

Aspects of the ecology of macaroni penguins (*Eudyptes chrysolophus*) around South Georgia



Macaroni penguins (*Eudyptes chrysolophus*) at the Fairy Point study colony on Bird Island, South Georgia. © Tom Hart 2005

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Macaroni penguins (*Eudyptes chrysolophus* Brandt) feed when diving during long foraging trips away from their breeding colony. Their foraging behaviour is constrained by a number of factors including their physiology, their condition, the condition of their partner, and the condition of their chick. This thesis concerns aspects of their foraging ecology in relation to prey availability. Time Depth Recorders and other archival tags are increasing in their capacity, battery life and durability at the same time as getting cheaper and smaller and are frequently used to explore foraging behaviour. However, development and application of techniques to analyse these resulting data sets lags behind our ability to collect the data. This thesis develops new methods and uses data from 129 macaroni penguins collected over 6 years to determine patterns of foraging behaviour. In particular:

1. Appropriate identification of sex is essential to any study of foraging ecology, in particular during the breeding season. In response to an identified problem with morphometric sexing, I conduct a validation test between molecular and morphometric sex tests and make conclusions about the nature and identity of error in these tests.
2. I use summary statistics to characterise dives. This work suggests that time series techniques may provide insights that have been lacking from previous analyses. I apply time series techniques to the data to model non-independence in data sets and to compare results obtained using auto-correlation methods with findings obtained from (1) above. The time series approach allows a comparison of different temporal elements of dives, in particular correlations between features of successive dives and how correlation between dives decays with time.
3. I use Hidden Markov Models as a clustering algorithm to provide a statistically robust description of patterns in dives that may outperform the widely used concept of bouts of dives. I then use this method to determine whether such clustering of dives exists in my data. Characteristics of bouts and types of dives vary with year, the stage and sex of the individual.
4. Hidden Markov Models do not provide a direct replacement for the concept of bouts. Rather than identify bouts as summary statistics of diving activity, I take daily summaries of activity. I then use these to overcome the non-independence of dives and to determine the relative periods of dive activity and travel or searching. Comparison between the adult and chick fledging weights revealed a strategy of investment in the chick at the expense of the adult body mass.

The application of time series techniques has led to new insights about the timing of decision-making, but this thesis reveals that further advances also require positional data to be combined with temporal activity data.

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Chapter 1: Introduction

The need for behavioural studies of marine predators

Understanding how animals find food is vital to understanding their population processes. Animals need to find food with a sufficient rate of return to exceed their energy expenditure incurred in foraging in order to allow growth and reproduction. Changes in food availability or distribution can therefore alter individual survival, reproductive success and consequently, the population growth rate. Changes in food availability and abundance influencing predator behaviour have been reported as a result of human interference including fishing (Otley et al. 2007, Davies et al. 2009) and climate change (Charissin & Bost 2001, Croxall et al. 2002, Durant et al. 2005, Forcarda & Trathan 2009), with resulting conservation concerns (Caro 1999).

Marine predators are of particular concern because of documented global declines (IUCN 2008, Birdlife 2008) and because of the difficulty in obtaining suitable data to model their populations and prey availability. The Southern Ocean has undergone decades of study, but the foraging ecology of most species remain insufficiently understood to predict their responses to changing prey conditions.

Most predator biomass in the Southern Ocean is found in land-based predators (Atkinson et al. 2001), that breed on land, but forage at sea (Croxall et al. 1984). These species include seals, penguins and a number of flying birds, mainly albatrosses and petrels (Croxall et al. 1984) and most of which are in decline (Forcarda & Trathan 2009, Birdlife 2008).

Southern Ocean food webs are short (Atkinson et al. 2001) and dependent on, or centred around one species; Antarctic krill (*Euphausia superba*; Everson 2000,

Atkinson et al. 2001) which is hereafter referred to as *krill*. Predators' reliance on a common prey species makes them vulnerable to prey shifts, especially as krill are the focus of a sustained and increasing fishery (Hill et al. 2007) and apparently sensitive to climate change (Loeb et al. 1997, Atkinson et al. 2004).

The decline of Southern Ocean predators and shifts in krill make foraging studies more urgent, but collecting suitable data remains difficult. Penguins provide an excellent model for the challenges associated with studying foraging behaviour in a marine vertebrate. While they nest and moult on land, they spend the larger part of their lives foraging at sea (Bost et al. 2009). Consequently, ecologists have struggled to piece together a complete picture of their diet and foraging strategies, and also their migratory habits (Bost et al. 2009). However, a number of novel techniques have been developed in recent years that now enable some comparison of foraging strategies to be made between species and locations.

Activity recording tags, or *biologging* devices have emerged as the best way to study free-living animals and their foraging behaviour. A large number of studies have used these tags to investigate how animals use their environment and the decisions that underlie this (Jonsen et al. 2006, Wikelski et al. 2006). These studies have usually focussed on foraging, where the rules underlying the observed pattern are inferred by the pattern of when and where changes in behaviour occur (Jonsen et al. 2005, Jonsen et al. 2006). Advances in bio-logging technology have also facilitated studies of marine predators in terms of physiology (Green et al. 2002), foraging (Bost et al. 2007), migration (Block et al. 2001) and navigation (Biro et al. 2007). This has been driven by technological advances through innovation or new data recorders (Hooker et al. 2002, Shepard et al. 2009), miniaturisation (Boyd et al. 2004, Kooyman 2003) and increasing memory or battery life (Boyd et al. 2004, Kooyman 2003).

Determining how and when marine predators such as penguins feed remains a challenge, despite these technical advances (Bost et al. 2007). Before reviewing these challenges, I consider the types of data that can be obtained by different tags.

Types of Bio-logging device

Biologging devices are numerous and record a range of data that can be broadly summarised into the following groups:

1. Movement or motion

Understanding movement has been crucial in understanding how animals search, catch prey, navigate and migrate (Croxall et al. 2005, Teo et al. 2007, Guilford et al. 2009). Different types of remote tags have helped researchers gain insight into each one of these processes. Position recorders such as satellite tags or GPS tags have enabled inferences about home range (Dewar et al. 2004, Börger et al. 2006). Motion recorders such as Time Depth Recorders (TDR) have recorded the depth profile of dives in diving predators for a number of years (Wilson et al. 1986, Croxall et al. 1988, Naito et al. 1990) and have afforded insights into diving in a range of diving animals such as whales (Tyack et al. 2006, Soto et al. 2008), turtles (Hays et al. 2006), penguins (Tremblay & Cherel 1999, 2000), shags (Wislon et al. 2008) and petrels (Bocher et al. 2000). Accelerometers have allowed the orientation of animals to be inferred (Wilson et al. 2008), which has enabled researchers to calculate wingbeat frequencies, (Wilson et al. 2006, Sato et al. 2007, Wilson et al. 2008, Shepard et al. 2009) and also to calculate the energy budgets of different types of behaviour (Wilson et al. 2008, Shepard et al. 2009). Finally, angle loggers such as bill loggers have enabled the recording of prey capture events in penguins (Wilson et al. 2002, Takahashi et al. 2004a) or attempted capture in a range of other animals (Bost et al.

1997, Wilson et al. 2002).

2. Physiology

Physiology is a key determinant of space use and movement behaviour. In order to understand the physiological drivers of observed behaviour, researchers have deployed a range of physiological recorders. These can be used to monitor heart rate (Green et al. 2002), which has enabled calculations of metabolic rate in foraging penguins (Green et al. 2005), seals, (Boyd et al. 1999) and albatrosses (Bevan et al. 1994, Weimerskirch et al. 2002). Internal temperature loggers have also proven useful in elucidating metabolic rates and recovery from diving (Handrich et al. 1997, Halsey et al. 2008).

3. Physical measurements

Aspects of the environment can impact physiology or decision-making. Recorders that measure the oceanographic or physical environment that animals encounter have been useful in physiological, behavioural and oceanographic studies (Fedak 2004). Ocean Temperature Profiles have been used to infer the presence of a thermocline in relation to diving (Ropert-Coudert et al. 2006) and foraging preferences (Block et al. 2001).

Light levels have been used to infer the visual acuity of diving animals and light levels at different wavelengths can be used to infer chlorophyll concentration (Teo et al. 2009). Animal-borne cameras have also enabled researchers to directly see what the animal experiences (Hooker et al. 2002, Hooker et al. 2005, Takahashi et al. 2004b), which enables the formulation of new hypotheses (Hooker et al. 2002), and elucidation of the prey field (Hooker et al. 2005). Acoustic tags have been used to infer prey detection (Johnson et al. 2004, Zimmer et al. 2005) and social structure in cetaceans (Watwood et al. 2004).

4. Networks/encounter loggers

One of the key elements of understanding animal behaviour is sociality and interactions with other animals. Group behaviour is one of the most conspicuously absent factors from biologging studies, but may soon be addressed by contact and network tags. Group behaviour is important for our understanding of a number of social animals, from ibex that migrate or forage together to wolves that live and hunt in a pack (Conradt & Roper, 2000; Ruckstuhl & Neuhaus, 2001). It is highly plausible that our understanding of penguins is limited by our failure to investigate group dynamics (Naito 2007), as anecdotal examples of synchronous diving have been found in two different species of penguin (Tremblay & Cheral 1999; Takahashi et al. 2004b).

5 Combination tags

While it is straightforward to group the types of data recorded into the categories above, recent advances have come from deploying multiple sensors in the same tag. For example, combining temperature loggers with TDRs has enabled the detection of thermoclines. Animal-bourn tags are increasingly used in oceanography (Costa et al. 2008), and can now collect high-quality hydrographic information (Biuw et al. 2007, Charrassin et al. 2008). These instruments have the potential to collect information about the oceans that is not only relevant to studying the ecology of tagged animals (Block et al. 2002, Biuw et al. 2007), but also for studying the physical structure of the oceans (Lydersen et al. 2002, Boehme et al. 2008, Charrassin et al. 2008, Costa et al. 2008).

Similarly, combining data sets permits many comparisons that are not possible by tagging one group of animals. Tagged animals collect useful data wherever an animal is, but tell us nothing about the gaps. Therefore, while bio-logging provides

excellent physical, chemical and biological information to the ocean science community, a strategy is necessary to fill these gaps. The use of combination tags and free exchange of data between physical and chemical oceanographers and the bio-logging community should therefore be a priority.

The welfare of tagged animals

Penguins have been tagged for decades to answer questions about reproductive biology, foraging ecology and migration (Williams & Rodwell 1992). However, any device placed on free-ranging animals has the potential to affect their welfare and alter their behaviour (Murray & Fuller 2000, Wilson & McMahon 2006). Monitoring penguins through the use of flipper tags has already been shown to be problematic as tagged captive penguins swim at lower speeds and expend more energy swimming compared to control animals (Culik et al. 1993). In wild birds, tags have also been shown to increase mortality (Froget et al., 1998; Gauthier-Clerc et al., 2004). Since publication of these studies, the use of permanent tagging and flipper-banding has greatly declined. Since the decline of flipper tags, subcutaneous RFID tags have been used for individual identification, which can be read without capture (Green et al. 2006).

Comparisons of instrumented penguins with non-instrumented controls have shown a range of effects of instruments on penguins (Culik & Wilson 1991, reviewed in Ballard et al. 2001), through pecking and preening (Wilson et al. 1989), increased energetic expenditure (Culik and Wilson 1991, Bannasch et al. 1994) and decreased nesting success (Watanuki et al. 1992). Recently, these effects have been considerably reduced by the use of smaller and more streamlined instruments deployed on the lower back behind the area of greatest girth, where the thickest boundary layer (the area of slowest flow velocity and resulting lowest drag) is found (Bannasch et al.

1994).

The time-depth recorders (TDR; MK7, Wildlife Computers, Redmond, USA) used in this study were embedded in epoxy resin and hydrodynamically shaped following recommendations by Bannasch et al. (1994), and attached according to recommendations in Wilson et al. (1997) in the turbulent region of water flow around a diving penguin, so that this impacted minimally on the diving performance of the bird (Wilson & McMahon 2002).

Antarctic krill (Euphausia superba); a keystone prey species

In addition to studying space use and movement behaviour of predators, insight into foraging can be aided by knowledge of the distribution of prey. This thesis will focus on macaroni penguins, which feed on krill. Krill are a vital central link in most Southern Ocean marine food webs (Everson 2000, Atkinson et al. 2001, Croxall et al. 2002). Around South Georgia, krill are taken by a number of mammalian and avian land based predators, of which most biomass is found in macaroni penguins and Antarctic fur seals, which are the top avian and mammalian predator, respectively (Croxall et al. 1984, Boyd 2002). The effect of these predators on prey populations is well documented both in terms of biomass (Hill et al. 1996, Reid et al. 1996, Reid et al. 1999) and behaviour (Alonzo & Mangel 2001, Hernández-León et al. 2001), and the importance of krill has been shown to affect recruitment in penguins and seals (Reid et al. 1999). Antarctic Fur Seals and macaroni penguins both impact the behaviour of krill, which show diurnal (Pütz et al. 1998, Bost et al. 2002) and seasonal migration (Atkinson et al. 2004), although the response to predation pressure remains poorly characterised. Because prey are often patchily distributed (Everson 2000), the ability to assess and move between prey patches is a vital and fundamental life history trait of seabirds (Tremblay and Cherel 2003), which

is evidenced by a suite of behaviours used in foraging. This enables foraging in less predictable habitats such as deep and offshore waters (Trathan et al. 2008).

Krill is found throughout the surface waters of the Southern Ocean. They have a circumpolar distribution, with the highest concentrations located in the Atlantic sector. They are shrimp-like crustaceans of the order *Euphausiacea* that form large swarms which can reach densities of 10,000–30,000 individual animals per cubic metre (Miller & Hampton 1989, Everson 2000). Krill feed on phytoplankton, and in turn become food for a large number of Southern Ocean predators. Krill reproduce under the ice sheets (Everson 2000), where they feed on algae on the lower surface until the pack ice melts, after which they disperse or are advected to sea (Everson 2000). Movement is driven by the circumpolar current around the Southern Ocean as far north as the Polar Front. Currently, there is little knowledge of the precise migration patterns of krill since individuals cannot yet be tagged to track their movements, but krill found around South Georgia are known to arrive on a northerly current from the Antarctic Peninsula (Atkinson et al. 2004, Murphy et al. 2004, Shreeve et al. 2005). Compared to the biomass of krill, there is currently very little exploitation by fisheries, especially in the area around South Georgia, although this may increase in the future. As a food source for penguins, krill may be in decline due to sea ice loss (Loeb et al. 1997, Atkinson et al. 2004) and possible response to acidification of ocean waters (Sabine et al. 2004) which leads to problems of exoskeleton deposition, although little is known about the ability of organisms to adapt to these changes (Fabrey et al. 2008).

The Macaroni penguin (Eudyptes chrysolophus)

History

Macaroni penguins were described by Johann Freidrich von Brandt in 1837

and are one of six species of penguin in the Eudyptid family. There is good evidence to suggest that there was a large diversification of penguins that coincided with the last major ice sheet expansion out of Antarctica (Baker et al. 2006) and as such the Eudyptes family diverged relatively recently from other families (Figure 1).

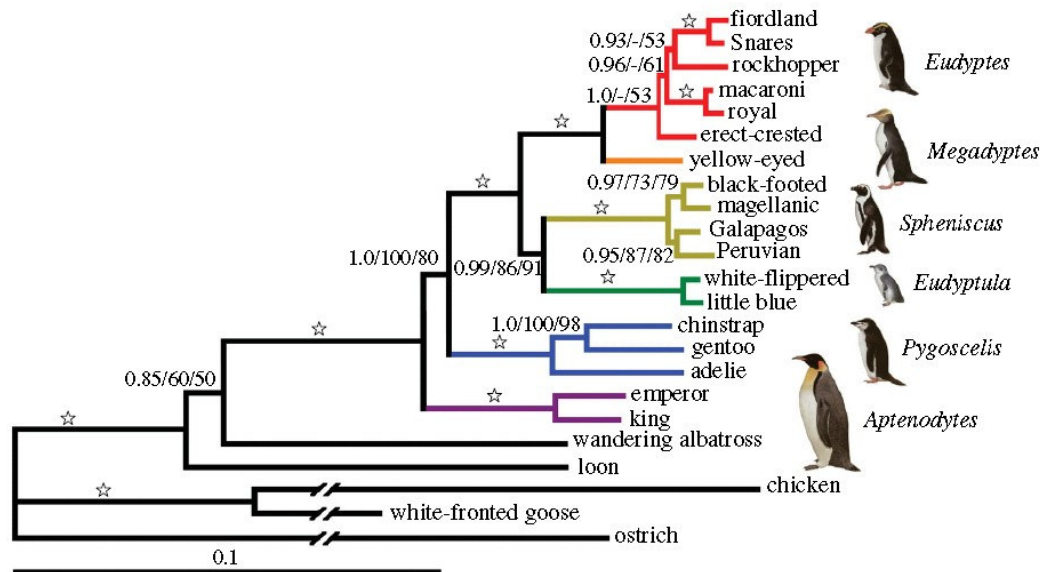


Figure 1: Bayesian estimate of phylogenetic relationships of modern penguins. Phylogenetic reconstruction was based on 2802 bp of RAG-1 and 2889 bp of mitochondrial 12S and 16S rDNA, cyt b and COI, excluding gaps and ambiguously aligned positions. Numbers above branches are Bayesian posterior probabilities/ML bootstrap proportions/MP bootstrap proportion, which are represented as open stars when (1.0/100/100). Branches for more distant outgroups were shortened for graphic purposes. A bar represents the expected number of DNA substitutions per site. Each genus is colour-coded. Penguin drawings were modified from del Hoyo et al. (1992), with permission, from Lynx Edicions, Barcelona, Spain. Figure reproduced with permission from Baker et al. (2006)

There is some evidence to suggest historical exploitation of macaroni penguins, with some reports of ships and expeditions using penguins for fuel rather than food. However, due to their unpalatable taste, they have rarely been eaten (Headland, 1990; Landis, 2003), although eggs may have been more extensively collected. It is likely that the populations have been disturbed in two main ways; by

consumption for fuel and also by removal of Antarctic fur seals (*Arctocephalus gazella*). Penguins and seals are thought to be in competition for around South Georgia (Barlow et al. 2002), and therefore it is likely that historical exploitation of seals positively impacted upon penguin recruitment and survival. More recently, the cessation of seal hunting and the subsequent recovery of the Antarctic fur seal population are likely to have had a negative impact on the macaroni penguin population through resource competition (Barlow et al. 2002).

Current status

Macaroni penguins have an estimated world population of 9 million breeding pairs, which makes this species the most numerous of all seabirds (Brooke 2004; Woehler 1995). The largest colonies are found on the islands of South Georgia, Crozet, Kerguelen, Heard, McDonald, Prince Edward and Bouvetoya (Woehler et al. 2001).

While penguins (subfamily *Spheniscinae*) may have been hunted by man in the past, presently the main threat to many species are the effects of global climate change (Forcada & Trathan 2009). Many of the 17 (IUCN 2008) or 18 (Birdlife International, 2008) species of penguin are currently in decline. These include the 7 species of the genus *Eudyptes*, all of which are listed in the International Union for Conservation of Nature (IUCN) extinction risk categories as Vulnerable (inc. macaroni penguins) or Endangered (IUCN 2008). Macaroni penguins are the most numerous species of penguin in the Southern Ocean but are now listed as Vulnerable as a result of marked declines in populations in areas such as South Georgia (Atlantic sector of the Southern Ocean, Figure 2) and Marion Island (Indian Ocean sector; Woehler et al. 2001). This is contrasted by apparent stability or even small increases in numbers of individuals at other localities such as Iles Kerguelen (Indian Ocean

sector; Woehler et al. 2001). Such contrasting trends in population size in different geographic localities raises questions as to whether shifts in geographic distribution are occurring amongst penguin populations through migration or whether observed changes are a result of increases or decreases in reproductive success and survival in colonies from different areas.

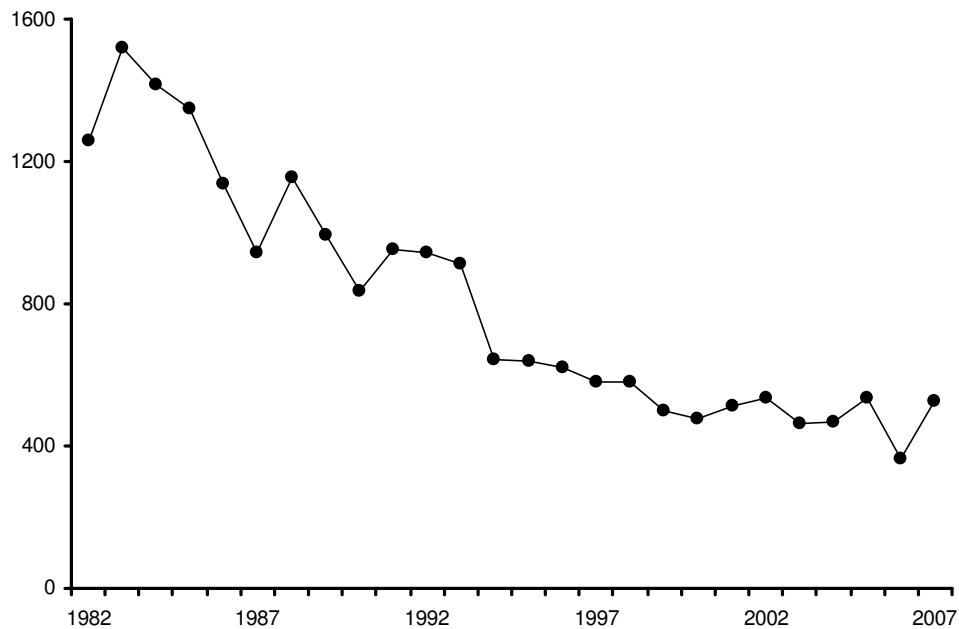


Figure 2: Numbers of macaroni penguin nests observed at the Fairy Point study colony on Bird Island, South Georgia between 1982 and 2006.

This study focuses on the population of macaroni penguins at Bird Island, South Georgia, which falls into FAO statistical sub-area 48.3 of the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR). The macaroni penguin is a priority species for the CCAMLR Ecosystem Monitoring Program (CEMP) because it is one of the most numerous krill dependent species in the Southern Ocean. The relevance of predator studies is rapidly increasing in the face of

the mounting reliance on Southern Ocean fisheries that now provide an increasing proportion of the world's protein consumption. Recently, there has been increased interest in the krill fishery around South Georgia, as well as massive investment in the krill fishing fleet (Nichol & Foster 2003).

Life history

Little is known about the natal dispersal of immature macaroni penguins. They first attempt to recruit at age 5 or 6. After this time they have high site-fidelity and strong pair-fidelity (Williams & Rodwell 1992). Breeding is highly synchronous within colonies, but timing differs between colonies, even between closely located colonies.

Males generally arrive at the breeding colony several days before the females. Egg laying is highly synchronised within a colony (Williams 1995). Macaroni penguins lay two eggs; an A egg and a B egg, but raise only a single chick. The chick is almost always derived from the second egg (B), which is typically 80% larger than the A egg. The A egg is generally rejected before the B egg is laid (Williams & Croxall 1991; Williams 1995). Crested penguins are unique among birds in showing both reversed egg size and reversed hatching asynchrony (Warham 1975). It is not understood what purpose the A egg serves (St Clair 1992, 1996), but variation in the levels and timing of the rejection and hatching suggest that the behaviour is adaptive to different environments (Massaro & Davis 2004, Poisbleau et al. 2008). While the underlying triggers are not known, studies have suggested that the degree of hatching asynchrony is under parental control by placing the A egg in a thermally disadvantaged position (St Clair 1992, Massaro & Davis 2004).

Parents take turns incubating the egg for several days at a time until the chick hatches and the female forages while the male stands guard (broodguard). When the

chick becomes large enough to be thermally independent, both adults forage during daytime, and return each evening to feed the chick. When the chick fledges, the adults replenish their body reserves with a long foraging trip prior to moulting, after which they disperse to sea over winter (Figure 2).

Relatively little is known about macaroni penguin at-sea distribution over winter, although recent work at Îles Crozet suggests that they associate with the polar front outside of the breeding season (Bost et al. 2009). In contrast, the breeding chronology is well understood and relatively constrained (Williams & Croxall, 1991, Figure 3).

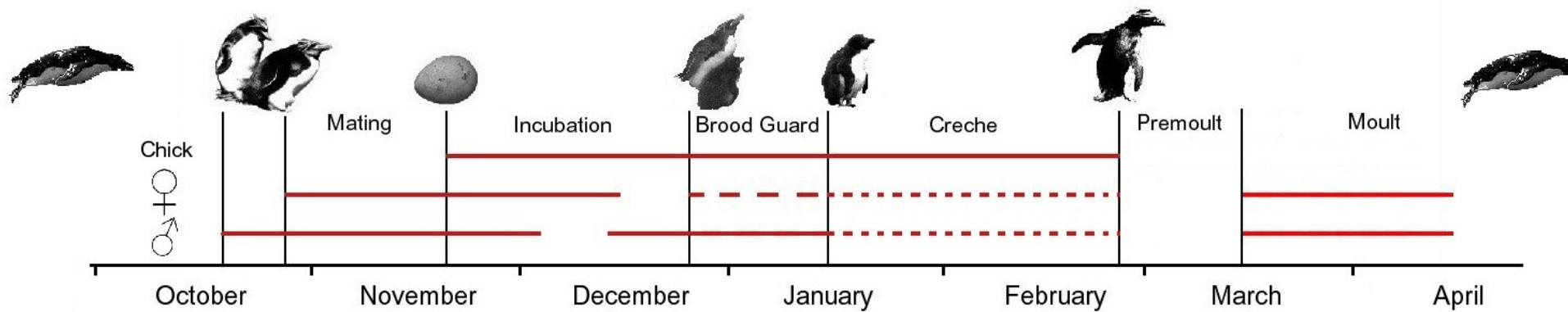


Figure 3: The breeding phenology of macaroni penguins on Bird Island, South Georgia. Lines indicate presence at the colony for males, females and chicks. Different stages in the breeding cycle are means taken from several years (Williams & Croxall, 1991), with significant variation between breeding seasons.

Diet

One of the obvious questions about foraging ecology concerns what animals eat. Early studies of macaroni penguins looked at stomach contents from penguins killed for the study (Downes, 1959; Warham, 1972). More recently, studies have tended to use stomach lavage to flush the stomach contents of living birds (Croxall et al. 1984 Croxall et al. 1997). These studies have two main limitations. Firstly, they discount any digestion at sea, so will tend to be biased towards the latter part of trips. Secondly, there is a lot of temporal averaging. We cannot determine when prey capture occurs within a foraging trip and stomach contents inevitably reflect the more recently eaten prey. To overcome this, we need a direct measure of prey capture, which has been attempted by the use of stomach-loggers (Handrich et al. 1997), bill angle recorders (Wilson et al. 2002; Takahashi et al. 2004a) and cameras (Takahashi et al. 2004b). Other studies have inferred prey capture events from aspects of the dive profile (Wilson et al. 2005).

