APPROACHES TO UNDERSTANDING THE POPULATION DYNAMICS AND BEHAVIOUR OF *SEPIA APAMA* IN NORTHERN SPENCER GULF

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Nicholas Leslie Payne
October 2010

Cover image: a pair of giant Australian cuttlefish, *Sepia apama*, at the Point Lowly breeding grounds. Photo credit: Nicholas Payne.

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ABSTRACT

Understanding the behaviour, physiology and energetic status of organisms in their natural environment is fundamental to ecology. For the unique breeding aggregation of giant Australian cuttlefish *Sepia apama* in northern Spencer Gulf, South Australia, a poor understanding of population dynamics and field energetics represents a hindrance to effective management. We used a combination of approaches to address these shortcomings, and to provide for a greater understanding of this species at the individual and population levels.

Although the operational sex ratio (OSR) of the aggregation is strongly male-biased, it is not known whether this is a result of disparate residence times between sexes, or a male-biased adult sex ratio (i.e. a relative paucity of females); such information is critical for predicting population viability. Using acoustic telemetry, we compared residence times between sexes, and found that the adult sex ratio is likely unbiased, with the skewed OSR due to gender differences in breeding durations. Regardless of sex, the relative brevity of residence times (compared to the 4-month breeding season) suggests that annual density-based population surveys may significantly underestimate population size.

Given the semelparous, protein-catabolising reproductive strategy of *S. apama*, knowledge of energy expenditure rates should provide for estimates of maximum breeding durations. With a view to better explain the relatively short breeding durations, the emerging ‘accelerometry’ technique (which measures partial body acceleration as a proxy of metabolic rate) was used to estimate metabolic rate and describe activity patterns of *S. apama* during breeding. Daily energy budgets allowed protein catabolism rates to be estimated, and these rates correlated well with observed breeding durations. Accelerometry also revealed significantly higher activity levels during the day, which is consistent with the visual mating strategy of this species.

Examining rhythmic activity patterns is common among marine ecologists, and with acoustic telemetry, a leading approach is to search for rhythmic patterns in the relative frequency of acoustic detections. We compared patterns in detection frequency from tagged cuttlefish with those of fixed-location reference tags, and found that strong diel patterns seen in cuttlefish tags were also seen for the fixed-location tags. We used these results to highlight

the danger of making inferences about behaviour without controlling for external factors such as wind speed, water currents and biological noise.

Whilst acoustic telemetry and accelerometry can provide valuable information for mature individuals, alternative techniques are required for understanding the dynamics of early life-history stages of cephalopods. Significant advances in chemical mass-marking techniques have recently been made for juvenile and larval fish, but little attention has been paid to developing similar techniques for cephalopods. We evaluated the use of ^{137}Ba for mass-marking *S. apama* eggs, finding that several combinations of isotope concentration and immersion time produced high mark success rates, but that the mark was only incorporated in later developmental stages. The development of this technique will hopefully provide the impetus for field application of mass-marking techniques to *S. apama* and cephalopod populations globally.

In summary, several different approaches were described in an effort to improve our understanding of the population dynamics, energetics and behaviour of the *S. apama* breeding aggregation. Observing the dynamics and behaviour of marine animals has traditionally been difficult, but further development of techniques such as those employed throughout this thesis promises to provide important insights into the operation of cephalopods and fish worldwide.

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CHAPTER 2

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CHAPTER 3

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CHAPTER 4

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CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Understanding the behaviour, physiology and energetic status of unrestrained organisms in their natural environment is fundamental to basic and applied ecology (Cooke *et al.* 2004). In part, this is because many ecological processes that determine population size and dynamics are explicitly behavioural (Chesson & Rosenzweig 1991; Houston & McNamara 1997; Levin *et al.* 2000). Despite widespread recognition that behaviour can profoundly influence population dynamics, studies that empirically link behaviour to demography are scarce for many systems (Anholt 1997; Gerber 2006), and in the aquatic realm, difficulties associated with field observation have traditionally complicated the ability to create these linkages. In more recent years however, a suite of indirect-observational methodologies have been developed that have provided significant advances in our understanding of behaviour, population dynamics, and movements of aquatic animals.

