were also restricted to the penguin breeding season, being replaced by other species in the non-breeding, winter period. Seven species of fish were consumed by little penguins in winter (July 1985) and four of these were eaten only at this time.

TABLE 10.5 Frequency of occurrence (FOO) and numbers of prey items identified from little penguin stomach samples from Albatross Island

Prey item		Number	% of fish diet	% of total diet	FOO %
FISH					
Blue grenadier	*Macruronus novaezelandiae*	5 898	50.1	25.1	23.5
Post larval fish	Unidentified species	2 808	23.9	11.9	18.3
Barracouta	*Thyrsites atun*	700	5.9	3.0	35.9
Leatherjacket	*Meuschenia* sp.	534	4.5	2.3	12.4
Warehou	*Seriolella brama*	509	4.3	2.2	23.5
South Australian garfish	*Hyporhamphus melanochir*	507	4.3	2.1	15.0
Red cod	*Pseudophysis bachus*	483	4.1	2.0	12.4
Big bellied sea horse	*Hippocampus abdominalis*	70	0.6	0.3	2.0
Blue sprat	*Spratelloides robustus*	66	0.6	0.3	4.6
Spotted trevalla	*Seriolella punctata*	61	0.5	0.3	8.5
Pilchard	*Sardinops neopilchardus*	59	0.5	0.2	9.1
Atherinid	Atherinidae species	32	0.3	0.1	2.6
Jack mackerel	*Trachurus declivis*	15	0.1	0.1	3.3
Anchovy	*Engraulis australis*	9	0.1	<0.1	2.0
Pipefish	*Stigmatopora* sp.	5	<0.1	<0.1	0.6
Sprat	*Clupea bassensis*	3	<0.1	<0.1	0.6
Silver trevally	*Pseudocaranx dentex*	2	<0.1	<0.1	0.6
Gemfish	*Rexia solandri*	2	<0.1	<0.1	0.6
Silverside	*Argentina australiae*	1	<0.1	<0.1	0.6
Dragonet	*Bovichthys variegatus*	1	<0.1	<0.1	0.6
		Number	% of cephalopod diet	% of total diet	FOO %
CEPHALOPODS					
Gould's squid	*Nototodarus gouldi*	3 351	92.1	14.2	94.8
Octopod	Unidentified species	286	7.9	1.2	17.6
		Number	% of crustacean diet	% of total diet	FOO %
CRUSTACEANS					
Krill	*Nyctiphanes australis*	6059	75.0	25.7	32.7
Stomatopod larvae	Unidentified species	1832	22.7	7.8	13.1
Brachyuran megalopa	Unidentified species	191	2.3	0.8	19.6
		Number	% of gastropod diet	% of total diet	FOO %
GASTROPODS					
Larvae	Unidentified species	43	100.0	0.2	0.6

TABLE 10.6 Number of fish removed each month from little penguin stomach samples from Albatross Island. ANOVA indicates significance of differences in size of fish within species measured from samples between months (ns not significant; * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$)

Species	Sep 1984	Dec 1984	Mar 1985	Jul 1985	Sep 1985	Nov 1985	Jan 1986	ANOVA
Blue grenadier	1997				3899	2		***
Post larval-fish	1527	563	339				379	
Barracouta		134	160			45	361	***
Leatherjacket			34				500	ns
Warehou	1	62				419	27	***
South Australian garfish	2		503	2				***
Red cod	451	7	7		1	10	7	***
Big bellied sea horse		70						
Blue sprat				66				
Spotted trevalla		20				39	2	ns
Pilchard		24		6		3	26	*
Atherinid				32				
Jack mackerel						13	2	***
Anchovy				3		2	4	**
Pipefish		1		4				
Sprat				3				
Silver trevally		2						
Gemfish		2						
Silverside							1	
Dragonet						1		

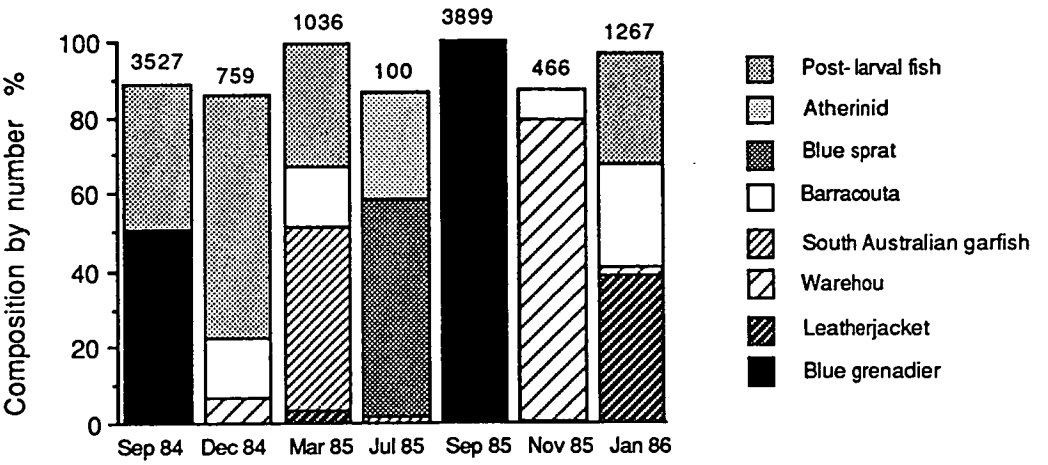

FIGURE 10.8 Composition by numbers (%) of the fish component of the diet of little penguins at Albatross Island. Inclusion of fish species restricted to those species/groups which comprised > 25 % of the fish diet in at least one month of sampling. Total numbers of these fish species shown for each month.

Of the 11 765 fish identified, measurements could be made of 33 % of the otoliths (n = 3 921), the remainder being too digested to allow reliable estimates of length and mass. The lengths of the measured otoliths and the estimated fish sizes are shown in Table 10.7. Individuals of most fish species were small, mean lengths ranging between 11.5 and 80.5 mm, and mean masses ranging between 0.03 and 5.2 g. The largest fish consumed was an atherinid which was estimated as 141 mm (standard length) and 31 g mass. Ten of the identified fish species were consumed in more than one month of sampling, and in eight species the size of fish showed significant seasonal variation (Table 10.6; Appendix 3).

The size-frequency of blue grenadier eaten by little penguins was bi-modal, and the sizes of individuals consumed in September 1984 and 1985 were significantly different (ANOVA: $F_{1, 2052} = 3\ 269.6$, $P < 0.0001$) (Figure 10.9). All of the blue grenadier consumed ranged between 17 and 76 mm total length (mass: 0.01 - 1.41 g), but those consumed in 1984 were considerably smaller (1984 mean TL: 27.2 ± 3.8; 1985 mean TL: 49.4 ± 10.2). In September of both years there were no significant differences between the sizes of otoliths which were recovered from the stomachs either loose or still encased in the crania (1984: t = 1.02, df = 767, ns; 1985: t = 1.36, df = 1 283, ns). Similar comparisons of otolith size were made on other species of fish and in no case was there a significant difference.

10.3.5.2 Cephalopods

Of the 3 637 cephalopod beaks, 7.9 % (n = 286) were from octopus and 92.1 % (n = 3 351) from squid (Table 10.5). Ninety-two per cent (n = 3 070) of the squid beaks were measured since evidence of digestion was infrequent. After close inspection and measurement, further identification of squid beaks was problematic due to the small size of beaks, 99.3 % (n = 3047) of those measured having a lower rostral length (LRL) of < 2 mm. The squid beaks with a LRL > 2 mm, and also intact specimens which were recovered from the stomach contents were identified as Gould's squid (*Nototodarus gouldi*) and the general appearance of the smaller beaks suggested that they probably belonged to the same species. I assumed, therefore, that all squid beaks belonged to Gould's squid, although it must be recognised that a species complex may be present among the smaller individuals. Similar problems of small size occurred with the octopod beaks and so no further breakdown can be given.

Octopods were most numerous in September and December 1984, when they represented between 25 and 30 % of the cephalopods in the diet. Very few were present at other times of the year. Gould's squid were present in the diet during each month of sampling with highest numbers occurring between December and March (Table 10.8).

TABLE 10.7 Summary of otolith length (OL), fish length (standard length SL, total length TL, fork length FL) and mass of fish removed from little penguin stomach samples from Albatross Island

Species	Code *	n	OL mm			Length mm				Mass g		
			Mean	S.D.	Range		Mean	S.D.	Range	Mean	S.D.	Range
Blue grenadier	a	2054	1.3	0.5	0.5 - 2.5	TL	41.1	13.6	17.5 - 75.7	0.3	0.3	0.01 - 1.41
Barracouta	b	552	1.6	0.5	0.7 - 3.8	FL	46.1	21.1	13.3 - 146.9	0.6	1.3	0.01 - 12.16
Leatherjacket	b, e	259	-	-	-	SL	15.5	3.4	10.0 - 29.0	0.1	0.1	0.03 - 0.55
Warehou	b	479	2.1	0.7	0.7 - 4.4	FL	48.8	17.4	13.6 - 110.3	3.0	3.4	0.04 - 25.60
South Australian garfish	b	126	1.0	0.3	0.7 - 2.2	SL	69.5	16.4	51.3 - 131.1	0.4	0.6	0.10 - 3.47
Red cod	c	216	1.4	0.6	0.9 - 4.8	TL	11.5	9.6	5.3 - 80.4	0.03	0.2	9.8E-5 - 2.4
Blue sprat	b	66	0.9	0.4	0.4 - 1.9	TL	50.3	22.6	21.3 - 104.6	1.3	1.7	0.03 - 9.12
Spotted trevalla	b, f	58	1.9	0.3	1.2 - 2.8	FL	43.1	8.5	25.1 - 65.9	1.6	1.0	0.26 - 5.22
Pilchard	b	45	1.5	0.5	0.8 - 3.0	SL	57.2	23.0	23.7 - 141.3	3.0	4.7	0.12 - 31.30
Atherinid	a, g	32	2.5	0.3	2.0 - 3.0	TL	74.1	7.8	59.4 - 89.9	2.4	0.5	1.37 - 3.49
Jack mackerel	d	15	2.1	0.5	1.4 - 3.1	-	-	-	-	-	-	-
Anchovy	a	9	2.2	0.5	1.7 - 3.0	SL	80.5	13.5	67.6 - 102.9	5.2	3.0	2.62 - 10.84
Pipefish	d	4	-	-	-	TL	72.8	6.6	65.0 - 80.0	-	-	-
Sprat	d	3	1.5	0.2	1.3 - 1.7	-	-	-	-	-	-	-
Gemfish	d	2	1.9	0.1	1.8 - 2.0							
Dragonet	d	1	-	-	-	SL	95.0	-	-	-	-	-

* Codes a - f define degree of precision of equations used for predicting fish length and mass (Appendix 2)
a: HIGH: equations based on data which includes range of predicted fish lengths, equation data base sample size > 20.
b: MEDIUM: equation data base includes maximum but not minimum length of predicted fish length, equation data base sample size < 20.
c: LOW: equation based on small sample (< 10) large individuals only
d: No predictive equation for species
e: Mass predictions based on equation of related species (Bridled leatherjacket *Acanthalutheres spilomelanurus)*
f: Length and mass predictions based on equation of related species (Warehou *Seriolella brama)*
g: Length and mass predictions based on equation of related species (Silverfish *Atherinason presbytoides)*

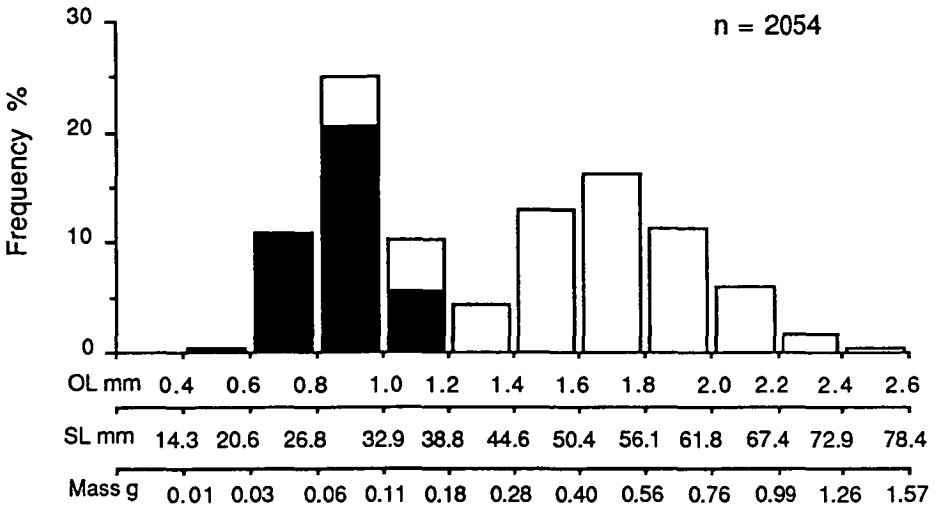

FIGURE 10.9 Length-frequency distribution of blue grenadier eaten by little penguins at Albatross Island during September 1984 (closed bars) and September 1985 (open bars). Estimated standard length (SL) and mass of fish given on separate scale. n refers to total number of otoliths measured.

TABLE 10.8 Number of octopods and squid, and summary of lower rostral length (LRL), dorsal mantle length (DML) and mass of squid removed from little penguin stomachs from Albatross Island

Month	Octopod Total n	Squid Total n	Squid Measured n	LRL mm Mean	LRL mm S.D.	LRL mm Range	DML mm Mean	DML mm S.D.	DML mm Range	Mass g Mean	Mass g S.D.	Mass g Range
September 1984	91	266	259	1.0	0.4	0.3 - 2.4	40.8	17.5	11.3 - 100.1	2.4	1.6	0.3 - 8.9
December 1984	168	396	361	0.9	0.3	0.3 - 2.6	36.6	14.5	11.3 - 108.6	2.0	1.3	0.3 - 10.2
March 1985	0	1 796	1 629	0.6	0.3	0.2 - 2.2	25.9	13.4	7.1 - 91.7	1.2	1.0	0.2 - 7.8
July 1985	2	134	134	1.1	0.4	0.4 - 2.8	45.3	18.8	15.5 - 117.0	2.8	2.0	0.5 - 11.4
September 1985	1	51	50	0.6	0.2	0.3 - 1.5	23.9	9.3	11.3 - 62.0	1.0	0.7	0.3 - 4.2
November 1985	23	161	159	1.0	0.3	0.4 - 2.1	39.9	12.8	15.5 - 87.4	2.2	1.1	0.5 - 7.2
January 1986	1	547	478	0.8	0.3	0.2 - 2.4	31.5	13.0	7.1 - 100.1	1.6	1.0	0.2 - 8.9
Total	286	3 351	3 070	0.8	0.4	0.2 - 2.8	30.8	15.4	7.1 - 117.0	1.6	1.3	0.2 - 11.4

Most of the 3 070 squid beaks measured were < 1 mm LRL (72.8 %, n = 2 235), the mean size being equivalent to a dorsal mantle length (DML) of 30.8 mm and mass of 1.6 g (Table 10.8). The largest squid taken by a little penguin was estimated to be 11.7 cm (DML) and 11.4 g. The size-frequency distribution of Gould's squid is shown in Figure 10.10 which shows the predominance of small animals. There was a significant seasonal variation in the size of squid eaten by little penguins at Albatross Island (ANOVA: $F_{6, 3063} = 102.2$, $P < 0.0001$). The size-frequency distributions, plotted on a monthly basis (Fig. 10.11), show that the largest individuals occurred in July 1985, and the smallest in March and September 1985. There were also annual differences, the squid consumed in September 1984 being significantly larger than those consumed at the same time in 1985 (Fishers PLSD: $F = 0.101$, $P < 0.05$).

10.3.5.3 Crustaceans

The contribution of the three identified types of crustaceans have already been shown to vary between sites and season (Table 10.3, Fig 10. 6). The brachyuran megalopa and stomatopod larvae could not be identified to lower taxonomic levels, but all the krill were identified as *Nyctiphanes australis*. The numbers of crustaceans present in the diet of little penguins at Albatross Island during each month of sampling are shown in Table 10.9, and within the crustacean component of the diet, krill predominated in four of the seven months.

Of the 6 059 krill identified from Albatross Island samples only 411 (6.8 %) could be reliably measured due to advanced digestion. The size-frequency distribution of these individuals is shown in Figure 10.12. Most individuals measured (75.7 %, n = 311) were between 15 and 18 mm total length. The size of krill consumed during the sampling period showed significant seasonal variation (ANOVA: $F_{4, 406} = 61.7$, df = 410, $P < 0.0001$), and a summary of the sizes recorded are shown for each month in Table 10.9. The largest krill were found in September 1984, prior to a significant decrease in size in December 1984 (Fishers PLSD: $F = 0.09$, $P < 0.05$). Detailed analyses of changes in size over the sampling period were thwarted by extremely small sample sizes in March 1985 and January 1986, and the results from these periods may not reflect real trends. The largest krill consumed was estimated at 20 mm total length.

10.4 DISCUSSION

10.4.1 INTERPRETATION OF RESULTS

Stomach contents of seabirds tend to be highly digested upon recovery when the birds return ashore, and this makes analyses of dietary samples subject to a variety of biases. These problems have been addressed by a number of workers (e.g., Croxall *et al.*, 1985, 1988; Duffy & Jackson, 1986; Adams & Klages 1987; Brown & Klages, 1987; Gales 1987*b*, also Chapter 3) and yet there is no clear solution to overcoming

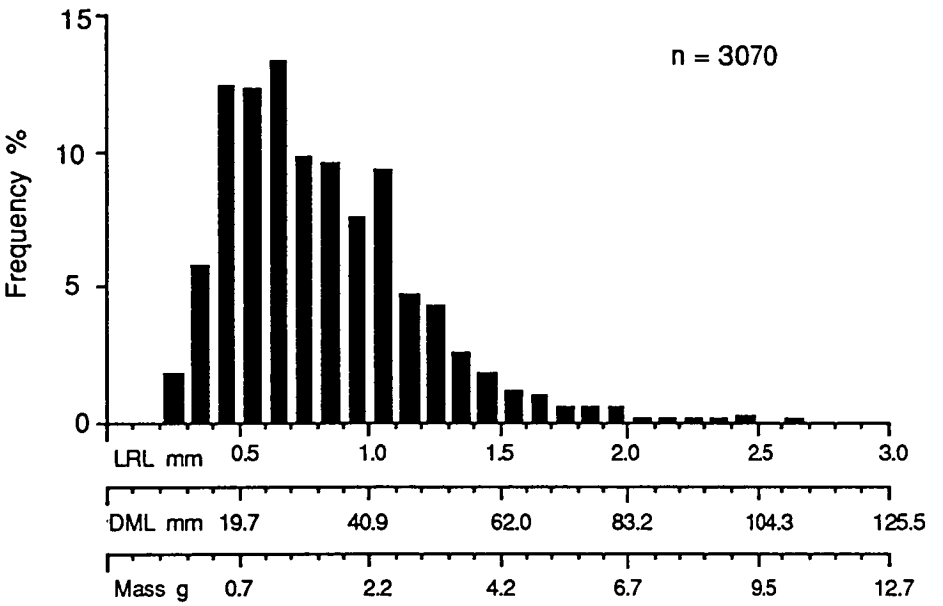

FIGURE 10.10 Length-frequency distribution of lower rostral beak lengths (LRL) of Gould's squid consumed by little penguins at Albatross Island. Estimated dorsal mantle length (DML) and mass of whole squid given on separate scale

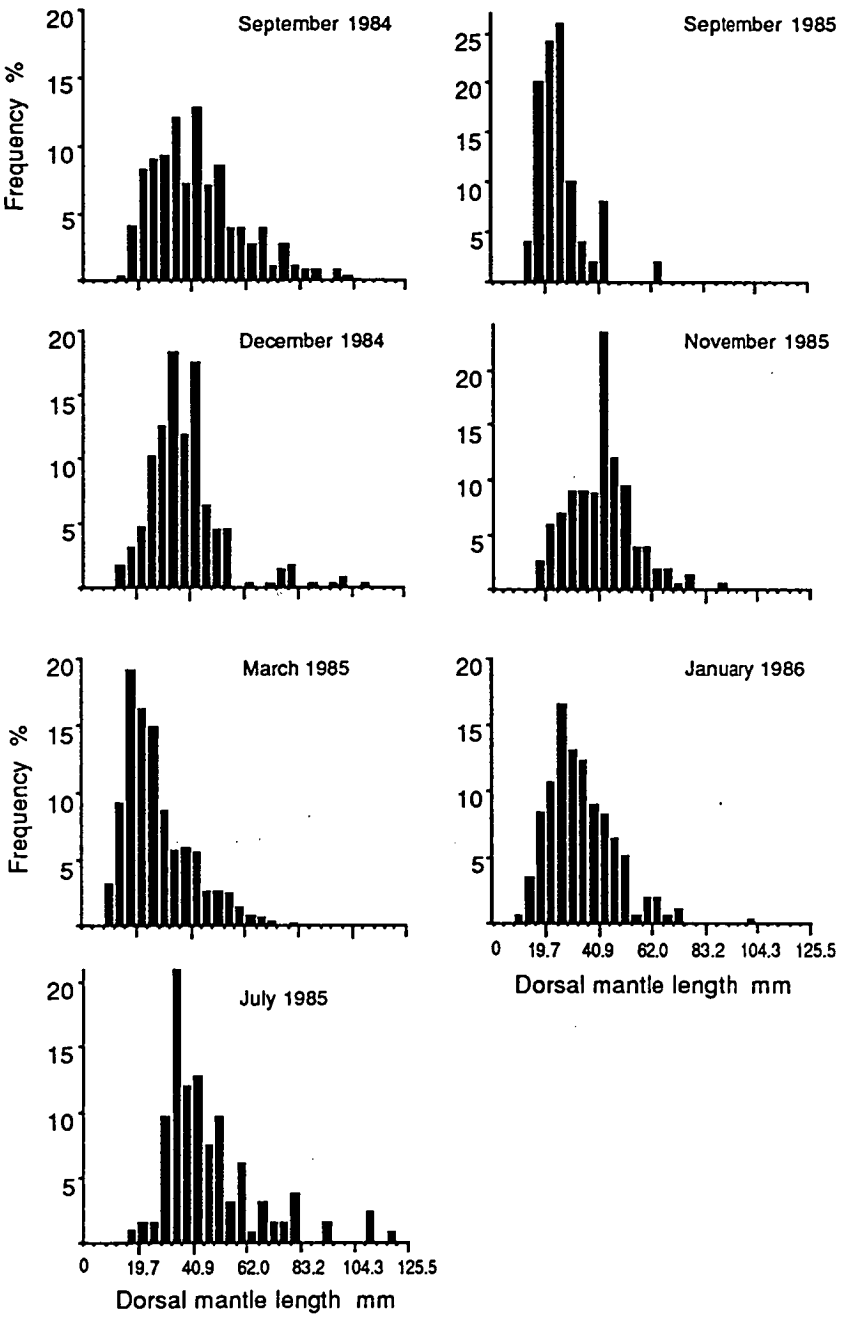

FIGURE 10.11 Length-frequency distribution of estimated dorsal mantle length of Gould's squid consumed by little penguins from Albatross Island during each month of sampling

TABLE 10.9 Number of crustaceans and summary of carapace length (CL) and total length (TL) of krill removed from little penguin stomachs from Albatross Island

Month	Brachyuran megalopa n	Stomatopod larvae n	Krill		CL mm			TL mm		
			total n	measured n	Mean	S.D.	Range	Mean	S.D.	Range
September 1984	0	151	633	144	5.1	0.3	4.2 - 5.9	17.1	0.9	14.3 - 19.7
December 1984	26	126	1 785	162	4.8	0.3	3.5 - 6.0	16.1	1.1	12.1 - 20.0
March 1985	101	0	34	5	4.3	0.5	3.5 - 4.7	14.6	1.5	12.1 - 15.9
July 1985	55	0	0	0	-	-	-	-	-	-
September 1985	0	27	19	0	-	-	-	-	-	-
November 1985	4	1 528	3 468	98	4.3	0.5	3.0 - 5.4	14.7	1.7	10.5 - 18.1
January 1986	5	0	120	2	4.1	-	4.0 - 4.2	14.0	-	13.7 - 14.3
Total	191	1 832	6 059	411	4.8	0.5	3.0 - 6.0	16.1	1.5	10.5 - 20.0

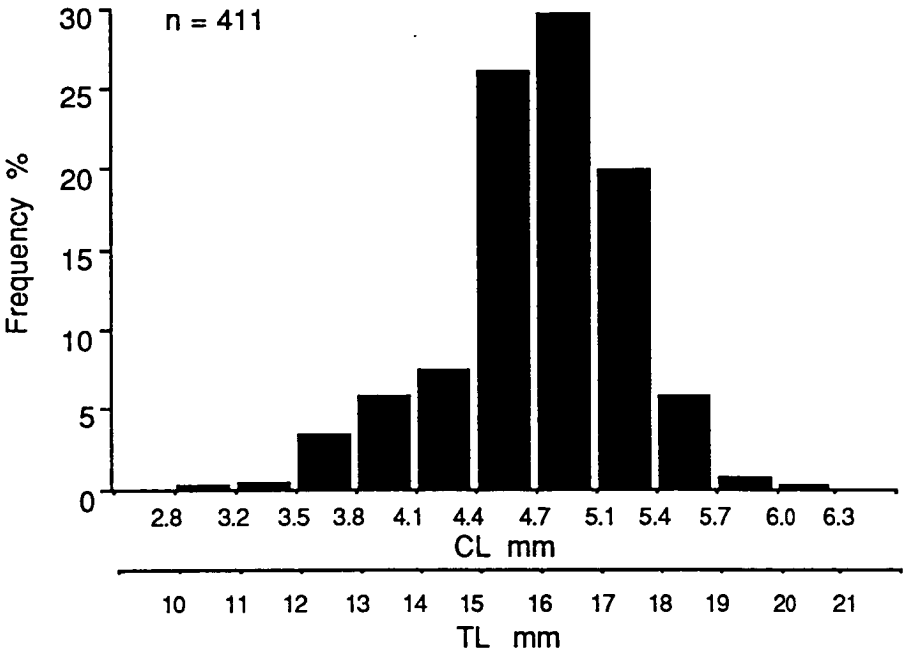

FIGURE 10.12 Length-frequency distribution of krill *Nyctiphanes australis* eaten by little penguins at Albatross Island where CL is measured carapace length and TL is estimated total length.

the inherent biases introduced by differential digestion. Consequently, there is a clear need to report the methods of dietary analyses precisely, together with an assessment of biases which are to some extent species specific, depending on the prey and digestive physiology of the predator. This approach is necessary to facilitate accurate comparisons between studies.

In the present study, attempts were made to minimise errors in interpretation by sorting complete stomach samples rather than subsamples to avoid the problems associated with heterogeneous samples. The composition of the diet has also been expressed in a number of ways since each of the different methods of analysis has its own form of bias (see Hyslop, 1980; Duffy & Jackson, 1986).

Problems associated with differential retention and digestion of diagnostic prey remains are inherent in any study of seabird diet analyses. It has been shown that the digestion and passage rates of otoliths are subject to variation in little penguins and other marine piscivores, and this variation is affected by stomach fullness and time of retention, the calorigenic effect of feeding, the degree of heterogeneity of the diet, the activity of the animal, and the relative size and thickness of otoliths, which varies both between and within fish species (Bigg & Fawcett, 1985; Wilson *et al.*, 1985; Jobling & Breiby, 1986; Gales, 1988*b*; and references therein; also Chapter 4).

Some seabird species, including temperate penguin species, consume both fish larvae and post-larval fish (Wilson, 1985*a*; Montague & Cullen, 1988; van Heezik, 1988; this study), and these present problems with species identification as well as possessing extremely small, and relatively undifferentiated otoliths which succumb rapidly to digestion. Further, some types of fish (e.g., Syngnathidae and Monacanthidae) consumed by little penguins in Victoria and Tasmania (Montague & Cullen, 1988; this study) have characteristically small otoliths which are difficult to quantify and measure being prone to rapid digestion.

Following increasing numbers of studies of digestion rates, it is generally assumed that the otoliths recovered from penguin stomach contents represent the accumulation of fish caught on one foraging trip regardless of condition of the otoliths. In the present study, I have documented the contribution of fish, distinguishing between numbers of loose otoliths and those still enclosed in fish crania and this, together with experimental evidence, suggests that all otoliths recovered from little penguin stomach contents were obtained within one day, with no long term accumulation (Gales, 1988*b*, also Chapter 4). Further, given the predominance of fish with small, fragile, hyaline otoliths in the diet of little penguins, it is probable that some fish prey may have been digested completely and so not included in calculations

of numbers of fish consumed. It is likely, therefore, that in the present study the number of fish and their contribution to the diet has been underestimated.

In estimating fish length and mass from otolith length, accurate estimates rely on the use of non-eroded otoliths, as any otoliths which have been even slightly digested, will result in significant underestimates of fish size (Gales, 1988*b*, also Chapter 4). In the present study, only otoliths unaffected by digestion were measured for estimates of fish size. Further, the equations relating OL to fish length and mass should ideally be based on data encompassing the size range of fish consumed by the predator. This was not possible for all fish species in this study, and so equations were coded in order to identify the level of precision of estimates for each species (Table 10.7).