Foraging studies

Where penguins forage is relatively easy to determine, and a number of studies have used satellite transmitters (Trathan et al. 2006) and GPS (Global Positioning System) tags (Trathan et al. 2008) to investigate foraging areas. Until now, these studies have been mostly limited to summer foraging behaviour, although winter distribution studies have recently become technically possible (Bost et al. 2009).

Many studies have also been concerned with aspects of dive behaviour, such as the depth, timing and nature of different dives within a foraging trip. These studies have determined how time of day and duration of the trip determine the nature of the dive patterns. Several studies have tried to characterise the nature of dive clustering in penguins (Tremblay & Cherel, 2003; Boyd et al. 1994; Mori 2001) and described the mean structure of dive trips (Tremblay & Cherel 2003) or how local clusters impact behaviour (Green et

al. 2002).

To understand the decisions and trade-offs made by foraging animals, we need to include a spatial and temporal element, so that we know not only what animals are eating, but also where and when they are taking this food. Penguin foraging ecology is only just getting to the stage where it is possible to instrument birds to collect these data. Until now, we have not been able to simultaneously record time, depth and position because the tags were too large to be able to put more than a TDR (time-depth recorder) or a satellite transmitter on a single individual. Recent studies (Wilson et al. 2005; Takahashi et al. 2004) have had some success in directly recording what the animals are feeding on, which is likely to revolutionise foraging studies once these tags become smaller and more reliable.

One of the outstanding remaining questions in foraging ecology is how to determine a change of behaviour. At the moment, foraging studies reduce the data set by averaging or blocking data into time periods. We need to determine the points of change in behaviour so that we can investigate on what information animals base their decisions. Only then can we understand how animals use their environment.

Sexual dimorphism in penguin foraging studies.

Macaroni penguins show limited sexual dimorphism. Females are usually shorter and lighter, but morphometry between sexes shows considerable overlap (Downes 1959, Warham 1972). A number of studies show sex-specific foraging differences, but this is complicated by the breeding chronology of macaroni penguins (Figure 3), in which only females forage during the broodguard phase, and further complicated by recent observations that broodguard is a period when there is the greatest abundance of krill (Saunders et al. 2007). Some data suggest that males competitively exclude females during the crèche period, when both sexes forage sympatrically. Barlow and Croxall (2002) found

that males forage closer to the colony than females, and Green et al. (2007) suggested that this results in females being forced to forage at greater, less efficient depths. Similar findings have been reported for Adélie penguins (Clarke et al. 1998).

Aims of the thesis – what is left to infer in penguin studies?

A number of studies have investigated macaroni penguin reproduction and foraging behaviour around South Georgia (Croxall et al. 1988, Croxall et al. 1993, Barlow & Croxall 2002, Green et al. 2003). My study is distinct from all others for two main reasons. Firstly, it involves a much larger data set than any other comparable study, and as such is able to investigate inter-annual differences in more detail and with more statistical power than previous studies. Secondly, this data set also has more complete coverage of the breeding seasons than any other study.

Within this thesis, I aim to answer important questions in penguin ecology and to bridge the gap between the analytical techniques currently employed in diving studies and those that are necessary for a fuller understanding of penguin foraging ecology. In many respects I start from first principles to identify what are the important questions in light of previous studies and current techniques. I then develop several new approaches that reduce dive data to independent units and use time series techniques to analyse these units. It is my hope that a number of these approaches will become more widely used – in particular the use of autocorrelation to broadly characterise time series (Chapter 3) and Hidden Markov Models to characterise states of behaviour (Chapter 4) and the daily allocation of time (Chapter 5).

Analysis of foraging patterns could be described as the problem of investigating clustering in time and space. This comes down to identifying what the different types of

behaviours are, when changes occur between behaviours and what influences these changes. Once we understand these rules, we can

- 1) Determine the units of behaviour;
- 2) Describe the transitions;
- 3) Determine what influences the transition over a range of timescales.

Penguins at sea have to make a decision to start diving, followed by decisions of whether to continue with a set behaviour or change strategy, with a final decision to stop diving and return to land. We are interested in overall trip decisions and how behaviours are moderated during daily dive periods.

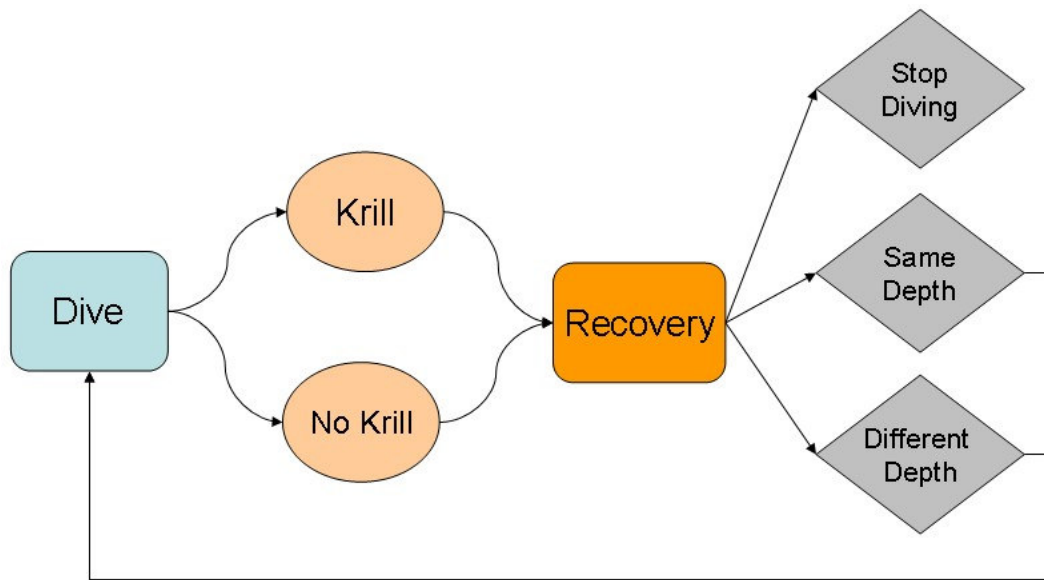


Figure 4: Decision flow-chart of penguins foraging. During each dive, the forager encounters prey at a rate that allows it to estimate the local prey abundance, which can inform the next dive and lead to patterns in the sequence of dives.

To generalise, consider the decision pathway in Figure 4. Information is gathered about krill density and depth on a dive and the number of prey caught. There is a reaction to the dive, which is the surface interval. The surface interval may include outside

influences such as predator avoidance. The information (krill) gathered in the dive, informs a decision of whether to continue diving with or without modifying behaviour. The decision to stop diving could be based on a time limit, being full or the rate of gain falling below a critical threshold.

What possible strategies are there?

We need an analysis, or a range of analyses, that will capture all of the possible variation and strategies that may exist. As such, we need to make an educated guess as to possible patterns in the data to ensure that the techniques used are sufficiently powerful to detect them. We use this simple thought experiment to see whether our current analytical tools are likely to be able to answer the questions we posed in the introduction.

Firstly, is the timing of dives fixed or are there bouts? A number of penguin foraging studies have commented on the existence of bouts in penguins (Naito et al. 1990, Green et al. 2002, Green et al. 2005, Naito 2007) Autocorrelation is a useful tool to look for periodicities and to describe them. Also plotting the depth (or the natural log of depth), duration and surface interval should indicate whether there are clusters. A range of cluster techniques such as Principal Components Analysis, k-means clustering or Hidden Markov Models can be used to define which dives are in which state.

Once bouts have been identified, there may be several possible dive patterns within bout. These are:

1. *Trends in dive depth*; does the depth increase, decrease or stay constant?
2. *Variance*; what is the variance in depth and does this change during a bout?
3. *Trends in variance*; does variance increase or decrease as predators influence the prey field by selection or elicit behavioural responses from prey?
4. *Repeating motifs*; are there alternating strategies between short and long dives, deeper and shallow dives.

Sources of Data

The data used were collected from the Fairy Point macaroni penguin study colony on Bird Island (54°00'30S, 38°04'21W, Figure 6). This colony is part of a long-term monitoring study for BAS, and is a small, circular colony of 400-600 pairs, approximately 20m in diameter. Data were collected following standard methods utilised as part of the CCAMLR Ecosystem Monitoring Programme (CEMP; www.ccamlr.org). One hundred and fifty-seven macaroni penguins were tagged from this colony during the austral summers of 1998/9 to 2004/5, a total of six breeding seasons. Individuals were tagged between November and March (during the breeding season) at various stages in the breeding cycle (Figure 3 and Table 1).

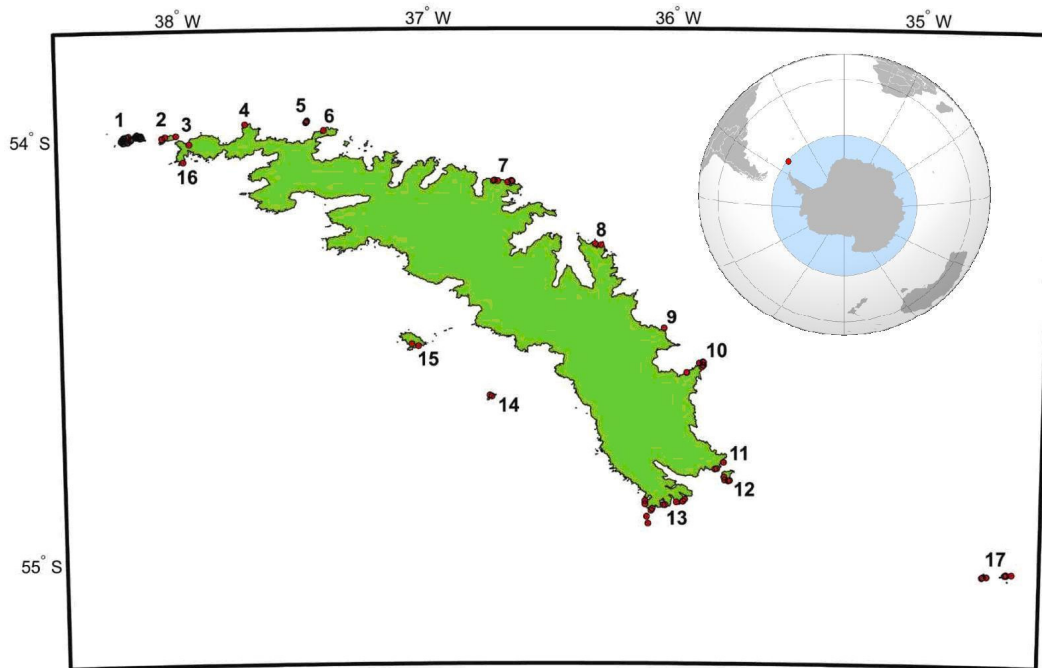


Figure 6: Positions of macaroni penguin colonies at South Georgia (Trathan et al., 1998). 1) Willis Island, 2) Bird Island, 3) Elsehul, 4) Cape North, 5) Welcome Isles, 6) Sheathill Bay, 7) Hercules Bay, 8) Rookery Bay, 9) Calf Head, 10) Cape Charlotte, 11) Cooper Bay, 12) Cooper Island, 13) Natriss Head, 14) Pickersgill Islands, 15) Annekov Island, 16) Cape Paryadin, 17) Clerke Rocks; reproduced with permission from Phil Trathan. Insert shows the Southern Ocean, with South Georgia represented by the red dot, public image.

Birds were tagged at the edge of the colony to avoid excessive disturbance. It is understood that there is the possibility that peripheral birds do not experience the same breeding conditions as central birds and may also be less experienced and/or younger (Ainley et al. 1983). They may therefore show different at-sea behaviour to mature, experienced birds. Deployment methodology is described in more detail in Chapters 3-5.

Of the 156 deployed tags all were recovered and data successfully downloaded from 129 of these. Zero Offset Correction (ZOC) identifies the surface as the running mean of the top of a series of dives. If the tops of these dives were too varied or showed large

trends, the tag was rejected on the assumption that the calibration of the pressure sensor had failed. Twenty-one tags were rejected were due to hardware or software failure, where the tag had not been set up correctly. A further six tags were rejected after ZOC. The number of usable data sets retrieved is given in Table 1 below. Sample sizes in each year were a consequence of differing projects underway over the course of 6 years. These different objectives resulted in different sample sizes.

Table 1: Sample sizes for each stage per year. Grey cells indicate data for which no body mass measurements were recorded. Combined with two missing body mass measurements for 1998/9, this brings the total to 103 individuals with pre-trip and post-trip body mass measurements.

<i>Season</i>	<i>Incubation</i>		<i>Brood Guard</i>		<i>Crèche</i>		<i>Premoult</i>		<i>Total</i>
	♀	♂	♀	♂	♀	♂	♀	♂	
1998/9			1		2	2			5
2000/1	4		4		6	6	4	1	25
2001/2	4	3	20		7	7			41
2002/3			6		7	5			18
2003/4			13		3				16
2004/5			9		10	5			24
Total	8	3	53	0	35	25	4	1	129

Structure of thesis

While there have been numerous studies of diving behaviour in marine predators, few have focussed on the sequential nature of behaviours. Therefore, in this thesis I

employ a number of statistical techniques in order to characterise time-depth data in new ways; in particular to understand the temporal nature of diving and the decision process in foraging.

- 1) Given the reported sex-based differences in foraging studies, accurate sex tests are essential, especially when the relatively low sample sizes common in tagging studies are considered. Observations made on large numbers of penguins led to concern over the validity of morphometric sexing. I therefore adapted a molecular technique to facilitate greatly increased accuracy in sexing and to highlight where errors in sexing studies might lead to misleading results.
- 2) A good understanding of the patterns in dives is essential to adopting the right approach to extracting useful information. I therefore present a number of summary statistics from the dive data set and explore simple analyses to show where previous studies may have failed to present the full picture. Inferences from these summary statistics show that one main element missing from most foraging analyses is a time-series component. I therefore use autocorrelation and an analysis of the model fit to determine broad patterns.
- 3) Greater understanding from point 2 shows that the problem is well defined, but an approach that combines time-series analysis with the ability to include discontinuities (or decision-making events) is still lacking. I therefore develop a Hidden Markov Model to allow these events to be characterised.
- 4) Returning to first principles, using the dive definitions from the Hidden Markov Models in 3, I analyse daily cycles using the period of time spent foraging and travelling.
- 5) Finally, I summarise these new techniques and their addition to existing literature.

Chapter 2: Testing the validity of sexing tests in the macaroni penguin.

Published as: Hart, T., Fitzcharles, E., Trathan, P. N., Coulson, T. N., Rogers, A. D. (2009) Testing And Improving The Accuracy of Discriminant Function Tests: A Comparison Between Morphometric And Molecular Sexing In Macaroni Penguins. *Waterbirds* **32**: 437-443.

Abstract

Dimorphism in various morphological traits is widely used to sex birds. However, when a threshold is set and there is overlap in the distribution of traits between sexes, there will be an error rate. Macaroni Penguins show limited sexual dimorphism in size, with the distributions of most phenotypic traits overlapping between sexes, which make using morphological characters for sexing difficult, although bill depth is used in one discrimination test. Two existing DNA-based PCR tests that use different primer pairs (P2/P8 and 2550/2781) were adapted for use with fluorescently labelled primers to enable the size of fragments to be accurately measured via genotyping protocols and sex to be determined. The two molecular tests were applied to the same samples to estimate the error rate within and between tests. Whilst both primer pairs reproducibly identified the sex of the penguins under study with no inconsistencies between tests, the P2/P8 primer pair had a higher success rate of amplification. To estimate the error rates of morphometric tests, they were compared with molecular tests, revealing areas of disagreement. Structure in the error indicated a bias near the morphometric discrimination threshold. We suggest that larger sample sizes in discriminant function analyses and calibration with molecular data can be used to estimate the likelihood of misidentification for any particular value of the trait. A description of where error lies can then be used to target the more expensive and time-consuming DNA tests to check individuals that are most likely to be mis-assigned.

Introduction

Reliable sex tests are critical for studies of sexual selection, ecology, demography, conservation and evolutionary change (Sheldon and Ellegren 1997), and have been important in testing theories of sex allocation and behaviour in wild populations of a range of bird species (Sheldon 1998). Whilst most penguins are sexually dimorphic in some respects, these differences are small and often difficult to evaluate by eye. Previous studies have used behaviour within breeding pairs or dissection (Downes, 1959) to classify sex and identify either single morphological characters that are completely discriminatory (Warham 1972; Williams and Croxall 1991), or multiple characters that combine to give good discriminatory indices (Amat et al. 1993; Bertellotti et al. 2002). Morphometric tests are cheap, quick and give instant results in the field, but may give rise to errors (Coulson et al. 1983).

Many sexually monomorphic species can be sexed by behaviour, for example albatrosses in the Southern Ocean (Phillips et al. 2004). The sex of tagged individuals can be determined from database archives (despite sexual monomorphism) at times of the year when they do not exhibit sex-specific behaviour. Behavioural approaches to sexing have become unpopular in penguins because flipper bands cause increased mortality of tagged individuals (Froget et al. 1998; Gauthier-Clark et al. 2004). However, behavioural sexing may become more important as Passive Integrated Transponder (PIT) become more commonly used as non-harmful alternatives to flipper bands (Culik et al. 1993). For penguins and other seabirds, repeated observations are often limited, and identification of sex is often based on a single capture rather than repeat observations. Most morphometric tests have been developed from a small sample of known-sex birds and then extrapolation to a wider population. Usually they cannot be applied to juveniles, which lack sufficient dimorphism

(Bertellotti et al. 2002). Applying morphometric sex tests to individuals from outside of the calibrated sample (breeding pairs) assumes that the non-breeding population shares the same distribution of the relevant phenotype between the sexes. This need not be the case: non-breeders will often be younger, smaller males and females. Also, bill measurements are based on a threshold, where males (which are the slightly larger sex in Macaroni Penguins) have to grow through the 'female' range of dimensions before they are scored as male (Coulson et al. 1981, Hamer and Furness 1991, Bertellotti et al. 2002). Males that are unusually small or late to develop will therefore be scored as females. There is no equivalent error for females who cannot grow through the male regions. The only error for females is where the tail of the bill depth distribution overlaps with the male distribution. Therefore, morphometric tests are potentially prone to a bias.

DNA-based tests exist that should give an accurate indication of sex, but need to be optimised for new taxa (Bertellotti et al. 2002). DNA-based sex tests are now routinely used to sex birds and have revolutionised the field of sexual selection and sex allocation research by permitting sex determination of monomorphic species, whether for adults, juveniles or young (Sheldon 1998). The two most common tests utilise the size variation in intron lengths of the CHD genes present on the male and female chromosomes (females ZW, males ZZ). The P2/P8 test (Griffiths et al. 1998) uses two primers, which concurrently amplify CHD-W and CHD-Z gene intron fragments on the W and Z chromosomes. The introns on the W and Z chromosomes differ in length, so can be separated by gel electrophoresis. The 2550/2718 test (Fridolfsson and Ellegren 1999) utilises a different primer pair, which amplifies the same gene. The two tests differ in the length and identity of fragments that they produce. While the nature of the product is species-dependent, in most species the

P2/P8 test produces a W gene fragment that is shorter than the Z fragment (367bp and 384bp respectively). In contrast, the 2550/2718 primers tend to produce a shorter Z fragment (664bp compared to 744bp for the W fragment). Furthermore, the P2/P8 test amplifies shorter fragments than 2550/2718. In both tests, the size difference is small, and therefore products are better distinguished by acrylamide gel electrophoresis.

Our study addresses the question of whether the current morphometric sex test for Macaroni Penguins (Williams and Croxall 1991) is sufficiently reliable to sex birds in the field and if it can be improved in light of the genetic test results. From these results, we comment on how to improve the accuracy of similar discriminant function tests.

Methods

Sample Collection and Morphometric Test Methodology

A comparison between both molecular tests and the morphometric test was performed on a total of 147 blood samples from Macaroni Penguins at five locations around South Georgia: 65 samples from Goldcrest Point, Bird Island (54°00'30S, 38°04'21W), 52 samples from Fairy Point, Bird Island (54°00'39 S, 38°04'27 W). 14 samples from Rookery Bay (54°16'10 S, 36°18'56 W), 13 samples from Willis Island (54°00'33 S, 38°12'48 W) and 3 samples from Hercules Bay (54°07'36 S, 36° 40'05 W). Samples were taken during the austral summers of 2002/3 and 2003/4. Blood was taken from breeding adults present during the incubation or brood guard stage. Birds were sampled when both parents were present to avoid egg disturbance. For each sample, 0.1 ml of blood was taken from the brachial vein and immediately stored in 95% ethanol. Measurements were made of bill length and bill depth, as shown in Figure 1. All blood samples were taken by the same researcher and where bill measurements were made, the observer was recorded. Birds were scored for sex by

bill depth according to Williams and Croxall (1991). Individuals were scored as female if the bill depth was less than 24mm and male if greater than or equal to 24mm. Bill length was measured to determine if including an extra morphometric measurement could increase the discriminatory power of the test. In addition, 24 blood samples were taken from juveniles at Fairy Point in 2005/6 to determine the applicability of molecular sexing to juveniles and non-breeders. There are no legal restrictions covering research on animals at South Georgia. However, BAS carries out all animal procedures in accordance with UK Home Office guidelines and subject to ethical review.

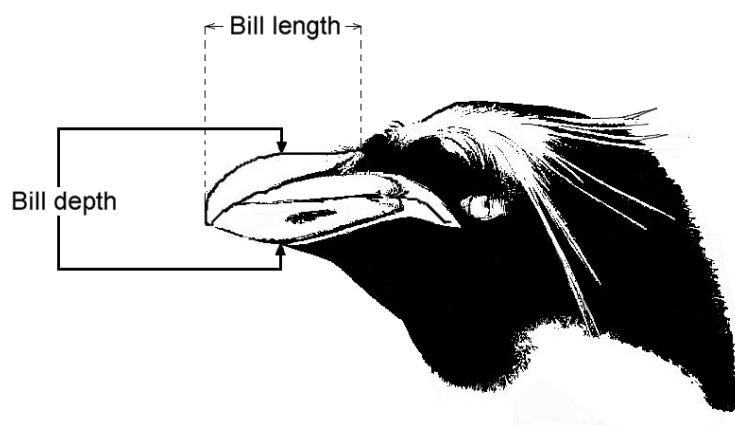


Figure 1: Macaroni Penguin head, showing the CCAMLR measurement of bill length and depth.

DNA-Based Sexing

In the laboratory, blood was centrifuged and excess ethanol removed. Total genomic DNA was then extracted from small (0.01ml) volumes of blood using DNeasy kits (Qiagen, Crawley, West Sussex, UK), following the manufacturer's instructions for animal tissue. The extracted DNA was resuspended in elution buffer and then DNA quantified using an Eppendorf Biophotometer (UV spectrophotometer, Eppendorf, Hamburg, Germany) by measuring absorbance at 260 nm. DNA was subsequently stored at -20°C until used for analysis.

DNA samples were amplified using Polymerase Chain Reaction (PCR) with fluorescently labelled primers. P2/P8 primer pairs: P8 (5'-CTCCCAAGGATGAGRAAYTG-3') and P2 (HEX-labelled-5'-TCTGCATCGCTAAATCCTTT-3'); 2550/2718 primer pairs: 2550F (GTTACTGATTCGTCTACGAGA) and 2781R (HEX-labelled-ATTGAAATGATCCAGTGCTTG). PCR was carried in a final volume of 10µl consisting of 1µl 10x NH₄ Buffer solution, 0.2µl 2mM dNTPs, 0.4µl 20mM MgCl₂, 0.5µl 1-4 diluted Taq (Bioline Ltd, London, UK), 0.3-0.4µl 1mM of both primers and 2µl Q solution (Qiagen Ltd, West Sussex, UK). Reactions were carried out using the following conditions: 93°C for 10 secs; 40 cycles of 94°C for 30 secs, 48.5°C (P2/P8) or 49°C (2550/2718) 1 min, 72°C for 2 min; 72°C for 10 minutes, followed by a slow cooling of 60°C, 50°C, 40°C, 30°C and 20°C for 1 hour each. Samples were run on a MegaBACE 1000 automated DNA sequencer and the size of the PCR product was estimated using Genotyper version 2.2 (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Samples were pre-injected to remove salts. ET-400 Rox (GE Healthcare) size standards were used for the P2/P8 test, and ET-900 Rox (GE Healthcare) for the 2550/2718 test.

Statistical Analysis

No within-test errors were observed in this study, so there can be no statistical comparison of the error rate between tests. Generalised Additive Models (GAMs) were used in R 2.3 (R Core Development Team, 2006) to determine the importance of variables in the study (Hastie and Tibishirani 1991; Wood 2006). A logit link function and a binomial error structure were used to test these effects. We assumed that the best DNA test should be that which has the lowest rate of disagreement between it and the morphometric sex. Sex scores from the DNA-based tests were combined with the data

for colony, bill depth, bill length and date of capture. Penguins were scored for agreement between the appropriate genetic test and the morphometric sex, with agreement scored as 1 and disagreement scored as 0.

Results

The P2/P8 DNA-based test had an approximately 70% success rate, whilst the 2550/2718 test had an approximately 30% success rate where success is defined as amplified DNA for which any peaks can be read by the MEGABACE sequencer (Table 1). In successful cases, peaks identified were 10,000 times the background fluorescence or greater (Figure 2). In all cases, re-running samples generated results for samples that had not originally worked. A comparison between the two DNA-based tests revealed no incidences of disagreement. In consequence, the comparison between genetic and morphometric tests was restricted to the P2/P8 test, as this test had a larger sample size that included all of the individuals sexed by the 2550/2718 test.

Table 1: Counts of agreement and disagreement between genetic and morphometric tests.

	P2/P8	2550/2718	Total
Same (agree)	110	43	153
Different (disagree)	15	5	20
Total	125	48	

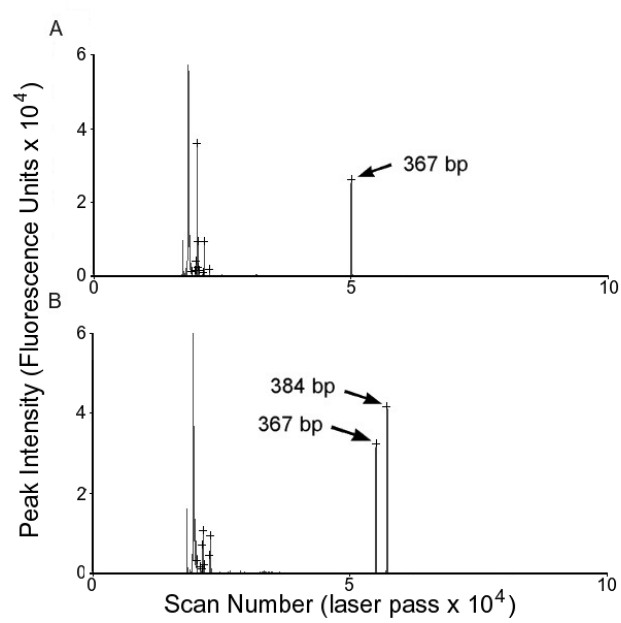


Figure 2: Output from genotyping using P2/P8 primers, showing peaks and fragment size for A) a male macaroni penguin, and B) a female macaroni penguin.