Bio-telemetry (or where data are logged, bio-logging) has emerged as a central tool for the remote monitoring of physiological and behavioural variables in a wide range of taxa (Cooke *et al.* 2004; Ropert-Coudert & Wilson 2005; Rutz & Hays 2009). The term bio-telemetry refers to the use of miniaturised tags that are attached to animals, and used to log or relay information about an animal's movements, behaviour, physiology and/or environment (Rutz & Hays 2009). For aquatic animals, developments in the field of telemetry have enabled a range of applications, including the identification of fish home ranges and habitat use (Bellquist *et al.* 2008; Papastamatiou *et al.* 2009), habitat connectivity (Pecl *et al.* 2006; Semmens *et al.* 2010), estimates of swimming speed, locomotor activity and costs of migration in the field (Rand & Hinch 1998; Hinch *et al.* 2002; Pon *et al.* 2009), and diel activity patterns (Andrews *et al.* 2009; Blumenthal *et al.* 2009).

Most animal taxa display rhythmic patterns of activity, and describing these patterns has been the focus of a wide range of marine research (Hunter *et al.* 2004; Sims *et al.* 2005; Hays *et al.* 2006; Shepard *et al.* 2006; Andrews *et al.* 2009). With acoustic telemetry, researchers typically examine the relative frequency of acoustic detections from tagged animals, and use these patterns to make inferences about animal behaviour (e.g. Heupel *et al.* 2006b; Collins *et al.* 2007; Collins *et al.* 2008; Kawabata *et al.* 2008). However, factors such as biological noise, wind and current speeds strongly influence detection efficiency (Heupel *et al.* 2006a;

Simpfendorfer *et al.* 2008), and these often occur with diel, tidal and seasonal rhythmicity. As such, correctly interpreting patterns in animal activity requires controlling for those factors (e.g. biological noise, wind speeds etc.) that influence detection frequency in the absence of animal behaviour. To date, no published studies report the use of controls to separate the influences of animal behaviour and environmental (i.e. abiotic) factors on detection frequency, and this compromises the interpretation of animal activity patterns. The emerging 'accelerometry' technique relies on the relationship between body acceleration and energy expenditure and has strong potential to estimate activity patterns, field metabolic rate and swimming speed in a range of taxa (Wilson *et al.* 2006; Green *et al.* 2009; Halsey *et al.* 2009a). Most accelerometry studies have focussed on humans or birds (Eston *et al.* 1998; Yoda *et al.* 2001; Mathie *et al.* 2004; Johansson *et al.* 2006; Wilson *et al.* 2006; Green *et al.* 2009; Halsey *et al.* 2009a; Halsey *et al.* 2009b), and whilst two recent studies have evaluated its use with captive sharks (Whitney *et al.* 2007; Gleiss *et al.* 2010) the technique is yet to be used to estimate field metabolic rate for aquatic taxa.

The complexity of data and logistical requirements vary widely across bio-telemetry/logging techniques, from the relatively simple presence/absence data recorded from acoustic curtains and arrays (Pecl *et al.* 2006; Collins *et al.* 2008; Semmens *et al.* 2010) to the more complex heart-rate and accelerometry techniques (Green *et al.* 2001; Hinch *et al.* 2002; Green *et al.* 2009). The potential applications of bio-telemetry are broad, with a variety of hypotheses tested on a range of taxa (Cooke *et al.* 2004). However, given that transmitters should represent no more than 2% of the animal's mass (Guillemette *et al.* 2002; Bridger & Booth 2003; Cooke *et al.* 2004), bio-telemetry studies remain confined to larger species, and cannot be applied to the early-life history stages of many aquatic species.