The retention of cephalopod beaks can result in overestimates of cephalopods in the diet and is recognised as a problem in dietary analyses, particularly in predators with catholic diets, such as little penguins (Furness, Laugksch & Duffy, 1984). There have been no studies of the rates at which cephalopod beaks are retained in penguin stomachs and most researchers assess the number of cephalopods present by the total number of beaks (upper or lower) in stomach contents (e.g., Offredo & Ridoux, 1986; Adams & Klages, 1987; Brown & Klages, 1987; Montague & Cullen, 1988). Others, however, attempt to avoid over-estimation of cephalopods by only including beaks which are still associated with flesh (Hindell, 1988).

Most cephalopod beaks present in the stomach contents of little penguins were extremely small (LRL < 2 mm), showing little or no signs of digestion, and in this study, I calculated the number of cephalopods consumed as the sum of all loose lower beaks and those still enclosed in buccal masses. These two groups were counted separately and were found to be equally represented in stomach contents from two sites, but there was a predominance of loose beaks in stomach contents collected at Fort Direction (Table 10.3). Thus, if loose beaks were excluded from analyses, the proportion of cephalopods would be significantly less, but at present there is no evidence to justify such exclusion.

Retention rates of the chitinous exoskeletons of crustaceans in penguin stomachs are also unknown, and in the present study all crustaceans were counted, including traces represented by eyes, most of which were still attached in pairs. Many of the crustaceans were well digested, particularly krill, which were smaller and had less robust exoskeletons than the stomatopod larvae and brachyuran megalopa. Another potential source of error, is the possibility that some crustaceans present in little penguin stomachs may have been of secondary origin as some intact cephalopods

in the stomach contents were found to contain traces of krill in their stomachs. This, however, is not considered to pose a major problem in this study, particularly as at the times when krill were found to be abundant and relatively undigested, they occurred in penguin stomach contents in the absence of cephalopods.

A method which has been employed in some studies in order to reduce some of these biases, is to estimate the original size of each prey item and then to calculate the original meal size by combining the calculated masses of all ingested prey items (e.g., Croxall *et al.*, 1985; Adams & Brown, 1987; Brown & Klages 1987). The requirements for this process have been outlined by Croxall *et al.* (1988) and these include: 1) identification of all prey taxa to species level, 2) accurate means of assessing the number of individuals of each species in the sample, 3) accurate biometric data relating size of diagnostic prey remains of all species to original length and mass. If all these requirements cannot be met, then the different sources of error will be compounded and the result may be very misleading (Duffy & Jackson, 1986; Croxall *et al.*, 1988). In the present study this approach was not attempted, given the difficulties of specific identification within some prey groups (e.g. post-larval fish), the limited biometric data for estimating original prey size for some species, and the problems associated with retention of hard parts within the three main prey taxa.

In spite of the problems and biases which act to favour the under-estimation of the relative importance of fish and the over-estimation of cephalopods and crustaceans, fish were still the dominant prey taxon of little penguins, with cephalopods being the next most important taxa in terms of wet mass. When the energy content of prey are taken into account, the differences in contribution to the diet by the different taxa have enhanced significance, with fish being higher in energy content on a wet mass basis than either squid or krill (e.g., blue grenadier 6.3 kJ g^{-1}, Gould's squid 5.4 kJ g^{-1}, and *N. australis* 3.0 kJ g^{-1}) (Chapter 6).

10.4.2 STOMACH CONTENT MASS, MEAL SIZE AND FOOD REQUIREMENTS

The mean wet masses of stomach contents ranged between 31 and 47 g for the three sites around Tasmania. These values are lower than the mean mass values recorded in Victoria (80 g; Montague & Cullen, 1988) and Western Australia (57 g; Klomp & Wooller, 1988a). Little penguins are larger in Western Australia than elsewhere (Klomp & Wooller, 1988b) and so, in terms of per cent adult body mass, the mass of food brought ashore translates to 3 - 4 % in Tasmania, 4 % in Western Australia and 7 % in Victoria. In all three studies, the adults showed seasonal variations in body mass, increasing in mass prior to moult, before significant declines during moult with subsequent gradual increases prior to breeding (Fig. 10.3, this

study; Montague, 1982; Klomp & Wooller, 1988*a*). The mass of food brought ashore also showed significant seasonal variations, with maximum amounts being obtained in the breeding season when adults were feeding chicks.

As discussed, the mass of food bought ashore does not represent the total amount actually consumed during the foraging trip, even when foraging on a daily basis. In New Zealand, estimates of reconstituted meal mass were calculated from stomach samples flushed from little penguins which were returning to the colony to attend eggs or feed chicks (van Heezik, 1988). In that study, the calculated mean meal size of 26 birds was 19.8 g (S.D. = 26, range = 1 - 82). This figure is surprisingly low, being considerably lower than even the mean wet mass of food recovered from the stomach contents of penguins during incubation or chick rearing periods in Tasmania (Table 10.10), and elsewhere in Australia (Montague & Cullen, 1988; Klomp & Wooller, 1988*a*).

As an alternative approach to estimate daily food consumption rates, at Albatross Island I conducted a simultaneous study of the free-living energetics of little penguins using isotope turnover techniques to calculate the energy and food requirements over the annual cycle (Chapter 8). This study produced estimates of food consumption rates of little penguins, which showed a seasonal variation parallel to that exhibited by the mass of food in stomach samples. These figures are shown in Table 10.10 and while the seasonal variation is evident in both parameters, the mass of food recovered from stomach contents is consistently and markedly lower that the estimated daily food consumption rates at all times of year. Even the maximum amounts of food in stomach contents are consistently lower than the daily food consumption figures (Table 10.10). This discrepancy indicates that the bulk of the food consumed on foraging trips is completely digested prior to the birds' return to land.

The estimates of daily food consumption rates assessed by isotope turnover constitutes all food consumed by the parent, including that portion which is subsequently fed to chicks on return to the nest. The increase in the amount of food recovered from stomach contents during chick rearing is, therefore, probably almost solely for provisioning chick feeding, the adult having digested the food required to meet the energy demands of increased food consumption and foraging intensity necessary to rear chicks. The maximum figures registered on food mass brought ashore during chick rearing at Albatross Island (140 - 264 g) compare favourably to the maximum figure obtained by Hodgson (1975) in Tasmania who recorded a maximum of 156 g food being fed to a single little penguin chick by one parent.

TABLE 10.10 Wet mass of food bought ashore in stomach samples and daily food consumption rates as determined from isotope turnover of little penguins at Albatross Island.

Stage	Date	Wet mass of food bought ashore (g)			Mean daily food consumption * (g/day)	
		Mean	S.D.	Maximum		
Post-moult	Mar 85	30	17.8	60	164	
Winter	Jul 85	11	16.9	52	77	
Courtship	Sep 84	13	16.5	70		
	Sep 85	21	24.9	68	295	
Incubation/chick rearing	Nov 85	100	78.7	264	325	(early chick stage)
	Dec 84	50	48.0	144	581	(mid chick stage)
	Jan 86	57	55.5	140	754	(late chick stage)

* Data from Chapter 8. Chick stage data pooled from Nov 85 and Jan 86 data depending on age of chicks being fed by adults.

There was not only significant seasonal variation in the mass of food brought ashore, but also considerable variation between masses of samples collected in the same month and this short term variation is a common feature in studies of little penguin diet (Montague & Cullen, 1988; Klomp & Wooller, 1988*a*; this study). In Victoria, local weather conditions were not found to have a significant effect on the mass of food in stomach contents (Montague & Cullen, 1988), however, this relationship has not been examined at other sites. In Tasmania intra-month variability was greatest during the breeding season (Fig. 10.3). This may partially reflect the range of demands on the adults in terms of the variety of the ages of the chicks being fed during this time, i.e., the need to return to the colony with undigested food in order to feed rapidly growing chicks. In Western Australia, high levels of short term variability were also evident during the breeding season and were more protracted throughout the year than in Tasmania. In Western Australia, however, the breeding season is also considerably more protracted than in Tasmania. In any variation between individuals, however, there must also be an element of difference in foraging efficiency between individuals, and also in the proximity to the colony of the feeding grounds used by different individuals. These sources of variation operate both in the breeding and non-breeding seasons.

10.4.3 FISH

Fish was the most dominant prey taxon in terms of numbers and wet mass and appeared in the diet of little penguins in every month of sampling at all sites in Tasmania. There were, however, significant differences between sites in terms of the contribution of fish to the diet, with penguins at Fort Direction having a diet that consistently comprised almost exclusively of fish, with seasonal shifts between prey taxa being evident at both Marion Bay and Albatross Island (Figs. 10.4 & 10.5). The increase in the abundance of fish consumed by little penguins during the breeding season at Marion Bay and Albatross Island sites, relative to cephalopods and crustaceans, probably represents an increase in the availability of fish around the colonies at this time, and the level of increase may not be consistent in magnitude from year to year. These local and seasonal differences indicate that the little penguin diet reflects a non-uniform distribution and changing availability of fish species.

The major features of the fish diet of little penguins from Albatross Island are summarised in Tables 10.5 and 10.6. The fish prey of little penguins at Albatross Island is dominated by blue grenadier, a large merluccid species. The adults live on the continental slope off southern Australia and New Zealand at about 500 m depth, showing diurnal vertical migration, whereas juveniles are found near the edge of the continental shelf and also in the inshore waters of southern and western Tasmania (Last *et al.*, 1983; Bulman & Blaber, 1986). A major spawning ground occurs off

north-western Tasmania and spawning occurs between mid-May and late September. After spawning the adults disperse in spring, the pattern of juvenile movement being unclear at present (Kenchington & Augustine 1987; and references therein).

The location and timing of spawning of blue grenadier is reflected in the presence of the species in the diet of little penguins at Albatross Island where all but two of the 5 898 individuals counted occurred in the diet in September of both years. The small size of the blue grenadier eaten by little penguins (mean TL = 41 mm, Table 10.7) confirms that the individuals consumed were exclusively the product of the most recent spawning event, given the growth rate of young and that blue grenadier juveniles mature at about 70 cm body length (Kenchington & Augustine, 1987; Gunn *et al.*, 1989). The size-frequency distribution of blue grenadier eaten by little penguins (Fig. 10.9) shows that significantly smaller individuals were eaten in September 1984 than at the same time in 1985. This is consistent with the annual variation ($\approx$ 1 month) in the timing of the commencement and duration of spawning of blue grenadier which was observed in Tasmania in these years (Gunn *et al.*, 1989).

The next most abundant fish species consumed was barracouta (*Thyrsites atun*) which is an oceanic, pelagic species, the centre of its Australian distribution being in the south-eastern waters of Australia, particularly Bass Strait. Spawning times vary with location; off central Victoria and northern Tasmania spawning occurs from October to March, with the peak from November onwards (Blackburn & Gartner, 1954). These life-history data are consistent with the timing of occurrence of barracouta between November and March in the little penguin diet (Table 10.6). The small size of these fish (mean FL = 46 mm, Table 10.7) again indicates that the fish comprise 0-group individuals from the recent spawning event. Barracouta reach 300 mm in length after 1 year (Cowper, 1966), the largest individual taken by a little penguin (145 mm) being approximately half this length.

The four fish species which comprise between 4 and 5 % of the number of fish consumed (Table 10.5) are species that are abundant in Tasmanian waters and exhibit a pelagic, schooling phase in their life history.and the individuals consumed comprised predominantly post-larval and juvenile cohorts (Table 10.7; Last *et al.*, 1983; P. Last personal communication). All other fish species contributed < 1 % of the fish diet by numbers, and these species were generally consumed in the absence of large numbers of the more dominant species in the diet (Tables 10.5 and 10.6). Although much less abundant in the diet, the individuals belonging to the less common species were generally larger than the individuals of the more dominant species, and were not solely the products of recent spawning events (Table 10.7). The size of pilchards (*Sardinops neopilchardus*) consumed ranged between 24 and 141 mm in

length which translate to ages of < 1 to 4 years of age (Blackburn, 1950*a*), indicating that both juvenile and adult pilchards were eaten. The largest individuals consumed, in terms of mean length and mass, were anchovy (*Engraulis australis*) which ranged in length between 67 and 103 mm. These fish, therefore, were all mature individuals ranging between 1 and 3 years of age (Blackburn, 1950*b*).

A feature in common with all the fish prey of little penguins at Albatross Island is that all were consumed during their schooling, pelagic phase although not all species remain in the pelagic zone later in life. The dominance of blue grenadier and other post-larval fish, including barracouta, can be explained by the proximity of their spawning grounds to Albatross Island, and also the vast abundance of a single species which must be evident during post-larval dispersion, following concentrated and synchronised spawning.

10.4.4 CEPHALOPODS

The level of contribution of cephalopods to the diet was highly site specific and showed seasonal variations which were not consistent between sites or between years. At Marion Bay and Albatross Island cephalopods were present in the diet in every month of sampling and represented in excess of 80 % frequency of occurrence, 14 % by number and 20 % wet mass. At Fort Direction, the only one of the three sampling sites which is not situated on the open coast, however, cephalopods were infrequently consumed, occurring in only 11 % of all samples and comprised < 1% of numbers and mass. Also, loose beaks and buccal masses were equal in proportion in samples from Marion Bay and Albatross Island, but loose beaks accounted for almost 70 % of instances of cephalopod occurrence at Fort Direction (Table 10.3). This suggests that cephalopods are consumed well before penguins return to the colony at Fort Direction, possibly at a greater distance from the colony than is the case at Marion Bay and Albatross Island.

As discussed previously, all squid in the little penguin diet were designated as Gould's squid (*Nototodarus gouldi*), although other species may have some, probably small, contribution. Gould's squid is the most abundant cephalopod in Tasmanian waters and is a coastal and oceanic squid with a distribution extending from the Great Australian Bight to southern Queensland, the principal stocks occurring in western Bass Strait (C.C. Lu, personal communication). It is thought that Gould's squid breeds throughout the year, with peak spawning in June, September and February, juveniles growing to 15 cm (DML) in the second month after spawning (Wormuth, 1976; Harrison, 1979; Machida, 1983; Smith, 1983). To date, spawning grounds have not been located, the seasonal movements of juveniles are unknown and there are virtually no data concerning the larval stages. Squid were most abundant in

the diet of little penguins at Albatross Island in March with fewest occurring between July and November. However, the persistence of squid in the diet and the small size of these individuals (Table 10.8) indicates a protracted breeding season. The largest squid consumed was 12 cm (DML), which indicates that all the squid eaten by little penguins were consumed within three months post-spawning.

The size frequency distribution of Gould's squid taken by little penguins at Albatross Island (Fig. 10.10) is similar to that reported from little penguin stomach contents in Victoria by Montague & Cullen (1988) who also reported only slight changes in the size of squid consumed at different times of year. The rapid growth of *Nototodarus* (Roberts, 1982) and a protracted breeding season would serve to obscure the growth of age cohorts of squid, although interpretation of trends in seasonal size-frequency distribution in this study is made more difficult by some uncertainty of the specific status of the very small animals. At Albatross Island there was a significant seasonal variation in the size of squid taken by little penguins with the smallest individuals occurring in March and September 1985 and the largest in July of the same year (Table 10.8, Fig. 10.11). There was also a yearly variation with significantly larger squid being consumed in September 1984 than at the same time in 1985. These differences in the peaks of squid abundance between sites, and in squid size between years, are consistent with what is known about the spawning of Gould's squid, the timing of spawning differing in different parts of Bass Strait and neighbouring Tasmanian waters, and that in some years it may be more concentrated than in others (Harrison, 1979).

10.4.5 CRUSTACEANS

Crustaceans were the least represented of the three prey taxa in the little penguin diet in Tasmania, but nonetheless they featured in the diet at all three sites, their abundance showing both local and seasonal variation. Crustaceans were more significant in the diet at Marion Bay and Albatross Island than at Fort Direction where they occurred in fewer numbers, constituting only 5 % of all prey items at this site (Tables 10.2 - 10.4). The three types of identified crustaceans were fairly evenly represented at Marion Bay, but at Fort Direction brachyuran megalopa dominated in numbers, as did *Nyctiphanes australis* at Albatross Island.

Crustaceans were infrequent and erratic in samples from Fort Direction, but occurred in every month at the other sites, with concentrations between the months of August and November at Marion Bay and September and December at Albatross Island (Figs. 10.4 & 10.5). Little is known regarding the biology of stomatopod and brachyuran larvae in Tasmanian waters, and in this study occurrence of stomatopod larvae in the diet was highly seasonal, with peaks occurring between September and

December at both Marion Bay and Albatross Island. The occurrence of brachyuran megalopa was more protracted at Marion Bay, with peaks in abundance between January and February at Fort Direction, while at Albatross Island they were more abundant in March and July (Fig. 10.6, Table 10.9). Brachyuran megalopa are also eaten by little penguins in Victoria (Montague & Cullen, 1988) and both stomatopod larvae and brachyuran megalopa were found in the diet of short-tailed shearwaters (*Puffinus tenuirostris*) feeding in Tasmanian waters (Skira, 1986).

There is considerably more information concerning *N. australis* in Australia, and many of the features of its behaviour and biology are reflected by the nature of predation by little penguins on this species. *N. australis* is restricted to the shallow, neritic waters of the continental shelf of New Zealand and it is also the most important neritic euphausiid in south-eastern Australian waters (Blackburn, 1980). *N. australis* is a major component of the zooplankton biomass and this, together with its swarming behaviour results in it being an important dietary item of many cephalopod, fish and bird species. Characteristics of the biology and swarming behaviour of *N. australis* in Tasmanian waters pertinent to the predation by little penguins include: 1) aggregations are predominantly composed of adults (>11 mm TL); 2) there is evidence for both night and daytime surface swarming which occurs throughout the year around Tasmania; 3) breeding is continuous over most of the year, with seasonal peaks, possibly a prolonged post-winter peak extending over the summer;. 4) maturity is attained in 3 - 4 months, and the life span is approximately 12 months (Jillett, 1971; Blackburn, 1980; Hosie, 1982; Ritz & Hosie, 1982; O'Brien, 1987).

The widespread distribution and abundance of *N. australis* in Tasmanian waters is clear from the euphausiid literature, but the availability to little penguins is clearly not uniform. The reason why *N. australis* is only rarely consumed by little penguins at Fort Direction is unclear as *N. australis* occurs in high densities in the Storm Bay region throughout most of the year (Hosie, 1982). A patchy distribution of *N. australis* in Storm Bay has been documented by Hosie (1982) who found that while the water column was devoid of *N. australis* in winter, mass swarms and strandings occurred in adjacent areas at the same time of year. The well developed, high density swarming behaviour, characteristic of *N. australis* adults, can result in vast areas of low density, or complete absence of individuals, with encounter of predator and prey becoming a matter of chance.

N. australis were most prevalent in the diet of little penguins at Marion Bay between the months of July and November of both years, and later, between September and January at Albatross Island, with higher densities occurring in 1984 than 1985 at both sites. The seasonality of occurrence of *N. australis* at these sites is in

agreement with the evidence of a protracted breeding season, with a prolonged post-winter peak extending over summer, and that swarming is probably related to reproduction. The yearly variation is consistent with other evidence which suggests broad-scale fluctuations in the distribution and abundance of *N. australis* between sites and years as its distribution is tied to advections and eddies of water masses and major current systems (Blackburn, 1980).

The size distribution of *N. australis* consumed by little penguins from Albatross Island (Table 10.9, Fig. 10.11) shows that the individuals consumed are exclusively adults, ranging in TL from 11 to 20 mm. Further, the mean sizes taken by little penguins in each month of sampling ranged between 14 and 17 mm TL, and this is consistent with the range of mean lengths of adult individuals between different swarms, 12 - 17 mm, and the low degree of variation (S.D. ≈ 1.3 mm) in the lengths of individuals within schools (O'Brien, 1987). Homogeneity in length within, and heterogeneity between swarms of krill has also been documented by Mauchline & Fisher (1969), and the statistically significant difference in sizes of *N. australis* in the little penguin diet through the year probably reflects the heterogeneity between swarms and differences in the time and place of foraging, rather than significant seasonal shifts, given that all krill consumed were adults. The largest *N. australis* taken by a little penguin was 20 mm which is close to the maximum of 21 mm documented by Hosie (1982) for *N. australis* in Tasmanian waters.

10.4.6 Size of Prey

The results of the present study indicate that main prey species consumed by little penguins appear to be dictated by obligate schooling within specific size classes/age cohorts. There also appears to be an apparent maximum size which can be effectively handled by little penguins as many of the species consumed continue to school in coastal, surface waters when they have exceeded the size range taken by the penguins. As discussed, little penguins consume the full size range of *N. australis* adults, as it is adults which predominantly form schools and this species grows to a maximum size of 21 mm (TL). Gould's squid, however, can grow in excess of 400 mm (DML), yet the maximum size consumed by little penguins in Tasmania was 117 mm (DML). This is consistent with the maximum size of Gould's squid taken by little penguins in Victoria (100 mm DML) and of *N. sloani* in New Zealand which were all under 20 g mass (Montague & Cullen, 1988; van Heezik, 1988).

Fish prey were also generally small, the largest fish consumed in the present study being a barracouta measuring 147 mm (FL), with most fish prey being less than 100 mm. Similarly, in Victoria most fish prey of little penguins were less than 100 mm in length, in Western Australia little penguins mainly consumed fish measuring

between 60 - 100 mm, and in New Zealand all fish consumed were less than 50 mm (Klomp & Wooller, 1988*a*; Montague & Cullen, 1988; van Heezik, 1988). It appears that, in association with behaviour of prey species appropriate to predation by little penguins, fish and cephalopod prey are primarily within the length range of 20 - 100 mm, with crustacean prey measuring between 10 - 20 mm, and that individuals within these size ranges represent the optimal return for effort for little penguins, with an upper handling threshold of ≈ 150 mm.

10.4.7 Comparison with other studies and species

Other studies of the diet of little penguins have shown that local, seasonal and annual differences are apparent, and a summary of the prey species identified from little penguin stomachs is shown in Table 10.11. In all locations fish were found to be the dominant prey item in terms of relative abundance, although at certain times of year, the importance of other prey taxa increases. The broad pattern which emerges from the extensive species list in Table 10.11 is that little penguins feed on a range of inshore or neritic prey organisms and that, although a wide range of species are consumed, a small number dominate the diet, with additional species occurring infrequently and in low numbers. Further, all species which are consumed in significant numbers are characteristically schooling species, and as discussed previously, the individuals consumed are small in size. The species composition of prey items therefore probably reflects the local distribution and abundance in the vicinity of the breeding colonies. The absence of Gould's squid and *N. australis* in the diet of penguins in Western Australia, for example, reflects the eastern distribution of these species in Australia.

The diet of the little penguin is broadly similar to that of other temperate penguin species, particularly the four spheniscid species which feed predominantly on schooling species of clupeid type fish, generally between 25 - 150 mm in length. Also, at least three of the spheniscid penguin species regularly consume small squid (25 - 160 DML; Wilson, 1985*a*; Wilson & Wilson, in press; and references therein). These penguins are inshore feeders, and the similarity of the spheniscid and eudyptulid diet characteristics, comprising small, pelagic school fish and squid, is striking in light of the approximately four-fold size difference between species of the two genera.

Other penguin species, particularly the eudyptid species, have also been characterised as opportunistic predators feeding principally on schooling or swarming zooplanktonic prey (Croxall *et al.*, 1985; Cooper *et al.*, in press), with intra-specific differences in prey species reflecting local distribution and abundance of prey. Certainly, many intra-specific differences in the diet of many, probably all, penguin species appear to relate to broad-scale differences in prey availability. Some degree of

Table 10.11 Summary of results of quantitative little penguin diet studies. Bold figures represent % number of each taxa, other numbers indicate % number of each species, + indicates species present in unspecified proportions, total number of prey items given where possible.

Prey item	Location:	AI - Tas	PI - W.A.	PI - Vic	PC - Vic	RI - Vic	CI - N.Z.
FISH		**50**	**100**	**80**			**95**
Blue grenadier	*Macruronus novaezelandiae*	25					1
Post-larval fish	Unidentified species	12					
Barracouta	*Thyrsites atun*	3		5	+	+	
Leatherjacket	Monacanthidae	2		6	+	+	
Warehou	*Seriolella brama*	2					
South Australian garfish	*Hyporhamphus melanochir*	2	17	1	+	+	
Red cod	*Pseudophysis bachus*	2		4	+		6
Sea horse	*Hippocampus*	<1		<1			
Blue sprat	*Spratelloides robustus*	<1	15			+	
Spotted trevalla	*Seriolella punctata*	<1			+		
Pilchard	*Sardinops neopilchardus*	<1	3	27	+	+	
Atherinid	Atherinidae	<1		1	+	+	
Jack mackerel	*Trachurus declivis*	<1					
Anchovy	*Engraulis australis*	<1	1	32	+	+	
Pipefish	Syngnathidae	<1		<1		+	
Sprat	*Clupea bassensis*	<1					
Silver trevally	*Pseudocaranx dentex*	<1				+	
Gemfish	*Rexia solandri*	<1					
Silverside	*Argentina australiae*	<1					
Dragonet	*Bovichthys variegatus*	<1					
Sandy sprat	*Hyperlophus vittatus*		61			+	
Sea mullet	*Mugil cephalus*		1				
Common bullseye	*Pempheris multiradiata*		1				
Rough bullseye	*Pempheris klunzingeri*		<1				
Flower of the waves	*Iso rhothoohilus*		<1				
School whiting	*Sillago bassensis*		<1			+	
Yellow fin sand whiting	*Sillago schomburgkii*		<1				
Ogilby's hardyhead	*Pranesus ogilbyi*		<1				
Scaly mackeral	*Sardinella lemura*		<1				
Southern Australian salmon	*Arripsis esper*		<1				
Common buffalo bream	*Kyphosus sydneyanus*		<1				
Yelloweye mullet	*Aldrechetta forsteri*		<1		+		
Gurnard	Triglidae			1	+		
Horse mackerel	*Trachurus mccullochi*			1		+	
Native trout	*Galaxias*			<1			
Clupeid	Clupeidae			<1			
Slender bullseye	*Parapriacanthus elongatus*			<1			
Wrass	Labridae			<1			
Sea moth	*Acanthopegasus lancifer*			<1			
Australian salmon	*Arripis trutta*			<1	+		
Blenny	Blenniidae			<1			
Flathead	Platycephalidae			<1	+		
Toadfish	Tetradontidae			<1			
Redbait	*Emmilichthys nitidus*				+		
Carangid	Carangidae				+	+	
Sweep	Scorpidae				+		
Dory	Zeidae				+		
Ahuru	*Auchenoceros punctatus*						88
Lantern fish	Myctophidae						<1
Unidentified species				2			
CEPHALOPODS		**16**	**<1**	**19**			**5**
Goulds squid	*Nototodarus gouldi*	14		13	+	+	
Arrow squid *	*Nototodarus sloani*						4
Warty squid *	*Moroteuthopsis ingens*						
	Loliolus noctiluca			1		+	
Calamari	*Sepioteuthis australis*			1	+	+	
	Idiosepius notoides		<1				
Argonaut	*Argonauta nodosa*			4	+		
Cuttlefish	*Sepia*				+		
Octopod	Octopodidae	1		<1			
	Octopus maorum						1
CRUSTACEANS		**34**	**<1**	**1**			
Krill	*Nyctiphanes australis*	26		1	+		
	Euphausia lucens				+		
Stomatopod larvae	Unidentified species	8					
Brachyuran megalopa	Unidentified species	1		1	+	+	
King prawn	*Penaeus latisulcatus*		<1				
GASTROPODS		**<1**					
Larvae	Unidentified species	<1					
TOTAL ITEMS		23 527	1 392	1 485			1 692

Locations and references: AI - Tas (Albatross Island, Tasmania; this study); PI - W.A. (Penguin Island, Western Australia; Klomp & Wooller 1988a); PI - Vic (Phillip Island, Victoria; Montague & Cullen, 1988); PC - Vic (Port Campbell, Victoria; Cullen & Montague, 1987); RI - Vic (Rabbit Island, Victoria; Cullen & Montague, 1987); CI - N.Z. (Codfish Island, New Zealand; van Heezik, 1988).
* indicates that these species were combined in quantitative analyses

intra-specific annual variation in the diet, in terms of the timing and abundance of prey have been detected in spheniscid, pygoscelid and eudyptid penguins, as well as *Eudyptula* (Croxall & Lishman, 1987; and references therein).

Differences in diet between the sexes of penguins have only been examined in a few species. No differences in prey species or size have been detected between the sexes of the dimorphic Macaroni penguins (*Eudyptes chrysolophus*), or in Adelie (*Pygoscelis adeliae*) and Chinstrap (*P. antarctica*) penguins, which have an 80 % overlap in beak size (Croxall & Lishman, 1987; and references therein). Dietary differences have been reported between the sexes of gentoo penguins (*P. papua*), which have a 10 % overlap in bill size, and it was suggested that this may be acting to reduce intra-specific competition, particularly over winter, as Gentoo penguins, unlike the other pygoscelid penguins, remain at the breeding colonies throughout the year (Trivelpiece *et al.*, 1983). Little penguins also generally remain in the vicinity of the breeding colonies over winter, males are usually heavier in body mass, and the sexes can be distinguished with 96 % accuracy, on the basis of beak length and depth (Gales, 1988*a*; also Chapter 2). In the present study, however, there was no difference in the diet between the sexes in terms of species composition or mass of stomach contents.