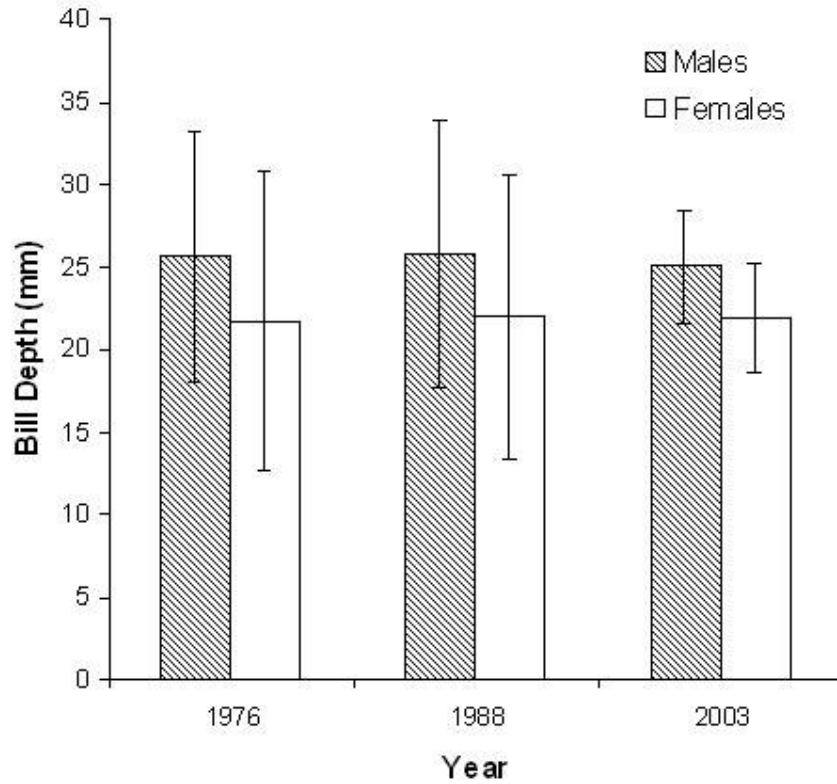


Figure 3: Mean bill depth from this study (2003) and those reproduced from Williams and Croxall (1991) for 1976 and 1988. Error bars show 95% confidence interval around the mean.

Figure 3 shows the estimate of the mean bill depths previously reported compared to this study. Figure 3 and Table 2 show that the increased sample size in this study has helped to increase accuracy by reducing the standard error. Figure 4 highlights the error rate by showing overlap between traits and enabling the tails of the trait distributions to be better estimated.

Table 3: Mean and distribution of bill depth and bill length for a range of sample sizes. Data for 1976 and 1988 is reproduced from Williams and Croxall (1991) and data from 2003 is from this study.

	Year	N	Depth	Range	SE	SD	Length	Range	SE	SD
Males	1976	15	25.7	24.3-27.8	1.0	3.87	61.3	56.5-65.1	2.3	8.91
	1988	21	25.8	24.5-27.5	0.9	4.12	55.5	52.0-59.0	2.0	9.17
	2003	77	25.0	21.5-27.3	0.2	1.36	58.0	52.7-64.3	0.31	2.75
Females	1976	15	21.7	20.1-23.8	1.2	4.65	53.7	50.9-56.1	1.6	6.20
	1988	24	22.0	20.0-24.0	0.9	4.41	49.9	45.0-54.0	2.4	11.00
	2003	71	21.9	18.0-25.9	0.2	1.35	52.6	46.7-57.4	0.5	3.89

Table 2: Counts of males and females scored correctly by the CCAMLR Standard Methods and the new threshold of 23.5mm for bill depth.

Sex scored by bill index				
	DNA sex	Correctly	Incorrectly	Total
CCAMLR Standard Methods	♂	60	16	77
	♀	66	4	71
(86.39%)	♂	68	9	77
	♀	63	7	71

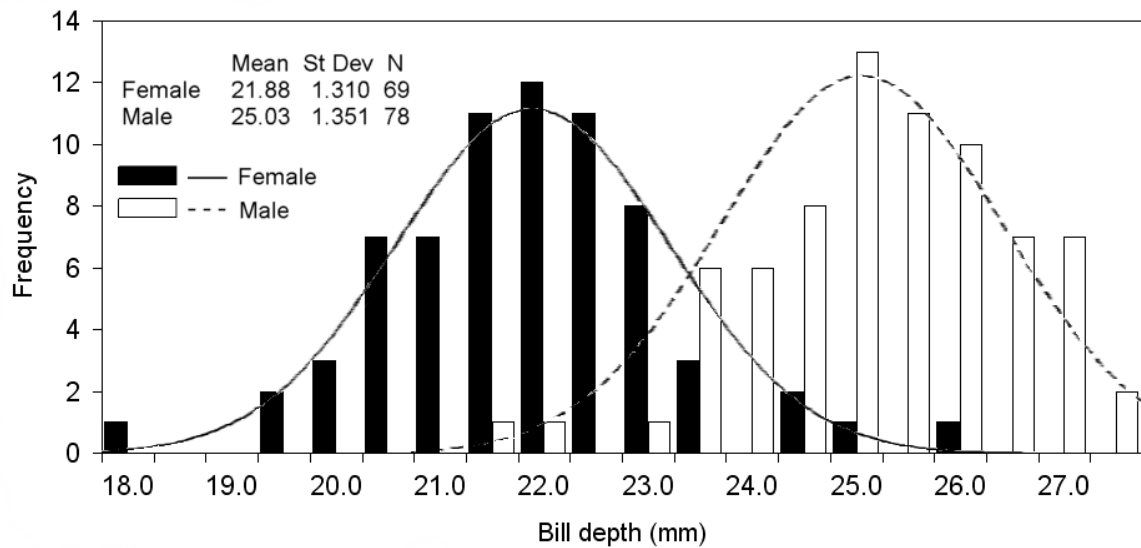


Figure 4: Histogram of bill depths for the two sexes as classified by DNA sex markers. Lines show a normal distribution fitted to the data. When a normal distribution is assumed, the larger sample size in this study as contrasted with previous studies shows considerable overlap of bill depths.

Agreement data between the molecular and morphometric tests were then analyzed using Generalized Additive Models (GAM) described above to determine what caused these errors (Figure 5). Data were analyzed for the effect of date, colony, observer and bill depth on the score. Date, colony and observer were not significant, so were dropped from subsequent analyses. For the P2/P8 test, bill depth was highly significant (edf = 2.733, Chi-square = 14.899, $p = 0.0014$, deviance explained = 22%, $n = 147$). The predicted values can be seen in Figure 5, which shows that the error rate is strongly linked to the distance from the threshold. One explanation for the error here being much greater than that observed by Williams and Croxall (1991), is that the error is due to variability between colonies. Therefore, all data from outside Bird Island were excluded and the analysis was repeated. The deviance explained increased slightly, but bill depth was still highly significant and the apex of the error curve remained the same (edf = 2.654, Chi-square = 11.735, $p=0.0059$, deviance explained = 25.8%, $n = 117$). We interpret this model and the fit line (Figure 4) as showing that there is only evidence for the bill depth affecting the error rate, and that the probability of agreement increases further from the threshold.

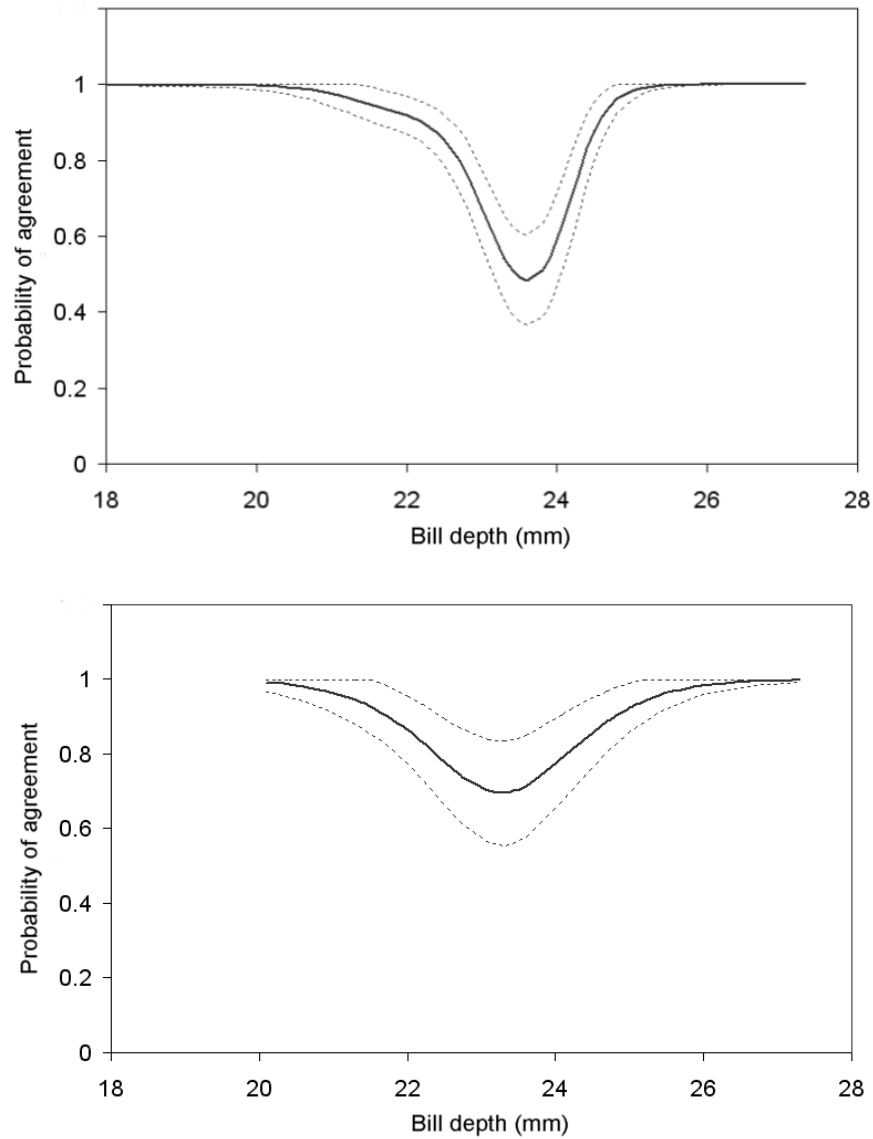


Figure 5: Predictions for the probability of agreement between the DNA and morphometric sex tests, A) P2/P8 and B) 2550/2718. Solid lines indicate the observed probability of agreement, and dotted lines represent the upper and lower 95% confidence intervals.

The P2/P8 test was carried out on the juvenile blood samples (the morphometric test was not intended for use on juveniles). The test sexed all individuals, with samples repeatedly tested using separate PCRs. Every individual was tested at least twice, with some repeated five times. These replicates showed no discordance.

Predicting Sex from Bill Depth and Bill Length

Binary logistic regressions were performed on the whole data set ($n = 147$) using the morphometric bill measurements to predict DNA sex and identify a new discriminant function. Bill length was not significant ($z = -1.777$, $p = 0.077$, $n = 147$), so should not be included in a discrimination index. A test on bill depth only was highly significant ($z = -6.44$, $p < 0.001$, $n = 147$), with the relationship: $\text{sex} = 34.573 - 1.4805 \times \text{bill depth}$. Values of zero or less score the individual as male while values of greater than zero score as female. The threshold for discrimination was a depth of 23.31 mm, with a 95% confidence interval of between 22.96 and 23.68mm, indicating that 23.31 is significantly different from the standard methods threshold of 24mm. When applied to these data, a threshold of 23.31 correctly assigned 89.18% of the individuals compared to 86.39% of the data when the standard methods were used. While not a dramatic improvement, the new estimate removed the systematic bias towards males being misidentified. The difference between the new threshold and that of the standard methods (CCAMLR 2004) should be tested on new data in future years to determine whether the relationship holds. The logistic regression was repeated excluding all individuals not from the Bird Island colonies. Bill depth remained highly significant ($z = -5.29$, $p < 0.001$, $n = 117$) and significantly different from the standard method with a bill depth threshold of 23.50 (CI 95% 23.12, 23.92).

Discussion

The larger sample size of our study than that of Williams and Croxall (1991), combined with the molecular verification, shows considerable overlap in traits between sexes that have not hitherto been reported in penguins. Both DNA tests were shown to be appropriate for identifying sex in Macaroni Penguins, but differed in success rates. Preferential amplification of smaller PCR fragments can occur under

some reaction conditions (Walsh, et al. 1992; Kopsidas et al. 2000), but the size difference between the W and Z intron fragments in both DNA based tests was too small to result in preferential amplification in this system. The 2550/2718 test showed a high failure rate and proved difficult to optimise, therefore most of the work was carried out using the P2/P8 primer pair set. Adaptation of this method to fluorescently-labelled primers worked well.

Concordance between the DNA test results and sexing according to morphometrics indicates that the poorest fit occurred at bill depths of approximately 23.5mm, and the binary logistic regression confirmed the threshold at 23.31mm for the pooled data, or 23.50mm for Bird Island colonies. The new threshold from the above analysis is below the standard cut-off of 24mm, indicating that the current threshold is potentially in the wrong place. Our results demonstrated that sexing by morphometry was less accurate than using molecular tests, and that the error rate increased near the cut-off. Furthermore, the error was skewed towards misidentifying males, indicating a systematic bias rather than any mislabelling or misidentification. There were insufficient data to calibrate the test for each colony and test for significant differences between the thresholds, but the increased deviance explained when the GAM was restricted to the Bird Island data suggests that they may well have different thresholds. Such differences are plausible if bill shape is adaptive for feeding as found in other species (Peterson, 1993) or for defending a nest site and there are local optima in the selection pressure. Therefore, we suggest that more data should be collected to calibrate the morphometric test on new colonies, and where accuracy is needed, DNA sexing should be used.

Observer error was not significant in this analysis, although observer error was included as a post-hoc hypothesis from information recorded in the field. As such,

observers were not randomised in the experimental design and operated in pairs, often with experienced researchers. The parameter estimates from the analysis showed that, while not significant, inexperienced observers deviated from the model fit more than experienced researchers. We conclude with caution that observer error was not important in this study, but might be in other studies such as long-term monitoring. William and Croxall (1991) did not present their results as a discriminant function, but their data has been cited and applied as such (CCAMLR 2004). Furthermore, the threshold has been applied outside the original data and colony without calibration, which has been previously identified as a problem (Barrett et al. 1989; Hamer and Furness 1991).

We suggest that the original data did not show an overlap between the sexes for two reasons; the data were collected by experienced researchers using behaviour at breeding to assign sex rather than bill depth. Moreover, morphometric measurements were published as correlational data rather than as a predictive relationship (Williams and Croxall 1991). The bill measurement test has been inferred from these data since the original publication (Williams and Croxall 1991). Secondly, using the raw data to infer overlap is misleading, because small sample sizes under-represent outliers (McCleery et al. 2007). A good estimation of the distribution of the bill depth is needed to identify a clear discriminant function. The standard deviation is linked to the degrees of freedom, which corrects for small sample sizes when estimating the distribution of a population. When the standard deviation is used (Table 3 and confidence intervals in Figure 3), the distribution of male and female bill depths are seen to overlap much more, which should be noted for all discriminant function studies.

The larger sample size in our study shows considerable overlap (Figure 4). We

suggest that the current methodology of taking biometric measurements is correct (Figure 1), but that the interpretation of these data should be revised. A more accurate method for determining the threshold is a binary logistic regression, which can also be used to assign subsequent individuals' group membership, with associated p -values. Figure 5 illustrates how calibration between molecular and morphological sexing can be used to show where the morphometric test is sufficient and where it is not. Our approach could be used to calibrate a discriminant function for any species for which a molecular sexing test exists.

The error rate of the morphometric test is much higher than previously thought (Williams and Croxall 1991, CCAMLR 2004), so DNA testing should be used more in field studies where the interpretation of such studies assumes a low error rate, such as where there is likely to be a small effect size between sexes. DNA testing would be advisable for previously unstudied colonies to recalibrate the discriminant function and in studies with poor replication, where a misclassification would have a disproportionate effect. The authors understand that the DNA method of sexing is expensive, and should therefore be applied to juveniles where tagging and subsequent determination of sex cannot be made. The suggested new threshold is more precise than most researchers can measure in the field, while still not being 100% accurate. If discriminant function tests in other species show a similar error rate, then DNA sex testing should be used to calibrate tests, and for individuals that lie within the region of the trait that overlap between sexes.

Chapter 3: Autocorrelation of dive data

.Hart, T., Coulson, T. & Trathan, P. N. Time series analysis of archival tagging data: new approaches to larger data sets. *Animal Behaviour*, DOI: 10.1016/j.anbehav.2009.12.033

Abstract

The nature of how behaviour at a time step influences the next is of great interest to behavioural ecologists, but rarely used for comparisons between animals. Time Depth Recorders (TDR) and other archival tags have been widely used to infer patterns of diving and foraging. However, while we can extract variables that describe individual dives, how runs of dives may indicate behaviours and how one dive influences the next, are not fully understood. Treating TDR data as time series, we examine patterns of autocorrelation to investigate structure in the timing of behaviour. We fit an oscillating best-fit curve to the autocorrelation and use the parameters of this curve to investigate differences in strategy of 129 foraging macaroni penguins (*Eudyptes chrysolophus*) of both sexes. We find inter-annual differences in autocorrelation parameters as well as differences between reproductive stages. In contrast to other studies of macaroni penguin diving based on depth analysis, we find no differences between the sexes. We mimicked changes in the various parameters by simulation of dive profiles, and used these to infer biological meaning from the parameters. As this technique makes very few assumptions about how to identify a dive or cluster of dives, we suggest that it is a useful first characterisation of diving or cyclical behaviour in a wide range of animals.

Introduction

Archival tags are extremely powerful tools that allow biologists to record the activity or behaviour of animals (Kooyman 2003, Naito et al. 2007, Guilford et al. 2009). They are now routinely deployed on diverse taxa of marine predators such as penguins (Tremblay & Cherel 2005), albatrosses (Croxall et al. 2005), seals (Boyd et al. 2002, Austin et al. 2006) and turtles (Hays et al. 2004). This results in extremely large data sets that are temporally auto-correlated (Fox & Long 1990, Crawley 2007), which means that a data point from time t is correlated with a data point from $t + n$. As an example, consider a marine diving animal that dives to feed, but which periodically surfaces to breathe. When n is small – measured in seconds – it is likely that the animal will have remained at approximately the same depth between sensor readings. This would mean that for small n , depth data are positively auto-correlated. However, when n becomes larger – measured in hours or days – there may be no correlation between data from time t and from time $t+n$. Intermediate comparisons between t and $t + n$ may be negative if there are cyclical patterns in the data and the comparison between t and $t + n$ results in the periodicity being out of phase (Hamilton 1994, Crawley 2007). While autocorrelation can be removed statistically, it is of interest in its own right. Characterising the nature of this autocorrelation and these cycles with increasing time intervals between data may provide hints about how individuals behave, as well as about the foraging strategies they follow.

Many of the classic statistical tests that biologists use, including general and generalised linear modelling, assume that data are temporally independent, i.e. not temporally auto-correlated (Hurlbert 1984, Crawley 2007). If these methods are applied to temporally auto-correlated data without an autoregressive component, parameter estimates, statistical significance and consequently biological inference can be biased or compromised (Hurlbert 1984, Grafen & Hails 2002). Ecologists have developed ingenious approaches to remove

temporal auto-correlation from data so that the classic statistical tests can be applied. This approach has provided some important biological insights. For example, Tremblay & Cherel (2003) sub-sampled a number of dive profiles to allow a daily and trip-long comparison of dive behaviours to be made between foraging rockhopper penguins (*Eudyptes chrysocome*).

Another approach is to visually examine data and to group runs of data that show repeating patterns. Early attempts to cluster dives used a threshold in the surface interval between dives to identify the start and end of a bout of dives (Naito et al. 1990). Once again, this approach has provided valuable insights. A number of researchers such as Boyd et al. (1994) and others (Green et al. 2005, Austin et al. 2006) made the somewhat ad-hoc approach of visual grouping more statistically robust, by setting a threshold surface interval below which groups of two or more dives are determined to be the start of a bout. Thereafter, a *t*-test is performed on the surface intervals until a significant difference is encountered. This has led to insights into the feeding (Boyd et al. 1994, Austin et al. 2006), resting (Green et al. 2005) and physiological (Green et al. 2002) tradeoffs of diving animals. While this approach has provided insight into clustering in a range of diving animals (Burns et al. 2004, Baird et al. 2005, Humphreys et al. 2006, Soto et al. 2008), multiple iterative testing and non-independence between successive dives may mean that the clustering process may be undermined by the very non-independence that this approach is attempting to overcome (Hurlbert 1984, Grafen & Hails 2002). Alternative approaches have used maximum likelihood approaches to cluster dives (Langton et al. 1995, Luque & Guinet 2007), which do not suffer from these problems, but do not provide a measure of the nature of non-independence.

Powerful statistical methods exist to analyse temporally auto-correlated data (Royama 1992, Powell & Steele 1994). The simplest of these methods seek to characterise the autocorrelation itself. Probably the most widely used descriptive methods are autocorrelation

and partial-autocorrelation regressions, whereby the description of interest is how the correlation between data from time t and time $t+n$ changes with n . These have been used in biology to provide insight into fields such as population ecology (Fox & Long 1990, Bjørnstad et al. 1996, Berryman & Turchin 2001), economics (Enders 2003) and disease progression (Meier et al. 2007) and have now started to be applied to behavioural ecology (Shazifzadeh et al. 2005). If we consider strategies of foraging as a sequence of searching and feeding events, then the temporal element may contain additional ecological information, such as how penguins shift from one behavioural state to another (Johnson et al. 2006). In this paper, we specifically focus on the autocorrelation in data in an attempt to provide novel inferences about penguin foraging behaviour.

The temporal nature of behaviour does not just include time of day, but also the persistence of a behaviour and time since the last decision (Dawkins 2007). Diving in air-breathing vertebrates is by nature discrete or cyclical, as an animal has to balance two resources: oxygen at the surface and food at depth (Houston & Carbone 1992, Soto et al. 2008). Because diving predators such as penguins can only perform breath-hold dives for a few minutes and are unlikely to exhaust their prey in this time, foraging behaviour may not be comprehensively characterised from the analysis of temporally-independent sub-sampled data (Mori 1997). For example, searching is unlikely to be a feature of a single dive, but a series of dives to different depths. Moreover, if a predator does not encounter a prey item on every dive (Takahashi et al. 2004), then it will take a number of dives to assess the quality of a patch. Therefore, a unit of behaviour may consist of one or many dives (Boyd et al. 1994, Mori 1997). Analyses that do not incorporate what happens before and after a given dive are unlikely to give a complete understanding of foraging patterns.

We can consider dives as a series of periodicities that cover different temporal scales, ranging from annual, seasonal, trip and day/night cycles to individual dives and even small

undulations at the bottom of each dive (Wilson 2003, Simeone & Wilson 2003, Takahashi et al. 2004). If the persistence of behaviours is interesting in its own right (Austin et al. 2006, Johnson et al. 2006), then runs of dives that cluster together (Boyd et al. 1994, Austin et al. 2006), or fluctuations of shallow and deep dives, could be quantified as another of these periodicities. Fitting a curve to the autocorrelation function (ACF) and comparing the parameters of that curve may be useful as descriptive summary statistics (Pinheiro & Bates 2000). Such parameters could be generated as summary statistics of an entire data set. Analysis of raw data can generate directly comparable summary statistics that are defined by the data rather than definitions of diving behaviour that can differ between fields and studies. ACF also describes behaviour at a range of time scales. In this study, we use parameter estimates from ACF best-fit curves to compare diving behaviour within foraging trips.

Methods

Definitions:

Surface: As the depth is calibrated relative to the surface, this calibration can fail or drift over time. We define ‘surface’ as the mean of the top of a series of dives (Zero-offset correction, ZOC), which helps reduce error introduced into the depth records by waves at the surface. If the surface could not be easily identified and ZOC failed to correct pressure trends, the tag was rejected. ZOC generally only affected depth records in the upper 3 metres of each data set.

Dive: The period from leaving the surface to resurfacing. When birds departed the surface for only one record (i.e. 2 seconds), this was not counted as a dive to avoid travelling dives and wave noise. Within each dive, depth, duration and undulation activity were calculated in accordance with conventions of other researchers (Boyd et al. 1994, Tremblay & Cherel 2000, Tremblay & Cherel 2003).

Stage: The phase of reproduction, which is identified by a change in behaviour or a

life history event, such as egg-laying or hatching. The stages in this study are: *incubation* (from egg-laying to egg-hatching), *broodguard* (where the male parent guards the young chick and the female forages), *crèche* (where both parents forage and the chicks form clusters in the colony guarded by few adults) and *premoult* (where the parents leave the chicks for a long trip to gain weight prior to moulting).

Trip: The period of time away from the chick or nest site.

Protocol

A total of 156 macaroni penguins from the Fairy Point study colony (54°00'30S, 38°04'21W) on Bird Island, South Georgia were tagged using Time Depth Recorders (TDRs). Tagging took place during the austral summers of 1998/9 and 2000/1 to 2004/5, a total of six breeding seasons (Table 1). Individuals were tagged between November and March of the breeding season. During the 2000/1 season, birds were tagged during all 4 reproductive stages. In other years, birds were primarily tagged during the broodguard and crèche stages to investigate the effect of chicks on foraging behaviour.

The archival tags used were Wildlife ComputersTM Mark 7 Time Depth Recorders. These tags measured 95 × 15 × 15 mm and weighed 50g in air, which corresponds to <0.5% of the bird's cross-sectional area and up to 1.5% of its body mass. TDRs recorded depth and time every 2 seconds. All TDRs were equipped with a saltwater switch so that records only reflected dive behaviour whilst the birds were at sea. TDRs were deployed by attaching them to the tips of feathers in the midline of the penguin's back with TesaTM tape and 2-part quick-drying epoxy glue (using methods modified from Wilson et al. 1997; Trathan et al. 2008). Various morphometric variables were recorded during deployment, such as body mass and bill dimensions (to determine the sex of birds). As birds returned, the devices were downloaded and redeployed on other animals.

Birds were tagged at the edge of the colony to avoid excessive disturbance. It is

understood that there is the possibility that peripheral birds do not experience the same breeding conditions as central birds and may also be less experienced and/or younger (Ainley et al. 1983). They may therefore show different at-sea behaviour to mature, experienced birds.

Table 1: The sample size of tags used in this study by year and stage.

	Incubation	Broodguard	Creche	Premoult
1998/9		1	4	
2000/1	4	4	12	5
2001/2	7	20	14	
2002/3		6	12	
2003/4		13	3	
2004/5		9	15	

Penguins were recaptured on their return to land and the TDRs removed before birds entered the colony. Data were downloaded, decoded using Wildlife Computers Ltd software (www.wildlifecomputers.com) and stored as text files ready for analysis in R 2.5.2 (R Project Core Development Team 2006). Raw data were summarised into dives in MATLAB® (The MathWorks Inc, www.mathworks.com) using the definitions above (Boyd et al. 1994, Tembley & Cherel 2000, Tremblay & Cherel 2003).