The study of otolith chemistry has emerged as a leading approach to inferring the movements, life-history and behaviour of a broad range of marine and freshwater fishes at a variety of life-history stages (Gillanders *et al.* 2003; Elsdon *et al.* 2008). Forming part of the inner ear in teleost fishes (statoliths are analogous to otoliths, but occur in cephalopods), otoliths are paired, calcium carbonate-based structures that are metabolically inert, such that many elements accreted onto the growing surface of the otolith are permanently retained throughout the life of the animal (Campana 1999). The calcium carbonate and trace elements that the otoliths/statoliths are composed of are derived primarily from the water (Campana 1999; Milton & Chenery 2001), so heterogeneity in the geo-chemical composition of aquatic environments often manifests itself in the composition of otoliths/statoliths across habitats. This property of otoliths has been the basis of studies discriminating stocks and sub-populations of fish (Edmonds *et al.* 1992; Campana *et al.* 1994), to identify anadromy (Kalish 1990; Rieman *et al.* 1994) and to determine connectivity between populations (Gillanders & Kingsford 1996; Campana *et al.* 2000; Thorrold *et al.* 2001; Gillanders 2002a). However, disadvantages of using natural otolith/statolith chemical signatures are that numerous source populations must be characterised (Gillanders 2002a), signatures are often temporally variable at a given location (Gillanders 2002b; Cowen & Sponaugle 2009), and the spatial scale over which heterogeneity in physio-chemical environments occur is too coarse to discriminate between sub-populations (Elsdon *et al.* 2008). Intentional elemental marking of calcified structures has been recognised as a useful method of mass-marking fish for some years (Ennevor & Beames 1993; Brown & Harris 1995), and field applications of mass-marking techniques has seen rates of dispersal and natal homing quantified *in situ* (Jones *et al.* 1999; Jones *et al.* 2005; Almany *et al.* 2007). Enriched stable isotopes (such as ^{137}Ba) have several advantages over other mass-marking techniques, including the relatively low concentrations required to produce marks (Munro *et al.* 2008), a lack of stress imposed by the marking procedure (Munro *et al.* 2009; Williamson *et al.* 2009b), the unequivocal nature of the marks produced (Munro *et al.* 2008), and the broad range of life-history stages that the technique could be applied to.

Whilst analysis of natural otolith/statolith chemistry and elemental mass-marking techniques may provide for inferences on movement and life-history behaviour, the temporal and spatial resolution of this information is generally far lower than for techniques such as bio-telemetry (Jones *et al.* 2009), and many physiological parameters (such as metabolic rate) cannot be estimated. As such, a combination of shorter-term bio-telemetry techniques for adult stages and otolith/statolith marking of juvenile stages will likely provide the most comprehensive understanding of population dynamics and behaviour throughout the life-history of a given species.

The giant Australian cuttlefish *Sepia apama* (Gray) is the largest cuttlefish species in the world, and forms the only known cuttlefish breeding aggregation. From May to August of each year, hundreds of thousands of *S. apama* aggregate over a restricted area of sub-tidal rocky reef near Point Lowly, South Australia (Fig. 2.1), to breed. The density of cuttlefish at Point Lowly has been recorded at up to 105 individuals per 100 m^2 (Hall & Hanlon 2002), and the operational sex ratio (OSR) is strongly biased towards males (mean 4:1, max 11:1; Hall & Hanlon 2002). This represents one of the strongest OSR skews recorded for any cephalopod population. The resulting competition between males has led to the development of spectacular and complex mating strategies and displays, and these have been well studied (Norman *et al.* 1999; Hall & Hanlon 2002; Naud *et al.* 2004; Hanlon *et al.* 2005). The sex ratio of *S. apama* in greater northern Spencer Gulf outside of the spawning season is close to unity (Hall & Fowler 2003), but it is unclear whether the skewed OSR at Point Lowly is due to a paucity of females (i.e. more males take part in the breeding aggregation than females; the adult sex ratio, ASR, is skewed), or shorter breeding durations for females relative to males. Since the adult sex ratio is a strong determinant of population and genetic viability (Gerber 2006; Vargas *et al.* 2007), understanding the vulnerability of the *S. apama* population to stochastic events is underpinned by knowledge of the ASR.