10.4.8 Foraging parameters and diet

It is clear that, across their range, little penguins feed in the neritic zone and almost exclusively on schooling, pelagic prey species. During the breeding season, little penguins attend their eggs or chicks on a daily basis, and so during this time are central place foragers with feeding restricted to areas in the vicinity of the breeding colonies. Estimates of the foraging range of two little penguins carrying electronic activity recorders were between 2 and 13 km from Albatross Island (Gales *et al.*, in press; also Chapter 9), and in Victoria most foraging of little penguins is restricted to within 5 to 10 km of the coast (Weavers, 1987).

A diurnal foraging pattern, a limited anaerobic capacity, and the evidence that penguins are visual hunters (Sivak, 1980), results in limiting foraging time and restriction to the upper layers of the water column as light is essential for detection of prey. This is consistent with the principal prey species which are all found predominantly in inshore areas in relatively shallow water, or alternately exhibit daytime surface swarming behaviour. All little penguin prey are characteristically schooling or swarming species, which results in a patchy distribution but with a potentially high yield upon encounter by a predator. The foraging behaviour of little penguins has been categorised into travelling, searching and feeding patterns, with feeding behaviour being characterised by bouts of rapid, shallow diving at high

swimming speeds. The foraging bouts are interspersed with periods of rest, travelling and searching (Gales *et al.*, in press; also Chapter 9). This behaviour pattern is consistent with a predator searching for a spatially unpredictable prey, with a rapid and sustained attack rate upon encounter. Blaxter & Hunter (1982) and Wilson (1985*a,b*) have summarised the swimming speeds of clupeids and clupeid-type fish, and these, together with the swimming speeds of *N. australis* (O'Brien, 1987) are less than those which are achieved and sustained by little penguins (Gales *et al.*, in press; also Chapter 9). The shallow diving behaviour of little penguins, reflecting their limited anaerobic capacity, is also consistent with the depth distribution of their pelagic fish prey (Last *et al.*, 1983), and the surface swarming behaviour of *N. australis*. Although Gould's squid adults are known to undergo diurnal vertical migrations, it is likely that concentrations of juveniles at least occur in shallow water, as is the case with other squid species (Nemoto *et al.*, 1985).

From simultaneous measurements of little penguin metabolic rate and foraging behaviour around Albatross Island, it was estimated that in order to remain in energy balance, if all dives were successful in prey capture, 0.3 g of food would be consumed per dive (Gales *et al.*, in press, also Chapter 9). It is interesting that blue grenadier were the most dominant prey species in the present study and that the individuals consumed had a mean mass of 0.3 g, requiring that at least one post-larval blue grenadier be captured on each dive. The mean mass of the individuals of all fish species in which original mass estimates were possible, however, was 1.6 g, identical to the mean mass of Gould's squid consumed. Capture of prey of this size would require that only 17 % of dives be successful in prey capture. Conceivably, this success rate would accommodate a component of searching dives plus some "misses" resulting from the anti-predator behaviours exhibited by the prey species, pelagic shoal fish becoming highly polarised in order to reduce predator efficiency (Neill & Cullen, 1974) and *N. australis* eliciting a variety of elaborate responses to evade predators (O'Brien, 1987). Encounter success rate would therefore be affected by the type of prey and the size and density of the school/swarm.

10.4.9 Little penguin diet in relation to prey availability and fishery operations in Bass Strait

The dominant fish species in the little penguin diet are target species of commercial fishing operations. In Victoria, where the principal fish prey species are clupeids, these are fished commercially in the area (Montague & Cullen, 1988). At Albatross Island, the most important species in the little penguin diet is blue grenadier which is the target of a considerable, and still growing, commercial trawl fishery. Barracouta, the next most important fish species, is also targeted by commercial operations. Gould's squid are important in the diet of penguins across Bass Strait, and

observations suggest that this species is potentially the basis of the largest single-species fishery resource in south-eastern Australian waters (Roberts, 1982; Collins, 1987).

Unfortunately there are no data by which direct comparisons can be made between little penguin diet species and the abundance and availability of the prey. In Bass Strait there are more than 10 pelagic fish species which are targeted by commercial fishing operation and the juveniles of most of these species are consumed by little penguins. In Victoria, pelagic fish landings from Bass Strait ranged between 7 000 and 16 000 t per annum over the last decade (Jones, 1986), and this catch rate is not considered to be limited by abundance. Since the discovery of spawning grounds and the development of a winter bottom-trawl fishery off north-western Tasmania the blue grenadier fishery has increased dramatically; the current landing is approximately 1 500 t per annum and the maximum prudent yield estimated at 3 600 t per annum (Kenchington & Augustine, 1987). Gould's squid have been fished in Bass Strait for many years, although on a small scale. The total catch in 1987 was 334 t, although historical catch records by foreign jigging fleets have indicated that up to 6 500 t are available each year, the area of greatest abundance being situated at the western approaches of Bass Strait, in the vicinity of Albatross Island (Roberts, 1982; Collins, 1987).

From an analysis of population size and food consumption rates, it has been estimated that a total of *ca.* 37 000 t of food are consumed by little penguins in Bass Strait annually (Chapter 8). Given the restrictions imposed by central place foraging, feeding activity would be concentrated around the breeding colonies, particularly during the breeding season. In the absence of reliable estimates of the distribution and biomass data of prey stocks, conclusions regarding competition between little penguins and commercial fishing operations are speculative. However, the prey consumed by little penguins is predominantly post-larval and, given the greater size of the individuals taken by fishing vessels and the present relatively low level of the fishing operations in the Strait, there is little scope for direct competition between little penguins and fisheries at present. In indirect terms, competition may also be of low significance; given the abundance of post-larval fish it is unlikely that little penguin predation would seriously affect recruitment into the adult population. In Western Australia, however, there is direct overlap between the species and size of fish caught by little penguins and by fishermen in the same area, and thus they appear to be in direct competition (Klomp & Wooller, 1988*a*). Given this, and in view of the lack of data concerning prey biomass, there is no room for complacency as any localised competition from commercial fishing, even at a low level, may have a significant and long lasting deleterious effect on penguin breeding and survival .

Of particular relevance to Bass Strait, which is the stronghold of little penguins in Australia, is the fact that the distribution of the major prey species are associated with water masses and current systems. The three major water masses which exert an influence in Bass Strait are cool sub-antarctic waters from the south, warmer eddies of the East Australian Current and the 'North Bass Strait Water' which is possibly an extension of the Leeuwin Current. It is likely that any unseasonal aberrations in the movements of these currents, which serve to disrupt the breeding and dispersal of juveniles of the dominant prey species, would have serious effects on little penguins, and it is such events which are probably responsible for the occasional large-scale mortalities of little penguins.

10.5 SUMMARY

The diet of the little penguin was examined quantitatively over a period of two successive breeding seasons at three sites around Tasmania. Fish were the dominant prey item at all three sites, with the contribution of cephalopods and crustaceans varying between sites. The results showed local, seasonal and annual changes in the availability of prey species. In Bass Strait, the area of highest concentration of little penguins in Australia, the diet was analysed in terms of the species composition and size of prey. A wide variety of species were identified, the dominant species within each taxa being blue grenadier *Macruronus novaezelandiae* (fish), Gould's squid *Nototodarus gouldi* (cephalopod), and krill *Nyctiphanes australis* (crustacean). The maximum size of prey consumed was 147 mm in length and 31 g in mass. Almost all fish and cephalopods consumed were juveniles, whereas all krill were adults. Prey were characterised as being small, schooling species which occur in relatively shallow water, consistent with the foraging behaviour of little penguins. The degree of competition between little penguins and commercial fishing operations varies with location and season, and may be important at some times. Aberrations in the movement of major water masses and currents which affect the breeding and dispersal of prey stocks are thought to be a major factor in the occasional, but severe, large-scale mortalities of little penguins.

CHAPTER 11

GENERAL DISCUSSION

A knowledge of the nature of predator-prey relationships is fundamental for understanding the functioning of any ecosystem. Such knowledge relies on information about diet, distribution and bioenergetics of both predators and prey, and on the detailed nature of their interactions (Croxall *et al.*, 1985; Croxall, 1987). In the marine environment, the fact that wide-ranging predators operate in a vast, three-dimensional environment has made studies difficult, and so previously, in the absence of a complete quantitative data base, the role of seabirds in the marine community has been assessed through the application of elegant models (e.g. Furness, 1978; Croxall *et al.*, 1984; Wiens, 1984). These energy flow models have suggested that seabirds consume substantial quantities of marine prey, and that competition may occur between seabirds and commercial fisheries (Furness, 1982). However, large errors in model outputs can result from inaccurate estimates of the model parameters, including predator population size, predator energy and food requirements, and the composition, energy content and size of prey species.

Over the last decade there have been significant advances in the understanding of energy fluxes in marine ecosystems, and with the realisation that penguins are important consumers of marine resources, the energy and food requirements of this group of seabirds have been the focus of increasing attention. The recent development of appropriate field techniques, together with the refinement of laboratory analytical procedures, have allowed the direct study of food and energy requirements, and the foraging behaviour of free-living penguins. However, due to the pelagic nature of many of the southern penguin species in the non-breeding season, it has not been feasible to assess the entire energy budget quantitatively, including breeding and non-breeding periods of these species. In contrast, in the present study it was possible to examine the food and free-living energetics of little penguins over the entire annual cycle and thereby to assess the role of little penguins in the marine ecosystem more accurately than has been possible in the past.

In the General Introduction (Chapter 1), four questions were posed as being central to the understanding of predator-prey interactions, and through the course of this study these have been addressed in reference to the little penguin. The framework relating little penguin energy and food requirements to population demands and resource dynamics is shown schematically in Figure 11.1 and this framework serves to illustrate the structure of the present study. The methods by which this was achieved

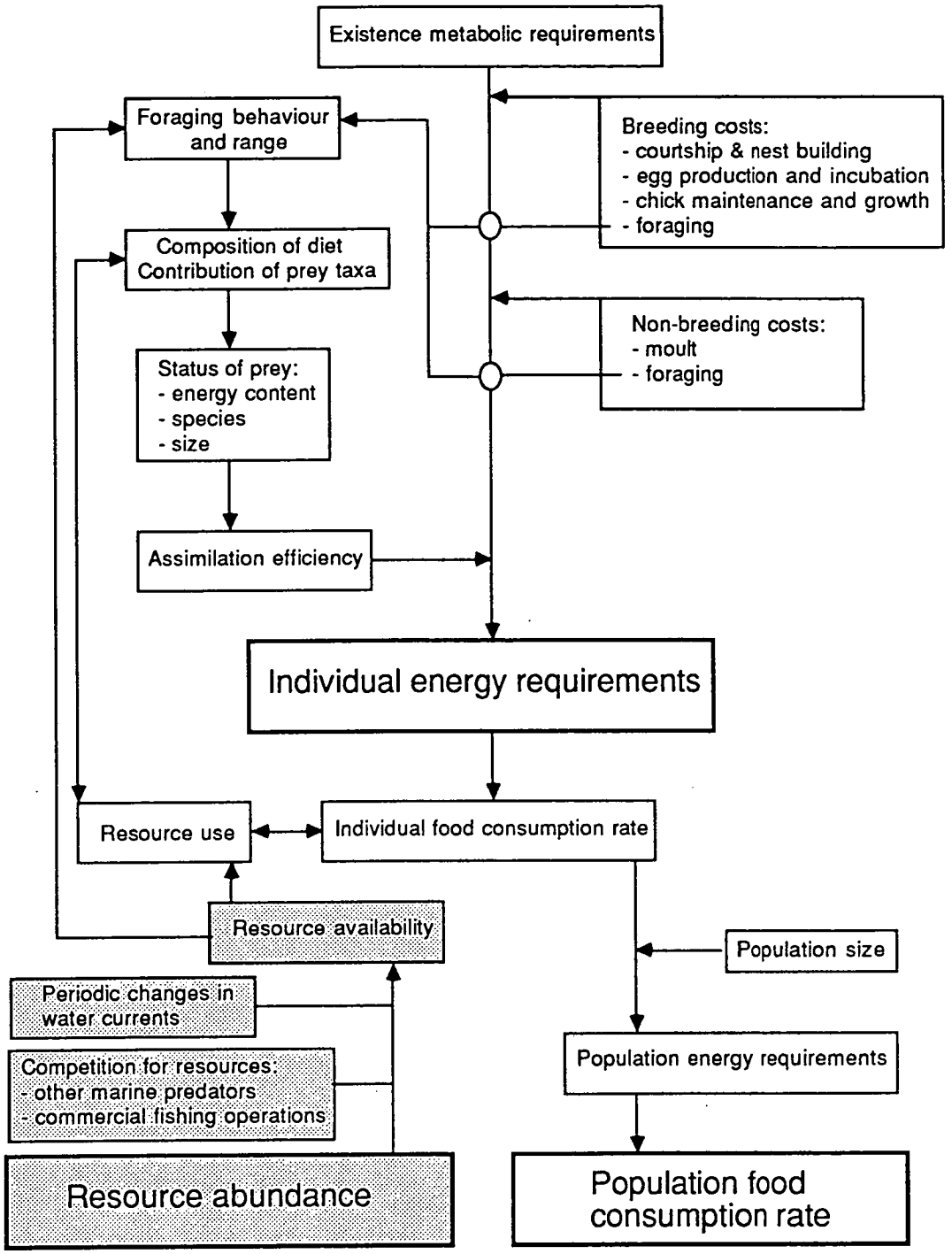

FIGURE 11.1 Framework relating components of little penguin energy and food requirements to population demands and resource dynamics as quantitatively assessed in this study. Components in stippled boxes could not be assessed in this study.

and the major findings of the field studies can now be synthesised in an attempt to more precisely define the role of the little penguin in the marine environment.

Diet studies are essential when addressing the question of the role of penguins in the marine environment. Until recently, stomach contents have been obtained by killing penguins, or by emetics and stomach pumps, the effects of which may be fatal and which may not provide complete, or indeed representative samples (Horne, 1985; Montague & Cullen, 1985). The development of the water off-loading technique by Wilson (1984) offered an alternative, but the efficacy of the technique required validation before its application could be accepted (Lishman, 1985). This was done on three species of penguins by way of feeding trials and examination of stomach contents obtained via stomach flushing (Gales, 1987*b*; Chapter 3). This study proved that the technique is effective in obtaining stomach contents of penguins, and so offers great potential in long-term dietary studies which are required to examine seasonal and annual variation in diet.

The examination of stomach contents subsequent to the feeding trials also provided the opportunity to assess the validity of using otoliths in stomach contents to accurately determine the number and size of fish consumed by penguins. The potential of using otoliths from predator stomach contents in the identification of fish prey species has been widely acknowledged, although the value of otoliths for accurate, quantitative evaluation of predator feeding habits has been a source of contention (Prime, 1979; DaSilva & Neilson, 1985; Jobling & Breiby, 1985). In the present study, I found that the digestion and passage rate of otoliths is rapid and subject to variation. If this is ignored, it will result in significant underestimates of the numbers of fish consumed and of original fish size (Gales, 1988*b*; also Chapter 4). This problem can be partially solved by inspection of otolith condition and restricting calculations of fish size to otoliths unaffected by digestion, and the realisation that some otoliths may have been completely digested, thereby causing an under-estimation of the contribution of fish to the diet.

The biases inherent in seabird diet studies are a result of the heterogeneous diet and differential digestion of prey types, and were discussed in Chapter 10. The results of the 20 month diet study at three sites around Tasmania are also discussed in Chapter 10, and despite the inherent biases in analyses, it is clear that fish were the dominant prey taxon at all sites, with the contribution of cephalopods and crustaceans varying between sites. There were, however, significant seasonal, annual and local variations in the diet, probably reflecting changes in the local availability of prey species, and a degree of opportunism in the diet of the little penguin. The seasonal shifts in prey species were consistent with the behaviour of prey, the synchronised breeding seasons

of prey and the proximity of spawning grounds of the dominant prey species to the penguin colonies, as evidenced by the bulk of the fish (e.g., blue grenadier, *Macruronus novaezelandiae*) and cephalopod (e.g., Gould's squid, *Nototodarus gouldi*) prey comprising juveniles in the early stage of their pelagic dispersal. The crustaceans consumed by little penguins at Albatross Island were predominantly adults of the euphausiid, *Nyctiphanes australis*, and the predation by little penguins on this species is consistent with the daytime, surface swarming behaviour of the adult krill.

A consistent feature that emerged from detailed analyses of the little penguin prey species and size classes, is that the prey consumed consisted almost exclusively of individuals below a maximum handling size of ≈ 150 mm in length, and which occur in dense aggregations in relatively shallow water in inshore regions. These pelagic, schooling species exhibit predator avoidance strategies to reduce the chances of detection and encounter, and the little penguin exhibits a foraging strategy which effectively exploits these species.

The foraging behaviour was examined using a new archival activity recorder which simultaneously measures swimming speed and depth against time (Gales *et al.*, in press; also Chapter 9). Analyses of the data recorded by these meters resulted in the first integrated data of little penguin activity at sea, which can be categorised as travelling, searching and feeding patterns. The foraging behaviour of little penguins is constrained by a number of features including: 1) a limited anaerobic capacity (Mill & Baldwin, 1983) which restricts the depths of the water column available for exploitation, a restriction in the vertical plane; 2) short nest relief periods which restricts the distance from the colony which can be traversed in periods of < 1 day, a restriction in the horizontal plane; and 3) a relatively small gape which restricts the size of prey available. These limitations can be effectively overcome by exploitation of small, aggregated prey in the surface waters, enabling visual hunting with rapid and frequent replenishment of oxygen stores. Little penguins achieve this by fast travelling and searching in the upper layers, interspersed with bouts of rapid, shallow diving at swimming speeds greater than that of the prey. This behaviour reflects a predation strategy which maximises return upon encounter when feeding on a spatially unpredictable, but aggregated, and possibly frequently encountered, prey.

A calculation of foraging efficiency from the assessment of the number of dives and food consumption rate, determined that if every dive is successful in prey capture, each would theoretically result in the ingestion of 0.3 g of food. This value is the same as the mean mass of blue grenadier individuals consumed by little penguins, this fish species representing the dominant prey species in this study. However, the mean mass of all fish and squid individuals consumed was 1.6 g which would require only

17 % of dives to be successful in prey capture. This rate would accommodate a component of searching and unsuccessful dives, encounter success rate presumably being affected by the type and size of prey, as well as size and density of prey aggregation. This approach to a diving efficiency index requires integration of information concerning: 1) diet, 2) foraging behaviour, 3) energy content of prey items, 4) predator assimilation rates and 5) free-living metabolic rate of the predator. The latter three parameters are also essential in determination of meal size and food consumption rates, as rapid digestion precludes reliable determinations based solely on stomach content analyses. Further, estimates of energy flow through seabird communities rely on accurate determinations of the composition and energy content of prey species, and unless these criteria are precisely known, results may be misleading if the predators took disproportionate amounts of high or low energy prey.

Determinations of the energy available to little penguins were assessed in this study by analyses of the composition of 19 species of fish, two species of cephalopod and one species of krill (Chapter 6). On a wet mass basis, fish was the taxon containing the highest energy levels, although there was variation between species. When the energy contents of prey are taken into account, and coupled to the little penguin prey composition information, the dietary importance of fish assumes an enhanced significance, and that of krill, although consumed in large numbers, diminishes in importance.

However, not all the energy ingested can be utilised by the predator, and so for accurate determinations of rates of energy and food consumption, there must also be precise information on the energy assimilation levels which are species specific and may vary with different food types. A materials balance trial was conducted feeding little penguins both fish and squid diets and the birds showed higher dry matter assimilation and energy assimilation levels when being fed squid (Gales, 1985; also Chapter 5), so this effectively enhances the importance of squid in the diet in terms of energy return. The levels of assimilation efficiencies obtained in this study are the lowest recorded for penguins and at the lower limit of seabirds, and such species specific variation in these parameters can significantly affect estimates of energy and food consumption rates.

Until recently, information on the energy metabolism of penguins has come primarily from studies of birds in captivity or restricted to periods when penguins are ashore, as conventional procedures were not appropriate for measuring energy budgets of free-ranging animals. Recently, isotope-turnover techniques were refined and it became economically viable to apply this technique to free-living penguins, the result being the first quantitative measurements of energy and food consumption rates,

effectively revolutionising our understanding of the functioning of penguins in their natural environments. Specifically, the combined use of three isotopes, tritium (^3H_2O), sodium-22 (^{22}Na) and oxygen-18 ($H_2{}^{18}O$), allows the assessment of field metabolic rates, and the partitioning, via water and sodium influx rates, of food and seawater consumption rates.

In designing and interpreting studies of the energetics of free-living animals it is important to know the magnitude of errors which may occur. However, despite the increasing attention being paid to the free-living energetic cycles of avian species, there are few validation studies of the isotope-turnover technique in birds, and prior to the present study, none in seabirds. Not only do seabirds scale differently from other birds in allometric analyses of field metabolic rate (Nagy, 1987), but the use of ^{22}Na had only been validated in one bird species. Consequently, in this study, the accuracy of measurements of the three isotopes was examined in captive little penguins by comparing simultaneous isotopic turnover rates to materials balance measurements, prior to the field based research (Gales, 1989; also Chapter 5). On average, ^3H_2O derived estimates of water intake were 6 % higher than measured water intake, ^{22}Na underestimated dietary sodium intake by 5 %, and doubly labelled water ($^3H_2{}^{18}O$)-derived estimates of metabolic rate were not significantly different from materials balance estimates. Considering the additional sources of error which may arise in field situations, isotopic turnover measurements in little penguins are expected to be accurate to ± 8 % or better, which is consistent with other studies (Nagy, 1987). For these errors to be minimised, however, the appropriate equilibration periods and isotope turnover levels must be achieved, and these were identified and adhered to in the field studies.

A further source of error can arise when food consumption rates are based on water influx rates alone, and it has been generally assumed that ingestion of seawater is negligible and that all water is derived entirely from preformed and metabolic sources. However, by balancing water and sodium influx rates in this study, I found that the mean seawater consumption rate of little penguins was 7 % of total water influx, but in some individuals accounted for over 30 % (Chapter 8). Seawater ingestion by penguins is variable, both within and between species, and if ignored, food consumption rates based on water influx rates alone may be significantly over-estimated, and by variable degrees (Green & Gales, in press).

A significant feature of the present study is that I was able to simultaneously collect quantitative data on the diet, and energy and food consumption rates of free-living penguins over the complete annual cycle, and thus achieve an integrated bioenergetics result. The breeding season of little penguins varies considerably in

timing and duration (Gales, 1985), and in Tasmania they breed during summer and moult after breeding. The non-breeding activities of penguins include those associated with the moulting cycle, and the time spent subsequent to moult until the onset of the next breeding season. During the moult period when penguins are restricted to land, they decreased substantially in mass, increased in total body water levels and exhibited a field metabolic rate (FMR) of 1.5 times basal metabolic rate (BMR). The energy required to sustain a moulting little penguin is 15 % higher than required for a resting, non-moulting penguin during the non-breeding season (Gales *et al.*, 1988; also Chapter 7). During the land-based activities in the breeding season, i.e. courting, incubating or brooding, FMRs ranged between 1.3 and 1.5 times BMR and were not significantly different to those of moulting birds, although the duration of these fasting periods associated with breeding are shorter than the moult period. While the energy utilisation levels of moult are elevated above BMR, the major energetic cost of the moult process is probably met during the pre-moult foraging period, when the penguins must consume enough food to ensure that they lay down sufficient fat reserves to endure the moult fast. Following moult, the food consumption rates of penguins during the winter period were low (74 g kg^{-1} day^{-1}) despite relatively high FMRs. This situation indicates that the penguins were working hard for little gain in food, probably reflecting a reduced availability of prey in the vicinity of the colony. Further, across their range, little penguins return to the colonies less often during winter than during the summer breeding season, reinforcing the interpretation that there has probably been a decrease in the abundance, proximity and availability of schooling prey, which consequently requires the penguins to increase their foraging effort without a concomitant increase in rate of food acquisition.

Little penguins which were primarily engaged in foraging during the breeding season, had FMR and food consumption rates that were uniform from courtship through to the early chick rearing stage, indicating that during this period there was little change in food supply and demand, and that the additional food consumed to feed small chicks is not a significant burden on the adults. However, as the chicks increased in size, and both parents were committed to foraging to feed the rapidly growing chicks, food consumption and FMR of adults increased rapidly. They reached a maximum towards the end of chick rearing when food consumption rates of attending adults exceeded 60 % of adult body mass and FMR approached 6 times BMR (Chapter 8).

The use of isotope turnover to measure metabolic rates provides an integrated value of field metabolism, and by partitioning time spent on land and at sea, I was able to isolate the costs of foraging at sea. These analyses reinforced the observation that the cost of foraging increased rapidly during the chick rearing period, and the actual

cost of foraging is over 7 times BMR when feeding large chicks. This cost translates directly to the cost of swimming and pursuit diving, as little penguins on daily foraging trips are active for 95 % of their time at sea (Gales *et al.*, in press; also Chapter 9). These at-sea metabolic rates are similar to the costs of flapping flight (Ellis, 1984; Birt-Friesen *et al.*, 1989), contrary to previous assumptions that underwater swimming is less expensive than flying (Schmidt-Nielsen, 1982).

The estimates of energy turnover were used to construct energy budgets for comparison with time-activity budgets. The most energetically expensive period of the annual cycle of little penguins is the chick rearing period which occupies only 16 % of the total time budget, but accounts for 31 % of the annual energy budget. Also, from these analyses, by combining metabolic rate, food consumption and population data, it was possible to extend the analyses to assessments of individual and population requirements (Chapter 8). Applying the appropriate little penguin demographic parameters (Reilly & Cullen, 1982), a 'typical' little penguin would consume 830 kg of food during its lifetime. In Bass Strait, the stronghold of the species distribution in Australia, it is estimated that a total of 37 000 tonnes of food are consumed by the 285 000 little penguins annually, of which approximately 67 % would comprise fish, 30 % cephalopods and 3 % crustaceans.

Comparisons can now be made between little penguins and other penguin species. Within species, the sexes of gentoo and macaroni penguins have been compared in terms of the rates of energy acquisition and utilisation, and Davis *et al.* (1989) found that there were no differences between the monomorphic gentoos, but that there were significant differences between the dimorphic macaroni penguins. Little penguins can be reliably sexed in the field on the basis of beak measurements (Gales 1988*a*; also Chapter 2), but this dimorphism was not reflected in either dietary or energetic parameters (Chapter 8 and 10).

Detailed reviews and comparisons of the food and feeding ecology of penguins have been addressed by Croxall & Lishman (1987), and the diving behaviour of penguins by Kooyman & Davis (1987). The free-living energetics of penguins has also recently been reviewed by Green & Gales (in press) and the relevant comparisons between little penguins and other penguins have been made in the appropriate chapters of this study. In terms of their functioning in the marine ecosystem, little penguins are more similar to the spheniscid species, and perhaps gentoo and yellow-eyed penguins, than any of the other penguin species. This similarity is based on characteristics shared by these species, including the relatively inshore and shallow foraging habits, consumption of generally small prey of mixed taxa and constant attendance at breeding colonies, as opposed to the deeper diving penguins which have more extended

foraging habits in time and space, and desert their breeding colonies over winter (Table 11.1).

In light of the recent, and rapidly growing data base, many of the review studies are aimed at assessing the role of penguins in their marine environment. As a result, penguins have been identified as possible indicator species to monitor the status of the marine environment (Croxall *et al.*, 1988), as the most immediate threat to any natural system is commercial exploitation. In order to address questions regarding potential competition between penguins and commercial fishery operations, it is essential to understand the energy and food requirements of the penguins, and how their demands change, both in time and space.

Models of energy flow through seabird communities in the North Atlantic, North Pacific and South Africa have estimated that seabirds may consume between 20 and 30 % of the available fishery stocks in the vicinity of seabird colonies (Wiens & Scott, 1975; Furness, 1978, 1982; Furness & Cooper, 1982). In assessments of competition between commercial fisheries and penguins, it is essential to know whether both predators are taking prey of the same species and size, or alternatively, whether the fishery is taking adults, and the birds taking the juvenile cohorts. It is the latter case which exists with little penguins in Tasmania as, although the dominant fish and cephalopod species consumed by the penguins are also targeted by commercial fishing, the penguin prey are almost exclusively juveniles (Chapter 10) and thus the impact is diluted to some degree, given the many other factors which operate in the mortality of young fish and squid. The competition is then essentially, indirect. Fishery-seabird competition is most severe when both consume identical species of the same size classes, as has been shown to be the case with little penguins in Western Australia (Klomp & Wooller, 1988*a*). This situation is exacerbated given the limitations imposed on penguins by central place foraging, particularly during the breeding season.