Data were also summarised as means of variables per hour and averaged across stages and years to produce summaries of dives. These were combined to form data sets to make a comparison between a time series approach and a more traditional analysis. General Linear- and General Linear Mixed Models were fitted in R using the stats library.

The time series approach used in this paper was based on the raw depth data and involved five steps:

(1) Firstly, we performed an autocorrelation on the data set from each bird. (2) Next, we generated a best-fit curve that could be used to describe these autocorrelation signatures and (3) estimate the parameters that described this best-fit line for each bird. (4) We then compared the parameter estimates using linear models and finally, (5) we interpreted the parameters to determine the underlying biological meaning. This was achieved by manipulating a simulated data set to determine how variation in dive profiles affected the best-fit curve parameters.

1. Autocorrelation of time series

Raw data (depth and time) were analysed by autocorrelation as a time series in R. As the time series were long (multiple hours or days), the maximum time lag for analysis was set to 6000 lags (12000 s or 3 hr 20 minutes) to enable detection of longer-term cycles such as periodicity between bouts of dives (Boyd et al. 1994, Green et al. 2002). Also, data exploration revealed that this was long enough for the oscillating component of the ACF fit line to decay.

2. Modelling the autocorrelation and producing a best-fit curve

While the raw time depth data (Figure 4A) show a large amount of variation, the autocorrelation (Figure 4B) shows a number of common features. All autocorrelations (ACF) show an initial cyclic element which decays to smaller oscillations over time. A best-fit curve was determined that could capture these aspects of the ACF for comparison between data sets:

$$amplitude \frac{\cos(frequency \times time \times 2 \times \pi)}{\exp(decay \times time)} + y \text{ intercept} + gradient(time)$$

Where: *amplitude* = amplitude of the oscillating component, *frequency* = frequency of the oscillating component and *decay* = rate of decay of the oscillating component. These

names refer to components of the best-fit curve, and inferences of how these relate to dive parameters are derived from the simulations below. A linear regression was fitted to take account of longer-term correlations in the observed ACFs. The best fit curve was fitted using nonlinear least squares in R. As the short-term periodicity and non-independence of dives are of interest in this study, the *y intercept* and *gradient* represented a structural component of the model and were not used for comparison. It is possible that their use could be expanded in future studies to look at longer-term periodicity in the data, such as circadian rhythms.

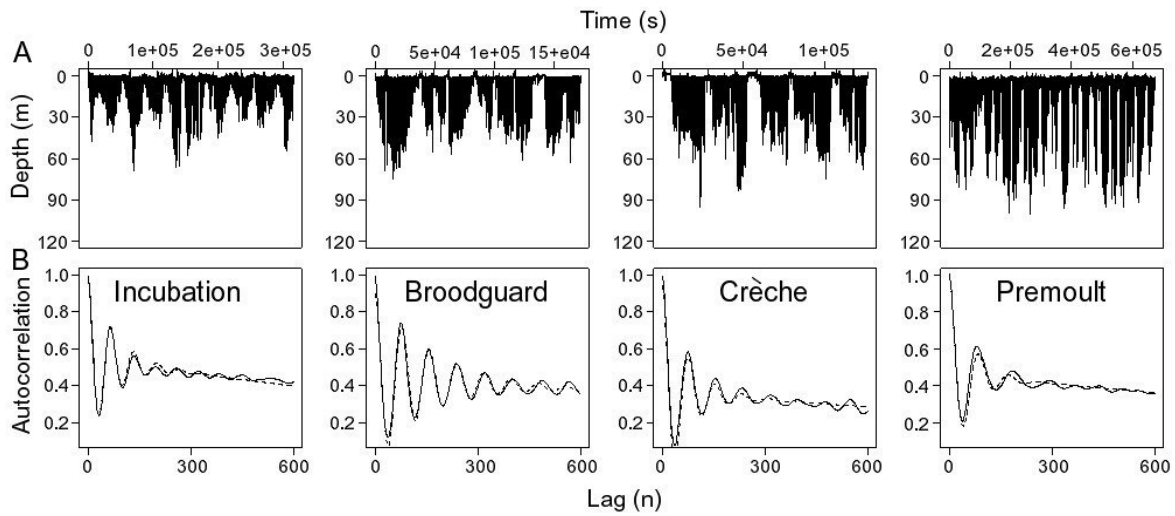


Figure 4: A) Dive depth (raw, unprocessed trace from TDR). B) The auto correlation over 600 lags to highlight short-term oscillations and the gradual decay of these oscillations. Solid line indicates the observed ACF for one bird from each stage, and the dashed line indicates the curve fit from the model. Autocorrelation aims to extract dominant periodicities from the data to show how one time step (or dive) relates to the next.

3. Fitting the curve to the ACF data and generating parameter estimates

For each individual, this model was fitted to the autocorrelation function in R using non-linear least squares regression to estimate each of the parameters. Goodness of model fit was examined using plots to show residuals from the best-fit line and the proportion of variance explained. The proportion of variation explained was calculated by fitting a null model to estimate the total variation (*total*). The error variation from the model of interest (*error*) was subtracted from the total to generate the amount of variation explained by the fit

line. The remainder from *total* - *error* was divided by the total variation to generate a proportion of the variation explained by the fit line (*explained*).

$$Explained = \frac{total - error}{total}$$

4. Comparing the parameters

Three separate analyses were carried out on the parameter estimates to determine the importance of season, stage and sex on *amplitude*, *frequency* and *decay*. Sex was nested in stage to overcome the effect of unbalanced data such as arises by only females foraging in broodguard. General Linear Models (GLM) were used to compare breeding stage parameter estimates (see results) with normal error.

5. Sensitivity analysis: deriving biological meaning from these parameters

Each of the parameters derived from the best-fit equation has a purpose in the equation, but may not relate directly to biological elements of dive behaviour. To determine biological meaning, we created dummy dive data sets and simulated variation in these data sets to evoke changes in the parameter values. The dummy data sets simulated had high or low variation in their maximum depth and high or low variation in their surface interval. The duration of an entire dive cycle (start of one surface interval to the start of the next) was altered, and the regularity of dives was changed to investigate the effect of localised clusters. Examples of how these simulations changed the autocorrelation signature are given in the results.

Results

Tag recovery

Of the 156 deployed tags all were recovered and data successfully downloaded from

129 of these. Zero Offset Correction (ZOC) identifies the surface as the running mean of the top of a series of dives. If the tops of these dives were too varied or showed large trends, the tag was rejected on the assumption that the calibration of the pressure sensor had failed. Six tags were rejected after ZOC, the remainder were rejected were due to hardware or software failure, where the tag had not been set up correctly. The number of usable data sets retrieved is given in Table 1 below.

Traditional dive analysis

Most of the variables used in these analyses were correlated (Figure 1 and Table 2). Depth, duration and undulation activity (the number of undulations in a dive profile) were quite strongly correlated, while surface interval shows a very low correlation with other variables. The analysis was performed on maximum depth only to avoid excessive hypothesis tests.

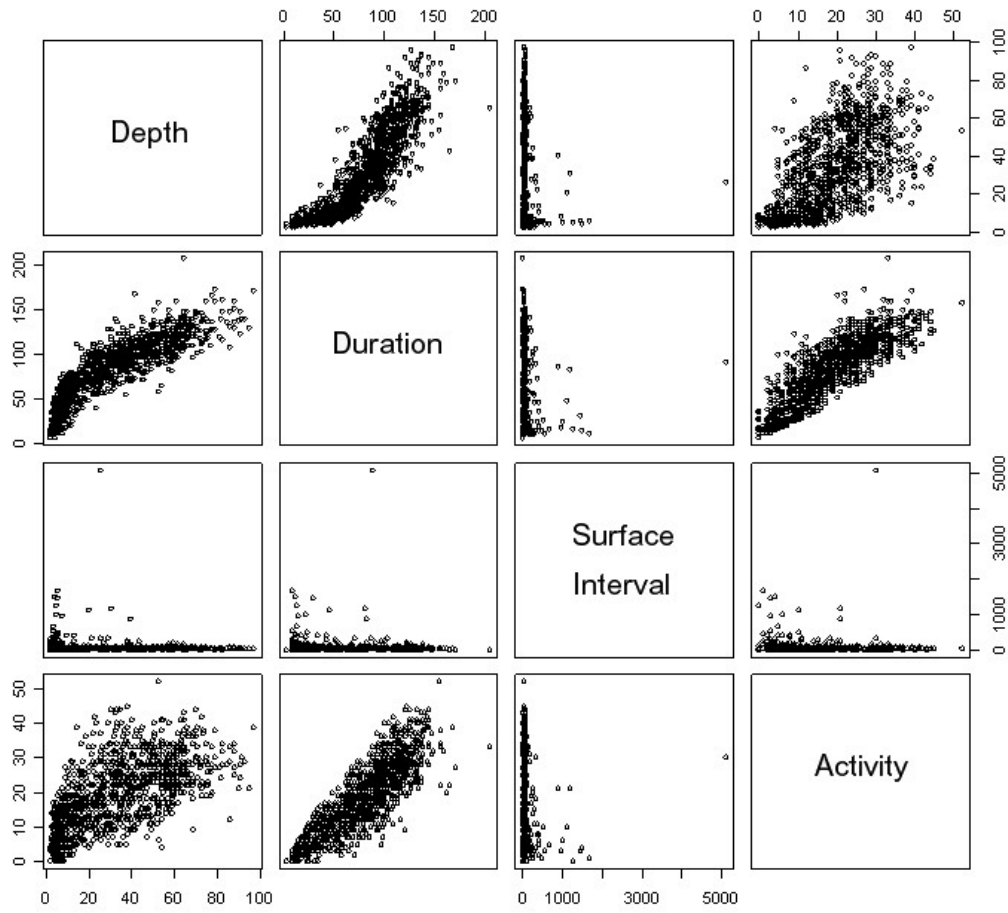


Figure 1: Correlations between dive depth, dive duration, surface interval and undulation activity.

Table 2: Correlation table of variables in Figure 1.

	Dive depth	Dive duration	Surface interval	Undulation activity
Dive depth	1.000	0.883	-0.054	0.698
Dive duration	0.883	1.000	-0.073	0.870
Surface interval	-0.054	-0.073	1.000	-0.052
Undulation activity	0.698	0.870	-0.052	1.000

Maximum depth

A General Linear Mixed Model of variables on maximum depth found that dive duration, undulation activity, surface interval, time of day, bird, stage and year were all significant (Table 3). Duration was the biggest predictor of depth and the model had an $R^2_{(adj)}$ of 75.2%. The patterns of depth, duration and undulation activity by time of day are shown in Figure 2, while the patterns of the same variables over years are shown in Figure 3. In contrast with previous work, sex was not significant in this analysis, which may mean that once stage and time of day are taken into account, the sex-differences are relatively small.

Table 3: GLM output for maximum depth. Crosses denote where an exact F-test was not possible.

<i>Variable</i>	<i>DF</i>	<i>F</i>	<i>P</i>	<i>Coeff</i>	<i>SE Coeff</i>
Dive duration	1, 2383	755.85	<0.001	0.053432	0.001943
Undulation activity	1, 2383	163.28	<0.001	-0.09393	0.007351
Surface interval (log)	1, 2383	286.74	<0.001	-0.19726	0.01165
Time of day (hour)	23, 2383	2.03	0.003		
Bird (Year Sex Stage)	103, 2383	19.52	<0.001		
Sex(Year Stage)	9, 2383	0.76 [†]	0.65		
Stage(Year)	8, 2383	3.14 [†]	0.003		
Year	6, 2383	4.58 [†]	<0.001		

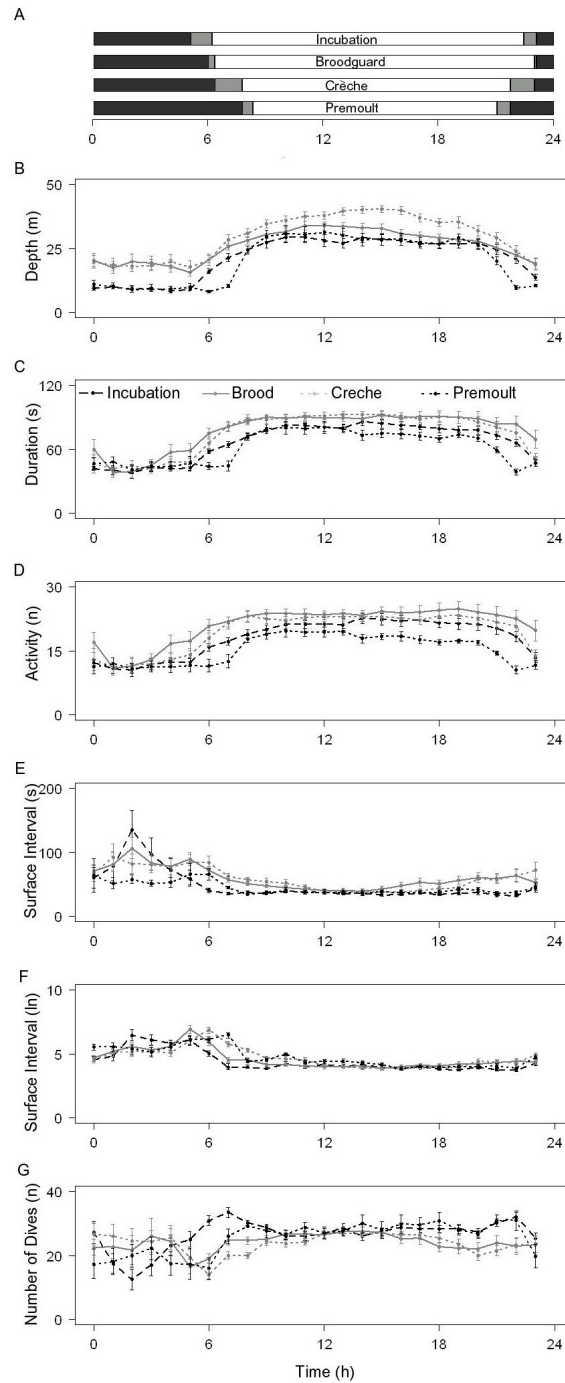


Figure 2: Summary statistics of diving by stage. A) The pattern of night and day for each stage, where dark grey indicates night, light grey indicates the variation in dawn and dusk over the period of the stage and white indicates daylight. B) Shows the mean dive depth in metres. C) Shows the mean dive duration in seconds. D) Shows the mean undulation activity per dive in number of undulations. E) Shows the mean surface interval in seconds, with intervals greater than 5 minutes removed. F) Shows the log of surface interval without any dives removed. G) Shows the number of dives per hour.

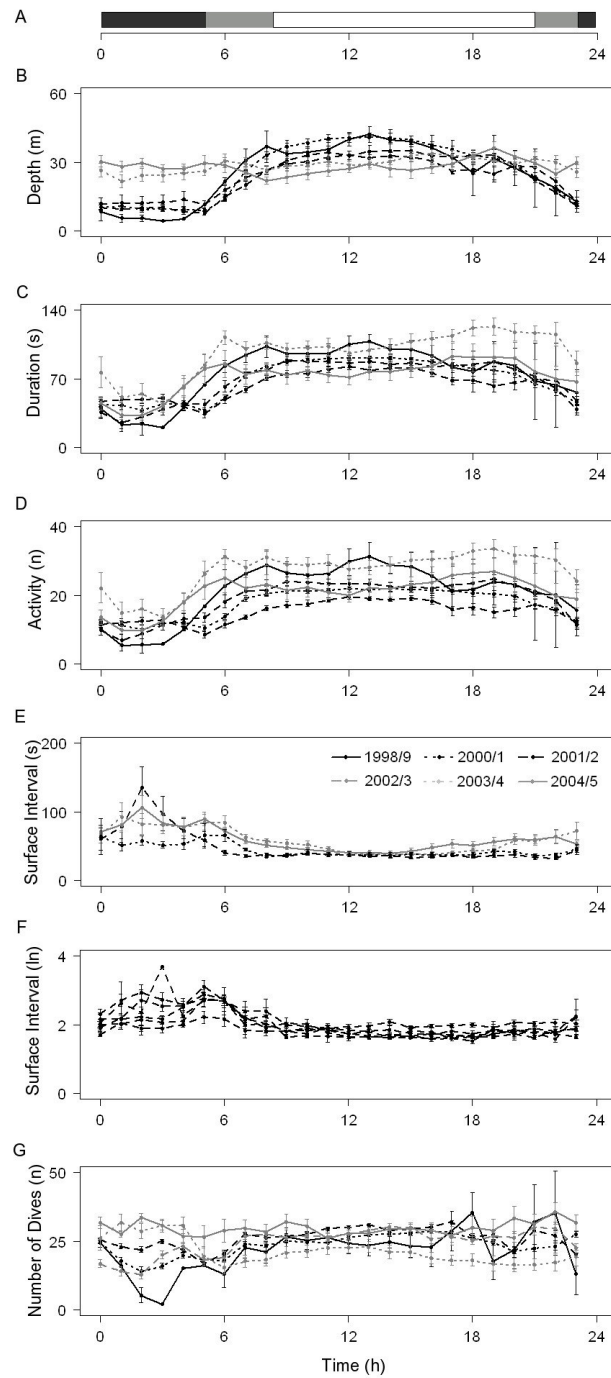


Figure 3: Summary statistics of diving by year. A) The pattern of night and day for each year, where dark grey indicates night, light grey indicates the variation in dawn and dusk over the reproductive period and white indicates daylight. B) Shows the mean dive depth in metres. C) Shows the mean dive duration in seconds. D) Shows the mean undulation activity per dive in number of undulations. E) Shows the mean surface interval in seconds, with intervals greater than 5 minutes removed. F) Shows the log of surface interval without any dives removed. G) Shows the number of dives per hour

Curve fit and parameter comparisons.

Randomly selected examples of the dive profiles and the associated ACF fit from 2000/1 are shown in Figures 4A and B. These show the strong cyclical element in the ACF, and the gradual decay of the correlations. The proportion of variation explained by the best-fit curve varied between 0.89 and 1.00.

Amplitude

General linear modelling showed that season and stage were both significant and influenced the amplitude of the fitted curve (season $F_{5,106} = 7.58$, $P = <0.001$; stage(season) $F_{9,106} = 3.21$, $P = 0.002$), while sex was not significant (sex (season stage) $F_{8,106} = 1.05$, $P = 0.407$). The full model explained 38.85% of the variance. The comparison of stage and season in Figure 5B shows that there are differences between reproductive seasons and all reproductive stages, but that broodguard and cr  che appear to have higher values of *amplitude* than incubation and premoult.

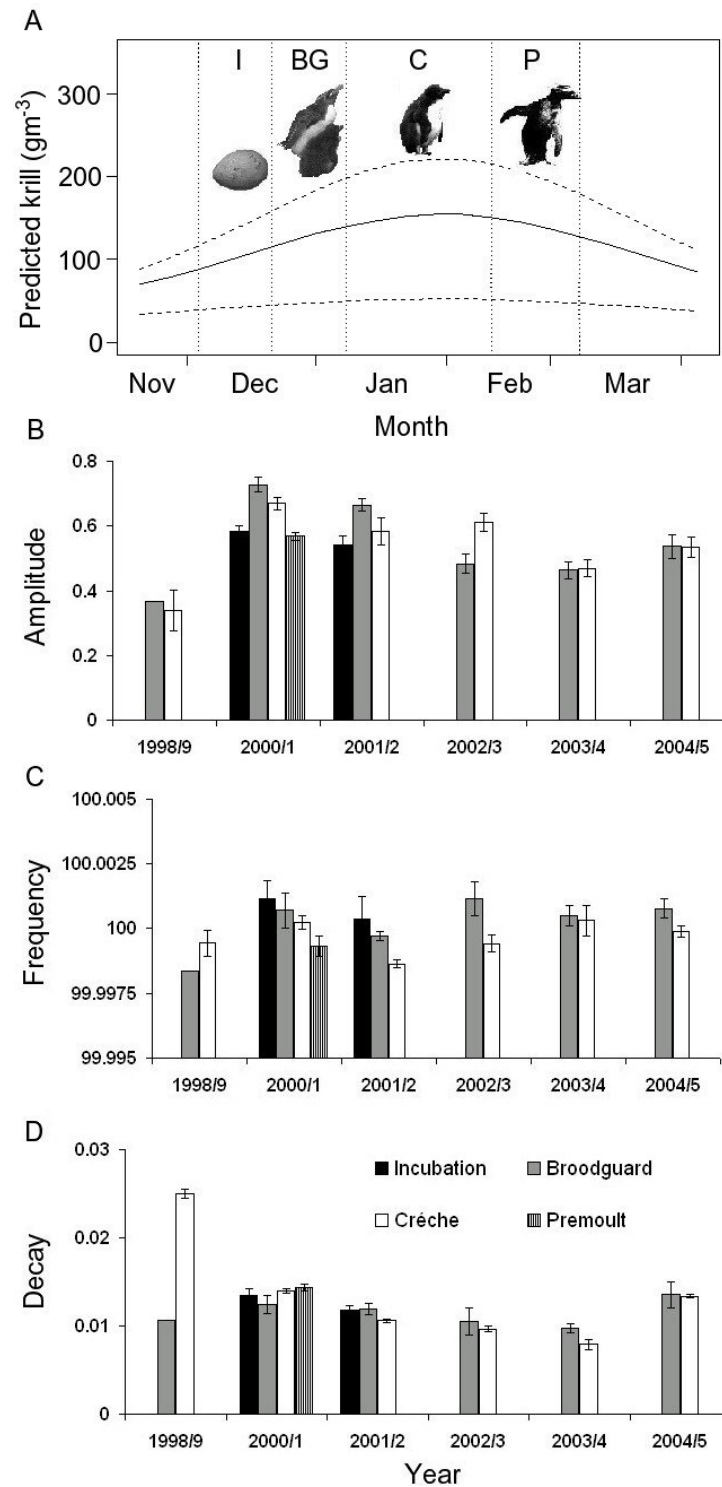


Figure 5: Observed parameter estimates in comparison with krill predictions. A) Predicted krill density (gm^{-3}) from Saunders et al. (2007). B) *Amplitude*. C) *Frequency*. D) *Decay*. Bars show the mean parameter estimate for each reproductive stage within each year, and the error bars represent standard error.

Frequency

The frequency of *amplitude* was significantly influenced by season and stage (season $F_{5,105} = 3.76$, $P = 0.004$; stage(season) $F_{9,105} = 3.61$, $P = 0.001$). Sex was not significant (sex (stage) $F_{8,105} = 3.35$, $P = 0.001$). The model explained 20.48% of the variance. The parameter estimate plots for each season and stage in Figure 5C show a decrease in *frequency* between brood and crèche for each year other than 1998/9, where there was only one broodguard individual tagged.

Decay

One extreme outlier 7.0 sd from the mean was removed from this analysis [did it influence results?]. Season significantly influenced the rate of *decay* in the autocorrelation (season $F_{5,106} = 4.98$, $P < 0.001$), while stage and season were not significant (stage (season) $F_{8,106} = 0.48$, $P = 0.884$; sex (stage) $F_{8,106} = 1.43$, $P = 0.193$) $R^2(\text{adj}) = 15.69\%$. Figure 5D shows differences between years and stage for *decay*.

Discussion

The approach used in this study compares time depth data at a range of temporal scales, with the data defining the clustering and producing summary statistics for an entire time series. Several of the parameters reported here represent new measures that have not been previously been used for analysis, such as the relationship between one dive and the next (*amplitude*) and the persistence of a correlation (*decay*). This shows that there is additional inference to be obtained from the persistence and cyclical nature of behaviours, which is usually removed by conventional analyses. Previously, autocorrelation between dive depths has been measured and noted (Tremblay & Cherel 2003) but not used for comparison. Comparing the autocorrelation reveals clear differences in behaviour during trips, which may

be due to physiology or strategy.

Summary statistics: conventional approaches

The effect of stage upon diving profile shows that macaroni penguins dive shallower, for less time and show less undulation activity at night than in daytime (Figure 2). Surface interval and undulation activity show a continuous distribution with no obvious step-changes to indicate distinct strategies (Figure 1). When the different seasons are plotted, there are distinct differences in the use of the water column that may be linked to strategy or prey availability. Analysis of the summarised data showed that time of day was significant with respect to depth, but not duration. The common element for all analyses undertaken is the strong link between depth and duration which emerges and is highlighted in Figure 1 and Table 2.

Interpretation of parameters

We carried out a sensitivity analysis for the range of observed parameter estimates to determine their influence on the best-fit line of the ACF. These are shown in Figure 6, and simulated data sets along with their ACFs in Figure 7.

Amplitude – the amplitude of oscillation in the ACF

Increasing values of *amplitude* increase the amplitude of the oscillating component of the ACF (Figure 6A). If these oscillations relate to sequential dives, then *amplitude* corresponds to the degree of correlation between one dive and the next. Simulated dives that were regular (Figure 7A) showed large values of *amplitude*, while those where dives were random between very shallow and maximum diving depth (Figure 7B) showed very low amplitudes. If *amplitude* reflects the degree of correlation between one dive and the next, *amplitude* might reflect the degree to which dives are to the same depth, or variation in depth.

This could reflect a continuum in behaviour between searching and uniform diving to one patch.

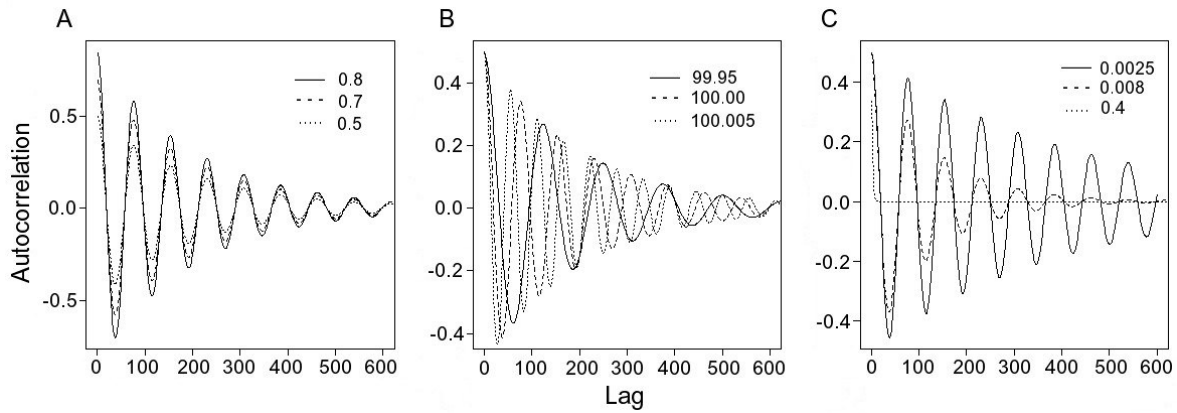


Figure 6: The effect of *amplitude*, *frequency* and *decay* on the fit-line for the ACF. A) Changing the value of *amplitude*, showing that the initial amplitude is reduced with smaller values of *amplitude*. The observed range of *amplitude* was 0.539-0.835. B) The effect of *frequency*, which influences the frequency of the oscillation; observed range of *frequency* was 99.16-100.012. C) The effect of *decay*, which reflects the persistence of the oscillating component. Observed range of *decay* was 0.004-0.458. When not specified, the default parameter values are: *amplitude* = 0.5, *frequency* = 100 and *decay* = 0.005

Frequency – frequency of the oscillations in ACF:

Changes in the value of *frequency* reflect the wavelength of the periodicity in the ACF. Across the range of parameter estimates obtained, increasing values of *frequency* led to longer periodicities (Figure 6B). Simulated data sets showed that when dives are less frequent (Figure 7C as contrasted with Figure 7A), there was a lower frequency of oscillation in the ACF. We therefore conclude that *frequency* is a measure of how the duration of a full dive cycle differs (from descent to the end of the recovery period or the start of the next dive). It will also include an indirect indication of dive depth, as this is linked to duration (Tremblay & Cherel 2000, Green et al. 2002). When variation in the duration of a single dive cycle is introduced (Figure 7F), the frequency of the ACF is disrupted.