Abundance in northern Spencer Gulf begins to increase in late April and by the end of August the vast majority of cuttlefish have left the spawning grounds (Hall & Hanlon 2002). However, this gives no indication of individual residence times. Indeed, given the lack of mate-pairing, habitat guarding or parental care by either sex (Hall & Hanlon 2002), it may be that individuals remain on the spawning grounds for only a short period of time. If so, then density-based biomass surveys may significantly underestimate population size. Methods for adjusting population size estimates to account for transience do exist (English *et al.* 1992; Hilborn *et al.* 1999), but are reliant on estimates of individual transience. Such estimates are not available for the *S. apama* aggregation.

Like many other cephalopods, *S. apama* are short-lived (generally 12-24 months; Hall *et al.* 2007) and semelparous, spawning once at the end of their life-cycle. Their anaerobic capabilities are poor compared to most aquatic vertebrates (Storey & Storey 1979), they have a limited capacity to store and oxidise lipids (Ballantyne *et al.* 1981; O'Dor & Webber 1986) and when food is lacking, they catabolise their own body proteins (O'Dor *et al.* 1984; Jackson *et al.* 2004). This protein catabolism occurs during the *S. apama* breeding season, with stomach content analysis suggesting fasting, and a significant decrease in somatic condition observed as the season progresses (Hall & Fowler 2003). Assuming they do not feed, and can catabolise no more than 50% of their body weight (as is the case for *Octopus vulgaris*; O'Dor & Webber 1986), then maximum *S. apama* breeding durations will be a function of their rate of energy expenditure. Rates of energy expenditure are unknown for individuals from the breeding aggregation, and given the implications for estimates of population size, such information is critical.

Measuring larval dispersal is recognised as one of the greatest challenges facing marine ecologists worldwide (Jones *et al.* 2005; Sale *et al.* 2005), and for *S. apama*, the extent of movements of juveniles from the aggregation area remains unknown. The vast majority of viable eggs hatch by early November (Hall & Fowler 2003), and cuttlefish density at the aggregation area is very low from after hatching until late April, which suggests that early life-history stages of *S. apama* leave the aggregation area following hatching. Many cephalopod species exhibit a pelagic phase following spawning, e.g. *Illex illecebrosus* (Dawe *et al.* 2000), *Nototodarus gouldi* (Stark *et al.* 2005), *Sepioteuthis australis* (Pecl *et al.* 2006), and *Sepia officinalis* (Challier *et al.* 2005), however no evidence exists to suggest this life-history strategy for *S. apama*. Similarly, no information exists to suggest a high degree of natal homing or whether the aggregation is made of recruits primarily spawned in other areas. Field application of mass-marking techniques has seen rates of dispersal and natal homing quantified *in situ* for several fish species (e.g. Jones *et al.* 1999; Jones *et al.* 2005; Almany *et al.* 2007), however a lack of method development for cephalopods has precluded similar field applications for this group. As is the case with most cephalopod populations worldwide, a paucity of information on movement and dispersal of the early life-history stages is an obstacle to understanding population dynamics of *S. apama*.

A lack of information on the adult sex ratio, actual population size, energy expenditure and dispersal of juvenile *S. apama* is a hindrance to effective management of the breeding aggregation. Targeted fishing of the aggregation increased dramatically throughout the 1990s, which is likely to have significantly decreased the aggregation biomass during this period (Hall 2002). In response to concerns over the vulnerability of the population, a partial fishing closure was implemented for the aggregation area in 1998, and this was extended temporally and spatially (to include the entire aggregation area, and remain in place year-round) in 2004. Despite this, annual density-based population surveys suggest an overall decline in biomass from 2001 to 2008 (Hall 2008), and interpreting the nature of this apparent decline is difficult without an understanding of population dynamics and behaviour.

