Age determination and establishment of reproductive condition from marine mammal biopsy sampling

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ABSTRACT

Management of marine mammals is often constrained by limited knowledge of reproductive variables and / or age-specific survival rates. I reviewed the use of analyses of tissue samples obtained through biopsy sampling to determine reproductive condition or age of marine mammals, free-ranging cetaceans in particular. Literature searches show that reproductive condition has been successfully determined from blubber biopsies for one species, while no study used such biopsies to determine ages of individuals. I suggest that hormonal assays using muscle or blubber tissue obtained from biopsies could easily be used to determine reproductive condition. However, a study needs to be conducted to define age-related changes in selected hormones if hormonal assays of tissues obtained from biopsies are to be used in marine mammal age determination.

Keywords: marine mammals, age determination, reproductive condition, biopsy sampling, conservation management.

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1. Introduction

Knowledge of marine mammal life-history and general biology is often limited. For instance, most of the current understanding of marine mammal physiology is based on observations of captive individuals (Dierauf & Gulland 2001). Understanding of free-ranging marine mammals has been limited largely to bio-geographical scales of marine mammal population structures (e.g Baker & Medrano-Gonzalez 2002; Hoelzel 2002). In some instances, particularly with the pinniped and delphinid species, demographic models are commonly used to study the dynamics of their populations (Barlow & Boveng 1991).

However, values for demographic variables of marine mammal species, in particular the cetaceans, are mostly educated guesses because of the difficulty of estimating these variables. For non-marine species, modellers have used species' populations with good data as surrogates to model another species' population (e.g. Gage & Dyke 1988). Few similar surrogate examples exist for marine mammals, but recently the National Institute of Water and Atmosphere (NIWA) has developed a surrogate population model for Maui's dolphin (*Cephalorhynchus hectori maui*) based on life-history knowledge of Hector's dolphin (*Cephalorhynchus hectori hectori*) (Sam Ferreira, Department of Conservation, Auckland, pers. comm.). There are likely to be some risks associated with surrogate approaches to population dynamics. For highly threatened species, such risks could affect the persistence of the species.

The Department of Conservation (DOC) has a mandate under the Marine Mammal Protection Act (1978) to conserve New Zealand's marine mammal populations. This is a daunting task considering that 45 cetacean species frequent New Zealand coastal waters (Baker 1999), of which at least three species or sub-species are threatened and endemic. Furthermore, some marine mammal species are taonga for some tangata whenua and are usually highly valued by New Zealanders in general. Some marine mammal populations are now also important economically, forming the basis of a number of tourism ventures (e.g. whale watching, swim-with-dolphin tours etc.). Maintaining populations of marine mammal species is therefore important from scientific, cultural and aesthetic perspectives.

Normally, management of threatened species focuses on factors affecting one or two key demographic aspects that are known to result in negative population growth rates. For instance, the establishment of a Marine Mammal Sanctuary at Banks Peninsula along the east coast of the South Island was directed at reducing mortality of both adult and juvenile Hector's dolphins (Dawson & Slooten 1993). Identifying the most obvious threats to marine mammals is often readily achievable (e.g. Martien et al. 1999), particularly for species with easily measurable demographic variables. In general, marine mammal species management is constrained by the aspects that are poorly known and not readily measurable and could benefit from the development of methods to determine age-specific variables such as survival and reproductive rates.

Recently there has been an increase in genetic studies using samples collected by biopsy techniques (e.g. Pfeiffer 2002). Following on from this, it has become

evident that other information could also be gained from these samples. For instance, biopsy sampling could be used for regular assessment of disease status in populations (Kennedy-Stoskopf 2001) or levels of toxic pollution (O'Hara & O'Shea 2001). Considering that demographic models explicitly represent age-structure (Barlow & Boveng 1991) and associated age-specific survival and fecundity schedules, it is likely that the potential exists to use biopsies to estimate such demographic variables for free-ranging marine mammals, cetaceans in particular.

I reviewed the literature on biopsy sampling and associated reproductive condition and chemical age-determination techniques. I found that reproductive condition was successfully assessed for one marine mammal species using hormonal assays, but could find no report of the age of marine mammals being determined using biopsy sampling. Here I report on my assessment and provide recommendations on the use of biopsy sampling to estimate reproductive condition and age.