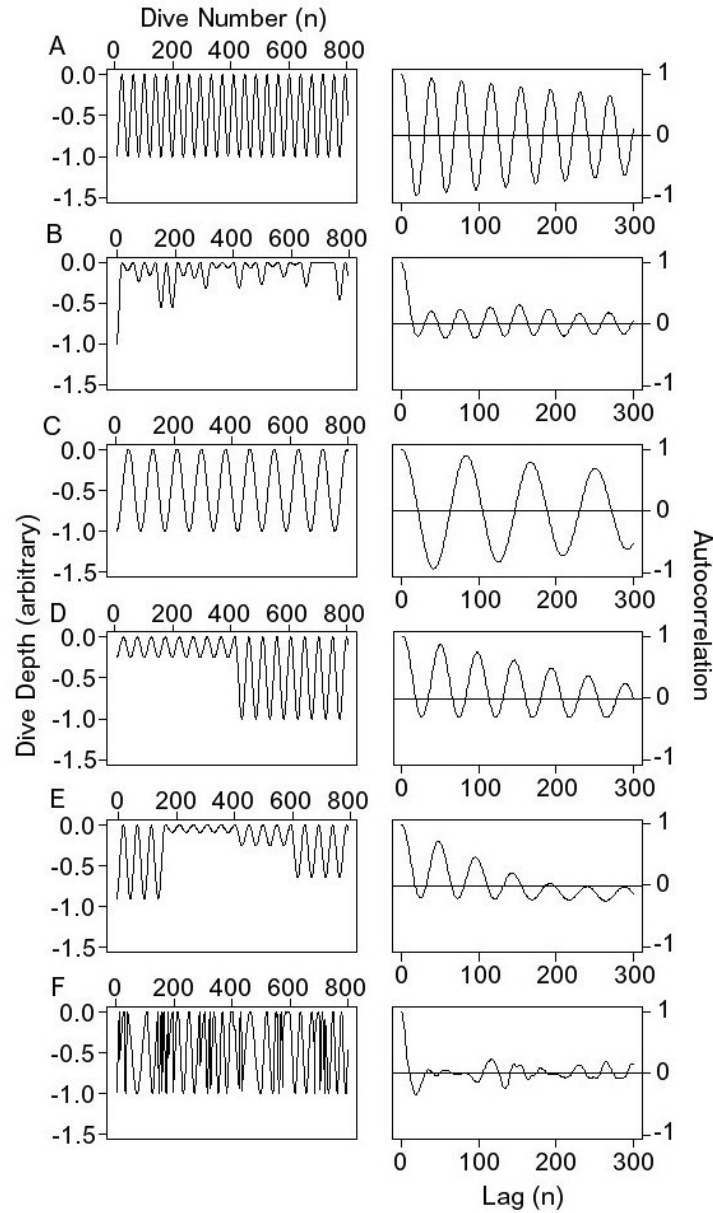


Figure 7: Examples of simulation of dive patterns and the autocorrelation patterns associated with them. A) shows a dive pattern that is relatively uniform, and with low variance. The auto correlation graph next to the dive pattern shows what this time series looks like as an autocorrelation. B) shows larger variation in the dive depth, which is reflected in the autocorrelation as smaller amplitudes. C) represents a longer dive cycle and a longer associated autocorrelation signature. D) shows a block of uniform dives and E) shows smaller units of uniform dives. Comparing A, D and E demonstrates that units of similar dives alter the rate at which the oscillations decay in the autocorrelations. F) Variation in the durations of dives for a constant depth disrupts the identification of a clear frequency in the ACF.

Decay- the persistence of oscillations

Variation in *decay* leads to changes in the persistence of the oscillating component of the ACF. Figure 6C shows that lower values of *decay* corresponding to the oscillations persisting longer in the ACF. Similarly, smaller blocks of uniform dives result in faster *decay*. Figure 7A shows uniform dives with a slow *decay*, Figure 7D shows blocks of uniform dives with a faster *decay*, and Figure 7E shows smaller blocks of uniform dives with even faster *decay*.

Conclusions about parameters

Amplitude and *frequency* were straightforward to simulate and interpret as the regularity of diving and the frequency of the dive cycle, while *decay* requires more careful consideration. We suggest that the biological meaning of *decay* is that it is related to the number of dives that are serially autocorrelated. The pattern of decay in the oscillating component is similar in inference to the patterns reported by Tremblay & Cherel (2003), who performed ACF on the depths of dives. In our analyses, *decay* reflects the regularity of timing, although there is likely to be a link with dive depth, as the dive interval depends upon the physiological recovery period (Green et al. 2003) and whether or not animals anticipate dive durations and load up on oxygen (Sato et al. 2002, Wilson et al. 2002) or adjust their recovery period (Green et al. 2003). If *decay* indicates the regularity of dives in terms of the timing, then this could be related to the patchiness of the environment and prey distribution. *Decay* could also be linked to the abundance of prey. If the size of a patch is large, then penguins may choose the optimal foraging strategy and maximise their intake, rather than breaking off from a patch in order to search for a new resource (Mori 1997). Higher correlation values between one dive and the next might imply a more consistent prey field with potentially less searching behaviour, and therefore a longer sequence of dives to the

same depth until the prey is exhausted or the density is reduced below a critical density (Mangel & Beder 1985, Alonzo et al. 2003).

The effect of year on parameter estimates

Breeding season (year) had an effect on amplitude, frequency and decay. There have been large inter-annual and seasonal differences reported in krill abundance and distribution around South Georgia (Trathan et al. 2003). It seems reasonable to assume that prey distribution will be reflected not only by individual dive profiles (Takahashi et al. 2004), but also in the strategies that penguins use to find them. We argue that this shows inter-annual differences in the sequential structure of dives: how one dive relates to the next, the frequency of the dive cycle and the number of dives that are serially autocorrelated. Inter-annual differences in diving behaviour have been shown in seals (Boyd 1999) and macaroni penguins from the same study colony used in this study (Barlow & Croxall 2002, Green et al. 2005) using the maximum depth of dives. Our study covers more seasons than have previous studies, which has been very useful in showing the extent of inter-annual variability in the behaviour of penguins.

The effect of stage on parameter estimates

Stage was significant for all parameter estimates, which indicates that there are differences in behaviour, physiology, or prey distribution at each breeding stage. We cannot determine the relative importance of these factors from this study, but it is interesting that the pattern of *amplitude* in Figure 5B matches the temporal abundance of krill around South Georgia (Saunders et al. 2007). Saunders and colleagues showed that the mean krill abundance peaks during broodguard or cr che. Therefore, the lower values of *amplitude* observed during incubation and premoult could reflect either lower abundances of krill, or a

greater proportion of searching behaviour. Figure 2 shows that incubation and premoult birds tended to perform shorter, shallower dives with lower undulation activity than during broodguard and crèche. The demands of chicks produce behavioural constraints, which have been well documented (Williams & Croxall 1991). Most obvious of these is the time spent at sea, as birds with a chick are constrained by the need to return to the nest, to swap duties with their partner and to feed the chick (Williams & Croxall 1991, Tremblay & Cherel 2005). Differences between those birds with and without a chick could therefore be partly due to the commuting time. Broodguard and crèche individuals must return to the colony overnight, and therefore some of the difference could be due to the relatively large proportion of travelling dives (Green et al. 2005).

Effect of Sex

Sex was not significant in any of the analyses. In part, however, this is confounded with breeding stage, as only females go to sea during broodguard. It is interesting that where both sexes are present, there are no detectable differences between males and females. This contrasts with observations in other studies as regards dive depth (Green et al. 2005) and location (Barlow & Croxall 2002, Mori & Boyd 2004). However, the sex identification used in this study followed Williams and Croxall (1991), which has an error rate (Hart et al. 2009) that may have led to small differences between sexes becoming non-significant.

The use of ACF parameters as summary statistics

The results of this study have shown interesting differences in the autocorrelation of diving behaviour in penguins. If autocorrelation is to become a useful measure of behaviour, these analyses should be refined to detect more local changes in behaviour within a time series (Sharifzadeh et al. 2005). Behavioural and physiological studies in humans have used local autocorrelation to assess the behavioural state of breathing and provide insight into the period of time over which ‘behavioural memory’ exists in anaesthesia (Brack et al. 1998) and

atrial fibrillation (Braunstein & Franke 1961), which is analogous to the cyclical nature of diving and the persistence of behaviour.

Considering TDR records as time series has clear advantages and has increased our understanding of diving behaviour in penguins, but the use of ACF does have limitations. ACF reports average periodicities of a data set, which loses local shifts in behaviour (local deviations from the dominant periodicities) that may be important in understanding how animals make decisions. In contrast, previous studies have summarised TDR data into individual dives before analysis (Tremblay & Cherel 2003, Ropert-Coudert et al. 2004). Focusing on single dives as data points provides valuable detail (Wilson et al. 1996) and is good at detecting local changes in behaviour, but may lose generality and the sequential structure of data. Time series are appropriate for looking at timing, though they do lose the detail of the individual dive. Dive analysis therefore needs a method that combines timing and depth. A useful next step would be to combine some form of ACF modelling with traditional TDR analysis.

Key features that we would like to capture in an analysis include: trends over time, periods between foraging dives, points of change in behaviour, alternating behaviours or repeating patterns of behaviour and clustering.

Ideally, new techniques in dive analysis would:

1. Identify break points in the data where behaviour changes,
2. Generate summary statistics that can be generalised between species and systems;
and,
3. Identify whether or not there are differences in behaviour between individuals,
stages and seasons, and how these relate to foraging decisions.

We suggest that this study has shown the applicability of our technique to points 2 and 3, but not point 1. Autocorrelation is excellent at showing mean periodic events, but not those

that are irregular. As such, this technique is useful for generating summary statistics for comparison, but poor at comparing timing of specific events in time series. As mentioned above, this could be overcome by taking sub-sets of TDR records or repeating the autocorrelation modelling approach on a moving window of the data (Brack et al. 1998).

We have fitted one model to our data which worked because there was one dominant periodicity across all data. If the dominant periodicity in a time series is different between data sets, then the model may exaggerate differences between groups by converging on a different dominant periodicity, because there is only one oscillating component in the best-fit curve. Fourier analysis may provide a more universal approach to identifying the dominant frequencies prior to using ACF (Safi et al. 2006), or by decomposing the TDR signal into dominant frequencies to show how each component contributes to the overall signal throughout the time series (Safi et al. 2006).

Conclusion

We suggest that trends and periodicity are important features of the data, from which key inferences can be drawn. Therefore time series analyses will need to be more widely adopted in the analysis of tagging studies. The autocorrelation modelling performed has shown how the sequential nature of dives differs during foraging activities. While it is clearly not a complete answer to the problems highlighted, ACF modelling does show potential to operate alongside other analysis techniques. As performed in this study, autocorrelation lacks the detail that we will need to look at variation within trips, and is poor at identifying differences in dive depth, which are likely to be important. Nevertheless, these parameters are well estimated by existing dive summary techniques, so a goal of the diving literature should be to find a way to combine techniques, or find a new one that is sensitive to timing and periodicity of events as well as parameters such as the depth and duration of dives. It is possible that ACF can be carried out on dive descriptors of processed dives, but the elegance

of ACF as used here is that it can produce inference without the need for conventions. Conventions such as the bottom period of a dive may differ between fields and studies, while methods that make inferences from the raw time series may be more universally comparable.

Chapter 4: Hidden Markov Models in Foraging Ecology

Hart, T., Mann, R., Coulson, T., Pettorelli, N. & Trathan, P. N. Behavioural switching in a central place forager: patterns of diving behaviour in the macaroni penguin (*Eudyptes chrysolophus*). *Marine Biology*, *in press*.

Abstract

Recording the activity of animals as they migrate or forage has proven hugely advantageous to understanding how animals use their environment. However, where animals cannot be directly observed, the problem remains of how to identify distinct behaviours that represent an animal's decision-making process. An excellent example of this problem is that of foraging penguins, which travel to sea to find prey to provision their young. Without multi-logger deployments or direct sampling of the prey field, we cannot directly calibrate what penguins are doing within dives. To overcome this, we use a Hidden Markov Model, which is a machine learning technique that seeks to identify the underlying states of a system from observable outputs. We apply HMM to determine classes of behaviour from repetitive dives. We take dive data from 103 breeding macaroni penguins at Bird Island, South Georgia, for which we have measures of weight gain over a trip. We identify two classes of behaviour; those of short-shallow and long-deep dives. Using these two behaviours, we calculate the transition probabilities between these states, and analyse these data to determine what predicts variation in the transition probabilities. We found that the stage, sex and year influenced the probability of transition between long-deep and short-shallow sequential dives. We also found differences in the hourly transition rates between the four reproductive stages (incubation, broodguard, crèche and premoult) over a daily cycle. We conclude that this new metric for behavioural switching is potentially useful for other species and other types of recorded behaviour.

Introduction

Understanding the patterns of habitat use is important to understand a species' niche (Jonsen et al. 2003), but it is the rules or trade-offs that govern how habitats and resources are used that are key to predicting behaviour. Within the broader context of current global environmental change, such an understanding is particularly relevant for making predictions about how resource use may vary with habitat alterations or climate change (Jenouvrier et al. 2009). Habitat and resource use are governed by animal decisions (Manly et al. 2002), which are influenced by the behavioural state of the animal at the time when the decision is taken as well as its previous experience (Burns 2005; Parejo et al. 2007; Stamps & Swaisgood 2007; Wolf et al. 2009). For example, whether or not a cheetah *Acinonyx jubatus* decides to chase a prey animal may be based on the local environmental conditions such as terrain (Bissett & Bernard 2007) and prey density (Cooper et al. 2007), its reproductive state (Cooper et al. 2007), or the time since its last meal (Caro 1994).

Unveiling the processes shaping patterns of habitat use can be challenging when direct observational data are difficult to collect. Data from activity loggers (or bio-logging tags) have improved our understanding of animal migration (Croxall et al. 2005, Guilford et al. 2009), navigation (Biro et al. 2007), foraging (Takahashi et al. 2004, Bost et al. 2007, Trathan et al. 2008) and physiology (Green et al. 2002, Green et al. 2003) in an increasing number of taxa (Ropert-Coudert & Wilson 2005, Wikelski et al. 2007, Sims et al. 2008). Marine diving animals provide an excellent example of where direct observations are difficult. Indirect data such as those from tags require calibration to understand how patterns of movement or activity relate to the behaviour and the biology of the animal (Jonsen et al. 2006, Patterson et al. 2008). When classes of behaviour cannot be directly calibrated by observation, data are used to define behaviours (Guilford et al. 2004, Jonsen et al. 2005,

Patterson et al. 2008). Methods include objective mathematical description of the shape and motifs present in individual dives (Wilson 1995, Halsey et al. 2007), cluster analysis (Safi et al. 2006) and machine learning (Roberts et al. 2004, Guilford et al. 2009). The nature and persistence of these behaviours are then described over time or between groups of interest (Guilford et al. 2004, Biro et al. 2007).

Dives form natural, discrete units of behaviour, as air-breathing marine animals on breath-holding dives must balance their access to oxygen at the surface with food at depth (Kramer 1988, Houston & Carbone 1992, Soto et al. 2008). If prey are not encountered in every dive (Takahashi et al. 2004, Naito 2007), or are not exhausted in a single dive, multiple dives will be needed to assess a patch. Therefore, dives can form aggregations, which may be regarded as a single behaviour (Boyd et al. 1994, *sensu* Dawkins 2007). Temporal clustering of dives has been investigated as *bouts* (Boyd et al. 1994, Luque & Guinet 2007), where a bout is defined by the rate of dives (Boyd et al. 1994), or by separate start and end criteria (Naito 1990). Temporal clustering can be used to infer the persistence of dives, and localised trends within this particular behaviour (Mori & Boyd 2004). While a number of clustering techniques have been used in the diving literature (Boyd et al. 1994, Luque & Guinet 2007), there is frequently a threshold imposed on dive density or timing that may lead to artefacts in the behaviours identified. As such, temporal clustering such as bouts could test hypotheses of patch and resource use. Hart et al. (*in press*) suggested limitations to this approach in penguins. Similarly, defining bouts in caribou (*Rangifer tarandus*) has recently proven controversial, with arguments about whether the scale of measurement may influence the patterns generated (Nams 2006, Johnson et al. 2006).

We argue that the successful identification and characterisation of behaviours in diving endotherms requires (1) an objective way to identify the number of distinct behavioural states defined by the data, (2) a method of clustering similar dives that are

considered part of the same behaviour at any point in time, and (3) analyses of behaviour based on transition points between these behaviours. A variety of models exist that have been used to identify change points in time series data, which include Kalman Filters (Kalman & Bucy 1961), Fourier processes (Safi et al. 2006), wavelet analysis (Cazelles et al. 2008), kmeans clustering (Rendell & Whitehead 2005, Arnold & Zuberbühler 2006, de Craen et al. 2006) and Hidden Markov Models (HMM) (Fanke et al. 2004, Roberts et al. 2004, Guilford et al. 2004, Macdonald & Raubenheimer 2007). Hidden Markov Models are well understood for temporal processes and have recently been applied to behavioural data (Fanke et al. 2004, Roberts et al. 2004, Guilford et al. 2004, Macdonald & Raubenheimer 2007).

In this study, we use data collected on adult macaroni penguins *Eudyptes chrysolophus* and HMM to determine change points, as our data may be regarded as time series with non-independence between sequential dives (Tremblay & Cherel 2003, Hart et al., *unpublished*) with k number of hidden states. We propose that dive depth and duration are related to a hidden behavioural state which may be feeding or not feeding. We wish to identify these states and describe transitions between them. When applied in this context, Hidden Markov Models can be thought of as a more sensitive version of Boyd's (1994) iterative t-test approach. The strength of Boyd's (1994) method is that it attempts to introduce 'state persistence', which is the core of the HMM approach and accounts for the potential non-independence of dives. We ask our model to find two states for three reasons, namely (1) to be consistent with previous studies of bouts (Boyd et al. 1994, Luque & Guinet 2007), whereby dives are either above a certain density and within a bout, or below that density and not in a bout; (2) we used the distribution of dive depths and durations to objectively identify the number of clusters for which the model should search: plotting depth and duration revealed strong bimodality in each of these variables as well as correlation between the two variables; and (3) running the model with more than two states revealed that only two states

were significantly occupied. The following predictions were then tested:

(H1): There are distinct states of behaviour defined by depth and timing of dives.

(H2): The amount of time spent in each state is related to observed weight gain.

(H3): There are different strategies for penguins in different reproductive stages and years

Methods

Data collection

We here use a data set based on 103 breeding adult macaroni penguins for which dive and mass data have been collected before and after each foraging trip. Monitored birds can be found in four different breeding stages, namely *incubation* (from egg-laying to egg-hatching), *broodguard* (where the male parent guards the young chick and the female forages), *crèche* (where both parents forage and the chicks form clusters in the colony guarded by a few adults) and *premoult* (where the parents leave the chicks for a long trip to gain mass prior to moulting). A trip is here defined as the period of time (in hours) away from the egg or chick (one continuous absence).

Macaroni penguins were tagged with Wildlife Computers™ Mark 7 Time Depth Recorders (TDRs) at the Fairy Point study colony (54°00'30" S, 38°04'21" W) on Bird Island during the 1998-2005 austral summers. Fairy Point is a small circular colony approximately 20m in diameter. Individuals were tagged between November and March at various stages during the breeding cycle before they went to sea to forage. All procedures conformed to the Scientific Committee of Antarctic Research (SCAR) Code of Conduct for Use of Animals for Scientific Purposes in Antarctica (2006).

Devices were glued with 2-part epoxy resin onto the tips of feathers along the spine between and below the scapulae, following methods modified from Wilson et al. (1997). The TDRs were 95×15×15mm in section and weighed 50g in air, which corresponded to <0.5 % of the bird's cross-sectional area and 1.0-1.5 % of body mass; within guidelines for maximum

device loads that these birds can potentially carry (Wilson et al. 2005, Wilson & McMahon 2006). However, any device placed on free-ranging animals has the potential to affect their welfare and alter their behaviour or reproductive success (Murray & Fuller 2000, Wilson & McMahon 2006), as has been found with flipper tags (Froget et al., 1998; Gauthier-Clerc et al., 2004). Other variables recorded were the mass of each bird on deployment and recovery, and the sex of each bird, determined using bill measurements and observations of marked pairs on the nest (Williams & Croxall 1991, but for caveats see Hart et al. 2009). Mass was measured using a balance, with the bird immobilised in a bag.

Birds were captured using a crook from the edge of the colony to avoid excessive colony disturbance. Birds had implanted TIRISTM tags (Texas Instruments Radio Identification System), to avoid the possibility of recapturing birds over successive years. Birds were recaptured on their return to land and the TDRs removed before the bird entered the colony. Birds were weighed before they had fed chicks. Replicates were obtained by recycling the TDRs over the season, so each TDR tag was deployed up to four times on different birds during each breeding season.

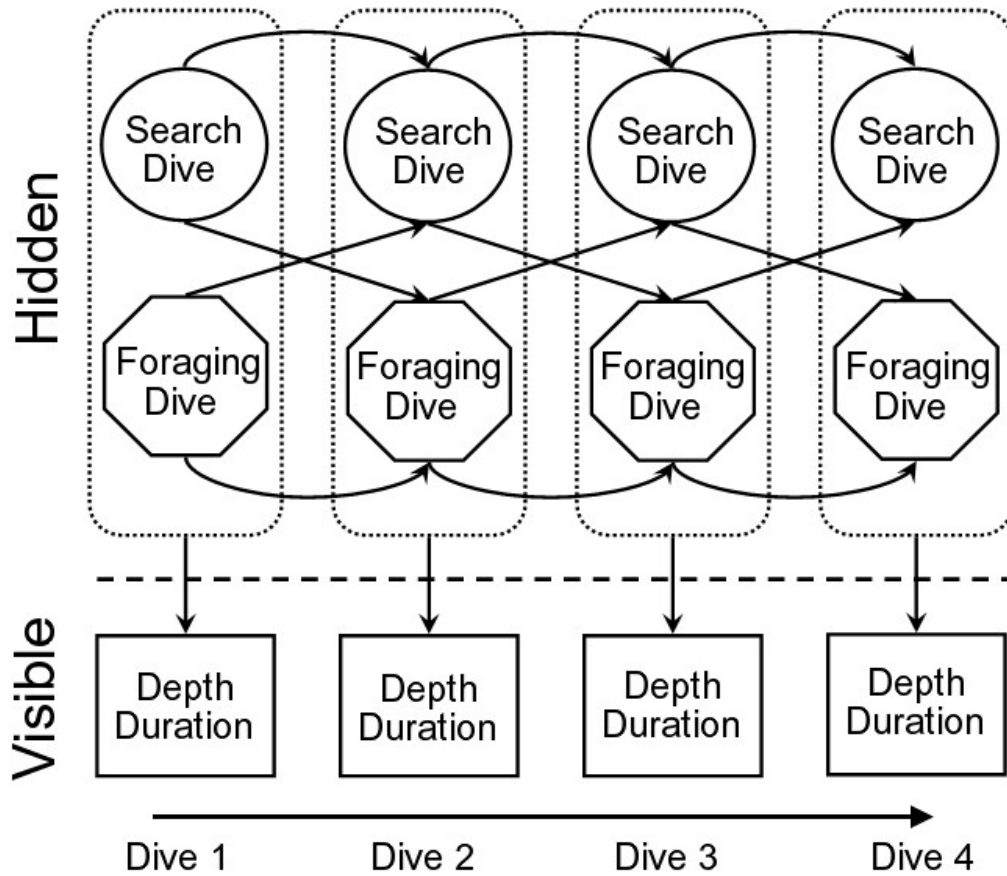


Figure 1: Schematic of a Hidden Markov Model as used for diving analysis. This representation shows a period of four dives (numbered 1-4). At each instance, the bird occupies one or other behavioural state (of searching or foraging) which is hidden (above dotted line). Each behavioural state is associated with a different ‘emission’ probability. As emissions from dive 1 to dive 4 are observable (below dotted line) and contain information about the hidden state, we can use the observed data to determine the likelihood of being in each hidden state. We assume that search dives will also include travelling dives as these will be short and shallow.

Data processing and Statistical Analysis

Data in this study have been treated in three steps: (1) processing of raw time series data into individual dives, (2) using a Hidden Markov Model (HMM) to identify two states of diving (Figure 1) and (3) statistical analysis using General Linear Models (GLM) or Generalised Linear Mixed Models (GLMM) to investigate rates of mass gain, and General Additive Mixed Models (GAMM) to determine what influences the nature of diving during a day.

Raw data were summarised into dives using our own scripts in MATLAB® (The MathWorks™, www.themathworks.com), which followed conventions of dive identification described in Tremblay & Cherel (2000). This processing included zero offset correction (ZOC) at the surface, which generally affected depth records in the upper 3m of each data set. ZOC identifies the surface as the running mean of the top of a series of dives over a minimum of 200 seconds-long enough for the penguin to have surfaced at least twice. If the tops of these dives were too varied or showed large trends, the tag was rejected on the assumption that the calibration of the pressure sensor had failed. The scripts then identified dives as the period from leaving the surface until the time of returning to the surface. For each dive, we recorded the time at the start and end of the dive, the maximum depth, dive duration and the interval between the end of a dive and the next dive. Where presented, the time of sunrise and sunset were calculated using the methods of calculation in Montenbruck & Pfleger (2005).

The Hidden Markov algorithm was applied in Matlab® using code previously developed to estimate the hidden states of navigation in homing pigeons (Roberts et al. 2004, Guilford et al. 2004). Using HMM on the data for each individual, HMM identified the state of each dive and a transition of the probability of moving between states for each penguin. HMM treats the time series as a series of states, and seeks to identify the states and the transition points between them (Markov 1971). The code used is a mathematical implementation of Occam's razor, whereby models need to balance noise in the data with explanatory power and simplicity. Models could be simple (not many states), but have large error around them, or complicated to the extreme case of one state per observation and no error. Under the method of variational learning used in this study, models containing parameters with large variation around them are down-weighted, and the number of parameters are also penalised, such that the final model chosen is the one most likely to

predict the next time step. For details of this approach, see Roberts et al. (2004).