The key objectives of this thesis are to:

- (i) provide a better understanding of the adult sex ratio and population size of the *S. apama* breeding aggregation,
- (ii) describe activity patterns and energy expenditure of *S. apama* in the field, and
- (iii) develop a mass-marking technique for the early life-history stage of *S. apama*.

Notes on chapter style

Chapters 2-5 of this thesis present original data written in a style suitable for publication in scientific journals. Whilst I have attempted to maintain a logical flow of ideas throughout the thesis, each chapter can be read independently. Tables and figures are imbedded within relevant chapters, and cited references are listed at the end of the thesis. The objectives of chapters 2-6 are as follows:

Chapter 2

Adult sex ratios and population size are fundamental population parameters, underpinning the genetic and demographic viability of a population. The highly transient *S. apama* breeding aggregation has a strongly skewed operational sex ratio (M:F = 4:1), and density-based survey estimates indicate significant inter-annual fluctuations in population size. However, a lack of temporal information (i.e. individual residence times) represents a significant barrier to accurately understanding population dynamics of this aggregation. We used acoustic telemetry to estimate breeding durations for male and female *S. apama*, with the aim of (i) estimating the adult sex ratio, and (ii) providing for more accurate estimates of actual population size.

Chapter 3

Understanding the energetic status of unrestrained organisms in their natural environment is fundamental to basic and applied ecology, but traditional methods of estimating energy expenditure in the field have significant limitations. We combined swim-tunnel respirometry with the accelerometry technique to estimate patterns of energy expenditure of *S. apama* during breeding. Our specific objectives were to (i) determine whether field metabolic rate approaches the aerobic maximum for this species during reproduction, a hugely expensive life-history stage, and (ii) create daily energy budgets to gain insights into the overall costs of reproduction.

Chapter 4

Acoustic telemetry has emerged as a prominent tool for identifying lunar, tidal and diel patterns of activity in aquatic animals. Typically, researchers examine the relative frequency of acoustic detections transmitted by animals over various temporal scales, and subsequently make inferences about animal behaviour. Of the suite of studies that do this, none report the use of controls (i.e. fixed-location transmitters) for separating the influences of animal behaviour and external (i.e. abiotic) factors on patterns in detection frequency. We compared patterns in detection frequency from transmitters attached to cuttlefish and those of fixed-location transmitters to show that a failure to employ adequate controls can lead to incorrect interpretations of animal behaviour.

Chapter 5

Development of techniques that investigate the movement of early life-history stages of fish and invertebrates is recognised as a high priority for marine ecologists. Little attention has been paid to the development of mass-marking techniques for the early life history-stages of cephalopods, but for fish, the use of enriched stable isotopes has emerged as a successful method of mass-marking the calcified structures of early life history stages. We evaluated the potential of marking the statoliths of *S. apama* hatchlings by immersing eggs in water enriched in ^{137}Ba. The specific objectives of this chapter were to examine the influence of (i) isotope concentration, (ii) length of immersion period, and (iii) egg developmental stage on the uptake of ^{137}Ba by the statoliths.

Chapter 6

In this chapter, I provide a brief discussion of preceding chapters, how they are associated, and directions for future research.

Scouting for subjects. Photo credit: Daniel Gorman.

CHAPTER 2

BREEDING DURATIONS AS ESTIMATORS OF ADULT SEX RATIOS AND POPULATION SIZE

CHAPTER 2 PREAMBLE

This chapter is a co-authored paper published in the journal *Oecologia*, and as such, is written in plural throughout. It is included with permission from Springer (see Appendix A), and can be cited as:

Payne NL, Gillanders BM & Semmens JM (2011) Breeding durations as estimators of adult sex ratios and population size. *Oecologia*, **165,** 341-347.