Single species of predators, however, should not be viewed in isolation and for a more complete, and realistic interpretation, the many other top level predators must also be considered in such analyses of competition with commercial fishing, and in assessments of the impact of predators on marine resources. Considering seabirds alone, in Tasmania there are in the order of 11 million pairs of breeding seabirds, representing 10 families, which are all united in their reliance on the sea for their food (Table 11.2). Little penguins account for less than 3 % of the total breeding population, although 82 % of the seabirds breeding in Tasmania are summer residents only. The diet of most of these migratory birds consists principally of the euphausiid crustacean, *N. australis*, together with fish and squid species which overlap directly

TABLE 11.1 Generalised breeding and feeding characteristics of the four groups of penguin species *

Group	Species	Mean mass (kg)	Breeding season	Age at first breeding (yrs)	Number of eggs laid (hatched)	Nest relief periods	Non-breeding distribution	Principal food	Feeding zone
1	*Aptenodytes forsteri*	30	long, non-annual	3 - 6	1 (1)	long	at sea	fish & squid	offshore pelagic
	A. patagonicus	15							
2	*Eudyptes chrysolophus*	4.5	short, annual	7 - 8	2 (1)	long - intermediate	at sea	crustacea	offshore semi-pelagic
	E. chrysocome	2.5							
	E. pachyrynchus	3							
	E. robustus	3							
	E. schaleri	3.6							
3	*Pygoscelis adeliae*	5	short, annual	3 - 7	2 (2)	intermediate	at sea	crustacea	offshore semi-pelagic
	P. antarctica	4.5							
4	*Pygoscelis papua*	5.7	short, annual	2 - 4	2 (2)	short	breeding colony	fish & crustacea	inshore
	Megadyptes antipodes	5.2						fish & squid	
	Spheniscus humboldti	4.2						fish	
	S. magellanicus	3.5							
	S. demersus	2.9							
	S. mendiculus	2.2							
	Eudyptula minor	1.1						fish & squid	

* Information compiled primarily from review articles (Stonehouse, 1975; Croxall, 1984; Croxall & Lishman, 1987; and references therein)

TABLE 11.2 Estimated number of seabirds breeding in Tasmania

Seabird species *		Breeding pairs	Status **	Reference ***
Little penguin	*Eudyptula minor*	250 000	P	1
Shy albatross	*Diomedea cauta*	8 000	P	1
Fairy prion	*Pachyptila turtur*	1 200 000	P	1
Sooty shearwater	*Puffinus griseus*	3 000	S	1
Short-tailed shearwater	*Puffinus tenuirostris*	9 100 000	S	2
White-faced storm petrel	*Pelagodroma marina*	30 000	S	1
Common diving petrel	*Pelecanoides urinatrix*	200 000	P	1
Australian pelican	*Pelecanus conspicillatus*	100	P	1
Australasian gannet	*Sula serrator*	3 000	P	1
Black faced cormorant	*Phalacrocorax fuscescens*	8 000	P	1
Pacific gull	*Larus pacificus*	3 000	P	1
Kelp gull	*Larus dominicanus*	1 000	P	1
Silver gull	*Larus novaehollandiae*	200 000	P	1
Caspian tern	*Sterna caspia*	100	P	1
Crested tern	*Sterna bergii*	5 000	P	1
White-fronted tern	*Sterna striata*	60	P	1
Little tern	*Sterna albifrons*	< 10	P	1
Fairy tern	*Sterna nereis*	100 - 150	P	3

* Seabirds as defined by Harrison (1983)
** Status: P = permanent resident, S = summer resident
*** Reference: 1 = N. Brothers (unpublished data); 2 = Skira (1986); 3 = Rounsevell (1983).

with those taken by little penguins (Skira, 1986; N. Brothers, unpublished data). Of the birds which remain in Tasmania throughout the year, little penguins constitute 13 % of the seabird population. However, these calculations do not allow for the additional numbers of juveniles and non-breeders which would add significantly, but to different extents, to all the families present. Despite the extensive data set which now exists on the functioning of the little penguin on land and at sea, given the lack of comparable information for the other seabird species, it is premature to attempt to extend the assessment of the role of all seabirds in the Tasmanian marine ecosystem.

In assessing and modelling the role of southern seabird communities in the marine ecosystem, the ecosystem approach has, to date, been most effectively achieved in the region of the Scotia Sea, around South Georgia, where it is estimated that consumption by the 36 million seabirds in South Georgian shelf waters alone may be between 3 and 5 million tonnes annually (Croxall *et al.*, 1984). Assessments of impact on marine resources also requires information, not only of the requirements of other predators, but also the dynamics of the distribution and abundance of the resources. There are currently no reliable data pertaining to the productivity of Bass Strait and the Tasmanian marine waters which could serve to satisfy this requirement. Certainly, at present, we lack the empirical information to further examine the impact of the seabird community in the Tasmanian marine ecosystem. The results of the present study, however, are a significant contribution towards this aim. The functioning of little penguins in their marine environment, in terms of the initial questions central to predators and their interactions with their prey: how many are there, what do they eat, how much energy and food they require, where and how they acquire that food, and how these parameters change in time and space, have now been satisfied. These questions were first outlined in the General Introduction (Chapter 1), and have subsequently been addressed in the body of this thesis, thus fulfilling the aim of the study.

The answers to these questions together define the little penguin as an effective predator which forages in the upper layers of the inshore regions around its breeding colonies, consuming schooling prey below a size of 150 mm length. The local, annual and seasonal variation in diet reflects the localised distribution of prey and a degree of opportunism in the diet. The three critical periods in terms of energy flux and food acquisition have been identified as the period towards the end of the breeding season when adults are rearing large chicks, the short period immediately subsequent to breeding when preparing for the rigours of the moult fast, and during the non-breeding, winter period. Little penguins which successfully breed require approximately 137 kg of food each year, although this figure may increase if: 1) lower energy prey sources are consumed, 2) if penguins breed more than once per year, or

3) if prey availability, and hence penguin foraging efficiency, is decreased as a result of competition with commercial fisheries or any unseasonal aberrations in water currents which serve to disrupt the breeding and dispersal of juveniles of the dominant prey species.

Synthesis of the different facets of this study, combined with the population estimates, allowed assessments of the food and energy requirements of little penguins, and how these needs change in time and space. These data enable little penguins to be viewed in the context of efficient marine predators, and allow for more confident management decisions. Only, however, with a comprehensive suite of data which answers similar questions of the other predators and the resource, can the situation be viewed in the broader context, and only then can we advance further towards an understanding of the dynamics which operate within the marine ecosystem in which little penguins play an integral role.

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APPENDIX 1

Example of the iterative calculation of water and sodium flux partitioning.

Estimation	Water influx ml	Estimated food in * g	Food Na ** mmol	Na influx mmol	Seawater derived	
					Na mmol	water ml
Proximate	256.0	301.2	18.1	31.5	13.4	28.5
Iteration 1	227.5	267.6	16.1		15.4	32.8
2	223.2	262.6	15.8		15.7	33.4
3	222.6	261.9	15.7		15.8	33.6
4	222.4	261.6	15.7		15.8	33.6

* Food intake estimated from water influx assuming available water content of 85 % in food (i.e., free and metabolic water).

** Assuming 60 mmol Na per kg food.

It is possible to calculate the amount of food and seawater ingested by the penguins while foraging, assuming, in this example, that the sodium and available water contents of the diet are 60 mmol and 850 ml per kg fresh mass respectively. A proximate calculation of food consumption can be made by assuming that food represents the only source of water influx and no sea water is ingested. This proximate estimate of food intake provides an estimate of sodium obtained via food and any discrepancy between this value and the measured sodium influx must therefore come from seawater ingestion. The mean sodium concentration of seawater is 470 mmol per litre, and therefore the volume of seawater ingested can be calculated. This volume can be subtracted from the water influx measurement to provide a revised estimate of food consumption, and therefore food, sodium and seawater ingestion. By the fourth iteration of these calculations, the estimates of food intake, seawater ingestion, and the measures of water and sodium influx effectively balance.
(Taken from Green, Brothers & Gales, 1988)

APPENDIX 2

Conversion factors for fish species, all dimensions in mm and g. Unless otherwise stated all regressions are based on locally caught fish, the range of fish size used in the regressions are shown, as well as sample size (n) and r^2. The relationships used to convert otolith length (OL) to fish length (SL standard length, FL fork length or TL total length) to fish mass (W) for fish species are shown below.

Blue grenadier *Macruronus novaezelandiae*

$TL = 32.87\ OL^{0.91}$ ($r^2 = 0.99$, n = 27, TL range = 8.2 - 395)

$W = 2.3 \times 10^{-6}\ TL^{3.08}$ ($r^2 = 0.99$, n = 13, TL range = 100 - 260) (Lalas, 1983)

Barracouta *Thrysites atun*

$FL = 22.06\ OL^{1.42}$ ($r^2 = 0.95$, n = 25, FL range = 173 - 1015)

$W = 2.33 \times 10^{-6}\ FL^{3.10}$ ($r^2 = 1.00$, n = 54, FL range = 108 - 1015)

Leatherjacket *Acanthalutheres spilomelanurus*

$W = 4.43 \times 10^{-5}\ SL^{2.80}$ ($r^2 = 0.99$ n = 45, SL range = 22 - 95)

Warehou *Seriolella brama*

$FL = 20.38\ OL^{1.14}$ ($r^2 = 0.95$, n = 45, FL range = 103 - 212)

$W = 1.25 \times 10^{-5}\ FL^{3.09}$ ($r^2 = 0.98$, n = 61, FL range = 89 - 300)

South Australian Garfish *Hyporhamphus melanochir*

$SL = 68.70\ OL^{0.82}$ ($r^2 = 0.98$, n = 42, SL range = 92 - 295)

$W = 3.27 \times 10^{-8}\ SL^{3.79}$ ($r^2 = 0.99$, n = 42, SL range = 92 - 295)

Red cod *Pseudophycis bachus*

$TL = 6.33\ OL^{1.62}$ ($r^2 = 0.94$, n = 9, TL range = 248 - 448)

$W = 1.90 \times 10^{-7}\ TL^{3.73}$ ($r^2 = 0.93$, n = 9, TL range = 248 - 448)

Blue Sprat *Spratelloides robustus*

$TL = 54.33\ OL^{1.02}$ ($r^2 = 0.88$, n = 23, TL range = 45 - 73)

$W = 3.54 \times 10^{-7}\ TL^{3.67}$ ($r^2 = 0.97$, n = 23, TL range = 45 - 73)

Pilchard *Sardinops neopilchardus*

$SL = 32.07\ OL^{1.35}$ ($r^2 = 0.91$, n = 24, SL range = 68 - 155)

$W = 6.43 \times 10^{-6}\ SL^{3.11}$ ($r^2 = 0.96$, n = 29, SL range = 68 - 154)

Atherinid *Atherinason presbytoides*

$TL = 29.30\ OL^{1.02}$ ($r^2 = 0.97$, n = 102, TL range = 26 - 101)

$W = -2.77 + 6.97 \times 10^{-2}\ TL$ ($r^2 = 0.89$, n = 66, TL range = 30 - 101)

Anchovy *Engraulis australis*

$SL = 45.64\ OL^{0.74}$ ($r^2 = 0.83$, n = 40, SL range = 58 - 126)

$W = 1.71 \times 10^{-6}\ SL^{3.38}$ ($r^2 = 0.99$, n = 24, SL range = 71 - 126)

Conversion formula for squid and crustaceans. Abbreviations are dorsal mantle length (DML), lower rostral length (LRL), carapace length (CL, standard length 6; Kirkwood, 1982) and total length (TL, standard length 1; Kirkwood, 1982).

Gould's squid *Nototodarus gouldi*

$DML = 42.30\ LRL - 1.405$ (n = 36, LRL range = 0.1 - 2.0) (M.Cullen, unpubl. data)

$W = 2.234\ LRL^{1.585}$ (n = 36, LRL range = 0.1 - 2.0) (M. Cullen, unpubl. data)

Krill *Nyctiphanes australis*

$TL = 1.04 + 3.16\ CL$ (n = 61, $r^2 = 0.93$) (D. Ritz, unpubl. data)

APPENDIX 3

Summary of size of otolith length (OL), fish length (SL standard length, TL total length, FL fork length) and mass of fish removed from stomach samples of little penguins from Albatross Island for each month of study.
Data are presented as mean ± S.D. (range) n, where n = sample size. * indicates species present but no measurements recorded.

Species	OL mm SL/TL/FL mm Mass g	September 1984	December 1984	March 1985	July 1985	September 1985	November 1985	January 1986
Blue grenadier	OL	0.8 ± 0.1 (0.5 - 1.2) n = 769				1.6 ± 0.4 (0.6 - 2.5) n = 1285	*	
	TL	27.2 ± 3.8 (17.5 - 38.8)				49.4 ± 10.2 (20.7 - 75.7)	*	
	Mass	0.06 ± 0.03 (0.01 - 0.18)				0.43 ± 0.24 (0.03 - 1.41)	*	
Barracouta	OL		2.0 ± 0.5 (1.5 - 3.8) n = 119	1.8 ± 0.4 (0.8 - 2.3) n = 113			2.2 ± 0.6 (0.9 - 3.4) n = 45	1.4 ± 0.4 (0.7 - 2.7) n = 275
	FL		56.7 ± 23.0 (39.2 ± 146.9)	50.8 ± 14.1 (16.1 - 72.0)			67.6 ± 24.8 (19.0 - 125.4)	34.8 ± 13.9 (13.3 - 90.4)
	Mass		1.2 ± 2.3 (0.20 - 12.20)	0.6 ± 0.3 (0.01 - 1.33)			1.6 ± 1.8 (0.02 - 7.45)	0.2 ± 0.3 (0.01 - 2.70)
Leatherjacket	SL			15.4 ± 1.1 (13.0 - 17.0) n = 19				15.5 ± 3.5 (10.0 - 29.0) n = 240
	Mass			0.1 ± 0.2 (0.06 - 0.12)				0.1 ± 0.1 (0.03 - 0.55)
Warehou	OL	1.1, n = 1	2.0 ± 0.6 (1.1 - 3.6) n = 55				2.2 ± 0.7 (1.0 - 4.4) n = 398	1.5 ± 0.4 (0.7 - 2.3) n =25
	FL	22.7	45.1 ± 14.2 (22.7 ± 87.8)				50.3 ± 17.6 (20.4 ± 110.3)	33.0 ± 10.5 (13.6 ± 52.7)
	Mass	0.19	2.2 ± 2.4 (0.20 - 12.60)				3.2 ± 3.6 (0.14 - 25.64)	0.8 ± 0.7 (0.04 - 2.61)
South Australian garfish	OL	1.5 ± 0.7 (1.0 - 2.0) n = 2		1.0 ± 0.3 (0.7 - 2.1) n = 122	1.9 ± 0.4 (1.6 - 2.2) n = 2			
	SL	95.0 ± 37.2 (68.7 ± 121.3)		68.3 ± 14.6 (51.3 ± 126.2)	116.1 ± 21.3 (101.0 ± 131.1)			
	Mass	1.4 ± 1.6 (0.30 - 2.56)		0.4 ± 0.5 (0.10 - 3.00)	2.4 ± 1.5 (1.29 - 3.47)			
Red cod	OL	1.2 ± 0.2 (0.9 - 1.7) n = 184	2.3 ± 0.6 (1.5 - 2.9) n = 7	1.2 ± 0.1 (1.0 - 1.3) n = 7		1.3, n = 1	2.5 ± 0.8 (1.7 - 4.1) n = 10	3.3 ± 1.0 (2.6 - 4.8) n = 7
	TL	9.0 ± 2.1 (5.3 ± 15.0)	24.3 ± 9.5 (12.2 ± 35.5)	8.4 ± 1.2 (6.3 ± 9.7)		9.7	29.5 ± 14.7 (15.0 ± 62.2)	44.5 ± 22.7 (29.8 ± 80.4)
	Mass	0.001 ± 0.001 (9.8E-5 - 0.005)	0.05 ± 0.05 (0.002 - 0.115)	0.001 ± 2.7E-4 (1.8E-5 - 0.001)		0.001	0.14 ± 0.26 (0.005 - 0.935)	0.67 ± 1.03 (0.06 - 2.42)
Blue sprat	OL				0.9 ± 0.4 (0.4 - 1.9) n = 66			
	TL				50.3 ±22.6 (21.3 ± 104.6)			
	Mass				1.3 ± 1.7 (0.03 - 9.12)			
Spotted trevalla	OL		1.9 ± 0.2 (1.5 - 2.2) n = 17				1.9 ± 0.4 (1.2 - 2.8) n = 39	1.8 ± 0.1 (1.7 - 1.8) n = 2
	FL		43.0 ± 5.9 (32.4 ± 50.1)				43.4 ± 9.7 (25.1 ± 65.9)	38.6 ± 1.8 (37.3 ± 39.8)
	Mass		1.5 ± 0.6 (0.58 - 2.23)				1.7 ± 1.2 (0.26 - 5.22)	1.0 ± 0.1 (0.90 - 1.10)
Pilchard	OL		1.7 ± 0.1 (1.5 - 1.9) n = 18		1.5 ± 0.1 (1.4 - 1.6) n = 6		1.0 ± 0.1 (0.9 - 1.0) n = 2	1.4 ± 0.6 (0.8 - 3.0) n =19
	SL		66.6 ± 6.8 (55.4 ± 76.3)		56.7 ± 4.9 (50.5 ± 60.5)		29.9 ± 3.0 (27.8 ± 32.1)	51.3 ±32.2 (23.7 ± 141.3)
	Mass		3.1 ± 1.0 (1.70 - 4.60)		1.9 ± 0.5 (1.28 - 2.23)		0.3 ± 0.1 (0.20 - 0.31)	3.5 ± 7.2 (0.12 - 31.30)
Atherinid	OL				2.5 ± 0.3 (2.0 - 3.0) n = 32			
	TL				74.1 ± 7.8 (59.4 ± 89.9)			
	Mass				2.4 ± 0.5 (1.37 - 3.49)			
Jack mackerel	OL						1.9 ± 0.3 (1.4 - 2.2) n = 13	3.1 ± 0.1 (3.0 - 3.1) n = 2
Anchovy	OL				1.8 ± 0.1 (1.7 - 1.9) n = 3		1.8 ± 0.0 (1.8 - 1.8) n = 2	2.7 ± 0.3 (2.3 - 3.0) n = 4
	SL				69.5 ± 3.3 (67.6 ± 73.4)		70.5 ± 0.0 (70.5 ± 70.5)	94.0 ± 7.6 (84.5 ± 102.9)
	Mass				2.9 ± 0.5 (2.62 - 3.46)		3.0 ± 0.0 (3.02 - 3.02)	8.1 ± 2.2 (5.58 - 10.84)
Pipefish	TL		*		72.8 ± 6.6 (65.0 - 80.0) n = 4			
Sprat	OL				1.5 ± 0.2 (1.3 - 1.7) n = 3			
Gemfish	OL		1.9 ± 0.1 (1.8 - 2.0) n = 2					
Dragonet	SL						95.0, n = 1	

APPENDIX 4

Reprints of published papers which form a part of this thesis:

Gales, R. P. (1987*b*). Validation of the stomach flushing technique for obtaining stomach contents of penguins. *Ibis* **129:** 335-343.

Gales, R. P. (1988*a*). Sexing adult blue penguins by external measurements. *Notornis* **35:** 71-75.

Gales, R. P. (1988*b*). The use of otoliths as indicators of little penguin *Eudyptula minor* diet. *Ibis* **130:** 418-426.

Gales, R. P. (1989). Validation of the use of tritiated water, doubly labeled water, and sodium-22, for estimating food, energy and water intake of little penguins, *Eudyptula minor*. *Physiol. Zool.* **62:** 147-169.

Gales, R., Green, B., Stahel, C. (1988). The energetics of free-living little penguins *Eudyptula minor* (Spheniscidae) during moult. *Aust. J. Zool.* **36:** 159-167.

Validation of the Use of Tritiated Water, Doubly Labeled Water, and ^{22}Na for Estimating Food, Energy, and Water Intake in Little Penguins, *Eudyptula minor*

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Abstract

The water, sodium, and energy turnovers of little penguins were examined by comparing estimates determined from tritium (HTO), sodium-22 (^{22}Na), and doubly labeled water (DLW) turnovers with estimates from simultaneous materials balance trials. Two diets were used to assess the effect of food type on assimilation efficiencies and on turnover rates. Energy assimilation efficiencies were higher with the squid diet than with the fish diet but were still the lowest recorded for any penguin species. All three isotopes had equilibrated with body pools between 2 and 6 h after injection and biological half-lives were 4.3, 3.4, and 2.9 days for HTO, ^{18}O, and ^{22}Na, respectively, with no difference between diets. On average, HTO-derived estimates were significantly higher than measured intake (6.5%), but DLW-derived estimates of metabolic rate were not significantly different from materials balance estimates. On average, ^{22}Na underestimated dietary sodium intake by 5.0%, and, although this difference was not significant, discrepancies were greater with the squid diet than with the fish diet. Changes of approximately 50% in isotope levels in the blood are required between injection and sampling to ensure reliable results from turnovers of all three isotopes.

Introduction

Of all the seabirds, penguins show the greatest degree of adaptation to the marine environment, and information on their energetics and feeding rates is essential to the understanding of energy flow in marine ecosystems. There have now been studies on the energetics of at least eight of the 17 penguin species (Kooyman et al. 1982; Davis, Kooyman, and Croxall 1983; Nagy, Siegfried, and Wilson 1984; Gales, Pemberton, and Green, unpublished

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data), including the little penguin, *Eudyptula minor* (Costa et al. 1986; Green et al., in press; Gales et al., in press).

The little penguin is the smallest penguin species and is restricted to the coasts of New Zealand and southern Australia. In these waters it is an important consumer of marine resources. Results of preliminary field studies in Tasmania have shown a combination of three isotopes to be appropriate for the investigation of feeding rates and foraging efficiencies of the species (Green et al., in press). Tritium (HTO) and doubly labeled water (DLW, $HT^{18}O$) were used to determine total body water, water turnover rates, CO_2 production, and energy metabolism. Sodium-22 (^{22}Na) was also used to measure the total Na flux and to partition the water and Na influx into intake via food and intake via ingestion of seawater.

Despite the increasing attention being paid to the free-living energetics of the little penguin and other avian species (summarized in Nagy [1987]), there are few validation studies of isotope turnover techniques in birds (e.g., LeFebvre 1964; Hails 1979; Degen et al. 1981; Williams and Nagy 1984*b*; Goldstein and Nagy 1985; Williams 1985; Williams and Prints 1986; Buttemer et al. 1986), and none in seabirds. Not only do seabirds scale differently from other birds in allometric analyses of field metabolic rates (Nagy 1987), but the use of ^{22}Na has only previously been validated in one bird species (Herd 1985).

In interpreting and designing studies of free-living energetics it is important to know the magnitude of errors that may occur and also the appropriate time required both for equilibration of isotopes with the body pools and for animal recapture. In this study, I have examined the accuracy of measurements of the three isotopes in captive little penguins by comparing simultaneous isotopic turnover to metabolizable intake measurements. These methods include a relatively new procedure for ^{18}O determinations with a mass spectrometer. In addition, I examined the effect of two different diets on assimilation efficiencies and isotope turnover.

Material and Methods

Animals and Experimental Conditions.

The three adult penguins used in this study had been captured from the east coast of Tasmania and held in outside enclosures for at least 4 mo before experimentation. At the beginning of each experiment the penguins were moved into plastic-lined wire mesh cages measuring $1.0 \times 0.8 \times 0.8$ m. They were housed in a controlled temperature room (15.0 ± 1.0 C), which was within the thermoneutral zone of this species (Stahel and Nicol 1982), with

a 12L:12D regime. Before the start of each experiment, the penguins were maintained in these conditions for 5 days in order to acclimate to the laboratory conditions and the feeding regimes.

Validation experiments of the isotope turnover technique were run in two series. The penguins were fed different diets in each series, one comprising a fish diet of whole specimens of sandy sprat (*Hyperlophus vittatus,* Clupeidae), and the other, a squid diet of Gould's squid mantles (*Nototodarus gouldi,* Ommastrephidae), both species occurring frequently in the natural diet of little penguins (Gales, unpublished data). The squid mantles and fish were stored frozen. Prior to feeding, the food was thawed in sealed plastic bags, rinsed in freshwater and blot-dried before weighing into 150-g batches. Samples of food were retained for analyses of water, Na, and energy content (see below).

Before injecting birds, blood was sampled for background isotope levels. Each penguin was weighed and given intraperitoneal injections of 1.0-mL tritiated water (HTO: 185 MBq), 0.5-mL sodium-22 (^{22}Na: 185 kBq), and 0.3-mL oxygen-18 (^{18}O: 95% + atom excess). The accuracy of these injection volumes was established gravimetrically, and none varied by more than ±0.7% from the indicated value. Blood samples of approximately 1 mL were taken from a brachial vein of each penguin, at 1-, 2-, 4-, 6-, 12-, and 24-h intervals after injection. Food and water were withheld for 12 h before the injections and during the initial 24-h period. Blood samples were obtained by nicking a brachial vein with a scalpel blade and collecting blood directly into nonheparinized plastic vials. They were then centrifuged, and the serum and red cell fractions were separated and stored frozen.

Each penguin was force-fed 150 g of squid or fish each day. They were not given access to drinking water. The isotope turnover trials ran for 6 days (from injection to final blood sample), and blood samples were collected after 2, 4, and 6 days. As the plastic cage liners were installed only after the final equilibration sample (24 h postinjection), the materials balance trial ran for only 5 days. The penguins were weighed with spring balances (±5 g) whenever blood was collected. The same three birds were used for both experiments, which were identical except for diet, and which were separated by a period of 3 mo.

Analytical Procedures

At the end of the trials, the cages and plastic liners were thoroughly swabbed with cotton wool and distilled water. The excreta (feces, urine, and salt-gland excreta) and samples of the food were then dried in a vacuum oven at 55 C until their mass was constant. Subsamples of the food and excreta

were then ground in a Wiley mill and stored. Dried samples of food and excreta were compressed into 0.5-g pellets and combusted in a Gallenkamp ballistic bomb calorimeter to determine energy content (in triplicate). Weighed subsamples (0.5 g) of the food items and excreta were also digested in concentrated nitric acid and then diluted with deionized water. The Na concentrations were measured in an atomic absorption spectrophotometer (Varian Techtron model 1000) with an air acetylene flame. Serum samples (5 μL) were diluted to 2 mL with deionized water for estimation of Na concentration.

The red cell fractions were lyophilized to complete dryness (Vaughn and Boling 1961). Samples of extracted water (10 μL) were added to 3 mL of PCS cocktail (Phase Combining System, Amersham) and assayed for tritiated water activity in a liquid scintillation spectrophotometer (Beckman LS 2800). I placed 50-μL aliquots of extracted water in Urey tubes together with a standard charge of CO_2 gas. The Urey tubes were incubated overnight at 80 C, after which the equilibrated CO_2 charge was removed and the ^{16}O:^{18}O ratio was determined with a VG Isogas 903 isotope ratio mass spectrometer. The serum samples were bleached with concentrated hydrogen peroxide and oven-dried overnight. They were then mixed with 3-mL PCS cocktail and assayed for ^{22}Na activity by liquid scintillation spectrometry (Green and Dunsmore 1978).

Computational Procedures

The penguins' gross energy intake (GEI) during each feeding trial was calculated by multiplying the amount of food consumed by the mass-specific energy content of the food. Dry matter assimilation (DMA) and energy assimilation efficiency (EAE) were calculated with the following equations (Gessaman 1972):

$$\text{DMA} = \frac{\text{dry mass food (g)} - \text{dry mass excreta (g)}}{\text{dry mass food (g)}} \times 100$$

and

$$\text{EAE} = \frac{\text{GEI} - \text{energy in excreta}}{\text{GEI}} \times 100.$$

Based on EAE, the amount of energy assimilated daily (DEA) was evaluated as the product of EAE and the amount of food eaten. The use of these calculations in this study assumes that gut-passage rate is less than 24 h. This is true

TABLE 1
Water, sodium, and energy contents of the fish and squid used in the validation trials

	Water Content (%)	Sodium Content (mmol/kg)	Energy Content	
			Wet Mass (kJ/g)	Dry Mass (kJ/g)
Fish (sandy sprat):				
Mean	72.85	75.9	5.47	20.27
SD	1.19	...[a]	.25	.73
n	49	...	10	10
Squid (Gould's squid):				
Mean	77.13	96.20	5.46	23.89
SD	.87	4.83	.32	1.17
n	6	6	6	6

[a] Pooled sample.

for little penguins on diets of both squid (Montague 1982) and fish (Gales 1988). Change in penguin body mass (%) was expressed as the change in mass as a percentage of the mean mass over the duration of the experiment.

Exchangeable Na and body water pool sizes (TBW) were calculated by comparing blood isotope levels at equilibration to standard dilutions of the injected isotopes. With ^{18}O standards, a sample of the standard diluent was also retained for mass spectrometry, as were the ^{18}O background samples. Isotope flux rates were calculated following Lifson and McClintock (1966), Nagy (1980), and Nagy and Costa (1980), assuming that changes in the pool size reflected body mass changes and that these changes were linear. The biological half-lives ($T_{1/2}$) of the isotopes were estimated from the formulae presented by Green and Dunsmore (1978).