2. Material and methods

I searched eight scientific journals including Marine Mammal Science, Journal of Mammalogy, Journal of Endocrinology, Journal of Molecular Endocrinology, Journal of Reproduction and Fertility, Reproduction, Reviews of Reproduction and Biology of Reproduction as sources for studies on age-determination and / or reproductive condition and focused on papers published since 1995. I expected that cross-references in manuscripts published in the above journals would provide further references.

In addition, I was aware that developing techniques are often not reported on in journals early in their development, and therefore conducted an internet search for potential additional sources of information. In some instances, my searches produced summaries of the interests of researchers that were relevant to my interests. I contacted some of these people and discussed with them potential uses of biopsy sampling techniques.

3. Results

3.1 AGE DETERMINATION

Age determination in marine mammals has been based on counts of growth layers deposited in persistent tissues such as teeth (e.g. Hohn et al. 1989; Oosthuizen 1997; Pinedo & Hohn 2000) or bone (Klevezal 1996; Marmontel et al. 1996). Body size has also been used as a rough estimate of age (Stevick 1999). These techniques are all suitable for age determination of dead or captive specimens, but are difficult or impossible to apply to free-ranging marine mammals, especially cetaceans.

I could find no evidence of age determination using some form of chemical technique that may be applicable to biopsy sampling. Molecular methods to determine gender are relatively well developed (see Shaw et al. 2003), while molecular methods (amino acid racemization) have been used for the determination of sample ages of dead tissue, e.g. forensic samples (Ritz-Timme et al. 1999). As far as I know, no attempt has been made to develop racemization techniques applied to biopsy samples to assess age of individual living marine mammals. The influence of aging on DNA polymerase *a* has been investigated in humans, but the results were influenced by diet (Srivastava et al. 1999). We therefore believe that molecular techniques will be of limited value in developing chemically-based age-determination techniques using biopsy samples from living marine mammals.

Hormonal assays have the potential to be developed into age-determination techniques. For instance, St Aubin et al. (1996) reported that concentrations of thyroid and adrenal hormones changed with age in Atlantic bottlenose dolphins (*Tursiops truncates*). However, St Aubin et al's (1996) analysis was based on blood samples and the exact relationship between age and hormonal concentrations has not been defined. One hormone which could be investigated as an age-determination method is growth hormone. Favier et al. (2001) reported that growth hormone concentrations change with age in young dogs. To date, I know of no studies on marine mammals that have assessed changes in hormonal concentrations as individuals get older.

3.2 REPRODUCTIVE CONDITION

Knowledge of cetacean reproduction is limited and is largely based on necropsies of stranded or killed whales (e.g. Iga et al. 1996), observations of individually identified free-ranging animals (e.g. Chapman & Mayo 1987; Steiger & Calambokidis 2000) or tissue samples from captured (e.g. Sawyer-Steffan et al. 1983; Robeck et al. 2001), killed (e.g. Kjeld et al. 1992) or stranded (e.g. Carballeira et al. 1987) animals. A number of tissues have been used for hormonal studies, but blood has most commonly been used for these (e.g. Sawyer-Steffan et al. 1983; Schneyer et al. 1985; Kjeld & Olafsson 1987; Kjeld & Theodorsdottir 1991; Kjeld et al. 1992). Reproductive condition has also been evaluated using analysis of milk (West et al. 2000) and urinary steroids (Walker et al. 1988; Robeck et al. 1993). For pinnipeds, analyses of saliva and faecal samples have proven useful for evaluation of steroids (Pietraszek & Atkinson 1994).

However, very little use has been made of hormonal assays using biopsy samples collected from live, free-ranging animals. Yoshioka et al. (1994) developed a technique to quantify muscle progesterone that successfully distinguished between pregnant and non-pregnant minke whales. The muscle technique is limited, as biopsy dart samples from free-ranging cetaceans often consist of skin and blubber (Brown et al. 1991; Brown et al. 1994), and the muscle-assay would therefore depend on development of a suitable biopsy technique that targets muscle tissue (Yoshioka et al. 1994).