Histograms of the log depth (Figure 2A), duration (Figure 2B) and the two variables together (Figure 2C) indicated that there were two strategies, a ‘short, shallow’ strategy and a ‘long, deep’ strategy. Plots of surface interval commonly showed one distribution with a long tail, so the natural log of the surface interval was used to correct this (Figure 2D). The HMM based on depth and duration was therefore programmed to find two clusters in the dive duration and the log of dive depth, and to classify each of the dives into one of these states, along with an associated probability. Long, deep dives are termed d , and short, shallow dives s . The probability of a dive being d was recorded for each dive as well as the global transition matrix between types of dive (Table 1). The transition matrix shows the probabilities of transition between successive dives. As we are concerned with patterns of foraging, we use the $p(dd)$ (Table 1) for comparison between individuals and groups, where $p(dd)$ reflects the relative continuity of long deep dives.

Table 1: The probability transition matrix from the Hidden Markov Models. The matrix records the observed probability of moving between dive types s and d in the current and next time step. Transition rate $p(dd)$ is used for analysis because this represents the probability of continued deep diving and inferred foraging behaviour.

		State ($i + 1$)	
		d	s
State (i)	d	p	$1-p$
	s	q	$1-q$

Specific *a priori* hypotheses listed in the introduction were tested using Generalised Linear Mixed Models (GLMM) in R 2.7.2 (www.r-project.com). Each model was checked with standard diagnostic plots. To determine what influenced the daily cycle of dive behaviour, Generalised Additive Mixed Models (GAMM) were fitted to HMM probability output summarised by hour, using the mgcv (1.4-1) library.

Results

Tag recovery

Of 156 deployed tags, all were recovered and data successfully downloaded from 129 of these, the remainder were rejected were due to hardware or software failure, where the tag had not been set up correctly. These were primarily in the 1999/2000 season, for which no usable data exist. Of these data sets remaining, 103 had records for pre- and post-trip weights. Six tags were rejected after ZOC, The number of usable data sets retrieved is given in Table 2 below.

Table 2: The sample size of tags used in this study by year and stage. Sample sizes for stage and sex comparisons are given in Figure 4.

	Incubation	Broodguard	Crèche	Premoult
1998/9	0	1	2	0
2000/1	4	7	9	5
2001/2	13	15	14	0
2003/4	0	7	2	0
2004/5	0	11	13	0

Identification and interpretation of HMM states

Using the HMM algorithm we identified two clear behaviours. An example is given in Figure 3, showing how these states relate to the depth and time, and the probability of being in each state. The justification for Hidden Markov analysis is that sequential dives are non-independent and exist in two distinct states, which represent two different behaviours. Figure 2 shows bimodality in both the depth and duration plots (which could easily indicate a four-state system), and Figure 3B shows that most dives fit very neatly into one of two states

identified by HMM with very few dives showing intermediate probability. Repeating HMM analysis with more than two states showed that only two states were readily occupied. In light of our prior hypothesis of two behavioural states, it seems likely that the states identified by HMM represent two behaviours.

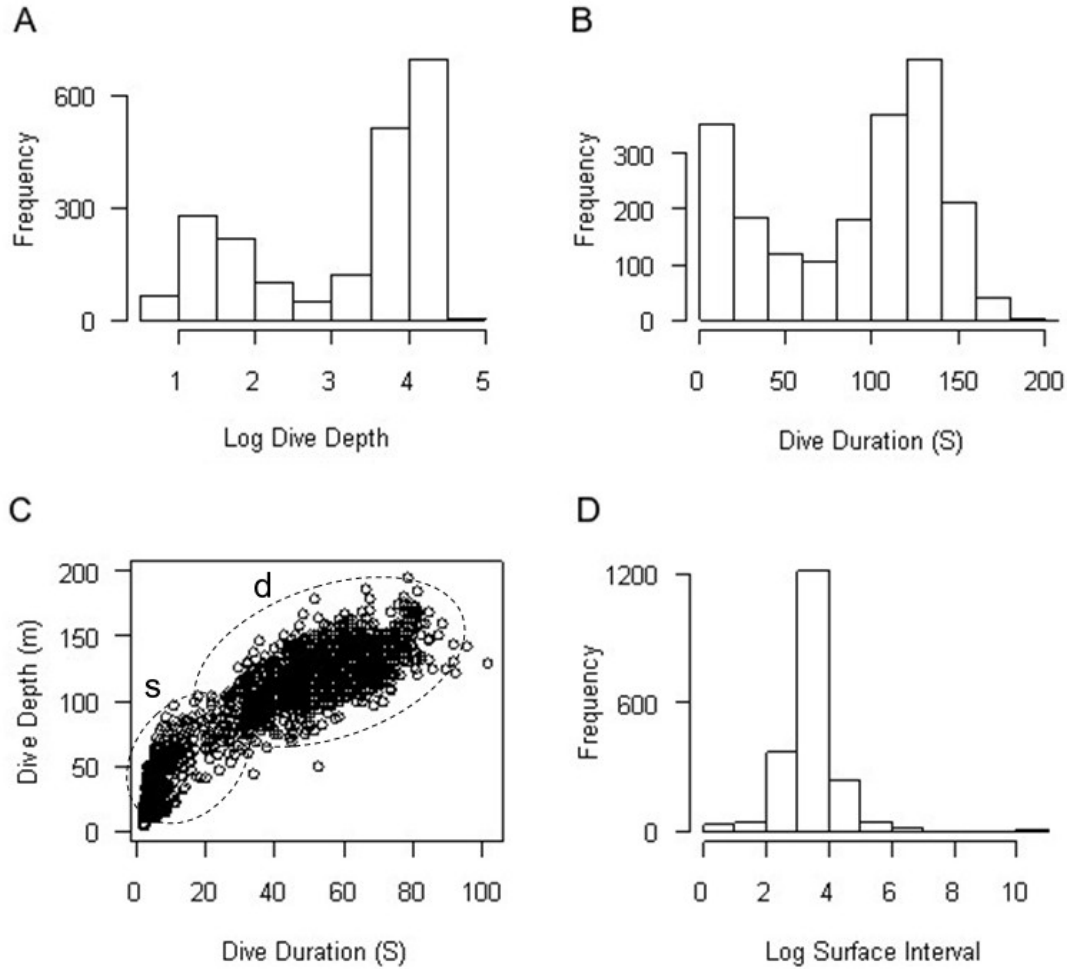


Figure 2: Figures showing the justification for selecting two states of diving based on the variables included in the Hidden Markov Model. Each graph shows dives from one individual: z30. A) Histogram of the log of dive depth. B) Histogram of the duration of dive duration. C) Scatter plot of dive depth versus duration, showing the relationship between the two, and the existence of two clusters, *s* and *d*. D) Distribution of surface intervals. The log of surface interval is used throughout this study because histograms of the surface interval commonly show a very long tail.

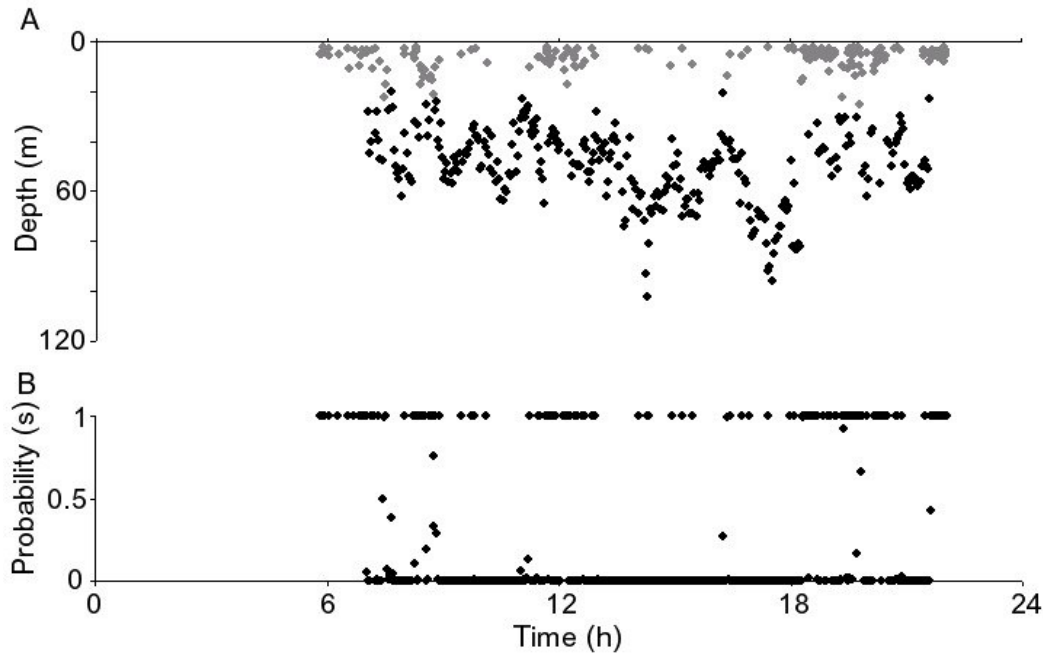


Figure 3: Output of the Hidden Markov Model for penguin z30 for a 24 hour period. A) The depth of dives over time, *s* (grey) or *d* (black) as identified by the HMM. B) The probability of being in type 1 in relation to the dives in (A). This shows that there are two clear clusters, with some intermediate dives.

The effect of behaviour and life history on weight gain.

There was considerable variation in the rate of weight gain (min=-0.0014563, max=0.000285) and $p(dd)$ (min=0.557779, max=0.992268). The probability of continued deep dives [$p(dd)$] was correlated with the rate of weight gain (Pearson = 0.260, $P = 0.008$), but a GLM of stage on $p(dd)$ revealed that this is because $p(dd)$ is confounded with stage ($F_{1,3}=3.01$, $P = 0.033$). The GLMM to investigate factors that predict the rate of weight gain showed that *dd* was not significant ($dd F_{1,88} = 0.18$, $P = 0.676$). Year, stage and sex were all useful predictors of the weight gain (*year* $F_{4,88} = 3.05$, $P = 0.021$; *stage* $F_{3,88} = 13.46$, $P < 0.001$; *sex(stage)* $F_{4,88} = 6.43$, $P < 0.001$, Figures 4 and 5). The full model explained approximately one third of the variance (R^2 (adj) = 36.65%).

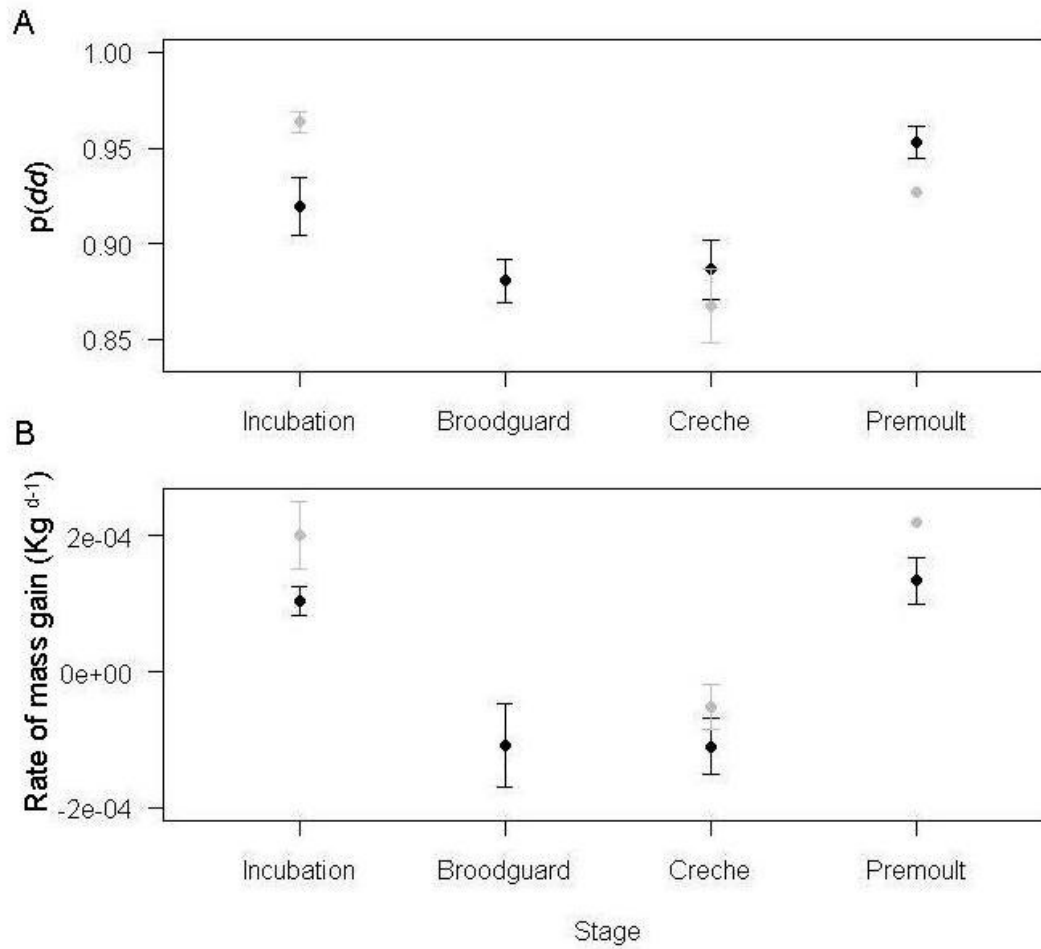


Figure 4: A) The state probability by hour for each of the four reproductive stages. Black points represent the means for females, and grey points the means for males and error bars represent the standard error of the means. Sample sizes are for females are: incubation=13, broodguard=39, crèche=21 and premoult=4. Sample sizes for males are: incubation=4, broodguard=0, crèche=20 and premoult=1.

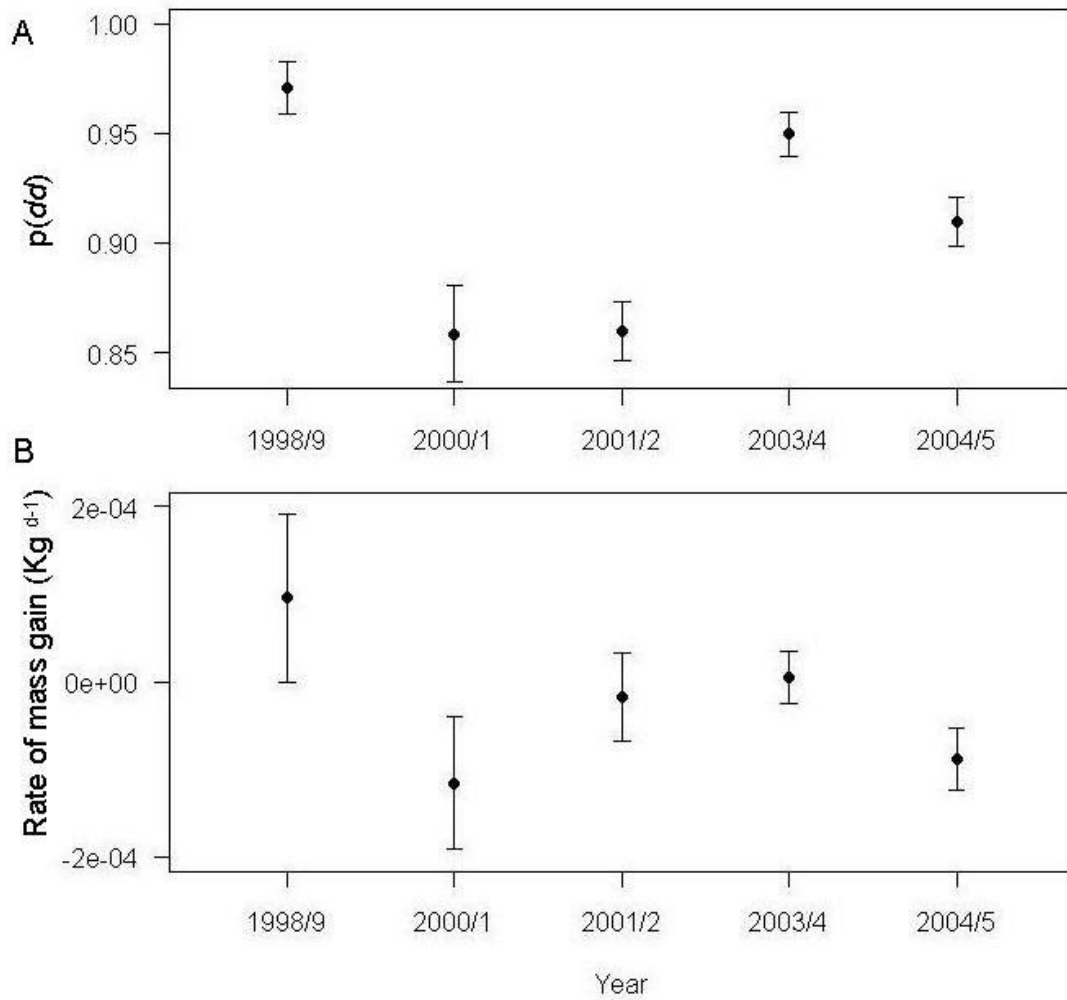


Figure 5: A) The probability of transition between state d for subsequent dives $p(dd)$ for each year. Black points represent and error bars represent the standard error of the means. Sample sizes are: 1998/9=3, 2000/1=25, 2001/2=42, 2003/4=9 and 2004/5=24. B) Represents the rate of mass gain for the same penguins.

Diurnal strategies between stages

Fitting a GAMM to the hourly probability of being in d shows that the hour of day was highly significant ($P < 0.001$) once the individual, sex, stage and year were taken into account. Plotting the fitlines for each stage (Figure 6), shows where the differences between stages lie. Incubation and premolt (Figure 6A and 6D) showed much lower mean probability of d dives

than broodguard and crèche (Figure 6B and 6C) overnight. All stages showed similar probabilities of d dives during daylight hours.

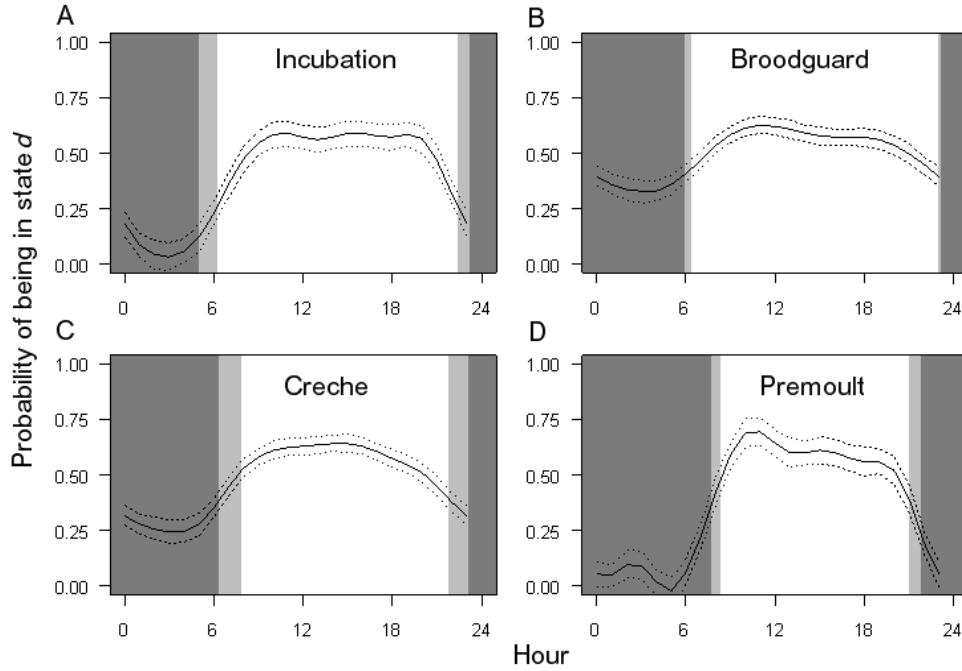


Figure 6: The state probability by hour for each of the four reproductive stages [A) incubation, B) broodguard, C) crèche and D) premoult]. The solid line represents the mean transition probability (deep-deep dives) for each hour, and dotted lines represent the 95% confidence interval around the line. All stages have similar mean transition probabilities for deep diving $p(dd)$ during the day, but differing probabilities during the night.

Linking surface interval to state

To test whether the post-dive surface interval was linked to the diving state of the previous dives, we took the mean of the previous five dives to show short-term average, and plotted this against surface interval (this is shown for one individual in Figure 7A). There were clear differences in the variances and ranges of results, but this may be linked to the number of data points over different mean probabilities (Figure 7B). To overcome this, the data frame of *mean p* (the mean probability of being in state d for the five preceding dives) was sub-sampled for each individual. Sub-sampling stabilised the variance over the range of *mean p* (Figure 7C), and a quadratic regression showed that there was a significant relationship

between *mean p* and log surface interval ($F_{2,1141} = 8.80$, $P < 0.001$), but that this explained very little of the variance ($R^2(\text{adj}) = 1.3\%$). This shows that dives that are strongly within either state *s* or *d* are more likely to be followed by a longer surface interval, so runs of dives that include dives not clearly of *s* or *d* are less likely to be at the end of a long run of dives. Figure 7D shows where these dives with intermediate *mean p* lie on the depth/duration curve.

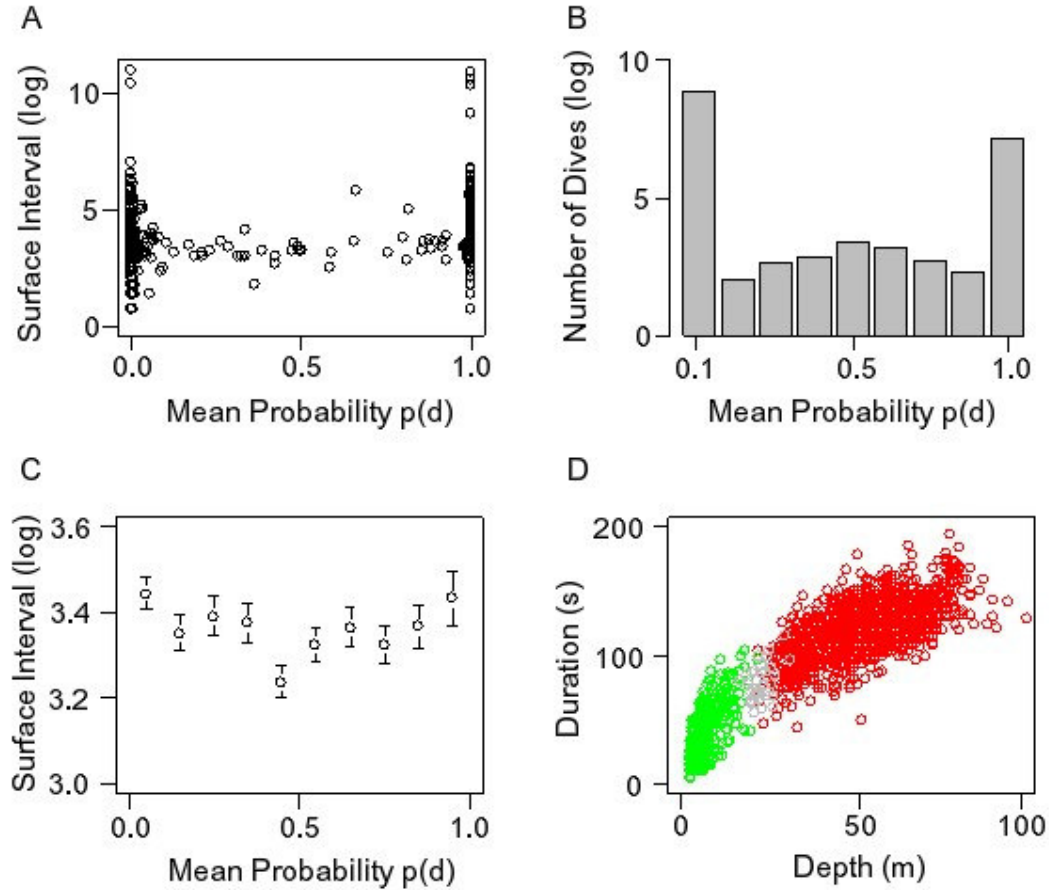


Figure 7: The effect of mean P on the surface interval of dives. A) Shows the log surface interval plotted on the mean probability of the previous five dives being *d* for a single penguin, z30. This shows that there is much greater variation in the surface interval for those dives that are strongly assigned to one state or the other. B) Shows the histogram of dives binned by the mean probability of the previous 5 dives. C) Shows the sub-sampled data of surface interval and mean P, while D) shows where dives of different probability lie on the depth/duration curve. Green represents dives with $p(dd)$ 0-0.05, grey = $p(dd)$ 0.05-0.95, and red = $p(dd)$ 0.95-1.

Discussion

The use of Hidden Markov Models in foraging ecology

The use of Hidden Markov Models to partition observed diving into behavioural states, then analysing the transition rates between behaviours, is novel. Other studies have placed an emphasis on spatial analysis in the lateral plane (Roberts et al. 2004, Jonsen et al. 2006), but HMMs can equally be applied to time series of any behavioural data such as diving. The behaviours identified are easy to visualise as use of the environment against time (Figure 7D), which aids interpretation. Recent advances in bio-logging have enabled more direct measures of energy consumption (Shepard et al. 2009) and prey capture (Takahashi et al. 2004) in single dives. Our approach can be used alongside these advances to better describe where behavioural changes occur and how these influence animals' energy budgets.

As discussed in the introduction, most definitions of bouts are unlikely to be applicable to penguins, because runs of dives are non-independent. Since HMMs produce an estimate of likelihood, this can be used to determine where there are notable changes in behaviour, or preferably, in light of the discussion above, the transition rates between behaviours.

One of the interesting features of this analysis is that there are a small number of dives that do not fall into clear states. When a dive falls between the clusters of normal behaviours, this could be because it was exploratory, accidental or abortive. There may also be interactions with predators or other occasional external influences that alter local behaviour, after which disturbance the animal returns to the previous behavioural state. There is therefore a trade-off between sensitivity and robustness. Clustering dives into bouts is quite sensitive, but loses robustness. Alternatively, using the probability of dives being within a certain state or the transition probability loses some sensitivity, but does preserve a measure of decision-making while reducing the influence of outlier dives.

The Hidden Markov Models used in this study revealed that there are two different behaviours, which would be masked by analysis of the unprocessed dive depths for the same data set. While we have focussed on depth and duration (to include both a spatial and temporal element), there are numerous attributes of individual dive that could be included in an HMM, short-term changes in depth within a dive (Halsey et al. 2007). We now address the remaining hypotheses of whether the behaviour links to weight gain, whether there are differences in strategies between stages, and whether there is inter-annual variation in foraging behaviour.

Do deep dives correlate with the amount of observed weight gain?

We tested the hypothesis that the amount of time spent in one state would relate to the observed weight gain in a foraging trip. We find no evidence for this, primarily because dive state use is strongly influenced by reproductive stage. Within each stage, there was no link between the amount of time spent in one state and the amount of weight gain, but this could be because there was relatively little variation in weight gain within stage compared to variation between stage.