For the fish diet, metabolic heat production was estimated from each penguin's rate of CO_2 production assuming 25.4 J/mL CO_2. This factor is based on the chemical composition of anchovy (19.7% protein, 5.2% fat, <0.5% carbohydrate; South African Fisheries Industrial Research Institute [1980], cited in Nagy et al. [1984]). Anchovy is of similar composition to sandy sprat (anchovy: 72.0% water, energy content 23.9 kJ/g dry mass [R. Gales, unpublished data]; and see table 1) and belong to the same order, Clupeiformes.

For the squid diet, the conversion factor was 24.9 J/mL CO_2, which is based on the composition of another Ommastrephid squid, Japanese flying squid (76.6% water, 4.19 kJ/g wet mass, Croxall and Prince [1982]; and see table 1). The conversion factors used in estimating oxidation water were 0.5 mL water per g of metabolized protein and 1.07 mL water per g metabolized fat (Schmidt-Nielsen 1975). I assumed that fat and protein had the same fractional assimilations and, as carbohydrates formed <0.5% of both diets, they were considered negligible. Unless stated otherwise, all mean values are presented ±SD, all Student's *t*-tests are paired, and the .05 level of probability was accepted as indicating statistical significance.

Results

Materials Balance

The water, Na, and energy contents of the two diets are shown in table 1. The penguins showed no significant changes in body mass during the experiments (fish diet t = 1.75, NS; squid diet t = 0.95, NS; table 2), which indicates that they maintained water, Na, and energy balance during the trials. The energy content in the penguins' feces after being fed the fish diet was 10.82 ± 0.01 kJ/g dry mass (n = 3) and after being fed the squid diet was 13.63 ± 0.77 kJ/g dry mass (n = 3). The food consumption, excretory output, apparent DMA, and EAE are shown for each penguin in table 2. When being fed the squid diet the penguins showed higher %DMA (t = 9.56; $P < .05$) and %EAE (t = 3.65; $P < .05$) than when on the fish diet.

The mean daily energy intake for the fish and squid diets were 728.0 ± 29.5 kJ/kg (n = 3) and 699.7 ± 13.1 kJ/kg (n = 3), respectively, and did not differ significantly from one another (t = 1.43, NS). There was also no significant difference between the two diets in terms of DEA (t = 0.15, NS).

Rates of Isotope Equilibration

The HTO had equilibrated in the penguin body pools by 2 h, and the levels in the samples taken between 2 and 12 h after injection remained relatively unchanged. This is reflected in the estimates of total body water (TBW), calculated from HTO dilution (fig. 1*A*). Samples taken only 1 h postinjection resulted in very high estimates of TBW. When TBW, determined at each of the equilibration sampling times, is expressed as a percentage of body mass at the time of injection versus at time of sample collection, the differences are minimal (i.e., <1%) until 6 h postinjection. Owing to loss of body mass these differences increased after 6 h, reaching 2.3% at 12 h, and 4.3% after

TABLE 2

Digestibility of food and energy intake of little penguins

Diet and Penguin No.	Initial Body Mass (kg)	Mass Change (%)	Total Food Consumption			Fecal Production		DMA[a] (%)	EAE[b] (%)	DEA[c] (kJ/kg day^{-1})
			Fresh Mass (kg)	Dry Mass (kg)	Energy (kJ)	Dry Mass (g)	Energy (kJ)			
Fish:										
1	1.085	−.9	.748	.203	4,115	113.6	1,246	43.8	69.7	531.3
2	1.165	−.9	.747	.203	4,115	108.9	1,176	46.3	71.4	506.7
3	1.180	−4.2	.748	.203	4,115	113.9	1,213	43.8	70.5	502.5
Mean ...	1.143	−2.0	.748	.203	4,115	112.1	1,212	44.6	70.5	513.5
SD	.051	1.9	...	...	...	2.8	35	1.4	.8	15.6
Squid:										
1	1.170	+.7	.750	.171	4,085	81.1	1,173	52.6	71.3	496.1
2	1.190	−.7	.750	.171	4,085	80.7	1,087	52.6	73.4	505.6
3	1.165	−3.7	.750	.171	4,085	80.6	1,051	52.6	74.3	530.4
Mean ...	1.175	−1.2	.750	.171	4,085	80.8	1,104	52.6	73.0	510.7
SD	.130	2.5	...	...	...	.3	63	...	1.5	17.7

[a] Dry matter assimilation.
[b] Energy assimilation efficiency.
[c] Daily energy assimilation.

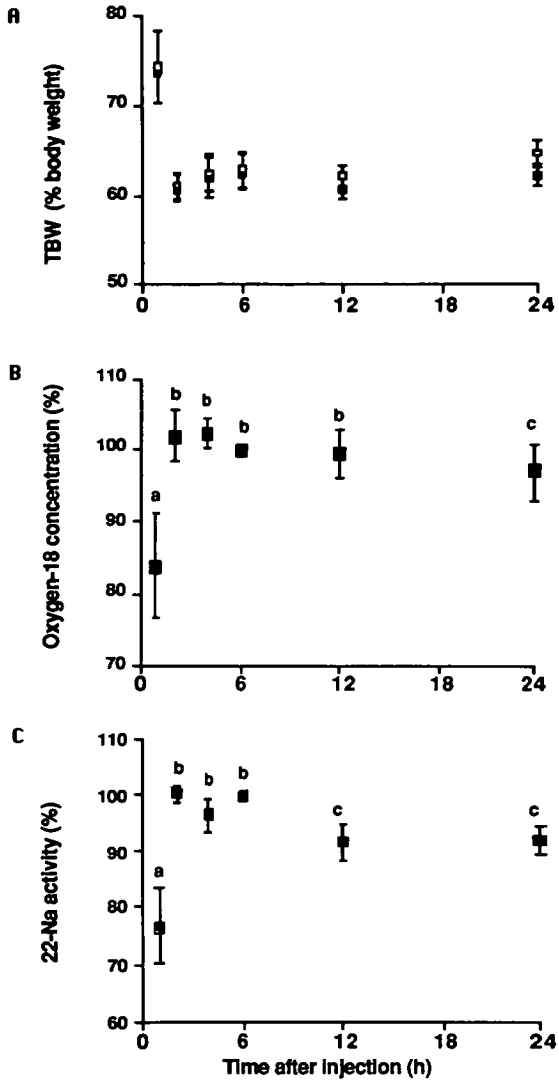

Fig. 1. A, *Total body water, calculated from HTO dilution, expressed as a percentage (±SE) of body mass at time of injection (closed box) and mass at bleeding (open box) for intervals up to 24 h postinjection.* B, *Mean ^{18}O concentration (±SE) expressed as a percentage of concentration 6 h after injection. Means with different superscripts are significantly different* ($P < .05$). *Sample size for each point is six, being the three penguins on the two diets.* C, *Mean serum ^{22}Na-specific activity expressed as a percentage of activity 6 h after injection. Means with different superscripts are significantly different* ($P < .05$). *Sample sizes as in* B.

24 h, and these differences are significant (12 h: t = 5.71, $P < .05$; 24 h: t = 12.65, $P < .05$). A period of between 2 and 6 h was therefore considered to be both adequate and convenient for equilibration of injected HTO with the body water pool.

As with HTO, ^{18}O was equilibrated with body water at 2 h and remained relatively constant, until 12 h postinjection. After 12 h, the concentration started to decline and was significantly lower than equilibration values at 24 h postinjection (t = 4.34, $P < .05$; fig. 1*B*).

The ^{22}Na concentration in the serum increased rapidly until 2 h postinjection (fig. 1*C*). Between 2 and 6 h ^{22}Na remained relatively constant but

started to decline after 6 h. Therefore, a period of between 2 and 6 h is appropriate for equilibration of injected ^{22}Na with the exchangeable Na in the little penguin. When equilibrated, the exchangeable Na was 43.6 ± 4.5 mmol (n = 6) or 37.6 ± 3.4 mmol/kg body mass. These levels are equivalent to 55 mmol/L body water.

Validation of Water Turnover Rates

TBW, determined from HTO equilibration, ranged between 57% and 71% of body mass (table 3). When TBWs were determined from both HTO and ^{18}O equilibration samples at 6 h after injection, the estimate derived from ^{18}O was only −1.6% ± 2.2% (n = 6) less than that determined from HTO, and this difference was not significant (t = 0.30, NS). The greatest discrepancy between the two methods was −5.0%, with ^{18}O giving the lower estimate. The biological half-life of HTO in the penguins was 4.3 ± 0.26 days (n = 6), and there was no significant difference between diets (t = 0.15, NS). There were also no significant differences between the water influx and efflux rates (table 3), indicating that the birds were in water balance (fish diet: t = 1.318, NS; squid diet: t = 0.948, NS).

The validation of the water turnover rates was assessed by comparing the estimated water intake rates of the two diets to the water influx rates estimated from the HTO turnover over the 6 days, at the completion of the trials. The preformed water provided by the fish diet was 0.728 mL/g fresh mass. The metabolic water obtained via oxidation of the metabolizable fat and protein was estimated as 0.154 mL/g fresh mass. Thus, the total water yield from the fish diet was 0.882 mL/g fresh mass. For the squid diet, the preformed water represented 0.771 mL/g fresh mass. The metabolic water provided by the squid diet was estimated as 0.09 mL/g fresh mass, the total water yield being 0.861 mL/g fresh food.

For little penguins on the fish diet, total water intake, as estimated by HTO over the 6-day period, was between +7.4% and +13.1% of the calculated value, while on the squid diet the range was between −8.6% and +12.3% (table 3). On average, HTO overestimated water intake by 6.5% ± 0.08% (n = 6). At only 2 days after injection, the HTO activity had decreased by 20.1% ± 3.3% from equilibration values (fig. 2*A*). The rate of water influx calculated from the 2-day HTO turnover was 26% lower than the estimated dietary water intake and differed significantly from evaluation of samples taken at 4 and 6 days postinjection ($P < .05$; fig. 2*A*). The HTO turnovers after 4 days overestimated water influx by 1.0% ± 0.1%, and this was not significantly different from the estimate derived over the 6 days.

TABLE 3

Components of the water metabolism and dietary water intake of the little penguins

Diet and Penguin No.	Mean Body Mass (kg)	TBW (mL/kg)	HTO $T_{1/2}$ (days)	Water Influx (I) (mL/kg day^{-1})	Water Efflux (mL/kg day^{-1})	Dietary Water Intake (II) * (mL/kg day^{-1})	Ratio (I/II)
Fish:							
1	1.080	650	4.15	109.3	110.2	101.8	1.074
2	1.160	608	4.17	101.8	102	94.8	1.074
3	1.155	709	4.60	107.7	112.7	95.2	1.131
Mean ...	1.132	656	4.31	106.3	108.3	97.3	1.093
SD	.045	51	.25	4.0	5.6	3.9	.033
Squid:							
1	1.174	567	4.69	83.8	83.0	91.7	.914
2	1.186	604	4.30	97.4	98.1	90.7	1.074
3	1.144	620	4.07	105.7	109.3	94.1	1.123
Mean ...	1.168	597	4.35	95.6	96.8	92.2	1.037
SD	.022	27	.31	11.1	13.2	1.7	.11

* Calculated as preformed and metabolic water.

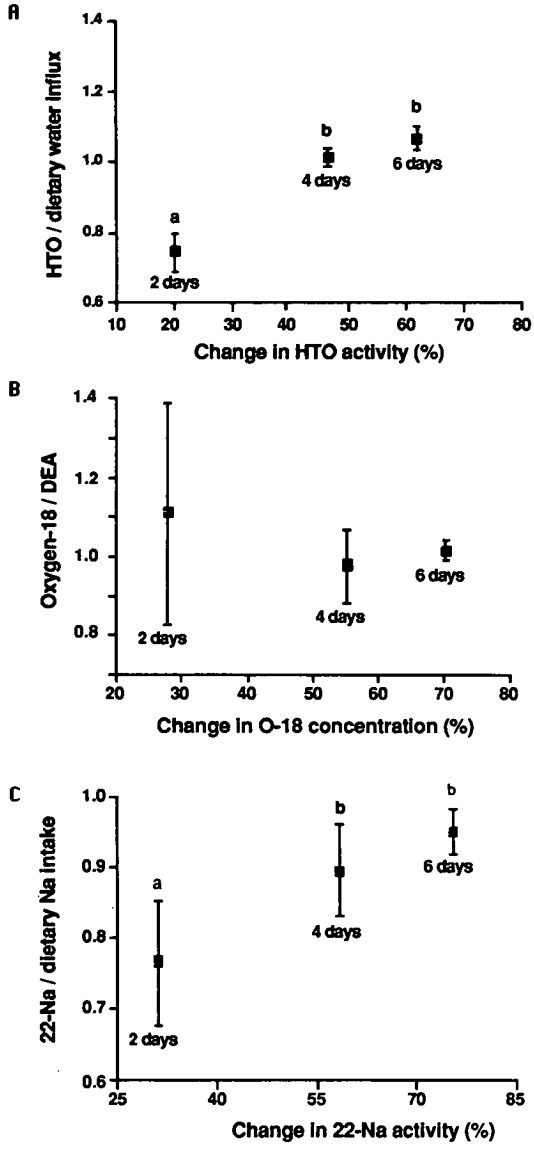

Fig. 2. A, *Water influx (mL/kg day^{-1}) derived from HTO turnover expressed as a ratio of dietary water intake (±SE) in relation to the percentage change in HTO activity from equilibrium (6 h) of samples after 2, 4, and 6 days. Means with different superscripts are significantly different (P < .05). Sample sizes are six for 2 and 6 days, being the three penguins on both diets, and five for day 4 as one sample was lost.* B, *Metabolic rate (kJ/kg day^{-1}) derived from DLW turnover as a ratio of DEA (±SE) in relation to percentage change in ^{18}O concentration from equilibration (6 h) to samples after 2, 4, and 6 days. Sample sizes as in* A. C, *Na influx (mmol/kg day^{-1}) derived from ^{22}Na turnover as a ratio of dietary sodium influx (±SE) in relation to the percentage change in ^{22}Na activity from equilibration (6 h) to samples after 2, 4, and 6 days. Means with different superscripts are significantly different (P < .05) and sample sizes as in* A.

Validation of Metabolic Rates

The biological half-life of ^{18}O during the two trials was 3.43 ± 0.12 days (n = 6), and the different diets had no effect on the half-life (t = 0.116, NS).

Estimates of CO_2 production from DLW turnover in the validation trial

TABLE 4
Validation of the DLW method as an estimate of metabolic rate and food consumption

Diet and Penguin No.	Metabolic Rate ($mL\ CO_2/g\ h^{-1}$)	Metabolic Rate ($kJ/kg\ day^{-1}$ [I])	Ratio (I)/DEA
Fish:			
1	.882	537.7	1.012
2	.825	502.9	.993
3	.927	565.1	1.125
Mean	.878	535.2	1.043
SD	.05	31.2	.071
Squid:			
1	.863	515.7	1.039
2	.836	499.6	.988
3	.841	502.6	.948
Mean	.847	506.0	.992
SD	.014	8.6	.046

using the fish diet averaged 0.878 $mL/g \cdot h^{-1}$, and the average was 0.847 $mL/g \cdot h^{-1}$ during the squid feeding trial (table 4). Thermal equivalents for CO_2 production were assumed to be 25.4 and 24.9 kJ/L CO_2 for the fish and squid diets, respectively (see Material and Methods). Mean metabolic rates were thus 535.2 $kJ/kg\ day^{-1}$ and 506.0 $kJ/kg\ day^{-1}$ for the fish and squid diets, respectively, and these values were not significantly different ($t = 1.675$, NS). When compared to mean DEA estimated from materials balance (table 2), DLW estimates of energy metabolism were 4.3% higher for the fish diet, which is not significantly different from the former value ($t = 1.053$, NS). For the squid diet the discrepancy between the average metabolic rate predicted by the DLW method and the material balance method was 0.8%, and the difference was not significant ($t = 0.346$, NS). On average, DLW overestimated metabolic rate by 1.8% ± 0.06%, and this is again not significant ($t = 0.677$, NS).

After 2 days, the ^{18}O concentration had decreased from initial equilibration levels by 28.1% ± 4.6%, by 55.0% ± 0.81% after 4 days, and by 70.3% ± 1.4% after 6 days (fig. 2*B*). Pooling data from both diets, metabolic rates

estimated from DLW turnover were 10.9% higher than that estimated from materials balance after 2 days, 2.4% lower than DAE after 4 days, and 1.7% higher after 6 days. The degree of variation in values within the three time periods were very different, and this would account for the lack of statistical difference between samples (fig. 2*B*).

Validation of Sodium Turnover Rates

The biological half-life of ^{22}Na did not differ significantly between diets ($t = 1.941$, NS) and averaged 2.95 ± 0.16 days ($n = 6$). There were no significant differences between Na influx and efflux rates associated with either the fish or the squid diets, suggesting that the penguins were in Na balance (fish diet: $t = 1.94$, NS; squid diet: $t = 1.17$, NS) (table 5).

The exchangeable Na pools of the penguins were consistently lower during the fish diet than during the squid diet. While on the fish diet, the penguins had an Na pool of 53.8 ± 0.21 mmol/L TBW, and during the squid trial, 67.6 ± 1.6 mmol/L TBW. The final estimates of Na influx by ^{22}Na turnover and estimated dietary Na intake were in closer agreement with the fish than the squid diet. On average, ^{22}Na turnover overestimated Na intake from the fish diet by 1.5%, but it underestimated calculated Na intake of the squid diet by 11.5% (table 5). When the data are pooled, there is no significant difference between estimates of ^{22}Na turnover and Na intake ($t = 1.726$, NS).

When sampled after 2 days, the ^{22}Na activity had decreased by 31.1% ± 7.4% from the initial equilibration value, decreased to 58.4% ± 5.9% after 4 days, and to 75.5% ± 1.7% by the final sample (6 d; fig. 2*C*). Na influx calculated from ^{22}Na turnover was 39.8% lower than the estimated dietary Na intake for the 2-day sample and was also significantly lower than the dietary values estimated for 4 or 6 days (fig. 2*C*). The ^{22}Na-derived estimate of Na influx at day 4 was not significantly different from that obtained after 6 days, although the variation in values was greater at 4 days.

Discussion

Energy assimilation efficiencies are essential for accurately modeling the food requirements of natural populations. Data for assimilation efficiencies of seabirds (summarized by Adams [1984]) are limited, and many bioenergetic studies rely instead on values from other species, and usually from chicks. It is important, however, that assimilation efficiencies can vary with species, diet type, and temperature (Wiens 1984).

TABLE 5

Components of the sodium metabolism and dietary sodium intake of the little penguins

Diet and Penguin No.	Exchangeable Sodium Pool (mmol/kg)	Sodium-22 Influx (mmol/kg day^{-1})	Sodium-22 Efflux (mmol/kg day^{-1})	Total Sodium-22 Turnover (I) (mmol)	Total Sodium Intake (II) (mmol)	Ratio (I/II)
Fish:						
1	34.80	8.84	8.90	57.3	56.8	1.009
2	32.67	8.10	8.15	56.4	56.7	.995
3	37.38	8.63	8.86	59.2	56.8	1.042
Mean ...	34.95	8.52	8.64	57.6	56.8	1.015
SD	2.36	.38	.42	1.4	...	1.024
Squid:						
1	39.38	8.73	8.72	61.5	72.1	.853
2	40.06	9.06	9.10	64.4	72.1	.893
3	41.51	9.56	9.82	65.6	72.1	.910
Mean ...	40.32	9.12	9.21	63.8	72.1	.885
SD	1.09	.42	.56	2.1	...	.029

In the present study, the squid had a higher energy content than the fish on a dry weight basis and the little penguins showed higher DMA and EAE when being fed the squid diet (tables 1 and 2). The EAE values for both diet types, however, were at the lower limit of the range for seabirds for which data are available (Adams 1984). The values of assimilation efficiency obtained in this study are the lowest recorded for penguins, and such species-specific variation in this parameter may importantly affect estimates of their food requirements. In their study of energetic requirements of little penguins, Costa, Dann, and Disher (1986) assumed an assimilation efficiency of 77.9% for an exclusively fish diet, a value obtained from jackass penguins (*Spheniscus demersus*) by Nagy et al. (1984). Substituting the EAE value determined in the present study for that used by Costa et al. (1986) produces a value of 4.64 kJ/g fresh matter available to the penguins, which is 10% lower than the 5.13 kJ/g based on jackass penguins. This discrepancy would compound in further calculations of estimated food intake and demonstrates the need, where possible, to use values appropriate to the species and diet type.

It is also important to know the period of time required for isotopes to equilibrate with the body pool as sampling before complete equilibration leads to errors in determination of pool size and subsequent turnover rates of the isotopes. Route of injection has been found to affect rate of isotope equilibration in mammals (Smith and Sykes 1974), but there was no difference in the rate of HTO equilibration in chukar partridges (*Alectoris chukar*) after either intramuscular (IM) or intravenous (IV) injections (Degen et al. 1981). For relatively small birds, where route of injection was either IM or IV, 1 h has been determined as sufficient for species ranging in size from 20 to 600 g (LeFebvre 1964; Degen et al. 1981; Williams and Nagy 1984*a*, 1984*b*; Williams 1985). In larger birds, however, equilibration periods have rarely been measured.

Equilibration time for HTO was determined in the gray-headed albatross (*Diomedea chrysostoma*, ca. 3.5 kg) and was complete in all cases by 2 h after IM injections (Costa and Prince 1987). With emus (*Dromaius novaehollandiae*, ca. 35 kg), plasma ^{22}Na concentration was stable from 6 h to 24 h after IM injections (Herd 1985). None of the isotopes used in the present study had equilibrated by 1 h postinjection, the route of injection being intraperitoneal (IP). By 2 h, however, all isotopes had equilibrated with the penguins' body pools, with HTO and ^{18}O remaining unchanged through 12 h and ^{22}Na through 6 h postinjection. An appropriate equilibration period for all three isotopes then, after IP injection, is 2–6 h for the little penguin.

In isotope studies, appropriate sampling intervals are important. If insufficient isotope turnover has occurred, or if isotope levels are too close to

background levels, large errors in estimating their turnover rates may result (Nagy 1983). Suggested recapture intervals are often presented as multiples of the biological half-life of an isotope, and this varies with taxon, size, and activity level of a given animal. Biological half-lives of HTO in birds have been summarized by Streit (1982), who presents a predictive formula that predicts a theoretical half-life of 8.6 ± 0.1 days for the little penguins of this study, double the value found in the present study. Using the formula presented for the biological half-life of ^{18}O in free-living birds (Nagy 1983), one finds that the theoretical half-life in little penguins is 2.1 ± 0.03 days, shorter than the 3.4 days determined in these experiments on captive animals. HTO is accurate for more than five half-lives, so calculated water fluxes are generally reliable for a longer period than are calculated CO_2 production rates, where reliable results are obtained between one and two half-lives of the ^{18}O isotope (Nagy 1983).

Published values of the biological half-life of ^{22}Na in birds range from 8.1 days for glaucous-winged gulls, *Larus glaucescens* (Roberts and Hughes 1984), to 18.9 days for emus (Herd 1985). These values are well in excess of the 2.9 days calculated for little penguins. These differences reflect the variety that may exist, not only between taxa, but also within species depending on body mass, metabolic rate, environment, diet, stage of breeding cycle, and experimental procedure (Streit 1982).

Mean TBW of little penguins in this study was 62.6% body mass, a figure that is typical of normally hydrated adult birds (Skadhauge 1981; Mahoney and Jehl 1984). This value agrees with TBWs determined via isotope dilution of adult little penguins (63%, Gales, Green, and Stahel 1988; 63.2%, Costa et al. 1986) and the value of 61% ± 2.3% determined by desiccation of five adult little penguins (Green, unpublished data). Errors in estimation of TBW by HTO in mammals have been summarized by Nagy and Costa (1980), and these range from −5.7% to +12.0%. Degen et al (1981) found that in chukar partridges and sand partidges (*Ammoperdix heyi*) there was no significant difference between desiccated and HTO-determined water space, although small differences were found in chukar partidges by Crum, Williams, and Nagy (1985), depending on methods of analyses.

In the present study TBW determined from HTO dilution was 1.6% higher than TBW determined from ^{18}O dilution, but the difference was not significant. TBW estimated from ^{18}O dilution in three species of sparrows (*Melospiza melodia, Zonotrichia albicolis,* and *Passer domesticus*) averaged 3.1% higher than values obtained by desiccation (Williams 1985) after correcting for the feather water pool. If no allowance is made for the water contained in the feathers, which is not penetrated by isotope, then the discrepancy

between TBWs determined by desiccation and isotope dilution decreases (Williams 1985).

A further source of discrepancy between desiccated and isotopically determined TBW is the time of weighing, as loss of body fluids having an isotope concentration lower than that at equilibration will result in an underestimation of TBW (Degen et al. 1981). This was reflected in the present study (fig. 1*A*) when, owing to loss of body mass, the discrepancy between TBWs calculated from mass at injection and bleeding increased after 6 h postinjection. Animals should therefore be weighed as close as possible to the time at which the isotopes equilibrate.

In this study, HTO overestimated actual water intake by 6.5% with a range of −8.6% to +13.1%. This is similar to the range of differences between measured and isotopically determined water intake in sand partridges (−9.3%–+13.3%) and chuckar partridges (−8.0%–6.4%) (Degen et al. 1981), and in a variety of mammals (−7%–+4%) (summarized in Nagy and Costa 1980). In most cases the water flux rates measured with HTO are expected to be within ±10% of the actual flux rates (Nagy and Costa 1980).

From this study, however, it is clear that timing of sampling importantly affects the reliability of results. When water intake was estimated from HTO turnover after only 2 days, or a 20% decrease in HTO activity, the discrepancy between estimated and actual water intake was significantly larger (26%) than at the end of the trial. In this study at least a 40% change in HTO activity had to occur before reliable estimates of water turnover could be estimated from HTO turnover. This is due to the fact that, where insufficient turnover has occurred, any analytical limitations and/or errors are greatly magnified. The reasons, however, for the consistent underestimates from HTO and ^{22}Na after 2 days (fig. 2A, 2C) are unclear. It could be associated with the deprivation of food during the 24-h equilibration sampling, resulting in the birds' not being in water and Na balance at the beginning of the trial. The balance may then have been regained after the 2-day sample—hence the closer agreement between estimates after this time.

There have now been a substantial number of studies of field energetics of mammals and birds using DLW (summarized in Nagy 1987), including studies on free-living penguins (Kooyman et al. 1982; Davis et al. 1983; Nagy et al. 1984; Costa et al. 1986). Using both respirometric measurement and energy balance techniques, the DLW method has been validated in several species of mammals and birds (Hails 1979; Nagy 1980; Williams and Nagy 1984*a*, 1984*b*; Weathers et al. 1984; Goldstein and Nagy 1985; Williams 1985; Williams and Prints 1986), and generally discrepancies are less than 10%. However, no validation studies have included seabirds, despite the

increasing use of DLW with this group (e.g., Flint and Nagy 1984; Nagy et al. 1984; Costa et al. 1986; Roby and Ricklefs 1986; Costa and Prince 1987; Gabrielsen, Mehlum, and Nagy 1987; Obst, Nagy, and Ricklefs 1987).

On average in this study, the difference between DEA and the DLW estimates was small, with DLW resulting in only a slightly higher (1.75%) value. The difference in values determined by the two methods ranged between −5.2% and +12.5%, and this range is typical in validation studies of birds (summarized in Williams and Prints [1986]). The sources of error are probably due to a combination of operator errors, small violations of underlying assumptions, and inherent analytical uncertainties in both methods being compared (Nagy 1980; Williams and Nagy 1984*b*; Goldstein and Nagy 1985).

While on average the agreement between energy balance and DLW values for metabolic rate was good at each sampling interval, the range of errors was greatest when sampled after only 2 days, or after only a 28% decrease in ^{18}O concentration. From this study, it appears that reliable results cannot be obtained until at least a 50% decrease in ^{18}O concentration has occurred, or until at least one biological half-life has elapsed.

Estimates of food intake from DLW averaged 1.4% above the measured amounts. An alternative method for determining food intake is the use of ^{22}Na, an isotope that is much less expensive than DLW and the analysis of which is relatively simple. This method has been examined in several species of captive mammals (Green 1978; Green and Dunsmore 1978; Green and Eberhard 1979; Williams and Green 1982) and has also been used to estimate food intake in free-living mammals (Green et al. 1978; Williams and Dudzinski 1982; Williams and Ridpath 1982; Green and Eberhard 1983; Tedman and Green 1987). This technique has been validated in mammals and lizards and has been shown to be a useful technique for determining food intake of emus (table 6). The value of using this isotope in seabirds, and in other marine animals, is that it allows the partitioning of water and Na flux into intake via food and intake via ingestion of seawater. Studies on seabird feeding rates have previously been based on the assumption that no seawater is ingested (Kooyman et al. 1982; Costa et al. 1986; Adams, Brown, and Nagy 1986; Costa and Prince 1987). However, through the use of ^{22}Na in free-living seabirds, it has been found that seawater ingestion does occur (Green, Brothers, and Gales 1989; Green and Brothers 1989) and can be of a sufficiently high magnitude that, if it is not taken into account, estimates of feeding rates may be significantly overestimated.