I am aware of one study that used blubber samples to determine pregnancy status (Mansour et al. 2002). In this study it was shown that progesterone can be extracted from the blubber of minke whales efficiently enough to allow pregnant and non-pregnant individuals to be identified. Information from this technique could be used in the management of a number of cetacean species.

4. Discussion

Information on age-distributions and reproductive conditions of free-ranging cetacean populations are limited because of the limited techniques available to estimate these variables. As a result, most cetacean management relies on some type of interaction model (e.g. Martien et al. 1999), or life-history information obtained from dead specimens, or incorporates survival and reproduction schedules in a single measure such as population growth rate.

However, cetaceans are primarily threatened through the effect of human activities on either survival or reproductive output. For example, commercial whaling (e.g. Evans 1987), subsistence whaling (e.g. Mitchell & Reeves 1980; Soegiarto & Polunin 1982; Hertz & Kapel 1986) or hunting of small cetaceans (e.g. Smith 1982; Perrin 1985; Price 1985) including live capture for dolphinariums (see Cornell 1984), are all activities that will significantly influence adult survival rate. In some situations smaller cetaceans are hunted or harassed because of their perceived effect on a common food source (e.g. Miyasaki 1983). Also, incidental catches during commercial fishing operations have been identified as one of the key factors causing declines in populations of some smaller cetacean species (e.g. Smith 1983; Gaskin 1984; Martien et al. 1999). These activities all influence populations primarily through increasing adult mortality.

Human disturbance (e.g. harassment resulting from marine mammal tourism) may have consequences for population dynamics because of effects on behaviour (e.g. Constantine 1999). This sort of disturbance can change feeding and breeding behaviour, e.g. by affecting reproductive schedules.

Since the 1980s, pollutant levels in cetaceans have been studied and reviewed (Gaskin 1982). The most severe effects of pollution may have consequences for adult survival, but at lower levels the most significant impacts may be on reproductive outputs.

Establishing ways to measure survival and reproductive schedules for free-ranging cetaceans may therefore greatly aid managers in pinpointing key aspects, such as harassment or pollution, affecting the persistence of species.

My review suggests that reproductive condition of individual marine mammals could be determined through hormonal assays of tissue (blubber or muscle) samples obtained using biopsy sampling techniques. This could allow productivity to be estimated for at least a broad adult age-class in a population. Reproductive condition information can also be used to determine age-classes of marine mammals. Note, however, that sample collection needs to be appropriate, as the assay may be restricted by the type of tissue sampled (Yoshioka et al. 1994).

In contrast to the successful determination and potential determination of reproductive condition, age determination of live, free-ranging cetaceans has never been attempted using biopsy sampling techniques. However, some associations of hormonal concentrations with age in terrestrial mammals suggest that assays of selected hormones present in tissue or blubber samples may have potential as an aging technique for marine mammals. At present, the lack of understanding of age-related changes in hormonal concentrations in marine mammals is the prime limitation to development of such a technique.

I believe that biopsy sampling can produce a snapshot of the demography of a population by providing information on age distribution, sex ratios, fecundity and survival. This could be useful to managers as it could easily contribute to a Leslie-Matrix or individual-based probability model predicting growth rates and variables in populations that are most sensitive to changes in environmental conditions.

5. Recommendations

It may be possible to use hormonal assays of tissue samples collected through biopsies to determine age of free-ranging marine mammals. I am aware of muscle and blubber hormonal assay techniques to determine pregnancy status in free-ranging cetaceans. I suggest that, as a first step to using hormonal assays to determine age:

1. A theoretical framework be developed for the likely hormonal changes from birth to death for marine mammals,
2. A study be initiated to determine the potential relationship between these hormonal concentrations and age of an individual. The study should have a two-pronged approach:
   a. Collection of tissue biopsies from known-age captive individuals (cetaceans in particular) across the world.
   b. Collection of tissue biopsies from stranded individuals in association with collection of teeth or bone samples to use in traditional aging techniques.
3. A study be initiated to develop hormonal assays (both muscle and blubber) from biopsies for a variety of species. This study should also have a two-pronged approach:
   a. Collection of biopsy samples from captive individuals (particularly cetaceans) where the reproductive status is known across the world.
   b. Collection of biopsy samples from stranded individuals in association with autopsy and necropsy results on reproductive status.

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