Given that Antarctic krill (*Euphausia superba*; the principle prey of macaroni penguins at South Georgia) show diel vertical migration (Everson 2000) and are found relatively deep during the day, the working hypothesis was that the deeper dives reflected foraging. A number of theoretical (Cresswell et al. 2008) and empirical (Croxall et al. 1999, Barlow et al. 2002, Hennicke and Culik 2005) studies have linked the foraging activity to trip length and weight gain in foraging penguins. However, few have commented on attributes within a trip that link to weight gain. The analysis in this paper shows that continuity of long deep dives (*dd*) is not directly linked to the weight gain over a trip, but that this is due to being confounded with stage. Therefore many of the differences in weight gain and behaviour in

foraging macaroni penguins appear to be due to the difference between stages, whereby incubation and premoult individuals may be more flexible to remain at sea overnight and forage further from the nest because they do not need to return to feed their chick.

While it seems likely that this method has successfully discriminated between foraging and searching or travelling dives, it cannot discriminate between successful and unsuccessful dives, as within each stage, the $p(d)$ does not link to observed weight gain. It is possible that prey are caught in relatively few dives, although other work based on bill opening and undulations in the dive profiles suggested that prey were caught in most dives (Takahashi et al. 2004). If macaroni penguins forage until they are full, we would not expect a relationship between the rate of weight gain and $p(d)$. Variation in the rate of weight gain predicted by stage suggests that they cannot be returning to the colony full.

Differences in behaviour between stages and year.

Broodguard and crèche individuals showed lower rates of dd than incubation and premoult birds (Figure 4A) and weight gain (Figure 4B), along with strong variation between years (Figure 5A). We offer two explanations as to why broodguard females and crèche parents dive more at night than other individuals (Figure 6); firstly that the timing of reproductive stages are confounded. Broodguard and crèche represent the brightest period of summer, and as such the period of dusk is much longer, and the ability to search for prey at night may be greatly enhanced. Secondly, this should be the period of greatest foraging demand on the parents. Broodguard represents the greatest rate of chick growth with only one parent foraging, so females in this stage should be under the greatest stress. Foraging females typically lose 12% of their body weight during broodguard (Cresswell et al. 2007). This period also represents the greatest abundance of krill (Saunders et al. 2007), so the lower, more consistent rate of diving may indicate a greater proportional success per dive. In an

evolutionary ecology context, the observed pattern is interesting, as it suggests that chicks hatch and grow at their fastest rate when krill are most abundant. It is also interesting that the chicks fledge while krill are still sufficiently plentiful to allow the fledglings to forage at first independence and put on weight for winter, during which their metabolic needs will increase (Green et al. 2002, Green et al. 2005).

The loss of body mass during this time is likely to reflect a shift from optimal diving to maximise the rate of net energy gain, to a strategy that optimises the gross energy gain that can be passed onto chicks. Parents may well return to feed the chick during the day, the rate of which could be confirmed by direct observation or sensing (Berrow & Croxall 2001). It seems very likely that the female should return to land to feed the chick during the day. Our data cannot answer this question, and it would be worth using GPS tracks to determine the frequency of feeding events. Dry points in the TDR data were insufficient to determine whether feeding had occurred, and therefore positional data is necessary to accurately quantify this.

Daily patterns in foraging

The patterns between night and day shown in Figure 4 are clear and make sense in light of penguins being visual predators (Wilson et al. 1993, Williams 1995). However, caution should be taken when interpreting the patterns linking diving behaviour to day/night cycles. Sunrise and sunset times reported are based on those observed for Bird Island. Incubation and Premoult individuals are likely to forage further from the colony, as they are not constrained by the need to return at night to feed a chick (Barlow & Croxall 2002). Foraging further from Bird Island will make these sunrise and sunset calculations less accurate. The relationship between daylight and the type and duration of bouts should be investigated with GPS tags as well as TDRs so that positional information can be used to accurately reflect day length (Bost

et al. 2009).

How do states of previous dives link to post-dive surface interval?

As discussed in the introduction, we are reluctant to convert state probability into runs of dives because the burden of proof for the cut-off is unresolved. We therefore used a running mean to determine how p links to surface interval. A running mean of five dives was chosen as this represented a short-term average, which is shorter than the bout structure reported previously (Green et al. 2003) and therefore more sensitive to small changes. Regression analysis showed that the post-dive surface interval was linked to the *mean* p of the previous five dives, and that being strongly associated with either state s or d slightly increased the post-final dive surface interval. We hesitate from over-interpreting this for three reasons: firstly, because the use of five dives for the running mean was a subjective choice, secondly, because long and short dive intervals may have a different mechanism, and thirdly, because long dive intervals are likely to be rare and therefore under-represented in this study.

It is interesting that observed surface intervals increase with the certainty of being in one state or the other (Figure 7C). It is still possible that the long tail in Figure 2D indicates two strategies, with longer surface intervals showing higher variation. Such variation is reduced in depth and duration because of the need to resurface. There have been many commentaries on dual or scale-dependent strategies of foraging seabirds (Weimerskirch 2007) both with (Sims et al. 2008) and without Lévy flight (Elliot et al. 2009, which has been shown to be an artefact under certain conditions (Edwards et al. 2007)). There are still many scale-dependent mechanisms that could be important to foraging penguins and using HMM has highlighted these. It is interesting that there are two distinct strategies in depth, duration and surface interval. It is possible that birds need distinct behaviours for accessing prey at different depths, but to determine this, we need more direct indicators of prey capture.

Conclusion

How units of activity cluster into behaviours is a ubiquitous problem in studies of animal behaviour, but as behavioural studies become more important in conservation (Sepulveda et al. 2007; Stamps & Swaisgood 2007), this type of problem only becomes more pressing. In this paper, we have determined a new way of disentangling the observed activity into a process that represents the underlying behaviour, or internal state of diving animals. We have also highlighted the problems of finding a method that is robust to occasional activities that do not fit into the normal suite of behaviours.

Of the questions we highlighted in the introduction, this study has made progress towards answering what influences the timing and nature of decision-making. We have characterised the behavioural plasticity of foraging penguins in more detail than previous studies. This study could be enhanced with a more direct estimation of prey abundance and the technique may be useful in future to link reproductive success to foraging activity. Increasing the number of simultaneous sensors and data descriptors has increased the discriminatory power of behaviours in other studies, such as the “daily diary” approach (Wilson et al. 2008), and it is likely that our approach would benefit from recording additional variables such as the geographic location of each dive, in particular to determine what goes on in a sequence of dives that are not clearly foraging.

Chapter 5: Foraging and movement patterns in macaroni penguins, reward reflects time budget

Hart, T, Coulson, T., Smith, J. and Trathan, P. N. Foraging and movement patterns in macaroni penguins, reward reflects time budget. *Marine Biology*, submitted.

Abstract

Understanding the rules and trade-offs that govern how penguins forage to provision a chick is important for a) understanding population dynamics and b) predicting how these species might respond to climate change and increased competition with fisheries. Breeding Macaroni Penguins (*Eudyptes chrysolophus*) forage on Antarctic krill (*Euphausia superba*) at sea returning to land to provision their chick. We deployed time depth recorders on 126 breeding Macaroni Penguins at Bird Island, South Georgia, to identify the relationship between time allocated to foraging and travelling and to examine how variation in these behaviours influenced adult and chick weight gain. Using an Hidden Markov Model, we identify shallow travelling type dives and deep foraging type dives, and take daily summaries of these two dive types to construct a simple time budget. We then compare these budgets between groups of penguins and against observed mass gain. Most adults (53.5%) gained mass during foraging, while 46.5% showed weight loss following foraging trips. Undulations in the dive profile, *activity*, is positively linked to the amount of time spent foraging, although this is not translated into weight gain. We find a negative correlation between mean annual adult weight change and the fledging weight of chicks. We conclude that adults may be mediating their investment in chicks at the cost of their own condition, dependant on their success during foraging trips.

Introduction

Most avian marine predators are central place foragers and therefore forage remotely from their young, which results in a trade off between foraging time and the provisioning rate of food to the young (Ricklefs 1983, Green et al. 2002, Durant et al. 2005). What informs the decision to return to the young may be the combination of distance from them and quantity of prey captured (Durant et al. 2005), the starvation tolerance of the young (Moreno et al. 1994, Heezik & Seddon 1996) and the need to pass food on to the chick before it is digested (Laugsh & Duffy 1986). Understanding these strategies is important in the face of habitat change and climate change (Furness & Nettleship 1990, Bost & Le Maho 1993, Jenouvrier et al. 2009, Fretwell & Trathan 2009). The need for this knowledge is heightened by potential increases in commercial fisheries in the area, especially the krill fishery with current catches relatively small compared to the precautionary catch limit (Hill et al. 2007).

Data from bio-logging studies have aided our understanding of aquatic behaviours (Naito 2007, Wilson et al. 2009, Bost et al. 2009). A range of bio-logging devices are now routinely deployed on marine predators including penguins (Ropert-Coudert et al. 2006), seals (Boyd et al. 2002, Austin et al. 2006) and flying birds such as albatrosses (Croxall *et al.* 2005) and shearwaters (Guilford et al. 2009). These devices record elements of behaviour such as position, depth and movement. If changes in behaviour can be identified from these data, then these devices can be used to inform as to how animals make foraging decisions.

Technological advances in battery life (Boyd et al. 2004), miniaturisation (Kooyman 2004, Pütz & Cherel 2005) and the number of variables that can be

simultaneously recorded (Boyd et al. 2004, Wilson et al. 2009) have revolutionised activity recorders that are placed on animals. Indeed, we may reach a point where we can directly record animal behaviour along with the timing and nature of prey capture (Hooker et al. 2002, Hooker et al. 2005) to determine how behaviour links to foraging success. Until such time, we can only infer behavioural decisions at a larger temporal scale and the influence of these decisions upon the rate of weight gain and reproductive success.

Extensive foraging studies on penguins over the last two decades have revealed many patterns of diving behaviour, but the answers to several important questions remain poorly understood (Naito 2007). In particular, we need a better understanding of how penguins find prey (Ropert-Coudert et al. 2006) and how prey availability influences condition and reproductive success (Reid et al. 1999, Miller et al. 2009).

An important behavioural decision that has not often been studied in wild birds is when animals cease performing a behaviour (Green 1984, Vásquez & Kacelnik 1998). In essence, how do individuals decide whether to stop foraging and feed their young, or to continue foraging? Factors which could influence this are the benefits of additional food to the adult and chick (Tremblay & Cherel 2005) and the chick starvation tolerance (Heezik & Seddon 1996) versus the optimal feeding pattern for growth (Tremblay & Cherel 2005). As adults are feeding remotely, if there is a time constraint due to chick starvation, there may also be a trade off between the time spent travelling and the time spent foraging (Cresswell et al. 2007).

Macaroni Penguins (*Eudyptes chrysolophus* Brant) are widely recognised as very important consumers of biomass in the Southern Ocean (Croxall et al. 1984, Croxall et al.

1999, Murphy & Reid 2001) being responsible for half of all biomass taken by land-based predators around South Georgia (Croxall et al. 1984). Despite being a highly important species in the South Georgian ecosystem, these birds are classed as ‘Threatened’ (IUCN 2008). It is therefore increasingly important to understand their life-history, and how they use their environment to forage and raise a chick in the face of climate change and competition with fisheries.

As most adults return to the colony overnight to feed their chick (Croxall et al. 1988) and because of the diurnal cycle in krill (Everson 2000, Wilson et al. 1993), a 24 hour period is an appropriate time-scale for decision-making in Macaroni Penguins. We use a Hidden Markov Model to split dives into foraging and travelling, and investigate decisions over this 24 hour cycle. We test the following hypotheses:

- 1) Foraging time is negatively correlated with commuting time.
- 2) The amount of time spent foraging correlates with adult weight gain.
- 3) Adult weight gain corresponds with fledging mass.

Methods

Macaroni Penguins have been monitored since 1963 at the Fairy Point colony on Bird Island, South Georgia. Mass data from 103 breeding adult macaroni penguins pre and post-foraging, were collected and linked with bio-logging data from Time Depth Recorders (TDRs or *tags*) to answer the above hypotheses over the four reproductive stages.

The *stage* of reproduction in a season, clearly identified by a change in behaviour or a life history event, such as egg-laying or hatching. The stages in this study were: *incubation* (from egg-laying to egg-hatching; approximately 35 days), *broodguard*

(where the male parent guards the young chick and the female exclusively forages; approximately 34 days), *crèche* (where both parents forage and the chicks form clusters in the colony guarded by a few adults; approximately 26 days) and *premoult* (where the parents leave the chicks for a long trip to gain mass prior to moulting; approximately 12-14 days). Durations of stages are means over a four-year period from Williams & Croxall (1991).

Data collection

Macaroni penguins were tagged with Wildlife Computers™ Mark 7 Time Depth Recorders (TDRs) at the Fairy Point study colony (54°00'30S, 38°04'21W) on Bird Island during the 1998-2005 austral summers. Fairy Point is a small circular colony approximately 20m in diameter. Individuals were tagged between November and March at various stages in the breeding cycle before they went to sea to forage. All procedures conformed to the Scientific Committee of Antarctic Research (SCAR) Code of Conduct for Use of Animals for Scientific Purposes in Antarctica (2006).

TDRs were deployed on 126 breeding adults over 6 seasons (1998/9, 2000/1, 2001/2, 2002/3, 2003/4 and 2004/5). Masses were not recorded in 2002/3 due to equipment failure, leaving 103 (82%) penguins with mass records. Devices were glued with 2-part epoxy resin onto the tips of feathers along the spine between and below the scapulae, following methods adapted from Wilson et al. (1997). The TDRs were 95 × 15 × 15 mm in section and weighed 50g in air, which corresponds to <0.5 % of the bird's cross-sectional area and 1.0-1.5 % of body mass; within guidelines for maximum device loads that these birds can potentially carry (Wilson et al. 2005, Wilson & McMahon 2006). The mass of each bird was recorded using a balance and bag on deployment and

recovery. The sex of each bird was determined by bill measurements and behavioural observations of marked pairs on the nest (Williams & Croxall 1991, but see Hart et al. 2009 for caveat).

Birds were captured, using a crook, from the edge of the colony to avoid excessive colony disturbance. More than half of the birds had TIRIS™ unique identifier tags, to discount the possibility of recapturing birds over successive years. Most birds were recaptured on their return to land and the TDRs removed before they entered the colony. It is assumed that birds were weighed before they fed chicks, although this is always uncertain which adds error to both broodguard and crèche measurements. Extra replicates were obtained by recycling the TDRs over the season, so each tag was deployed up to four times during each breeding season.

Fledging mass for a random sample of chicks was obtained using CCAMLR Ecosystem Monitoring Program (CEMP) standard methods (CCAMLR 2004). For each season, this represents the mean and standard deviation of a minimum of 100 chicks. January krill abundances were recorded from ship-based acoustic surveys, following methods described in Trathan et al. (2003).

Data processing and statistical analysis

The approach used in this paper follows four steps;

1. Firstly, we summarised the raw data into dives using our own scripts in MATLAB® (The MathWorks Ltd, www.themathworks.com) using standard definitions in dives (Gentry & Kooyman 1986, Tremblay & Cherel 2000). The surface was defined as the mean of the top of a series of dives (Zero-offset correction, ZOC), which helps reduce error introduced into the depth records by

waves at the surface. If the surface could not be easily identified and ZOC failed to correct pressure trends, the tag was rejected. Six tags (4.5% of successful deployments) were rejected in this way. The script then identified dives as the period between leaving the surface and the time of return to the surface. ZOC generally affected depth records in the upper 3 metres of each data set. For each dive, time was recorded at the start and end of the dive as well as the maximum depth and activity, duration and surface interval. Surface interval was calculated as the time between the end of one dive and the start of the next. Activity was calculated as the number of small undulations at the bottom of each dive. The bottom of the dive is defined as the region within 75% of the maximum depth (Tremblay & Cherel 2000), and an undulation as a change in direction of the dive trajectory.

2. The Hidden Markov algorithm was applied in Matlab® using code previously developed for positional entropy in birds (Roberts et al. 2004, Guilford et al. 2004) and previously applied to these data (Hart et al., *in press*). This algorithm identified two strategies; ‘short-shallow’ and ‘long-deep’ dives. Short-shallow dives were classed as *travelling* dives and long-deep dives were classed as foraging dives.
3. Behaviours were then split by their HMM classification and used to calculate daily foraging and travelling periods. Histograms of the surface intervals showed a positive skew, due to the inclusion of individuals that stopped diving overnight. These long surface intervals were rare (<0.5%), but contributed disproportionately to the mean. Surface intervals longer than 300s were truncated at 300s. Penguins

have been shown to adjust their post-surface recovery interval (Wilson et al. 2002, Green et al. 2003), in relation to the dive duration and depth (Wienecke et al. 2007). After a certain period, however, the dive interval no longer reflects this decision and is more likely to be a transition to resting or digesting, or prey have become inaccessible. Truncating unusually long dive intervals or removing these dives allows us to look at time spent foraging or travelling within these two behaviours that are more easily identifiable. Travel time was calculated as the total dive duration plus the total surface interval (after truncation) for short-shallow dives. Foraging time was calculated in the same way for long-deep dives.

4. Specific *a priori* hypotheses were tested using General Linear Models (GLM), which tested for linear relationships with a normal error structure, checked using standard diagnostic plots. Associations between mass gain, krill abundance and mean fledging mass (mean per year) were tested using Pearson's correlation coefficient.

Results

Is foraging time related to travel time?

A GLM on foraging time revealed that sex and travel time were not significant (Table 1). Stage, year and were activity were significant, with a positive relationship between *activity* and foraging time (Figure 1), indicating that when dives show more undulations on average, penguins spend a higher proportion of the day foraging. The travel and foraging time by stage are shown in Figure 2, along with how this relates to predicted krill availability over this period. Males have shorter travel and foraging times than females, and those birds with a chick (broodguard and crèche) show shorter foraging

times than those without (incubation and premoult). There is no evidence to suggest that birds with longer travel time spend any less time foraging than those with short travel times (Figure 3A).

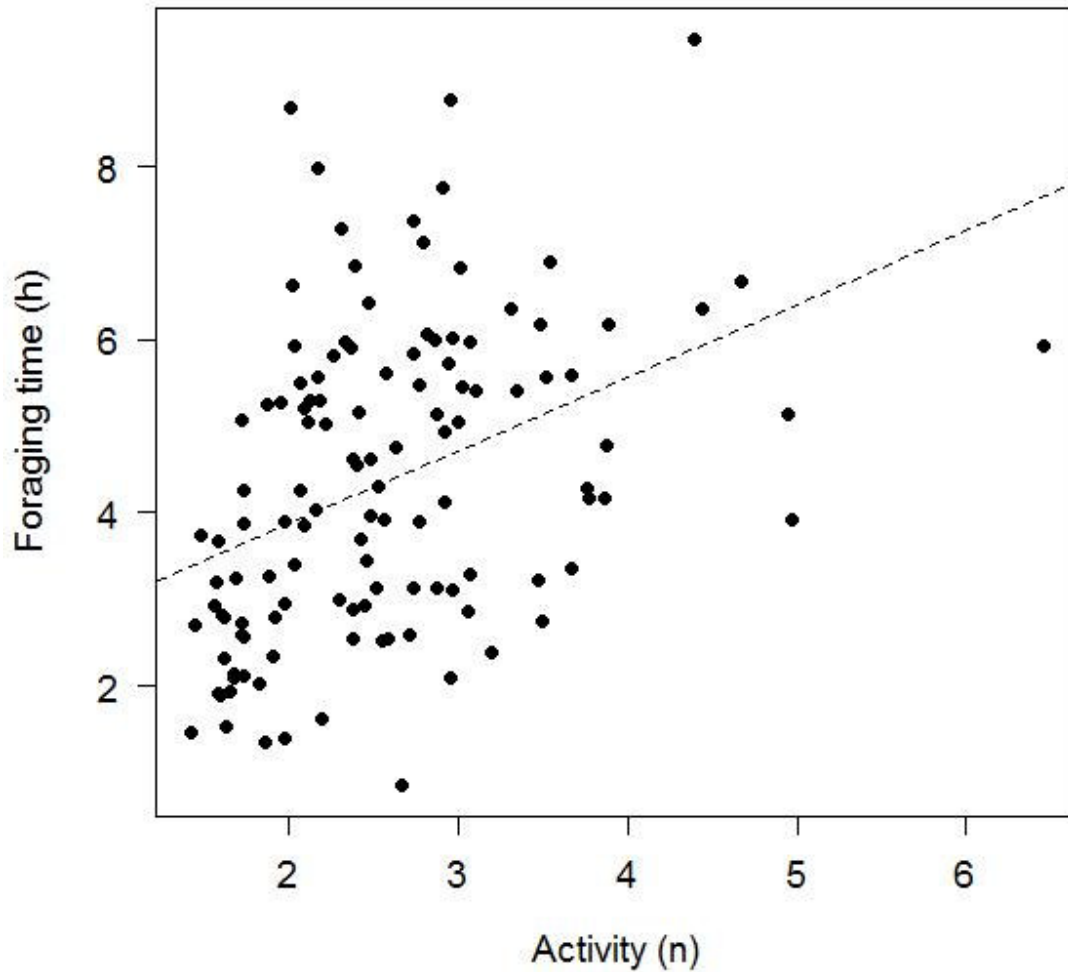


Figure 1: Regression plot showing the relationship between activity and foraging time. Points indicate the mean daily foraging time and activity of each penguin and the dashed line indicates the regression fit from the model in Table 1.

Table 1: GLM output of variables on time spent foraging, showing the degrees of freedom for the numerator and denominator, the F-ratio and P value for the GLM. Estimates of slope and the standard errors are given for continuous variables. Brackets denote variables that have been nested (variable outside brackets is nested within the variable in brackets). The $R^2(\text{adj})$ for this model is 44.99%.

Source	DF	F	P	Coeff	SE Coeff
Travel time	1,81	0.09	0.770	0.0298	0.101
Activity	1,81	28.53	<0.001	1.1143	1.225
Sex(stage)	9,81	1.41	0.214		
Stage(year)	8 ,81	3.44	0.001		
Year	5,81	2.47	0.030		

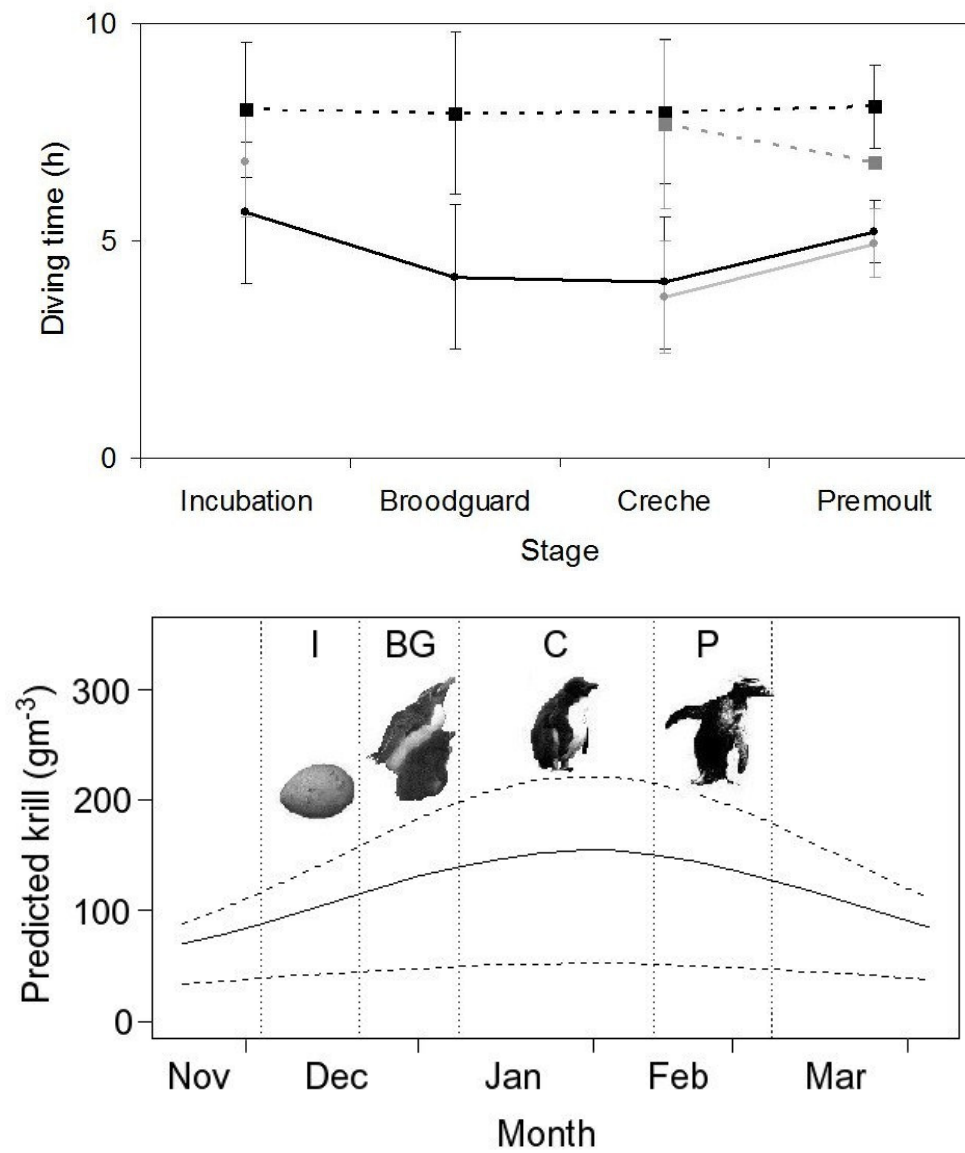


Figure 2: A) Mean travel and foraging times for each reproductive stage. Circles joined by solid lines show the mean foraging time for males (grey) and females (black). Squares joined by dotted lines represent the mean travel time for males (grey) and females (black). Bars denote the standard error of the mean. B) The predicted krill availability against time of year (data reproduced from Saunders *et al.* 2007). The mean timing of reproductive stages is overlaid (Williams & Croxall 1991), to demonstrate how reproductive stage is confounded with krill availability.