The turnover of ^{22}Na in little penguins agreed closely with dietary Na intake and supports the method as being useful for assessing rates of food intake. In most validation studies using ^{22}Na, there is only slight discrepancy between ^{22}Na turnover and dietary Na intake, but the differences in some

TABLE 6
Validation studies of the sodium-22 method

Species	Mass (kg)	Mean Error (%) (*n*)	Error Range (%)	Source
Skink (*Lampropholis guichenoti*)	.001	−7.6 (12)	−21.1 to +7.8	Gallagher et al. (1983)
Eastern quoll (*Dasyurus viverrinus*)	1.3	−15.7 (4)	−21.9 to −6.8	Green and Eberhard (1979)
Rabbit (*Oryctolagus cuniculus*)	1.8	−22.6 (11)	−28.9 to −12.4	Green and Dunsmore (1978)
Tasmanian devil (*Sarcophilus harrisii*)	3.8	−6.6 (6)	−18.8 to +2.4	Green and Eberhard (1979)
Dingo (*Canis familiaris dingo*)	16	+13.7 (10)	−25.4 to +5.8	Green (1978)
Buffalo (*Bubalus bubalis*)	293	+1.0 (5)	...	Williams and Green (1982)
Little penguin (*Eudyptula minor*)	1.15	−5.0 (6)	−14.7 to +4.2	This study
Emu (*Dromaius novaehollandiae*)	35	−2.6 (12)	−39 to +37	Herd (1985)

cases are relatively high (table 6). From this study, it was important that the turnover of ^{22}Na between samples was at least 50% before food intake rates were reliably estimated. This is consistent with results from emus, where samples should be taken between one and five half-lives to yield reliable results (Herd 1985).

Large discrepancies between measured versus ^{22}Na-estimated rates of food intake have been attributed to lack of absorbance of Na in the undigested fraction of the diet. In little penguins, when fed fish, the discrepancy between the two methods was small (+1.5%) but increased when the diet was changed to squid (−11.5%). Because dry matter assimilation was lower with the fish diet than with the squid, decreased digestibility of prey cannot be responsible for the difference. Herd (1985) takes the reflux of urine into the colon and the possible exchange of endogenous and dietary Na to account, at least in part, for the success of this technique with the emu. Little penguins also reflux urine into the lower intestine, but this cannot explain the difference in results with the two diets. Squid, however, have an appreciably higher concentration of Na than fish, and it may be some difference in the way in which Na is compartmentalized in the two prey types that affects its uptake during digestion. No other studies have investigated the effect of prey type of ^{22}Na turnover, and further work is required before the source of this difference can be understood.

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The Energetics of Free-Living Little Penguins *Eudyptula minor* (Spheniscidae), during Moult

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Abstract

Levels of circulating triglycerides and cholesterol in moulting little penguins in Tasmania were measured before, and throughout the moult. Levels at the initiation of moult were similar to those in breeding birds but increased by 2·5 times (triglycerides) and 1·8 times (cholesterol) during the moult. Water flux rates and field metabolic rate (FMR) were measured throughout moult with tritiated and doubly labelled water. TBW ranged from 54 to 70% body weight and increased during moult. Water influx rates were significantly correlated with rate of weight change. Mean FMR of moulting little penguins was 657 kJ kg^{-1} day^{-1}, or 1·5 times basal metabolic rate (BMR), and there was no difference between sites or sexes. The water influx rates of birds foraging immediately after moult were 11 times higher than in moulting birds. The energy required to sustain a moulting little penguin is 15% higher than that required for a resting, non-moulting penguin. Although the cost of moult is elevated above BMR, the main energetic expense is met during the pre-moult foraging period when birds must consume enough food to ensure that they lay down sufficient fat reserves to sustain the moult.

Introduction

All adult penguins undergo an annual moult during which the entire plumage is renewed. All species spend the duration of the moult ashore, and hence cannot feed but subsist on reserves of fat and protein accumulated during an intensive pre-moult foraging period. The moult period is one of starvation and rapid weight loss during which energy expenditure increases markedly from basal levels due to intense protein mobilisation for feather synthesis and to decreased insulation (Groscolas 1982). Changes in levels of plasma lipid during the moult of the emperor penguin, *Aptenodytes forsteri*, have been measured by Groscolas (1978, 1982) in order to assist in characterising energy metabolism during moult.

From studies of body composition of macaroni penguins, *Eudyptes chrysolophus*, and rockhopper penguins, *Eudyptes chrysocome*, Williams *et al.* (1977) have shown the changes in body composition during moult. Energy consumption rates of moulting penguins have been summarised for 13 of the 17 species by Croxall (1982). These data on energy consumption were calculated indirectly from rates of body weight loss, much of which was collected from captive birds, and from changes in body composition (Williams *et al.* 1977). These data show the high energy costs of replacing the entire plumage in a relatively short time. Laboratory studies with metabolic chambers have also shown that the energetic costs are elevated during moult in macaroni and rockhopper penguins (Brown 1985) and also in the little penguin, *Eudyptula minor* (Stahel 1984; Baudinette *et al.* 1986).

To date, however, there are no published studies on the energetics of moult of free-living penguins in their natural environments. The feasibility of estimating metabolic rates of free-living penguins by means of isotope turnover techniques has been demonstrated in: king

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penguins, *Aptenodytes patagonicus* (Kooyman *et al.* 1982); gentoo penguins, *Pygoscelis papua*, and macaroni penguins (Davis *et al.* 1983); and jackass penguins, *Spheniscus demersus* (Nagy *et al.* 1984). Recently, the energy requirements of four free-ranging little penguins, two fasting on shore and two foraging at sea, have also been reported by Costa *et al.* (1986). A preliminary study of the water, sodium and energy turnover rates in free-living little penguins in both adults and chicks has also recently been completed (Green *et al.* 1988), as well as a validation study of the use of isotopes in studies of little penguin energetics (Gales 1988).

The little penguin is the smallest of the 17 penguin species and its distribution is restricted to Australia and New Zealand. Descriptions of the changes in weight loss and plumage during the moult of the little penguin are presented by Richdale (1940) and Kinsky (1960). The seasonal timing of moult and location of the moutling site in relation to the breeding burrow has been reported for the species by Reilly and Cullen (1983). In the present paper, we report on the changes in plasma lipids and the energetic costs of moult in free living little penguins determined with tritiated and doubly labelled water.

Materials and Methods

The field study was carried out during March and April 1985 at Marion Bay, south-east Tasmania (42°50′S.,147°52′E.) and Albatross I., north-west Bass Strait (40°24′S.,144°32′E.). The colony of little penguins at Marion Bay is amongst sand dunes whereas that at Albatross I. is on rocky slopes and in caves. Birds were marked with flipper bands when they were first sighted ashore at the beginning of moult, within 1 day of their arrival. The sex of the birds was determined from beak measurements (Gales 1988). At Marion Bay, approximately 2 ml of blood was extracted from the brachial vein of moulting penguins for analyses of total triglyceride and total cholesterol levels. These blood samples were separated by centrifugation into red cell and serum fractions and stored frozen. Lipid assays were performed with an Abbott Biochromatic Analyzer (ABA-100). For this aspect of the study, the birds from which blood samples were collected were weighed, and categorised into one of four moult stages, as follows.

Pre-moult: initiation of moult, old feathers 'plumped' out.
Moult 1: beginning of loss of old feathers, new feathers just visible.
Moult 2: half of new feathers replaced, old feathers falling in sheaves.
End moult: new coat of feathers complete, birds about to return to sea.

Blood samples were also taken in the previous breeding season (December 1984), and levels of triglycerides and cholesterol were analysed. Water turnover rates and field metabolic rates (CO_2 production) were measured by means of tritiated water (HTO) and doubly labelled water (DLW, $HT^{18}O$) (Lifson and McClintock 1960; Nagy 1980; Nagy and Costa 1980; Degen *et al.* 1981).

Birds were caught at their moult roost sites and weighed (±10 g). Fourteen birds were then given an intraperitoneal injection of 1 ml of HTO (185 MBq) only, and 12 others were given 1 ml of HTO (185 MBq) and 0·3 ml of 95 atom % excess ^{18}O. Birds were then returned to their roost sites. After 5–6 h they were recaptured and a blood sample (*c.* 2 ml) was taken from the brachial vein. They were again returned to their roost site and were checked daily to determine any movement away from the original roost. After 3–14 days the birds were recaptured and weighed, and a blood sample was taken. They were then reinjected with 0·5 ml HTO (185 MBq) and a blood sample obtained 5–6 h later. Six birds, which were injected with HTO only at the completion of moult, returned to sea for 1–9 days. Blood samples were taken from these birds on their return from the sea in order to measure water fluxes of birds subsequent to termination of moult.

Blood samples were separated by centrifugation and were frozen until later analysis. Water was vacuum distilled from the red-cell fraction and the HTO levels determined by liquid scintillation specrometry using PCS (Phase Combining System, Amersham) cocktail and a Beckman liquid scintillation counter (Model LS2800). Subsamples of extracted water samples were also prepared for mass spectrometry by means of Urey exchange with standard carbon dioxide charges at 80°C overnight. The ^{18}O levels of the resulting equilibrated carbon dioxide samples were measured in an isotope ratio mass specrometer (V. G. Isogas, Model 903).

Rates of water flux and carbon dioxide production were calculated from the changes in isotope levels in the blood during the experimental periods (Lifson and McClintock 1966; Nagy 1980); it was assumed that mass specific body water pools remained constant and that any changes in body mass were linear.

Field metabolic rates (FMR, in kJ kg^{-1} day^{-1}) were converted from units of carbon dioxide production (ml g^{-1} h^{-1}) by means of the factor of 28·0 kJ per litre of carbon dioxide (Kleiber 1961).

Fresh, undigested samples of stomach contents were obtained by stomach flushing (Gales 1987) from seven little penguins arriving from sea at Albatross I. at the initiation of moult. These food samples were oven dried to constant weight at 55°C. Dried samples were then compressed into pellets of 0·5 g and combusted in a Gallenkamp adiabatic bomb calorimeter to determine energy content. Rates of food consumption required to sustain penguins through the moult process were calculated from values of energy content of the food samples brought ashore by penguins prior to moult, and the mean energy assimilation efficiency for the dietary components.

These seven food samples obtained from the penguins comprised mixed species of fish and squid. The mean water content was 74·5±2·4% and the dry matter energy content 22·1±1·1 kJ g^{-1}. The mean energy assimilation efficiency of little penguins feeding on fish and squid diets is 72% (Gales 1988). The food samples therefore contained metabolisable energy of 4·06 kJ g^{-1} fresh weight.

Data are presented as mean±SD. Student's *t*-tests (two-tailed) were used to test for significant differences between means, and the 5% level of probability was accepted as denoting statistical significance.

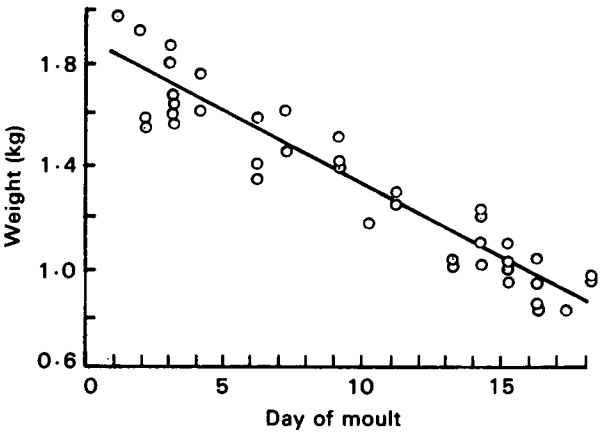

Fig. 1. Decrease in body weight of little penguins during moult.

Results

The pattern of weight loss during moult in little penguins exhibited a significant linear relationship ($r=-0·94$, $n=46$, $P<0·001$) (Fig. 1). For those birds in which the entire period of moult was monitored, the moult process lasted 16–18 days (16·7±0·79, $n=8$). The mean rate of weight loss was 55 g day^{-1}, and total weight loss during moult represented 46% of the initial weight.

The levels of triglycerides and cholesterols at the beginning of moult did not differ significantly from the levels obtained from breeding birds (triglycerides: $t=1·24$, $n=29$, $P>0·05$; cholesterols: $t=0·187$, $n=29$, $P>0·05$). During the process of moult, however, triglyceride and cholesterol levels incresed by 2·5 times and 1·8 times, respectively, from pre-moult to end moult (Fig. 2). In moulting birds, total body water (TBW) ranged between 54 and 70% (63±4·3%, $n=46$) of body weight. TBW expressed as a per cent of body weight increased during moult ($r=0·62$, $n=46$, $P<0·001$) and showed the relationship: $y=0·5x+58·8$, where y is TBW (%) and x is days after the beginning of moult.

Moulting birds showed a mean water influx rate of 16·9±3·6 ml kg^{-1} day^{-1} compared with a mean water efflux rate of 40·6±5·9 ml kg^{-1} day^{-1}. The rates of weight change and the water influx rates (Table 1) were significantly correlated ($r=-0·72$, $n=21$, $P<0·001$) and showed the relationship: $y=-4·0x+0·32$, where y is water influx (ml kg^{-1} day^{-1}) and x is weight change (% day^{-1}). The mean FMRs of little penguins moulting at

Marion Bay and at Albatross I. were not significantly different ($t = 0{\cdot}506$, $n = 12$, $P > 0{\cdot}05$). The difference between the FMRs of female and male little penguins were also not significant ($t = 0{\cdot}216$, $n = 12$, $P > 0{\cdot}05$). The mean energy cost for a penguin during moult was 657 ± 105 kJ kg^{-1} day^{-1} ($n = 12$) (Table 1). Changes in activity of injected isotopes over time were not sufficient to allow subdivision of energy costs within the moult process.

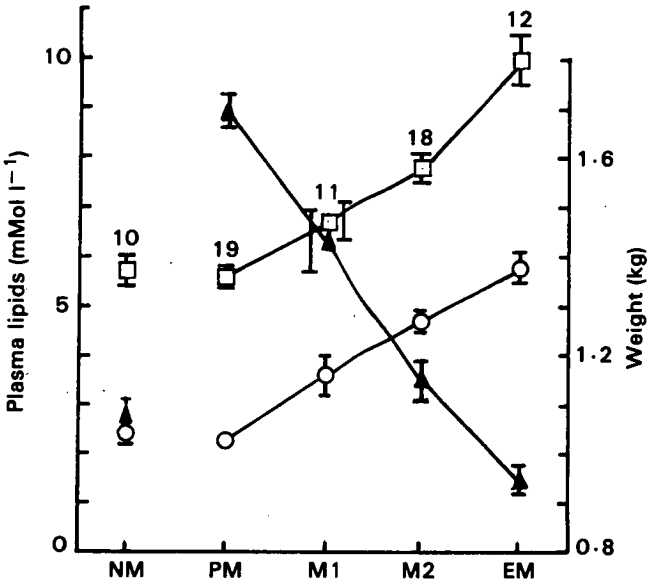

Fig. 2. Changes in levels of plasma lipids and weight during moult (mean ± SE; sample sizes are identical for all three categories). ▲ Weight. ○ Triglycerides. □ Cholesterol. NM, breeding birds; M1, moult 1; M2, moult 2; EM, end moult. Error bars for M1 weight and cholesterol shifted sideways for clarity.

The difference between the water influx rates of penguins that restricted movement to a minimum during moult (i.e. birds which were repeatedly located in the same roost sites) and those birds which were located in different roost sites, and were often seen traversing between these sites, was not significant ($t = 0{\cdot}311$, $n = 21$, $P > 0{\cdot}05$).

Table 1. Weight changes, and water and energy turnover in adult little penguins during moult

FMR, field metabolic rate

	Mean	SD	*n*
Initial weight (g)	1580	213	21
Final weight (g)	1109	194	21
Weight change (% d^{-1})	−4·2	0·7	21
Water influx (ml kg^{-1} d^{-1})	16·9	3·6	21
Water efflux (ml kg^{-1} d^{-1})	40·6	5·9	21
CO_2 production (ml g^{-1} h^{-1})	0·99	0·16	12
FMR (kJ kg^{-1} d^{-1})	657	105	12

The six birds which went to sea after completion of moult foraged for an average of 3 ± 3 days before returning to land and were in relatively stable energy balance (weight change, $-0{\cdot}26 \pm 1{\cdot}9\%$). The water influx and efflux rates of these birds were 183 ± 70 ml

kg^{-1} day^{-1} and 183 ± 64 ml kg^{-1} day^{-1} respectively. These levels are considerably higher (11 times and 4·5 times respectively) than the influx and efflux rates of moulting birds (Table 1).

Discussion

The little penguin, like most other species of penguins, moults after breeding, in late summer. The timing appears critical because the initiation of the moulting season, which ranges over 2 weeks, is less variable than is the onset of breeding, which ranges over about 10 weeks (Reilly and Cullen 1981). The pattern and rate of weight loss reported in this study is similar to those recorded elsewhere for the species (Richdale 1940; Kinsky 1960; Hodgson 1975) with approximately a 46% decrease from the initial weight during the 15–18 day moult period.

Changes in circulating lipids are often examined in order to characterise the physiological basis of phases of the annual cycle in birds (Berry *et al.* 1979; deGraw *et al.* 1979; Groscolas 1982). Triglycerides are the most important form in which dietary fat is transported in the Cape cormorant, *Phalocrocorax capensis* (Berry *et al.* 1979), a species which has a diet of predominantly small pelagic fish, broadly similar to the diet of the little penguin (R. Gales, unpublished data). In the emperor penguin, Groscolas (1982) reported that plasma lipids were depressed during the period of feather formation, as lipid levels were lower than those of non-moulting, breeding birds. In the little penguin, the levels of tryglycerides and cholesterols in birds which were breeding did not differ from the levels in birds which were initiating moult.

During moult of the emperor penguin all plasma lipids showed marked and identical increases in levels, and reached maxima at the end of the moult fast (Groscolas 1982). The magnitude of these increases was 1·85 times, a similar elevation to the 1·7 times increase recorded for plasma lipids during moult in eudyptid penguins (Tollu 1978, cited in Groscolas 1982). In the little penguin the magnitude of the elevation of cholesterol levels during moult was identical (1·8 times) but that of triglycerides was even higher (2·5 times) Groscolas (1982) concluded that the changes in plasma lipids in breeding and moulting emperor penguins are related to endocrine influences, rather than to starvation, provided fat stores are not depleted. In the Cape cormorant, Berry *et al.* (1979) showed positive correlations between the levels of plasma triglycerides and the total mass of fish ingested. During the moult fast in the little penguin, comparison with the linear decrease in body weight implies that there is an inverse relationship of plasma lipid levels and fat mobilisation, at least during this phase of the annual cycle (Fig. 2).

The mean TBW of moulting little penguins was $63 \pm 4 \cdot 3\%$ with a range of 54–70%. These are similar to the TBWs of gentoo and macaroni penguins reported by Davis *et al.* (1983) and for moulting eudyptid penguins (Williams *et al.* 1977). The mean water influx rate of little penguins whilst moulting was 16·9 ml kg^{-1} day^{-1} which is only slightly lower than the 18·1 ml kg^{-1} day^{-1} reported by Costa *et al.* (1986) for two fasting little penguins. Both these values are within the range of 13–25 ml kg^{-1} day^{-1} reported by Green *et al.* (1988) for three incubating and one moulting little penguin. It is assumed that this water is entirely metabolic water because the influx rates are low and show relatively little variation (Table 1). Although eudyptid penguins drink readily during the moult fast (Williams *et al.* 1977), little penguins, unlike some other penguin species, have never been observed to drink during moult (R. Gales, personal observation).

Many of the data concerning metabolic rates of moulting birds are derived indirectly from weight loss data and a fundamental difficulty in this technique is the lack of information on the exact composition of material lost (Croxall 1982). This problem, however, is alleviated when determining precursor-product relationships of FMRs from the isotope turnover technique (Kleiber 1961; Davis *et al.* 1983).

The composition of mass lost during moult has been determined for at least two species

of penguins, and ranges from 40·2% fat and 7·2% non-feather protein for rockhopper penguins to 36·4% fat and 4·9% non-feather protein for macaroni penguins (Williams *et al.* 1977). Using the energy equivalent of 28·0 kJ l^{-1} CO_2 in the conversion of CO_2 production to FMR assumes that all metabolic water is derived from oxidation of fat. From this assumption it can be calculated that a mean of 17·9 ml H_2O kg^{-1} day^{-1} would be produced, only slightly higher than the 16·9 ml H_2O kg^{-1} day^{-1} measured by HTO turnover. It is likely this difference is due to the oxidation of a small proportion of protein which liberates less water per millilitre of CO_2 than does the oxidation of fat (Schmidt-Nielson 1979).

It is common practice to describe costs of various activities or energy budgets as multiples of the basal metabolic rate BMR (King 1974; Ellis 1984). The BMR (post-absorbtive resting metabolic rate at thermoneutrality) of little penguins in the laboratory has been reported as 426 kJ kg^{-1} day^{-1} (Stahel and Nicol 1982). Slightly lower values were subsequently presented by Stahel *et al.* (1984), and these values were not substantially different from the BMR of 389 kJ kg^{-1} day^{-1} for a bird of similar size predicted from the equation of Aschoff and Pohl (1970). Baudinette *et al.* (1986) have reported a substantially lower BMR of 270 kJ kg^{-1} day^{-1} for little penguins. For consistency, the FMRs in the present study have been expressed as multiples of the BMR reported by Stahel and Nicol (1982) and as used by Ellis (1984), Brown (1984) and Costa *et al.* (1986).

The average FMR of moulting little penguins in the present study was 657 kJ kg^{-1} day^{-1}, or 1·5 times BMR. Baudinette *et al.* (1986), using metabolic chambers, reported that little penguins during moult increased oxygen consumption by approximately 40%. These moult-induced increases in energy turnover appear similar to those reported for other penguins. Brown (1985), using metabolic chambers, has reported elevations of 1·36 and 1·32 times resting metabolic rate for macaroni and rockhopper penguins, respectively (levels which can only be compared indirectly), but were slightly lower than those measured by Williams *et al.* (1977) for the same species (1·6 and 2·1 times BMR, respectively). Croxall (1982) summarised information on ratios of metabolic costs of moult (calculated from mass loss) to predicted metabolic rates for 13 penguin species. He concluded that energy costs during moult are approximately 2·0 times BMR, an increase of 0·7 times BMR above the value for fasting, non-moulting penguins (1·3 times BMR). Brown (1985) states that estimates of energy expenditure based on mass loss were approximately 30% greater than $\dot{V}(O_2)$ measurements in macaroni and rockhopper penguins.

To date, all these measurements of metabolic rates of penguins are derived indirectly either from weight loss data or from determination of $\dot{V}(O_2)$ in metabolic chambers. These methods are useful in that they can demonstrate patterns of energy utilisation during moult, rather than an integrated value. Groscolas (1978) has shown that moult in the emperor penguin is divisible into a number of phases, and these are associated with different biochemical events and rates of mass loss. The restrictive conditions of metabolic cages, however, may affect results, although Croxall (1982) presumes that moulting penguins tend to minimise movement. Indeed, Groscolas (1982) states that moulting emperor penguins are lethargic and that confinement has no effect on their behaviour. Brown (1985) concurs that moulting eudyptid penguins are invariably sedentary and concludes that it is only by minimising activity and movement that penguins can survive the rigours of moult.

This is not the case with little penguins. Whilst some of the little penguins in this study were relatively sedentary during the sampling period, others were seen to range between sites, some changing moult roosts every night. From observations of other birds, this was not a result of the disturbance involved in the isotope study, but a characteristic of some individuals. Recent observations also show that adelie penguins, *Pygoscelis adeliae*, and king penguins also move considerable distances during the moult fast (M. Whitehead, personal communication; R. Gales, personal observation). Sample sizes were not sufficient to assess the effect of movement on the FMR of little penguins. Therefore, the water influx rates of sedentary and mobile individuals were compared, following the assumption that all water

influx is metabolic water. The lack of significant differences between water influx rates suggest that, although the cost of moult is high, at least some movement during this time does not add a significant cost to the energetics of moult.

Given the range in BMRs presented for the species, and the artificial conditions inherent in laboratory measurements, it is logical to assess the cost of moult in relation to other studies of free-living, non-moulting, little penguins. The costs of incubation have been measured in three little penguins by Green *et al.* (1988) who report an FMR of 570 kJ kg^{-1} day^{-1}, similar to the 560 kJ kg^{-1} day^{-1} reported by Costa *et al.* (1986) for two fasting little penguins. The cost of moult in the present study is approximately 1·2 times higher than these values. An FMR of 1170 kJ kg^{-1} day^{-1} was measured by Green *et al.* (1988) for four penguins undertaking incubation shifts interspersed with foraging bouts. Thus energy expended during moult is approximately half that expended by these breeding birds. Further, in terms of water influx, for two penguins undertaking extended foraging trips, Green *et al.* (1988) reported an average of 567 ml kg^{-1} day^{-1} which is 34 times that of the water influx of moulting penguins. Given this information it is clear that the cost of moulting, although elevated above resting, fasting levels, is considerably less than the energy expended during foraging trips. Probably the most energetically expensive stage is the pre-moult foraging period when penguins almost double their weight to ensure sufficient reserves for the period when restricted to land. Penguins arriving ashore for the moult period are obese, but depart very lean and almost emaciated. Given that moult lasts on average 16·7 days and little penguins expend about 657 kJ kg^{-1} day^{-1} during moult, the total cost is 10972 kJ kg^{-1}. Incorporating the water content and metabolisable energy of food samples the penguins would need to take in approximately 2700 g of fresh food per kilogram extra during the pre-moult foraging period to supply adequate energy reserves for the moult.

If the penguins were simply resting on land for the same period, and were expending the 560 kJ kg^{-1} day^{-1} presented by Costa *et al.* (1986) for two fasting penguins, they would require approximately 2300 g of fresh food per kilogram. This value is 15% less than that required by moulting penguins. It should be noted that little penguins spend extended times ashore only during the moult.

When little penguins have completed moult, their energy reserves are depleted and the initial post-moult foraging trips are generally short. In the present study, the six birds monitored with HTO during these trips stayed at sea an average of 3 days, retained a stable weight and their water influx (183 ml kg^{-1} day^{-1}) mirrored water efflux (183 ml kg^{-1} day^{-1}). These influx rates are 20% higher than that measured by Costa *et al.* (1986) on two foraging birds, both of which were in negative energy balance. The post-moult influx rates are similar to the influx rates of birds foraging between incubation shifts, but lower than those of birds which are foraging and feeding young (Green *et al.* 1988). It appears then that, whilst recovering from moult, little penguins forage conservatively in energetic terms, compared with the foraging demands of pre-moult or chick rearing at least until energy stores and normal weights are attained.

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Validation of the stomach-flushing technique for obtaining stomach contents of Penguins

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The efficiency of the stomach flushing technique in obtaining complete stomach contents was tested on Little, Gentoo and Rockhopper Penguins. This technique was validated by feeding the penguins known amounts of fish and subsequently flushing their stomachs after specified time intervals. Examination of the contents showed that the method is effective and offers an alternative to killing penguins in order to obtain stomach contents. The effects of different states of stomach fullness on food recovery rates highlighted the necessity for multiple flushing. Quantitative information on the effect of time between feeding and stomach flushing on the recovery rates was also obtained. Where stomach contents were relatively undigested the rate of retrieval of fish was 90–100%, but this rate decreased with time and in no cases in which stomach contents were in advanced stage of digestion was the retrieval rate higher than 80%. Inclusion of the Gentoo and Rockhopper Penguins in the validation trials showed that the size of the penguin does not affect recovery rate.

Diet studies are essential when addressing the question of the role of penguins in marine ecosystems and there is mounting interest in the impact of penguins on marine resources (e.g., Croxall *et al.* 1984). Unlike many other seabirds, penguins do not regurgitate when disturbed and so most diet studies to date have required the killing of many penguins in order to obtain stomach samples (e.g., Ealey 1954, Volkman *et al.* 1980, Croxall & Furse 1980, Croxall & Prince 1980, Lishman 1985). Several methods have been developed to obtain stomach contents without killing the birds. Emetics are not only unsuccessful in some species but are sometimes fatal (Horne 1985, Montague & Cullen 1985). Stomach pumps (Emison 1968, Dahlgren 1982) do not provide complete, or indeed representative samples (Volkman *et al.* 1980, Croxall & Prince 1980, Horne 1985, Montague & Cullen 1985). More recently, the technique of stomach flushing has been applied to penguins (Randall & Davidson 1981, Wilson 1984, Ofredo *et al.* 1985). Randall & Davidson (1981) developed a flushing device for obtaining food samples from Jackass Penguins *Spheniscus demersus* but the technique was unsuitable for retrieving large food items and the associated trauma led to some nest desertion. An improved and simplified technique was developed by Wilson (1984) who claimed successful results with no associated nest desertions. The reception of this technique has been varied among penguin researchers, with Siegfried & Croxall (in press) claiming that it largely eliminates the need to kill birds while Lishman (1985) states that the method is ineffective because it does not always obtain the complete contents, particularly from birds with full stomachs.

The stomach flushing technique has now been used on at least seven species of penguins, and it is surprising that no comprehensive validation trials have been published to date. In the present study, efficiency trials were run on Little Penguins *Eudyptula minor* to address the following questions.