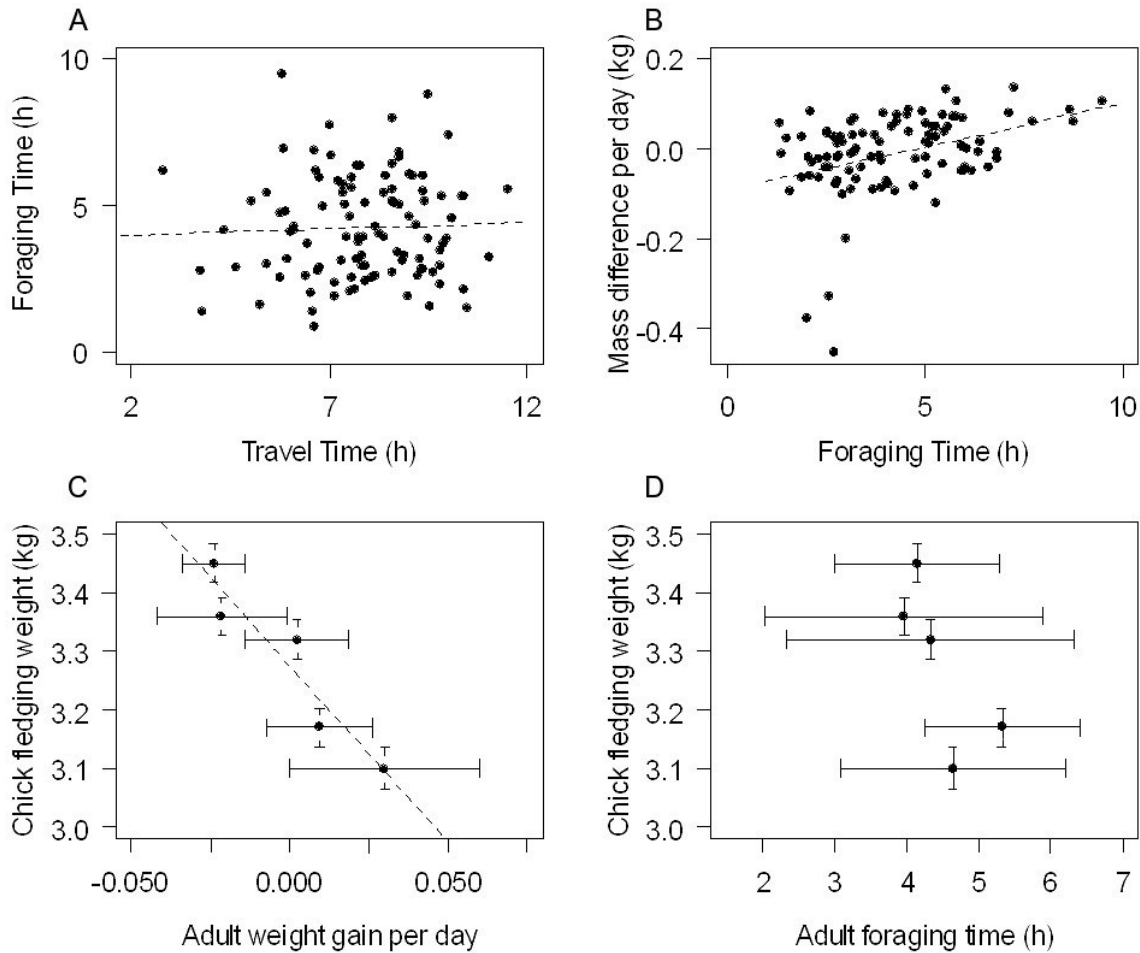


Figure 3: A) Foraging time against travel time. Points show the mean forage time and mean travel time for each penguin. Dotted line shows the regression of foraging time on travel time. B) The rate of change in mass of foraging penguins (kg per day) against the foraging time. Points show the mean for each penguin. This regression was not significant for this relationship. C) The relationship between the rate of mass change in foraging adults and the chick fledging mass. Points indicate means per year, and bars denote the standard error. The dotted line represents the regression fit line. D) The relationship between adult foraging times and the chick fledging mass. Points indicate means per year, and bars denote the standard error.

Is foraging time correlated with the rate of adult mass gain?

Of those individuals weighed before and after a trip ($n = 103$), 53.5% of adults showed a gain in weight and 46.5% showed weight loss over foraging trips. Foraging time, travel time and activity were not significant, while stage, sex and year were all

significant (Table 2A). This analysis revealed three extreme outliers, which were removed from the GLM (Table 2B). Removal of these data points affected the conclusions about sex and year, making them both significant. Birds with chicks in the broodguard and crèche stages showed lower or negative rates of mass gain than those without chicks in incubation and premoult stages (Figure 4).

Table 2: GLM output of variables on the rate of mass gain. A) Model performed on the complete data set. Brackets denote variables that have been nested in another (variable outside brackets is nested within the variable in brackets. B) The same model, but with three outliers removed to show their influence on the conclusions. The $R^2(\text{adj})$ for the model in Table 2A = 12.59% and Table 2B = 35.97%.

A)

Source	DF	F	P	Coeff	SE Coeff
Travel time	1	3.51	0.065	0.000046	0.000289
Forage time	1	0.43	0.516	0.000019	0.000029
Activity	1	0.45	0.504	0.000038	0.000057
Sex(stage)	5	1.81	0.123		
Stage(year)	7,66	2.49	0.025		
Year	4,66	0.51	0.725		

B)

Source	DF	F	P	Coeff	SE Coeff
Travel time	1	1.62	0.208	0.000018	0.000014
Forage time	1	0.06	0.805	0.000004	0.000016
Activity	1	0.07	0.797	-0.000008	0.000033
Sex(stage)	5	7.67	<0.001		
Stage(year)	7,63	5.27	<0.001		
Year	4,63	3.14	0.020		

Linking adult mass gain, fledging mass and krill abundance

In some years, adult birds put on mass, which is associated with lighter fledglings. The correlation between mean annual adult mass gain and the mean annual fledging mass

was negative and significant (Pearson correlation coefficient = -0.945, $P = 0.016$, Figure 3C). Inter-annual differences in chick weight is not linked to the amount of time adults spend foraging (Figure 3D). The correlation between January krill abundance and mean annual mass gain was not significant (Pearson = -0.729; $P = 0.162$).

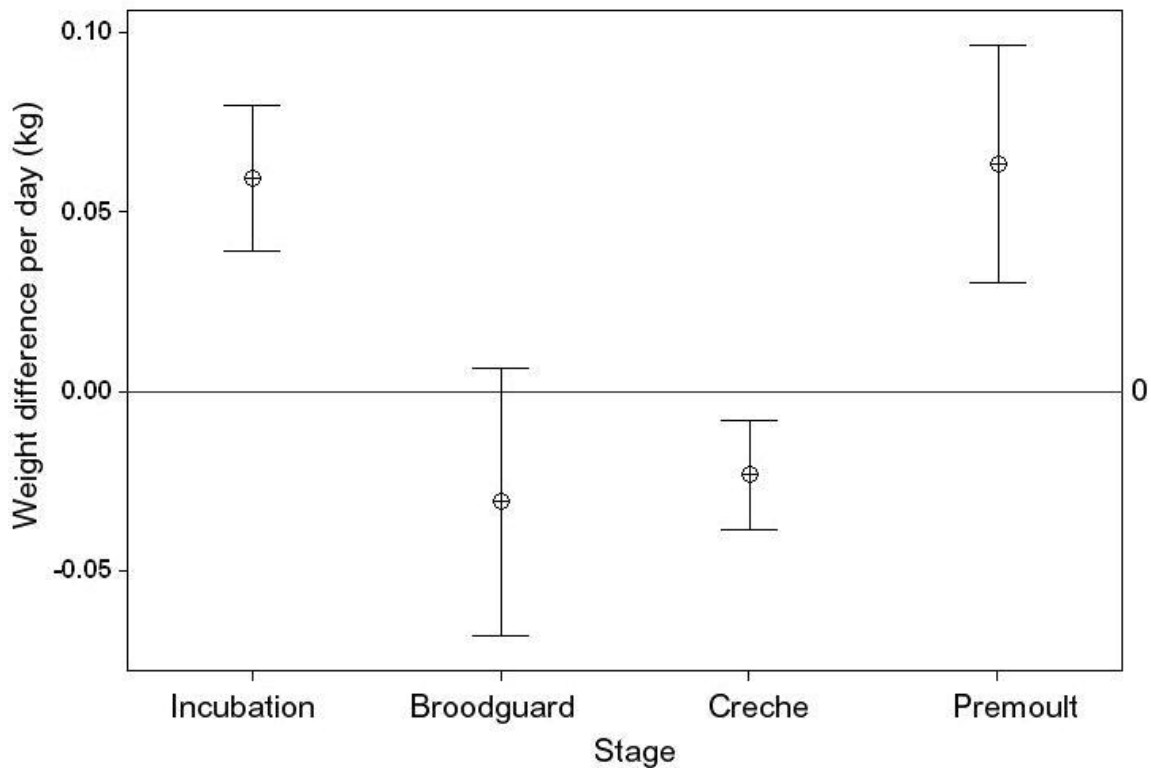


Figure 4: The effect of stage weight difference per day. Error bars show +/- the 95% confidence interval.

Discussion

Is foraging time correlated with travelling time?

The first hypothesis was that foraging time would be negatively correlated with the travel time. This would indicate that when birds are constrained by day length and the need to return to feed a chick, there is a possible trade-off between time spent travelling to the foraging ground and the amount of time spent foraging. Our data show no evidence

for this (Figure 3A and Table 1). Durant et al. (2005) argued that to raise young, a central place forager needs abundant food of sufficient quality, close enough to the origin to permit regular return trips. In the case of Macaroni Penguins, there is no evidence that the travel time is negatively linked with foraging time (Table 1) once stage is taken into account. Penguins are either not constrained by time overall showing that chicks starvation tolerance is high, or there is a particularly high cost of longer commute times. In contrast, more abundant prey could be found further away at the polar front or the shelf edge (Trathan et al. 2006).

Do birds stay out for longer in an attempt to gain more mass?

There is no clear pattern between foraging duration and mass gain, although this is partly confounded with krill availability (Figure 2) Adults with chicks (i.e.: those in broodguard and crèche) clearly have their dive periods limited by the need to return to land to feed their chick. In this study, the only indication of trip success comes from measuring weight. It would be very informative to be able to record the mass of meals given to a chick and thereby partition overall weight change into adult weight change and mass of food transferred, such as studies on albatrosses (Prince & Walton 1984). This would demonstrate the relationship between travel time, foraging time, and reward for the chick.

The effect of stage is significant, and shows the important constraint of a chick and the need to return to the nest, even if this results in a net loss of mass to the adult. The mean change in adult mass during both broodguard and crèche stages is negative (Figure 2B). This suggests that, as much of the food is passed onto the chick, birds in these stages, are losing condition (Williams & Croxall 1991, Cresswell et al. 2007). However,

given that incubation and premoult individuals choose to spend much longer diving, lower adult weights show that individuals in breeding stages with a chick are also incurring a foraging cost by returning early (Figure 3C & Figure 4). Adults have a certain degree of starvation tolerance and as such are able to lose mass (Cresswell et al. 2007). During foraging, the main goal may be to pass on as much food as possible to the chick, rather than to maximise mass gain. It is worth noting there will be some confounding effect of day length between stage, and this may be exacerbated by different day lengths over the different latitudes that birds are foraging in.

Incubation and premoult individuals are almost exclusively in the positive region of the mass difference per day (Figure 4). We suggest that these individuals deliberately spend longer at sea to maximise their rate of mass gain. When this pattern is compared to the modelled krill availability from acoustic mooring data around South Georgia (Saunders et al. 2007), it is apparent that broodguard and crèche individuals return under mass despite there being more krill available on average than during other stages. It is possible, that krill abundance does not directly translate into prey availability through failure to locate krill patches or if krill are deeper than penguins can dive.

Sex-differences in weight gain.

Previous studies have shown sex-specific foraging differences suggesting that males competitively exclude females during the crèche period, when both sexes forage sympatrically. Barlow and Croxall (2002) found that males forage closer to the colony than females, and Green et al. (2007) suggested that this results in females being forced to forage at greater, less efficient depths. Similar findings have been reported Adélie penguins (Clarke et al. 1998). We found no sex-based differences in the daily time

budget. Overall, there is no difference in the relative amounts of time travelling and foraging, although partly this is due to the relatively small sample size for males when divided into stages and years. Figure 2A would suggest that any sex differences were in accordance with the hypothesis of Green et al. (2007), whereby males show shorter travel times. This would need to be investigated with a larger sample size, or more balanced replicates between males and females. We did, however, find differences in the rate of mass change, with females losing mass faster than males.

Adult weight gain and chick fledging weight

Adult weight gain is negatively correlated with chick fledging mass suggesting that the adults forgo their own body condition in favour of investment in the chick, and therefore increased probability of chick survival. Activity is inversely linked to the foraging time, but not to adult weight gain, and as such is not linked to chick fledging weight. Other researchers have suggested that undulations are linked to prey capture events (Wilson et al. 1996, Halsey et al. 2007). If activity is linked to prey capture, then why is activity not correlated with weight gain? Do penguins stop feeding when they are satiated, or when they run out of time? If they stopped feeding when they were satiated then foraging time would be correlated with weight gain, for which there is no evidence. However if they stopped when they ran out of time then travel time would be correlated with foraging time which again there is no evidence for. Another possibility is that there is a trade-off between the amount of meal size delivered to the chick and the duration of absence from the chick. Adults that have an unsuccessful trip could return to their chick early to give a smaller meal but with a shorter starvation period.

Other researchers have used bill loggers to record the opening and closing of the

bill at depth (Takahashi et al. 2004, Wilson 2003) which could be a more reliable measure of foraging success per dive. Krill are known to be highly aggregating prey (Miller & Hampton 1989, Everson 2000), which swarm to avoid predators (Alonzo & Mangel 2001), so it is possible that large numbers of dives are searching dives. However, if penguins do relatively few exploratory dives compared to feeding dives and are good at finding krill, then variability in the encounter rate should be reduced.

Figure 3C shows that the fledging mass of young is highly correlated with the rate of mass gain of their parents during the chick rearing period. However, the relationship is negative; indicating that mean negative mass gain in adults resulted in the heaviest chicks. If heavier birds are not foraging more than light birds (Figure 3B), and those that are apparently more successful are spending less time foraging (Figure 3A), then it is likely that there is a missing component in the energy budget. This could be that adults decide how much to invest in their chick in any given year.

Either there is a cost to being fat (such as buoyancy) which prevents penguins gaining mass when food is abundant, or that investing in chicks is risky when it comes at a cost to the adult so that penguins seek to maximise their own condition when food is scarce. The link with chick fledging mass suggests that the latter is a more likely scenario. Adults could safeguard their own survival at the expense of their offspring's condition in bad years. If true, this would indicate that adults may be seeking to maximise their lifetime reproductive success. Modelling studies have suggested that weight loss in macaroni penguins is adaptive to maximise life-long reproductive success (Cresswell et al. 2007), but there is likely to be variation in strategy between good and bad years. There is a clear need more for data over more years to resolve whether or not this is the case,

and ideally more measures of krill abundance over the breeding season. Such a response has been shown in a range of flying birds in relation to predation risk (Gentle & Gosler 2001, Lilliendahl 1997) and environmental variation (Gosler 1996).

Conclusion

These analyses support the conclusions that there is variability in the duration of absence from the chick and rate of mass gain, and because the relationship between these was not significant we can conclude that the adults do not stop foraging due to being satiated. As travel time and foraging time are not correlated there is no time constraint on the adults to return to the chick and using comparisons of the stages with a chick against those without shows how much parents are constrained and must invest in chicks as they tend to lose mass despite an abundance of krill. The negative link between fledging mass and mean adult mass gain shows the investment, and how parents must make decisions based on their own condition and the environment.

Chapter 6: General conclusions

Sea level (Richardson et al. 2009) and ocean heat content (Barnett et al. 2005) have recently significantly increased. Ocean chemistry is changing more dramatically than at any other time in the past 20 million years (Sabine et al. 2004), and biological responses have been detected at all trophic levels (Buesseler et al. 2007, 2008). From phytoplankton upwards in food webs, oceanic species are showing shifts in distribution, abundance and behaviour. At the same time, fisheries have impacted massively on target and non-target species around the globe (Myers & Worm 2003, Daskalov et al. 2007).

Penguins are likely to be affected by such changes. As a group, they are tied to limited breeding areas in both space and time (Forcada & Trathan 2009), and are likely to be affected by climate change (Boersma 2008). Observing penguins foraging at sea is challenging, especially as the spatiotemporal distribution of their prey is poorly understood, there is a need to understand the basic foraging biology of penguins (Forcada et al. 2006) as well as how they might respond to changing climate and prey distribution (Forcada & Trathan 2009).

Macaroni penguins are in decline (IUCN 2008), but while the population declines are well documented at a number of colonies (Trathan 2004, Woehler et al. 2001), most of the important life history occurs at sea. This limits our ability to record and calibrate movement or activity with specific behaviour. A number of outstanding questions exist that cannot be answered with the techniques currently employed by latter day foraging studies. Therefore, much of this thesis has been concerned with finding suitable techniques and using them to answer questions about macaroni penguin foraging behaviour. In particular, what determines foraging success? This is an important issue in

light of macaroni penguin population decline (Trathan et al. 1998; Trathan 2004) and in the context of potentially expanding fisheries around South Georgia (Hill et al. 2007, Hill et al. 2009).

Chapter 2: Calibrating an appropriate sex test for penguins

The ability to identify sex is central to a wide range of studies, including behavioural ecology (Ellegren & Sheldon 1997), and has been central to testing theories of sex allocation and behaviour in wild populations of a range of bird species (Sheldon 1998). Given previously reported sex differences between on-land (Williams & Croxall 1991) and at-sea behaviours in macaroni and Adélie penguins (Williams & Croxall 1991, Clarke et al. 1998, Barlow & Croxall 2002, Green et al. 2007), there is a clear need to accurately determine sex in penguins.

This study (Hart et al. 2009), shows that there is a considerable overlap between morphological traits previously considered to be distinct. The larger sample size of Hart et al. (2009) than of Williams and Croxall (1991), combined with the molecular verification, shows considerable overlap in traits between sexes that had not previously been reported in penguins. We also demonstrate the importance of cross-validation in discriminant analyses. The relatively high rate of mis-classification of sex that we found (Hart et al. 2009), means that in a number of studies; birds scored as female may be younger or smaller males.

This study and others that have identified calibration as a problem (Barrett et al. 1989; Hamer & Furness 1991) help to identify ways in which the mis-assignment problem can be mitigated. Firstly, the sample size in calibrations should be large, and cross-calibrated with molecular sexing, which is inherently more accurate (Griffiths et al.

1998, Fridolfsson & Ellegren 2000). Genetic-based sex tests are derived directly from a locus linked to the sex-determination gene (Fridolfsson & Ellegren 2000), so should be used to calibrate morphometric tests at novel colonies if molecular sexing is not used exclusively. Molecular sexing is expensive and not possible in the field, so it is less useful in field studies than the quick and simple morphometric test. However, it is often simple to take a DNA sample in the field that can validate sexing at a later date. The error curve reported in Chapter 2 could be used to target this relatively slow and expensive test to those birds likely to be mis-assigned. In the absence of molecular testing, behavioural activity patterns at nesting must be used to calibrate morphometric tests.

Chapter 3: The use of Autocorrelation in diving behaviour.

Periodicity and change over time are defining characteristics of dive behaviour, but the temporal nature of diving behaviour has been greatly under-represented in diving studies. The impetus of this study (Hart et al. 2010) was to include the temporal nature of diving behaviour, which includes not just the time of day, but an analysis of how past states influence future behaviour. This was achieved by modelling the autocorrelation signature and comparing this with conventional analyses. The use of ACF is a novel approach for diving studies and behavioural studies in general.

While non-independence has been identified as a potential confounding problem in analyses, this non-independence has rarely-if ever, been modelled as a point of interest. We therefore modelled behaviour by autocorrelating sequential dives and comparing the parameters of this ACF fit. A number of these parameters varied between groups of foraging penguins. Namely these were the *amplitude*, *frequency* and *decay* (for definitions, see Chapter 3). These parameters represent new measures that been reported

(Tremblay & Cherel 2003), but not previously used for analysis, such as the relationship between this dive and the next (*amplitude*) and the persistence of a correlation (*decay*). This shows that there is additional inference to be obtained from the persistence and cyclical nature of behaviours not usually captured by conventional dive analysis. Comparing the autocorrelation reveals clear differences in behaviour during trips, which may be due to physiology or strategy.

The ability to successfully raise a chick depends upon the ability of the parents to find prey in sufficient numbers or density within range of the colony so that a chick can be fed at regular intervals (Cushing 1990, Tremblay & Cherel 2005, Durant et al. 2005). This study found that breeding season (year) had an effect on amplitude, frequency and decay. The effects of time of day and time during the breeding season have been well reported (Barlow & Croxall 2002), and while inter-annual effects have been noted in macaroni penguins (Green et al. 2005) and other species (Croxall et al. 1998, Reya Rey et al. 2007), few studies have successfully tracked at-sea behaviour over five years (Miller et al. 2009).

There have been large inter-annual and seasonal differences reported in krill abundance and distribution around South Georgia (Ried et al. 1999, Trathan et al. 2003, Saunders et al. 2007). It seems reasonable to assume that prey distribution will be reflected not only by individual dive profiles (Takahashi et al. 2004), but also in the strategies that penguins use to find them. It is therefore logical that we should employ statistical techniques capable of detecting these different strategies. Comparing the breeding phenology and foraging behaviour with the pattern of krill abundance reported by Saunders et al. (2007) suggests that differences in diving behaviour are likely to be

environmentally driven, and suggests that penguins match the timing of breeding such that the chicks hatch and grow at their fastest rate when krill are most abundant (Hart et al. 2010). It is also interesting that the chicks fledge while krill are still sufficiently plentiful to allow the fledglings to forage at first independence and put on weight for winter during which their metabolic needs will increase (Green et al. 2002, Green et al. 2005). It is important to note that this study found no sex differences between foraging penguins. This contrasts with observations in other studies of dive depth (Green et al. 2005) and foraging location (Barlow & Croxall 2002, Mori & Boyd 2004).

The autocorrelation approach used here is flexible to compare signals at a range of scales, with summary statistics for an entire time series, whether these time series consist of days, weeks or months. Since the deployments of TDRs used in this study, archival biologging devices have increased in their power such that they are able to record diving behaviour over a complete year (Green et al. 2005).

Modelling the autocorrelation of time depth data is a good way of producing a number of summary statistics, directly comparable between a range of taxa and studies. As such, it could provide a useful monitoring tool. Current indices of species vulnerability use environmental variables as a proxy for habitat change or degradation (Brooks et al. 2006). However, assessing behaviour can show important changes in behaviour that can highlight potential problems for species before changes become apparent in measures of population size or reproductive success.

While five years represents a short time-series, this is longer than other studies, with the recent exception of Miller et al. (2009). Continuing this data set, especially across a range of krill years from poor to good could allow the penguins in this study to

be used as sentinels of change in the prey abundance and distribution.

Chapter 4: Behavioural switching

How units of activity cluster into behaviours is a ubiquitous problem in studies of animal behaviour. If behavioural studies are to become more important for informing conservation management, then this type of problem must be addressed. While the autocorrelation technique discussed above is a flexible and useful tool for generating summary statistics of a range of behaviours, they average short-term deviations from the main periodic signals (Sharifzadeh et al. 2005). These deviations may be equally as important as the dominant signal for understanding the behavioural repertoire of foraging penguins.

Hidden Markov Models are a powerful analytical tool for understanding behavioural patterns and the transitions between these. They have been used to advance understanding of the mechanisms that underlie observed behavioural switches in the locust *Locusta migratoria* (MacDonald & Raubenheimer 1995), caterpillar *Helicoverpa armigera* (Zucchini et al. 2008) and homing pigeon *Columba livia* (Guilford et al. 2004), but have not been applied to behavioural studies in marine animals. The use of Hidden Markov Models to partition observed diving patterns into behavioural states, then analysing the transition rates between behaviours, is novel and could be applied to a wider range of behavioural data. Other studies have placed an emphasis on lateral spatial analysis on the surface of the earth (Roberts et al. 2004, Jonsen et al. 2006), but HMMs can equally be applied to time series of any behaviour in any dimension such as diving.

While this has identified two behaviours in diving penguins, it is limited by being two dimensional. Each dive had a probability of being in either state, and to avoid

repeating the problems of bouts identified (Hart et al. 2010), this study did not resolve groups of dives into clusters. One of the interesting features of this analysis is that there are a small number of dives that do not fall into clear states. As more variables are recorded, these dives could also be incorporated into models. For example, with the additional variable of mandible angle (Takahashi et al. 2004), a dive that does not fall clearly into a behavioural state could be explained as an abortive dive where a prey cluster was missed.

Secondly, there is the limitation of not being able to discriminate between success and failures on individual dives. Recent studies have successfully calibrated wing beat frequency with metabolic rate (Shepard et al. 2009), such that the metabolic rates of individual dives can be calculated. To produce a budget and decision matrix of foraging penguins, we need to determine how long birds persist with a behaviour when it is profitable compared to unprofitable to understand the cost-benefit trade-off and any marginal benefit (Mori & Boyd 2001). Without better indications of prey chase or prey capture, we are not able to determine this.

Ch 5: Daily foraging periods in macaroni penguins.

While a number of studies have tracked where penguins forage (Barlow & Croxall 2002, Trathan et al. 2006, Trathan et al. 2008), or the vertical foraging behaviour of penguins, there have been few attempts to quantify daily activity into a time budget, and look at how the daily strategies of foraging penguins vary. The Hidden Markov Models described above were useful to overcome the problem of classifying dives into runs of behaviour. Summing the amount of time spent in each dive state permitted three hypotheses to be tested about trade-offs made by foraging penguins. These were whether

foraging time is negatively correlated with commuting time, i.e.: whether there is a set time allocation at sea per day. Further to this, we tested whether the amount of time spent foraging correlated with adult weight gain and chick weight gain. Durant et al. (2005) argued that to raise young, a central place forager needs abundant food of sufficient quality close enough to the origin to permit regular return trips.

Partitioning observed diving into foraging and travelling revealed that incubation and premoult birds showed the longest foraging periods and the largest rates of mass gain. While there is an overall positive relationship between foraging time and mass gain, broodguard and crèche animals clearly have their dive periods limited by their need to return to land and feed their chick. Negative mean daily mass changes of both parents during broodguard and crèche suggests that birds are losing condition in these stages in order to raise a chick, as much of the food is passed onto the chick (Williams & Croxall, 1991, Cresswell et al. 2007). This is demonstrated by the link between annual chick fledging mass and the rate of mass gain of their parents during the chick rearing period. Years of mean negative mass gain in adults resulted in the heaviest chicks. If heavier birds are not foraging more than light birds and those that are apparently more successful are spending less time foraging, then it seems likely that adults adjust their investment in the chick in response to foraging success.

General conclusions

Given the increasing gap between what is understood about the biology of marine ecosystems and what needs to be urgently understood to combat the increasing threats to them, a step change is necessary in our understanding of marine species. This thesis applied several novel techniques to data that are very commonly collected. This shows

that even in a well-studied species such as the macaroni penguin, there are considerable advances that could be made through new types of analysis, in particular time series analysis.

Since the advent of bio-logging tags, advantages in these technologies have revolutionised the study of movements and feeding behaviour of marine predators. On-board processing, low-power satellite transmitters and miniaturized physical and biological sensors have enabled researchers to deploy increasingly advanced instrument packages on an increasing variety of marine species. While deployment packages have become more sophisticated and longer lasting, the average size of these devices has reduced and the application of animal welfare practice improved. Through bio-logging, we now have very useful information on the patterns of habitat use in time and space. However, we still need to determine the environmental drivers behind these observed behavioural patterns. The use of analytical tools such as Hidden Markov Models will allow us to elucidate the rules and trade-offs that govern how animals use their environment. As noted in Chapter 1, animal-based measurements can collect a suite of information wherever an animal is, but tell us nothing about the gaps. Therefore, while bio-logging provides excellent physical, chemical and biological information to the ocean science community, a strategy is necessary to fill these gaps rather than merely collecting more data in the same way. The progress made in this thesis towards understanding the behaviours underlying the pattern demonstrates the merit in looking to new analytical tools to augment those in common use.

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