(1) How effective is the stomach flushing technique at obtaining complete

stomach contents? (2) How does the state of stomach fullness affect the food recovery rate? (3) How does the time between feeding and stomach flushing affect the recovery rate?

Further validation trials were run on Gentoo Penguins *Pygoscelis papua* and Rockhopper Penguins *Eudyptes chrysocome* in order to broaden the scope of the trials and address the final question: (4) how does size of penguin affect recovery rate?

Methods

Apparatus and flushing method

The stomach flushing method used was identical in principle to that described by Wilson (1984). To simplify and speed up the process a commercial garden pressure sprayer (Hills 3·5 l) was adapted to pump water under pressure (2 l/min) into the stomach of the penguin. After filling the spray tank with seawater at ambient temperature the tank was sealed and pressurized by pumping. A plastic catheter (external diameter: 5 mm internal: 3·5 mm) attached to the nozzle of the spray gun was inserted into the penguin's mouth and pushed gently down to the base of the stomach ($\approx$30 cm). A switch on the spray gun was then released and seawater was introduced into the stomach under pressure until water flowed out of the corner of the mouth. The catheter was then quickly removed, the bird inverted over a bucket and pressure applied to the base of its stomach with the heel of the operator's hand while its throat was massaged with the fingers to dislodge any large food boluses which might otherwise have blocked the passage. Finally, the mouth of the penguin was sprayed to wash out any food remains which might have caught on the barbed tongue.

For the larger penguin species a multipurpose hand-operated hydraulic pump (Led Multipump) was utilized because it delivered water at a faster rate than the pressure sprayer. The subsequent procedure using this pump followed that described by Wilson (1984).

Validation trial protocol

The validation trials with Little Penguins were carried out on Albatross Island (40° 24′S, 144°32′E), Northwest Bass Strait, Australia in September 1984. During a two-week period 40 penguins were collected and used in the trials. These penguins had all been on land for at least 24 hours prior to capture and thus were unlikely to have had food in their stomachs (Wilson *et al.* 1985). The birds were force-fed pre-weighed thawed atheriinids *Atherisason hepsetoides* (mean weight $2{\cdot}8 \pm 0{\cdot}79$ g, $n = 50$) and then held in hessian sacks until stomach flushing. Time intervals between feeding and stomach flushing were 1, 2, 4, 8 and 16 hours and the number of fish per feed was 5, 10, 20 or 50. Thus, for each of the four categories of fish numbers there were five time periods, resulting in a total of 20 different treatments. Each treatment was duplicated and individuals were used only once.

When the prescribed amount of time had elapsed after feeding, the penguins were stomach-flushed as described above. Stomach flushing was repeated until the water ejected from a penguin contained no food material and was totally clear. The condition of food material and number of otoliths ejected with each flush were recorded, and the water was drained off prior to storage in 90% alcohol. After flushing, penguins were banded and held in sacks for at least one hour to ensure their recovery before release.

In addition to the validation feeding trial five Little Penguins were stomach-flushed as they came ashore at dusk on Wedge Island (43°08′S, 147°40′E), Southeast Tasmania, in August 1984. Stomach flushing was repeated on each bird until the last flush was clean. The penguins were then killed and stored frozen until their stomachs were dissected and examined for food material.

Validation trials on Gentoo and Rockhopper Penguins were carried out on Macquarie Island (54°30′S, 158°55′E) in November 1984. I first stomach-flushed 20) Gentoo and ten Rockhopper Penguins as they came ashore in order to familiarize myself with the technique on larger penguins. Six Gentoo Penguins and four Rockhopper Penguins were then fed pre-weighed amounts of school whiting *Sillago bassensis* (mean weight 84·7 ± 5·36 g, $n = 25$), held for a recorded period of time and then stomach-flushed until the last flush was clear. Stomach contents were sieved and stored in alcohol.

In the laboratory all samples were examined for sagittal otoliths which were removed, counted, sorted into left and right, and stored dry. These otoliths were used to calculate the number of fish obtained by stomach flushing. In order to describe fish condition an index of digestion (DI) was devised as follows.

Stage 0—fish intact without any signs of digestion.

Stage 1—fish not intact but scales present.

Stage 2—fish flesh in fillets only, vertebrae articulated, tails present.

Stage 3—only fragments of fish flesh, vertebrae not articulated, no tails, no scales, otoliths not eroded by digestion.

Stage 4—no flesh or bones, otoliths partially eroded, sulcus acusticus worn.

Stage 5—no fish remains, water stained green through bile secretion, empty stomach.

Results

No food remains were found in the dissected stomachs of the five Little Penguins which were sacrificed after being stomach-flushed, indicating that stomach flushing yielded 100% of the stomach contents. The penguins had been feeding on mixed diets of pelagic fish *Atherinason hepsetoides, Engraulis australia* and *Hyporhamphus melanochir*, squid *Nototodarus gouldi* and euphausid crustaceans *Nyctiphanes australis*.

Results for the validation feeding trial on Little Penguins are shown in Table 1. The rate of retrieval of fish was 90–100% in samples with digestion indices (DI) of 2 or less, i.e., where the fish remained relatively undigested (Table 2). In these cases the number of flushes required to empty the stomach ranged from three to ten (Fig. 1). As digestion progressed the retrieval rate decreased and no samples in which digestion was well advanced and the otoliths were worn (i.e., DI = 4) scored 100% retrieval. By definition, those samples with a DI of 5 were empty and thus had 0% retrieval. The number of flushes required to empty the stomach also decreased as digestion progressed (Fig. 1). For up to 2 hrs after feeding there was little difference in the proportion of fish retrieved between the different states of stomach fullness. After 4 hr the proportion retrieved increased with the stomachs of increasing fullness, and by 16 hr only penguins fed 50 fish had any food in their stomachs (Fig. 2).

Retrieval rates from Gentoo and Rockhopper Penguins were consistently high: 88–100% (Table 3). The rates of retrieval during the early phases of digestion ($DI \leqslant 2$) were similar to those of the Little Penguin despite the differences in body weight between the species (Gentoo: $\bar{x} = 5{\cdot}3$ kg, s.d. = 0·70, $n = 6$, Rockhopper:

Table 1. *Results of validation trials on Little Penguins: a and b are results for individual penguins*

Fish fed *n*	Time between feeding and flushing (hr)	Retrieval: Otoliths (*n*) a	Otoliths (*n*) b	Fish (%) a	Fish (%) b
5	1	10	9	100	100
	2	10	9	100	100
	4	7	6	80	80
	8	4	4	40	40
	16	0	0	0	0
10	1	19	18	100	90
	2	20	18	100	90
	4	19	17	100	90
	8	15	13	80	70
	16	0	0	0	0
20	1	36	34	95	90
	2	38	37	100	95
	4	39	38	100	100
	8	33	28	85	75
	16	0	0	0	0
50	1	100	100	100	100
	2	98	92	98	98
	4	94	90	96	96
	8	92	79	92	80
	16	63	42	62	42

Table 2. *Digestion indices and fish retrieval (%) from validation trial on Little Penguins*

Digestion Index (DI)	Fish retrieval (%)	*n*
0	90–100	5
1	90–98	3
2	90–100	12
3	75–100	8
4	40–80	6

$\bar{x} = 3{\cdot}4$ kg, s.d. $= 0{\cdot}42$, $n = 4$; Little Penguin: $\bar{x} = 1{\cdot}0$ kg, s.d. $= 0{\cdot}13$, $n = 40$). Weights of the fish meals fed to Gentoo Penguins were 14% of mean body weight, similar to the proportions by body weight of fish fed to Rockhopper Penguins (12%) and the 50 fish fed to Little Penguins (13%). The inverse relationships between the number of flushes and the time between feeding and stomach flushing were similar among species (Fig. 3). The rates of digestion of the larger penguins, however, were slower than that of the Little Penguin, and therefore high rates of retrieval were maintained for longer periods.

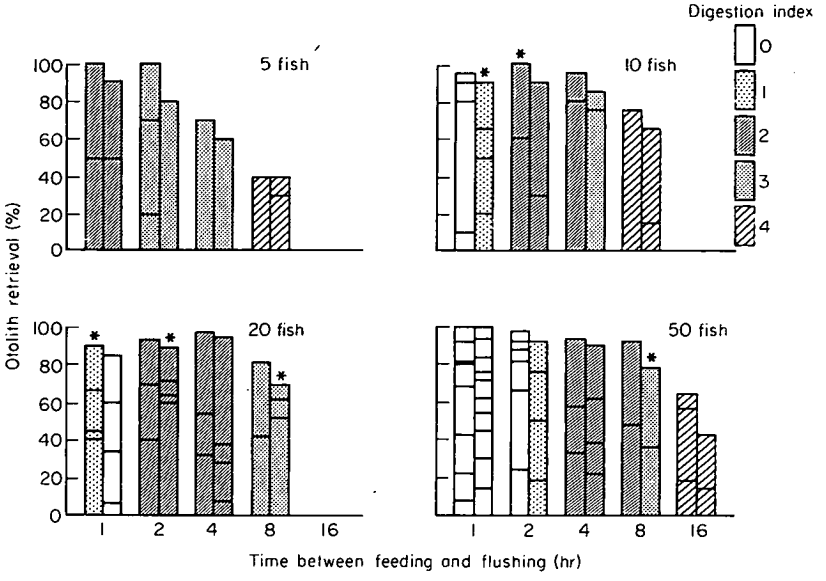

Figure 1. Results of validation trials on Little Penguins in relation to meal size. The state of digestion of stomach contents is indicated by shading. The number of flushes required to empty the stomach and the percentage of otoliths retrieved in each flush are indicated by the horizontal bars. Samples in which the first flush retrieved no otoliths are indicated by stars.

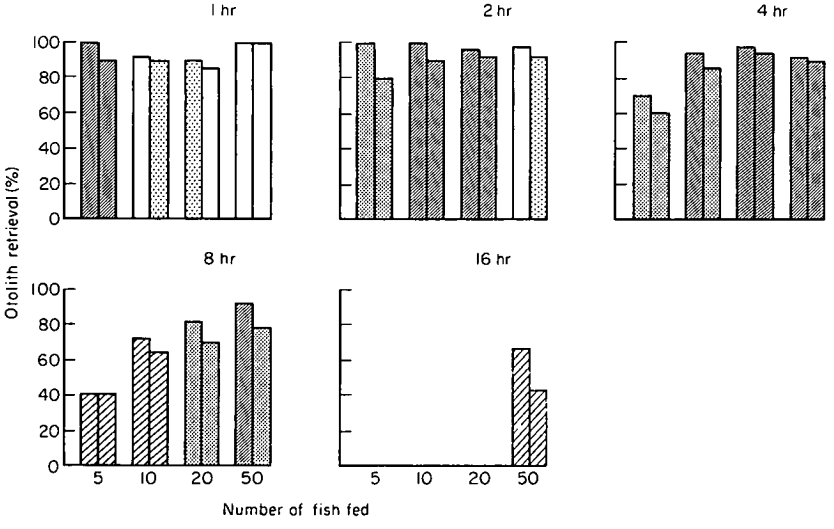

Figure 2. Results of validation trials on Little Penguins in relation to time between feeding and flushing. The state of digestion of stomach contents is indicated by shading, the legend being the same as in Fig. 1.

Discussion

The stomach-flushing technique has been shown here to be an efficient method of obtaining complete stomach contents. The validation feeding trials on the Little

Table 3. *Results of validation trials on Rockhopper and Gentoo Penguins*

	Fish fed *n*	Time between feeding & flushing (h)	Retrieval Otoliths (n)	Retrieval Fish (%)	Digestion Index (DI)	Flushes *n*
Rockhopper	5	4	10	100	1	6
		8	10	100	1	5
		16	10	100	3	3
		16	9	100	4	3
Gentoo	9	1	18	100	0	10
		2	17	100	1	8
		4	16	88	1	6
		8	18	100	2	6
		16	18	100	3	3
		16	16	88	4	2

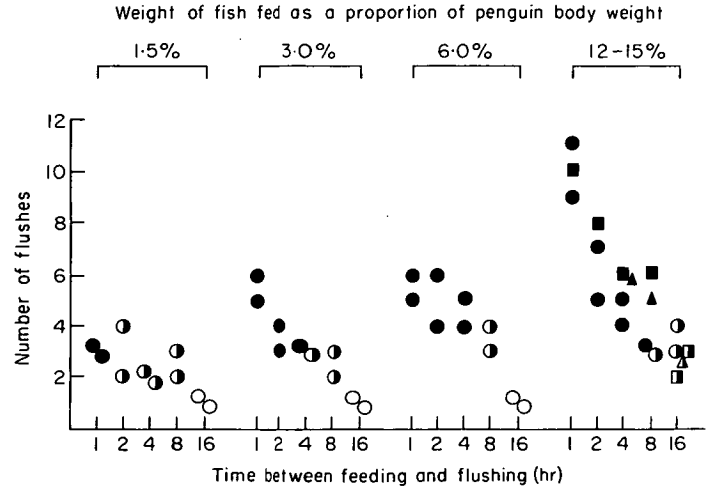

Figure 3. Relationship between number of flushes, digestion index and time between feeding and flushing. Circles represent Little Penguins, squares Gentoo Penguins and triangles Rockhopper Penguins. Digestion index depicted as: black symbol DI = 2; half-coloured DI = 3–4; open symbol DI = 5.

Penguins showed consistently high rates of stomach content retrieval and the efficiency of this technique was further verified by the subsequent killing of five birds. During a concurrent study of the breeding and feeding biology of the Little Penguin in Tasmania, 786 penguins were stomach-flushed between May 1984 and January 1986. No Little Penguins died as a result of the stomach flushing and no breeding birds which have been stomach flushed have deserted nests. The inclusion of the Rockhopper and Gentoo Penguins in the feeding validation trials further demonstrated the success of the technique across a five-fold variation in body size.

Inconsistencies in the methods of different workers using the stomach-flushing technique, together with a lack of precise detail in published accounts, appear to have

led to the conflicting of results reported and hence the lack of agreement regarding the efficiency of the technique. This is best exemplified by the question of the number of flushes required to empty the stomach. In the descriptive account by Wilson (1984) the number of flushes required was not stated, nor was it mentioned in many diet studies in which the technique has been used (LaCock *et al.* 1984, Wilson 1985, Offredo *et al.* 1985). In her work on Rockhopper and Royal Penguins *Eudyptes chrysolophus schlegeli*, Horne (1985) used the technique according to Wilson's specifications but flushed the birds only once. In the present study, it was shown that multiple flushes are essential to empty the stomachs of the three species of penguins tested. Indeed, the greater the fullness of the stomach, the greater the number of flushes required (Fig. 3). In cases where the first flush acts to loosen the contents but returns only cloudy water, if only a single flush were used the penguin would be erroneously recorded as having an empty stomach. In birds with full stomachs many consecutive flushes may be required until the complete stomach contents have been retrieved and the last flush is totally clear (Fig. 1).

The necessity for multiple flushing was also observed by Randall & Davidson (1981). Birds with empty stomachs are quickly identified by stomach flushing as the colour of the water from the first flush is tainted green by bile secretion (Randall & Davidson 1981, Wilson *et al.* 1985). In the present study, any bird which has not emitted green coloured water in the first flush was proved to have food in the stomach.

Horne (1985) estimated meal sizes of Royal Penguins from stomach samples obtained by single stomach flushes and compared results to meal sizes of Macaroni Penguins *Eudyptes c. chrysolophus* derived from the stomach samples from sacrificed birds (Croxall & Prince 1980, Croxall & Furse 1980). The Royal Penguins' average meal size ranged between 7% and 15% of those of the Macaroni Penguins. It is possible that this difference was an artefact of the stomach flushing being restricted to a single flush and thus only retrieving a portion of the stomach contents. Had the Royal Penguins been stomach-flushed until the last flush was clear the meal sizes might have been more comparable to those obtained by other workers who killed the birds.

It is also likely that a single flush produces biased results as the food items retrieved in the first flush represent those eaten most recently and/or the lighter prey remains. In instances where Little Penguin stomach contents contained crustaceans and fish, the crustaceans were usually retrieved in flushes before the fish. If a single flush is assumed to obtain the complete stomach contents the bias in the results would have far-reaching implications for interpretations relating to the importance of prey types. Further, restriction to a single flush may lead to errors in the interpretation of foraging ranges.

Digestion proceeds with time and so recovery rates decrease with time after food intake (Fig. 1). The duration of digestion increases with the fullness of the stomach and consequently high retrieval rates are maintained for a longer period (Fig. 2). Wilson *et al.* (1985) found that Jackass Penguins completely digested 50 g of anchovy in 10 hr and 100 g in 14 hr. These evacuation rates are comparable to those of the Little Penguins which digested meals of 5, 10 and 20 fish in 8–16 hr but meals of 50 fish (140 g) took in excess of 16 hr to be completely digested.

While the results of this study have verified that the stomach-flushing technique is reliable in obtaining complete stomach samples from Little Penguins, and shows great promise for Gentoo and Rockhopper Penguins, it is essential that the technique is validated in each species to which the technique is applied. This can be achieved by stomach flushing and subsequently killing the penguins and examining the stomachs for food items (Davies 1956). More profitable are validation feeding trials which,

although more labour intensive, also give indications of digestion rate (Wilson *et al.* 1985).

In the validation feeding trials on the Little Penguins the fish used were atheriinids, important prey items for the species in Tasmania (unpubl. data). It was not feasible in the present study to use local prey items in the Gentoo and Rockhopper Penguin feeding trials. The foreign nature of the school whiting used in these trials may have accounted for the slower rate by digestion of these penguins during the trials. It is preferable in validation feeding trials, where possible, to use food species which are important in the diet of predators. It would also be beneficial to use the different prey taxa (e.g., fish, squid and krill) and ideally, a combination of the prey types in feeding trials with species which prey on animals from different taxa.

In many studies penguins have been killed only to obtain stomach contents. These penguins are usually killed during the breeding season as they return to their nests from the sea. Consequently, not only are the individuals sacrificed but the breeding success of the colony is also decreased by the disruption of pair bonds and the concomitant death of embryos and chicks. The killing of relatively large numbers of penguins may be unacceptable either on ethical grounds or for practical reasons (e.g., of rare species) in concurrent breeding and population dynamics studies and when working on protected wildlife. Where a satisfactory alternative for obtaining stomach contents is available, killing penguins for diet studies is largely unwarranted. The stomach-flushing technique provides that alternative. This technique offers great potential for humane and long term penguin studies and in particular, makes the use of penguins as environmental monitors and indicators of resources more feasible.

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SEXING ADULT BLUE PENGUINS BY EXTERNAL MEASUREMENTS

By ROSEMARY GALES

In many species of penguins, the sexes differ in size (Croxall 1985), and in most, the males are about 10% heavier than females. This is apparent in Blue Penguins (*Eudyptula minor*) but the large annual variation in their body weight and the large overlap between sexes make weight unreliable for sexing.

The beak of *E. minor* is its most dimorphic character and the sexes of Blue Penguins of all the six subspecies can be distinguished by comparing the shapes of the beaks (O'Brien 1940, Kinsky 1960, Phillips 1960, Reilly & Balmford 1972, Kinsky & Falla 1976). These workers showed that in general the beak of the male is stouter and has a more acutely hooked tip on the upper mandible than that of the female. The female beak is more slender and tapered. However, this difference, although often described, has not been subjected to statistical analyses. In studying the Australian subspecies, the Fairy Penguin (*E. minor novaehollandiae*), in Tasmania, I have had to sex adults by their beak measurements and so could quantify the reliability of this sexing technique.

In Tasmania in 1984-1986, I sexed 136 adult Fairy Penguins either by dissecting freshly dead birds or by examining the cloaca for signs of swelling and distension at the time of egg laying (Serventy 1956). I measured the beak length (after Baldwin *et al.* 1931) and beak depth (after Warham 1975) of each bird to the nearest 0.1 mm. I analysed these data by Discriminant Function Analysis (DFA, Genstat) and calculated a discriminant score for each bird. DFA weights characters by their powers of discriminating between groups of unknown individuals, using data from individuals of known sex (reference, or known group).

With this technique I classified the sex of 107 Fairy Penguins (wild group), including 23 breeding pairs, which I measured in the field on Albatross Island (40° 24'S, 144° 32'E), Bass Strait, in the 1985/86 breeding season. By comparing the discriminant scores with the known group, I classified each bird as male or female. In addition, to examine the reliability of classifying sex by applying a single DFA, derived from the Australian subspecies, to penguins of a New Zealand subspecies, I calculated discriminant scores from the beak measurements of 40 Southern Blue Penguins (*E. minor minor*) of known sex. I had sexed these birds either by dissection or by cloacal examination (see above) in southern New Zealand between 1982 and 1984. The discriminant scores of these birds were then compared with the scores from the known-sex group of *E. minor novaehollandiae* and classified as male or female. The number which was

incorrectly sexed by the DFA method was then used to provide an index of reliability of using a single DFA between subspecies.

RESULTS AND DISCUSSION

The mean beak measurements of the known groups of the two subspecies of *E. minor* are shown in Table 1. In both subspecies the differences between male and female beak measurements were significant but nonetheless showed considerable overlap. The difference between the beak lengths of the two known-sex groups was not significant for males ($t = 1.50$, $df = 88$, $p > 0.05$) or females ($t = 0.82$, $df = 84$, $p > 0.05$). However, the groups showed highly significant differences in beak depth (males: $t = 4.89$, $df = 88$, $p < 0.05$; females: $t = 3.47$, $df = 84$, $p < 0.05$), with *E. minor minor* having the larger beaks in both sexes.

TABLE 1 — Beak measurements (mm) of reference and wild specimens of *E. minor*

SPECIMENS	CHARACTER	SEX	N	MEAN	RANGE	SD	t-statistic
E.m. novaehollandiae reference group	length	M	66	39.1	36.0 - 42.3	1.44	9.94*
		F	70	36.8	34.0 - 40.1	1.17	
	depth	M	66	14.3	12.6 - 15.8	0.67	17.25*
		F	70	12.4	11.2 - 13.9	0.60	
E.m. novaehollandiae wild group#	length	M	51	38.7	36.4 - 42.0	1.07	9.51*
		F	56	36.5	34.2 - 40.1	1.32	
	depth	M	51	14.5	13.5 - 16.0	0.59	19.54*
		F	56	12.4	11.2 - 13.4	0.07	
E.m. minor reference group	length	M	20	38.8	36.8 - 41.8	1.48	2.54*
		F	20	37.4	34.2 - 40.9	1.95	
	depth	M	20	14.9	13.5 - 15.8	0.55	9.93*
		F	20	13.2	12.1 - 14.0	0.54	

* indicates $P < 0.05$
\# sex classified by DFA

The classification formula which was derived from the *E. minor novaehollandiae* known-sex (reference) group was:

$$D = -83.10 + (10.06 \ln BL) + (17.99 \ln BD)$$

where D is the discriminant score, ln is the natural logarithm, BL is the beak length (mm) and BD is the beak depth (mm).

The sex of a Fairy Penguin can be determined by applying the bird's beak measurements to this formula. When D is positive, the penguin is classified as male, and when negative, female. Using this formula, of the 107 wild-group penguins measured in the field, I classified 56 (52%) as female and 51 (48%) as male (Table 1). As would be expected, there was no significant difference between the *E. minor novaehollandiae* known-sex group and the wild group (DFA classified sexes) in either beak length

(males: $t = 1.45$, $df = 115$, $p > 0.05$; females: $t = 1.58$, $df = 124$, $p > 0.05$) or beak depth (males: $t = 1.49$, $df = 115$, $p > 0.05$; females $t = 0.004$, $df = 124$, $p > 0.05$).

The differences between the discriminant scores of males and females within groups were all significant (Table 2) and the distributions of these scores are shown in Figure 1. Of the 136 birds in the *E. minor novaehollandiae* known-sex group, 128 were classified as the correct sex, giving a classification reliability of 94%. The eight penguins which were incorrectly classified by the discriminant formula were four males with relatively small beaks and four females with relatively large beaks.

The numbers of males and females of the 107 wild-group penguins whose sex was classified by the discriminant formula represent a female:male sex ratio of 1:0.91, which compares well with that of 1:0.86 for the same subspecies found by Hodgson (1975). Of the 23 breeding pairs, every pair was classified as a male-female pair.

When I used the formula derived from the *E. minor novaehollandiae* known-sex group to classify the sex of the *E. minor minor* group, the formula classified only 31 of the 40 New Zealand birds as the correct sex. This represents a classification reliability between subspecies of 78%. All nine of the misclassified birds were females, which were classified as males. The relatively low level of reliability is a result of the larger *E. minor minor* beaks, as in Table 1. This is also evident in the differences in the discriminant scores between the two subspecies (males: $t = 2.97$, $df = 84$, $p < 0.05$; females: $t = 4.57$, $df = 88$, $p < 0.05$).

Table 2 — Discriminant scores of *E. minor*

SPECIMENS	SEX	N	MEAN	RANGE	SD	t-statistic
E.m. novaehollandiae reference group	M	66	1.59	-1.23 to 3.51	1.016	17.82*
	F	70	-1.49	-3.68 to 0.68	0.992	
E.m. novaehollandiae wild group	M	51	1.702	0.15 to 3.51	0.879	18.94*
	F	56	-1.57	-4.00 to -0.03	0.893	
E.m. minor reference group	M	20	2.317	1.13 to 3.88	0.738	8.83*
	F	20	-0.316	-2.57 to 1.23	1.111	

* indicates $P < 0.05$

The differences in the beak dimensions between the sexes and between the six subspecies of *E. minor* were illustrated by Kinsky & Falla (1976). From their data and my results, the conclusion is that a discriminant formula derived from one subspecies cannot be used reliably to sex other subspecies. Juvenile birds may make the difference worse. The beaks of *E. minor* fledglings are on average only 91% of the adult length and 81% of the adult depth (Gales, 1987) and the age at which they reach adult dimensions is not known. However, the formula presented here for adult Fairy Penguins

in Australia gives a high reliability of classifying the correct sex from beak measurements. In practice, one can rapidly sex the adults of *E. minor novaehollandiae* in the field, at any time of the year, with 94% accuracy simply by taking the two beak measurements and calculating the discriminant score.

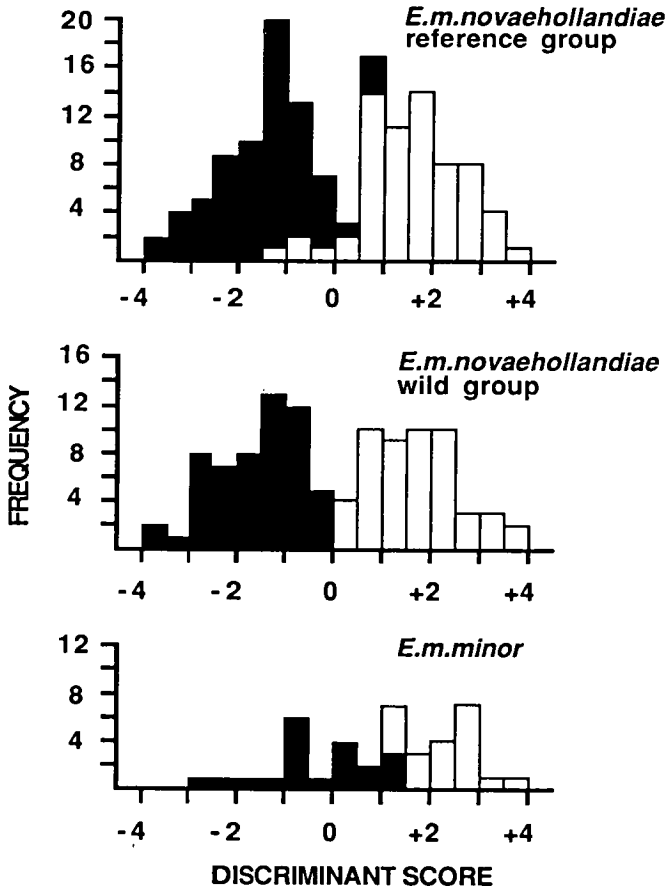

FIGURE 1 — Discriminant scores of female (solid) and male (open) reference specimens of *E.m. novaehollandiae* and of live specimens of *E.m. novaehollandiae* and reference *E.m. minor* classified as female or male

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★

SHORT NOTE

Southern Crested Grebes on a lowland coastal lake in winter

During the week of 18-24 July 1987, 52 lakes, ponds and lagoons throughout Canterbury were surveyed as part of the annual census of Southern Crested Grebes *(Podiceps cristatus australis)* and New Zealand Scaup *(Aythya novaezealandiae).* One hundred and seventy-six Crested Grebes were found, the highest number recorded in Canterbury since our counts began in 1981 (unpubl. data) and only 17 short of the total count from a South Island-wide survey in 1980 (Sagar 1981). The most notable feature of the 1987 count was the discovery of 20 Crested Grebes on Lake Forsyth, a lowland coastal lake near Christchurch. An additional grebe was seen on nearby Lake Ellesmere at Kaituna Lagoon on the same day. Only Lake Alexandrina had more grebes (59 birds).

Lake Forsyth (680 ha) is a coastal lagoon adjacent to Lake Ellsesmere. It is long and narrow and surrounded by steep hills which are part of Banks Peninsula. The waters are highly eutrophic, often being discoloured with high concentrations of algae.

The Crested Grebes were scattered over the whole lake but two concentrations of 10 and 6 birds were seen feeding in loose flocks. All birds were in full breeding plumage. "Head shaking" (pair maintenance display), aggressive displays and chases were observed. A large number of Black Shags *(Phalacrocorax carbo)* and Little Shags *(P. brevirostris)* was also present on the lake, indicating that a rich source of fish, an important food for Crested Grebes, was probably present.

The grebes were counted each week for six weeks and their numbers declined steadily until none was left on 31 August (K. Harrison). The weekly counts were: 24 July, 20 birds (CO'D, P. McClelland); 30 July, 19 (P. Reese); 8 August, 15 (CO'D, P. Dilks); 14 August, 11 (K. Hughey); 19 August, 8 (PMcC, A. Grant); 26 August, 3 (PMcC).

This is the first time that a large group of Crested Grebes has been recorded on the east coast of the South Island. Single grebes and pairs have been found on Lake Ellesmere, Lake Forsyth, the Avon-Heathcote Estuary and Brooklands Lagoon, but only during severe winters in the high country when most lakes have frozen over. However, winter 1987 was very mild, and no lakes were frozen during the July survey. It appears that the Forsyth grebes may have come from the Alexandrina lakes, some 190 km from Banks Peninsula. Counts on all other lake systems produced about the same number of grebes as were recorded in previous years when lake conditions were similar. Only the Alexandrina count (59) was much lower than the 1986-1987 summer high of 100 birds (R. Nilsson, pers. comm.) The 20 grebes at Lake Forsyth would largely account for most of the 30+ grebes absent from Lake Alexandrina. The occurrence of birds on Lake Forsyth is the first case of "mass" movement to the coast, a behaviour which is common in the nominate race, *P. cristatus cristatus*, of Europe (Cramp & Simmons 1977) and occurs in *P. c. australis* within Australia (Frith 1969). Such movements were not recorded in New Zealand by Sagar & O'Donnell (1982), who suggested that, apart from stragglers, grebes did not undertake long-distance movements from their favoured alpine and subalpine lakes.

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The use of otoliths as indicators of Little Penguin *Eudyptula minor* diet

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The validity of using otoliths from stomach contents quantitatively to determine the number and size of fish consumed was tested on Little Penguins. They were fed different meal sizes of known number and size of fish and the stomach contents were recovered after various time intervals. There were no differences in estimates of original fish size when calculated from otolith length or weight. Rate of digestion of otoliths tended to decrease with increased meal size but increased with time after ingestion. Digestion of otoliths proceeds rapidly and, if ignored, estimates of numbers of fish consumed and of original fish size can be significantly underestimated. This problem can be partially solved by inspection of otolith condition and restricting calculations of fish size to otoliths unaffected by digestion. Many factors introduce variations into rate of otolith degradation and further species-specific studies are required before appropriate correction factors can be applied.

In many studies of the diets of pelagic seabirds the stomach contents are in advanced stages of digestion with little readily identifiable materials (Brown *et al.* 1981, Croxall *et al.* 1985, Lishman 1985). Fish otoliths are increasingly being used in diet studies of piscivorous marine birds and mammals as diagnostic prey remains because otoliths are the most dense structure in teleost fish and are the most resistant to digestion (Treacy & Crawford 1981). Further, the shapes of the sagittal otoliths are species-specific and otolith dimensions can provide information on the age and size of fish (Fitch & Brownell 1968, Ross *et al.* 1979, Frost & Lowry 1981).

Until recently the influence of digestion on the analyses and interpretation of dietary studies has been largely ignored and most researchers have assumed that digestion rates were similar between prey types. Recently, however, differential digestion has been found to lead to errors in the determination of dietary importance (Furness *et al.* 1984, Da Silva & Neilson 1985, Murie & Lavingne 1985, Wilson *et al.* 1985). The retention and/or differential digestion of the diagnostic prey remains will also bias the interpretation of results. Common biases are the overestimation of the importance of squid in the diet due to the accumulation of the keratinous beaks in predator stomachs (e.g., Furness *et al.* 1984), and the underestimation of the importance of fish, because otoliths are both relatively small and subject to digestion (Prime 1979, Duffy & Laurenson 1983, Murie & Lavigne 1986).

The primary objective of the present study was to investigate the digestion of otoliths by Little Penguins *Eudyptula minor* to determine whether (1) otolith numbers can be used to calculate intake (meal or daily consumption) of food? (2) the rate of digestion of ingested otoliths is affected by meal size and/or time of retention; (3) the size of ingested otoliths can be used to calculate the size of fish, and which parameter of the otolith, length or mass, is most appropriate in such calculations.

Methods

Collection of samples

Forty wild Little Penguins (mean weight: 1·0 kg, s.e. = 0·02, $n = 40$) on Albatross Island (40°24'S, 144°32'E) NW Bass Strait, Australia, were used in feeding and stomach flushing trials during September 1984. The birds had been on land for at least 24 hours prior to the trials and so were unlikely to have any food in their stomachs. They were then force-fed thawed atherinids *Atherinason hepsetoides*, a common prey item of the Little Penguin in Tasmania (unpubl. data). These fish (mean length: 74 mm, s.e. = 0·66, $n = 100$; mean weight: 2·8 g, s.e. = 0·11, $n = 50$) were fed to the penguins in meals of five, ten, 20 or 50 fish per feed. After 1, 2, 4, 8 or 16 hours the birds were then stomach flushed with seawater and released. Thus, for each of the four categories of meal size there were five time periods resulting in a total of 20 treatments and two penguins were used for each treatment. Stomach samples were stored in 90% alcohol until otoliths were removed. For full details of experimental protocol see Gales (1987).

Analysis of reference material

Sagittal otoliths were removed from fresh atherinids, washed in detergent and stored dry. Fish standard length (FSL) was measured from the snout tip to the tip of the last caudal vertebra (±0·5 mm). To determine whether there were differences in otolith size within pairs, both otoliths were then measured along their maximum length (anterior–posterior) with a micrometer eyepiece (±0·05 mm), and then weighed on an electronic balance (±0·005 mg).

Relationships between otolith length (OL) and FSL, and otolith weight (OW) and FSL were investigated by regression analyses. Fish lengths in the reference collection extended from 31 mm (juvenile) to 97 mm (maximum length) (Last *et al.* 1983). Otoliths were also extracted from a sample of 100 fish of the size range fed to the penguins to provide a reference sample of the sizes of ingested otoliths.

Analysis of samples

The otoliths from the stomach samples were removed, washed in detergent, sorted into left and right where possible and stored dry. They were then measured and weighed as described above. The apparent standard lengths of the fish eaten were then calculated from the regression equations. In order to estimate the number of fish eaten, where the otoliths could be categorized into left and right, the maximum number was used, but where categorization was not possible, the total number of otoliths was divided by two.

By comparison with the reference material an index of otolith digestion (DI) was devised in order to describe otolith condition:

DI = (0) no apparent digestion.
- (1) slight digestion—margin crenulations less distinct but rostrum and sulcus acusticus still well defined.
- (2) moderate digestion—margin crenulations disappeared, rostrum and sulcus acusticus less distinct.
- (3) advanced digestion—no rostrum or sulcus acusticus apparent, otolith lost all diagnostic features.

Results

There was no significant difference within pairs of otoliths in either length ($t = 0{\cdot}327$, d.f. = 22, $P > 0{\cdot}05$) or weight ($t = 0{\cdot}825$, d.f. = 22, $P > 0{\cdot}05$). The relationships between otolith length (OL, mm) and FSL (mm) and otolith weight (OW, mg) and FSL were:

$$FSL = 21{\cdot}1\ OL^{1{\cdot}21} \qquad (r = 0{\cdot}97, n = 23, P < 0{\cdot}001)$$
$$FSL = 50{\cdot}5\ OW^{0{\cdot}41} \qquad (r = 0{\cdot}98, n = 23, P < 0{\cdot}001)$$

The number of recovered otoliths and the number of fish these represented are given in Table 1. The percentages of otoliths recovered over time are presented in Figure 1 which shows that the initial recovery rates were variable. One hundred per cent of ingested otoliths were recovered from at least one of the penguins which

received replicate treatments after the meals of five, ten and 50 fish within the first two hours. At no time were 100% of otoliths recovered after the 20 fish meal. After 8 hr post-feeding the proportion of recovered otoliths ranged from 40 to 79%, with more from the larger meal sizes. Only after meals of 50 fish were otoliths present in the stomachs after 16 hr. The variability of otolith recovery rates was indicated by treatments in which 20 fish were fed, where the recovery of otoliths was higher after 2 and 4 hr than after 1 hr. The variation was also evident in otoliths recovered from penguins which had received the same treatment, the incidence and magnitude of this variation increasing with time after ingestion (Tables 1 & 2).

It is unlikely that any otoliths were passed out in faeces. The penguins were held

Table 1. *Length of retrieved otoliths from Little Penguin stomachs and calculated fish standard length (FSL) with tests for differences in calculated FSL between replicates and between calculated FSL and ingested FSL. a and b are results for individual penguins*

Fish fed (*n*)	Time (h)		Retrieval otoliths fish (*n*)	(%)	Otolith length (mm) mean ± 1 s.e. (range)	Calculated FSL mean ± 1 s.e. (range)	*t*-statistic between replicates	*t*-statistic calc. FSL *v.* ingested FSL
5	1	a	10	100	2·7 ± 0·06 (2·5–3·0)	71 ± 1·9 (64–80)	0·088	1·227
		b	9	100	2·7 ± 0·02 (2·7–2·8)	71 ± 0·6 (70–73)		1·050
	2	a	10	100	2·6 ± 0·04 (2·5–2·9)	67 ± 1·4 (64–77)	2·010	1·254
		b	9	100	2·5 ± 0·02 (2·5–2·6)	65 ± 0·7 (64–67)		3·101†
	4	a	7	80	2·5 ± 0·09 (2·3–2·6)	63 ± 2·7 (58–67)	2·123	2·680†
		b	6	80	2·2 ± 0·07 (2·1–2·5)	53 ± 2·1 (52–64)		4·107‡
	8	a	4	40	1·8 ± 0·12 (1·3–2·3)	45 ± 3·4 (29–58)	1·823	11·124‡
		b	4	40	1·4 ± 0·25 (0·9–2·1)	32 ± 7·0 (19–52)		10·961‡
10	1	a	19	100	2·7 ± 0·03 (2·6–2·9)	70 ± 1·0 (67–77)	2·020	2·011
		b	18	90	2·8 ± 0·04 (2·5–3·0)	73 ± 1·2 (64–80)		0·451
	2	a	20	100	2·7 ± 0·05 (2·2–3·0)	70 ± 1·7 (55–80)	0·150	2·043*
		b	18	90	2·7 ± 0·05 (2·5–3·2)	71 ± 1·5 (64–86)		1·835
	4	a	19	100	2·2 ± 0·04 (1·9–2·5)	54 ± 1·2 (46–64)	4·809‡	11·154‡
		b	17	90	2·6 ± 0·08 (1·7–3·0)	67 ± 2·4 (40–80)		3·496‡
	8	a	15	80	1·5 ± 0·12 (0·8–1·9)	35 ± 3·3 (16–46)	2·212*	14·525‡
		b	13	70	1·8 ± 0·14 (1·1–2·1)	43 ± 4·2 (24–52)		12·047‡
20	1	a	36	95	2·8 ± 0·02 (2·6–3·3)	73 ± 0·7 (67–89)	1·224	0·879
		b	34	90	2·7 ± 0·03 (2·4–3·2)	71 ± 1·0 (61–86)		1·859
	2	a	38	100	2·7 ± 0·03 (2·4–3·1)	72 ± 1·0 (61–83)	0·699	1·740
		b	37	95	2·7 ± 0·02 (2·4–2·9)	71 ± 0·7 (61–76)		2·170*
	4	a	39	100	2·7 ± 0·03 (2·1–3·2)	72 ± 1·0 (52–86)	0·329	1·738
		b	38	100	2·7 ± 0·02 (2·3–2·9)	71 ± 0·7 (58–76)		2·173*
	8	a	33	85	2·2 ± 0·05 (1·4–2·6)	55 ± 1·4 (32–67)	3·115†	12·466‡
		b	28	75	2·4 ± 0·04 (1·7–2·7)	62 ± 1·3 (40–70)		7·917‡
50	1	a	100	100	2·7 ± 0·02 (2·2–3·1)	71 ± 0·6 (55–83)	0·196	3·681‡
		b	100	100	2·7 ± 0·02 (2·1–3·1)	72 ± 0·5 (52–83)		2·459*
	2	a	98	98	2·4 ± 0·02 (1·9–3·0)	60 ± 0·7 (46–80)	0·726	14·201‡
		b	92	98	2·3 ± 0·03 (1·9–2·9)	59 ± 0·8 (46–76)		14·110‡
	4	a	94	96	2·3 ± 0·02 (1·8–3·0)	57 ± 0·7 (43–80)	0·557	16·500‡
		b	90	96	2·3 ± 0·02 (1·6–2·8)	57 ± 0·7 (37–73)		17·529‡
	8	a	92	92	2·2 ± 0·02 (1·7–2·7)	54 ± 0·7 (40–70)	7·488‡	20·333‡
		b	79	80	1·9 ± 0·03 (1·4–2·3)	46 ± 0·8 (32–58)		26·404‡
	16	a	63	64	1·9 ± 0·04 (0·9–2·6)	45 ± 1·2 (19–67)	2·012*	22·396‡
		b	42	42	1·7 ± 0·07 (0·5–2·6)	40 ± 1·9 (9–67)		19·911‡

* = $P < 0.05$, † = < 0·01, ‡ = $P < 0.001$.

Table 2. *Weight of retrieved otoliths from Little Penguin stomachs and calculated fish standard length (FSL) with tests for differences in calculated FSL between replicates and between calculated FSL and ingested FSL.*

Fish fed (*n*)	Time (h)		Retrieval otoliths (*n*)	Otolith weight (mg) mean ± 1 s.e. (range)	Calculated FSL mean ± 1 s.e. (range)	*t*-statistic between replicates	*t*-statistic calc. FSL *v.* ingested FSL
5	1	a	10	2·36 ± 0·091 (1·99–2·81)	72 ± 1·1 (67–77)	0·425	0·648
		b	9	2·41 ± 0·073 (2·08–2·59)	72 ± 0·9 (68–75)		1·001
	2	a	10	2·06 ± 0·099 (1·60–2·60)	67 ± 1·3 (61–75)	2·087	2·089
		b	9	1·77 ± 0·046 (1·64–1·93)	64 ± 0·7 (62–66)		3·498‡
	4	a	7	1·64 ± 0·271 (1·18–2·12)	61 ± 4·2 (54–69)	1·517	3·009†
		b	6	1·14 ± 0·095 (1·39–2·01)	53 ± 2·0 (58–67)		4·126‡
	8	a	4	0·60 ± 0·117 (0·19–1·13)	39 ± 3·4 (26–53)	1·144	11·819‡
		b	4	0·35 ± 0·189 (0·07–0·89)	29 ± 7·0 (17–48)		13·551‡
10	1	a	19	2·34 ± 0·081 (1·96–2·98)	71 ± 1·0 (66–79)	1·690	1·405
		b	18	2·55 ± 0·094 (2·10–3·18)	74 ± 1·1 (68–81)		0·034
	2	a	20	2·25 ± 0·111 (1·21–2·96)	70 ± 1·5 (55–79)	0·807	2·283*
		b	18	2·34 ± 0·077 (1·94–3·06)	71 ± 0·9 (66–80)		1·490
	4	a	19	1·04 ± 0·070 (0·51–1·81)	51 ± 1·4 (38–64)	5·922‡	12·988‡
		b	17	2·09 ± 0·166 (0·62–3·13)	67 ± 2·4 (41–81)		3·356†
	8	a	15	0·41 ± 0·180 (0·34–0·74)	35 ± 2·3 (32–45)	2·427*	10·833‡
		b	13	0·64 ± 0·170 (0·41–1·50)	42 ± 2·7 (35–60)		9·846‡
20	1	a	36	2·52 ± 0·071 (1·82–4·56)	74 ± 0·8 (65–94)	1·465	0·332
		b	34	2·39 ± 0·055 (1·78–3·05)	72 ± 0·7 (64–80)		1·465
	2	a	38	2·47 ± 0·046 (2·00–3·23)	73 ± 0·6 (67–82)	1·661	0·792
		b	37	2·37 ± 0·034 (2·00–2·76)	72 ± 0·4 (67–77)		1·871
	4	a	39	2·46 ± 0·102 (1·02–4·95)	73 ± 1·2 (51–97)	1·296	1·095
		b	38	2·29 ± 0·062 (1·43–2·99)	71 ± 0·8 (58–79)		2·235*
	8	a	33	1·21 ± 0·066 (0·34–1·88)	54 ± 1·3 (32–65)	4·525‡	13·831‡
		b	28	1·66 ± 0·062 (0·67–2·25)	62 ± 1·0 (43–70)		8·422‡
50	1	a	100	2·42 ± 0·031 (1·57–3·24)	72 ± 0·4 (61–82)	0·825	2·055*
		b	100	2·45 ± 0·030 (1·55–3·16)	73 ± 0·4 (60–81)		1·699
	2	a	98	1·75 ± 0·056 (1·01–3·49)	63 ± 0·8 (51–84)	0·839	9·845‡
		b	92	1·74 ± 0·062 (1·05–3·63)	63 ± 0·9 (52–86)		10·288‡
	4	a	94	1·58 ± 0·050 (0·65–3·63)	60 ± 0·7 (42–86)	0·589	13·373‡
		b	90	1·53 ± 0·039 (0·92–2·61)	60 ± 0·6 (49–75)		14·962‡
	8	a	92	1·32 ± 0·045 (0·60–2·49)	56 ± 0·8 (41–73)	9·150‡	17·116‡
		b	79	0·78 ± 0·047 (0·17–2·13)	44 ± 1·0 (24–68)		24·569‡
	16	a	63	0·86 ± 0·052 (0·07–1·75)	46 ± 1·3 (17–63)	3·495‡	20·898‡
		b	42	0·60 ± 0·068 (0·01–1·57)	38 ± 2·1 (8–61)		20·752‡

* = $P < 0·05$, † = $P < 0·01$, ‡ = $P < 0·001$.

for at least one hour after being stomach-flushed and all faeces passed during this time were examined for evidence of otoliths. No otoliths were found in these faeces, nor in the faeces which have been examined from other Little Penguins (pers. obs.).

The mean length of otoliths extracted from the reference sample of fish fed to the penguins was 2·8 mm (s.e. = 0·02, range = 2·5–3·6) and the mean weight was 2·58 mg (s.e. = 0·6, range = 1·82–4·96). Otoliths recovered from the stomach samples decreased in both length and weight with time after ingestion (Tables 1, 2). The variation in size of otoliths retrieved from replicate treatments also increased with stomach residence time.

Using both the lengths and weights of recovered otoliths, apparent fish lengths were calculated using the regression equations from the reference group (Tables 1,

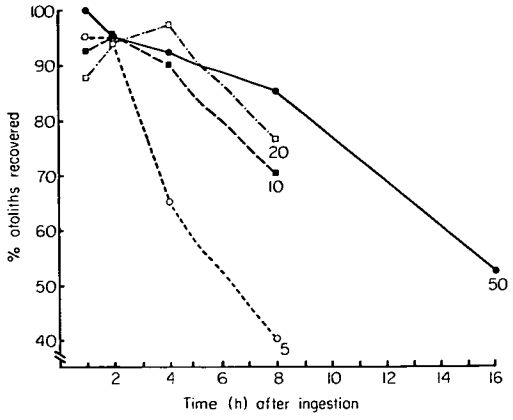

Figure 1. Per cent of retrieved otoliths from Little Penguin stomachs versus time for each of the meal sizes. Each point is the mean of the results of the replicate treatment for two penguins.

2). In no case were there any significant differences between the apparent fish lengths calculated from either otolith length or weight. There were no significant differences in calculated fish sizes between replicates for up to 2 hr post-feeding for any meal size. Variation between replicates was also insignificant at 4 and 8 hr post-feeding for the meals of four fish. This variation, however, tended to increase with both meal size and retention time and was significant after 2 hr when ten and 50 fish fed and after 4 hr when 20 fish were fed.

When apparent fish length, calculated from otolith size, is compared to the actual fish length ingested it is clear that the underestimation of fish size increased with time of retention (Fig. 2, Tables 1, 2). At 2 hr after ingesting meals of all sizes, in at least one of the replicates, the fish lengths calculated from length of retrieved otoliths were significantly smaller than the lengths of fish actually ingested. With the largest meal size of 50 fish this difference was significant in both replicates after only 1 hr post-feeding. After 16 hr the calculated fish length underestimated the actual fish length by 43%.

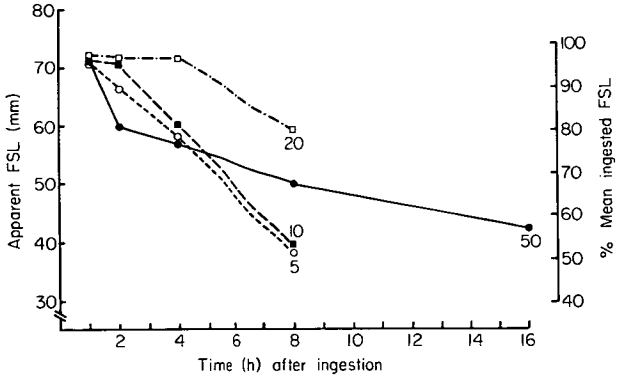

Figure 2. Apparent fish standard lengths (FSL) (mm) calculated from otolith lengths from Little Penguin stomachs versus time for each of the meal sizes. Apparent FSL is also expressed as the percent of the mean FSL actually ingested (74 mm = 100%).

Table 3. *Comparison of fish standard lengths calculated from otolith lengths from different Little Penguin digestion indices and ingested fish standard lengths*

Digestion index ($n = 50$)	Otolith length mean ± 1 s.e. (range)	Calculated FSL mean ± 1 s.e. (range)	t-statistic calc. FSL *v* ingested FSL
0	2·8 ± 0·02 (2·6 ± 3·3)	74 ± 0·6 (67–89)	0·385
1	2·3 ± 0·02 (2·0 ± 2·6)	58 ± 0·5 (49–67)	14·935‡
2	1·9 ± 0·03 (1·6 ± 2·3)	47 ± 0·9 (37–59)	22·753‡
3	1·5 ± 0·04 (0·8 ± 1·9)	34 ± 1·0 (16–46)	32·218‡

‡ indicates $P < 0·001$.

After the initial rapid rate of digestion which occurred during the first hour after being fed 50 fish, the rates of decrease of calculated fish lengths were rapid for the five and ten fish meals but slower for the 20 and 50 fish meals, respectively. This is evident from Figure 2 and, for example, for the apparent fish sizes after 8 hr post-ingestion of all meal sizes. After meals of five and ten fish, the calculated fish lengths were approximately 52% of the actual fish lengths, and were 79 and 68% for the 20 and 50 fish meals, respectively. Thus, subsequent to the first hour after ingestion, for the smaller meals which represented 1·4 and 2·8% of the penguin body-weight, digestion proceeded at a similar rapid rate, but for the larger meal sizes which represented 5·6 and 14% of penguin body-weight, digestion proceeded more slowly.

A sample of otoliths retrieved from stomach samples was described by the digestion index and their lengths and calculated fish lengths are shown in Table 3. No otoliths which were retrieved from the stomach samples but were still within skull cases showed any sign of degradation and hence were classified as DI = 0. However, some skull cases retained only one sagittal otolith indicating that there may be differential digestion within a pair, as most loose otoliths in the stomach contents scored 1 to 3 on the digestion index. When the calculated fish sizes are compared with the actual fish sizes, only those estimates derived from otoliths with a DI of 0 do not underestimate significantly the original fish length. The precision of the estimates decreased with increases in DI scores.

Discussion

Knowledge of the diets of seabirds is essential to an understanding of their role as predators in the marine ecosystem (Croxall *et al.* 1984) and the primary objective of diet studies is to determine quantitatively the composition of the diet by analyses which are free from bias (Hyslop 1980). The use of diagnostic prey remains to identify prey species and calculate original prey size is becoming increasingly common, but inherent in this practice is the assumption that digestion leaves hard parts unaffected. Squid beaks have been found to be affected by digestion (Bigg & Fawcett 1985) and may be retained in seabird stomachs for considerable periods of time (e.g., Furness *et al.* 1984). Thus, while the problem of squid beak accumulation is frequently discussed, little comment is made about the implications, or indeed the presence, of degraded otoliths in seabird stomach contents (e.g., Ainley *et al.* 1981, Jackson 1984, LaCock *et al.* 1984, Wilson 1985, Wilson *et al.* 1985). More attention is, however, now being focused on otolith digestion particularly by workers on seals

(e.g., Prime 1979, Bigg & Fawcett 1985, Da Silva & Neilson 1985, Murie & Lavigne 1985, 1986).

In this study, it was shown that atherinid otoliths are rapidly digested after ingestion by Little Penguins. Similarly, in a study of digestion in Jackass Penguins *Spheniscus demersus*, meals of 50 g of fish were completely digested and no otoliths remained, after 10 hr post ingestion, and meals of 100 g had been completely digested after 14 hr (Wilson *et al.* 1985). These meal sizes represent 1·7 and 3·0% of the Jackass Penguin body-weight and are equivalent to the five and ten fish meals fed to the Little Penguins which were completely evacuated between 8 and 16 hr after feeding. Murie & Lavigne (1985) found that in grey seals *Halichoerus grypus*, no otoliths remained in the stomach after 18 hr. They also found that 30% or more of the ingested otoliths had been completely digested by 3 to 6 hr post-feeding, a result which was reflected by the Little Penguins which digested between 20 and 60% of the otoliths by 4 to 8 hr after ingesting five fish.

A consistent element in these feeding studies is that the digestion and passage rates of otoliths are subject to variation. The Little Penguin data show that otolith digestion rates vary with stomach fullness and time of retention which together confound interpretation of results. Prime (1979), working with harbour seals *Phoca vitulina*, retrieved only a negligible proportion of ingested otoliths and concluded that no otoliths are retained for more than 48 hr. These observations demonstrate that if digestion of otoliths is not taken into account then any estimate of the number of fish consumed will be too low.

Not only are otolith numbers reduced, but otolith size is also affected by digestion. While otoliths can provide information on size and age of fish, any calculation of original fish size, when based on otoliths which are in any way affected by digestion, will underestimate the size of fish. In the present study calculations using retrieved otoliths resulted in significant underestimates of ingested fish length after only 1 to 2 hours post-feeding, and the difference between apparent and actual fish length increased rapidly until errors were in the order of 40%. In an effort to eliminate this latter bias, Frost & Lowry (1980) calculated the original fish size from only non-degraded otoliths. This also proved to be effective in the present study, as when otoliths with a DI of 0 were used to estimate size of ingested fish, there was no significant difference between apparent and actual fish length. In order to do this, however, a visual inspection under a microscope is necessary for each otolith and it is this requirement which makes otolith length a more time-efficient parameter than otolith weight. Although the two otolith measurements produce the same results in calculations, since otolith condition can be inspected simultaneously to measuring their lengths, considerably less time is required than for the double handling required when otoliths are both inspected and weighed.

Most workers do, in fact, use otolith length (Jackson 1984, Duffy & Laurenson 1983, North *et al.* 1984, Wilson 1985) and, hence, inspecting otoliths and thereby producing accurate estimates of FSL should be feasible. However, as noted by Murie & Lavigne (1985), this procedure may present sample size problems when used on predators whose prey have small, fragile, and hence readily digested, otoliths (e.g., Clupeidae).

The relative size and thickness of otoliths vary considerably between fish species (Hecht 1978) and there is evidence that small otoliths digest more rapidly than large ones (Prime 1979, Da Silva & Neilson 1985, Murie & Lavigne 1986). Therefore, it would be expected that otoliths from different age fish within a species, and from different species of fish, will digest at different rates. Some species of fish have highly morphologically variable otoliths (Morrow 1977) and so it is likely that these otoliths may also digest at different rates. Therefore, in partially digested meals, some prey

may have been digested completely and therefore not included in calculations of amount of prey consumed, and any differential digestion of diagnostic remains makes quantification increasingly difficult.

The absence of otoliths in the Little Penguin faeces suggests that unrecovered otoliths are completely digested. Otoliths are, however, occasionally found in Emperor Penguin *Aptenodytes forsteri* faeces (Green pers. comm.) and it may be that differences in otolith density and/or penguin digestive physiology account for this difference. The initial rapid rate of digestion during the first hour after being fed 50 fish may be explained by the caloric effect of feeding. After feeding the Little Penguin meals equivalent in size to the 50 fish meals in the present experiment, Baudinette *et al.* (1986) found that for an initial period the metabolic rate is almost doubled. Further variability in digestion and passage rates is introduced when mixed diets are consumed (Wilson *et al.* 1985) and also by the activity of the animal (Bigg & Fawcett 1985).

If a predator has a catholic diet which includes fish with small otoliths, there is considerable potential bias in results. Where specific data are not available, interpretation of piscivore diet analyses can result in underestimates of biomass of prey consumed and of prey age and size. The implications of these errors may be particularly important if the information from dietary studies is to be used in the assessment of prey resources.

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