In this paper, Bronwyn Gillanders and Jayson Semmens supplied funding and assisted with study design and intellectual development. I conducted the study, collected and analysed the data, and wrote the paper.

Signatures of co-authors:

Bronwyn M. Gillanders

Jayson M. Semmens

BREEDING DURATIONS AS ESTIMATORS OF ADULT SEX RATIOS AND POPULATION SIZE

2.1 ABSTRACT

Adult sex ratios (ASRs) and population size are two of the most fundamental parameters in population biology, as they are the main determinants of genetic and demographic viability, and vulnerability of a population to stochastic events. Underpinning the application of population viability analysis for predicting the extinction risk of populations is the need to accurately estimate parameters that determine the viability of populations (i.e. the ASR and population size). Here we demonstrate that a lack of temporal information can confound estimation of both parameters. Using acoustic telemetry, we compared differences in breeding durations of both sexes for a giant Australian cuttlefish *Sepia apama* breeding aggregation to the strongly male-biased operational sex ratio (4:1), in order to estimate the population ASR. The ratio of breeding durations between sexes was equal to the operational sex ratio, suggesting that the ASR is not strongly male-biased, but balanced. Furthermore, the short residence times of individuals at the breeding aggregation suggests that previous density-based abundance estimates have significantly underestimated population size. With the current wide application of population viability analysis for predicting the extinction risk of populations, tools to improve the accuracy of such predictions are vital. Here we provide a new approach to estimating the fundamental ASR parameter, and call for temporal considerations when estimating population size.

2.2 INTRODUCTION

Simulation models are increasingly used in conservation biology to evaluate the extinction risk of populations, and to better understand the demographics of endangered species (Brook *et al.* 2000; Reed *et al.* 2002). Population viability analyses (PVA) are generally accurate predictors of population persistence (Brook *et al.* 2000), but rely on the accuracy of demographic parameters such as population size and adult sex ratios (ASRs). The population ASR is of critical importance for population viability, with the probability of persistence and population size shown to be significantly reduced with increased biases in the ASR (Vargas *et al.* 2007). The ASR will also influence the genetic viability of a population, whereby small populations with skewed sex ratios will have a lower genetic viability than unbiased populations of the same size (Gerber 2006).

Despite the significance of the ASR for viability analysis, and indeed its role in the evolution of sex roles (Cluttonbrock & Parker 1992; Kokko & Jennions 2008), there is a tendency to either ignore the influence of the ASR, or incorrectly equate it to the operational sex ratio (ratio of fertilizable females to sexually active males at the site and time of mating, OSR) (Kokko & Jennions 2008). Trivers (1972) urged researchers to investigate the influence of the ASR on the evolution of mating systems, but his plea has largely been ignored, and studies continue to focus principally on the OSR (see review by Kokko & Jennions 2008).

Studies that calculate the OSR do so by quantifying the relative proportion of sexes ready to mate at a given time, or by using the concept of 'time-in' (the fraction of a reproductive cycle when an individual is ready to mate). With this method, the OSR is calculated as

$$\text{OSR} = \frac{\text{mean male 'time-in'}}{\text{mean female 'time-in'}} \times \text{ASR}$$

(Cluttonbrock & Parker 1992; Kvarnemo & Ahnesjo 1996; Parker & Simmons 1996). Parker & Simmons (1996) demonstrated that calculating differences in 'time-in' between sexes is sufficient to predict the direction of sexual selection provided that the ASR is unbiased. However, biased ASRs are common in nature (Kruuk *et al.* 1999), and measuring the parameter directly is often difficult. Although the above relationship is generally used to estimate the OSR (and therefore predict the direction and strength of sexual selection), it should be possible to estimate the ASR of a population if the OSR and time spent mating ('time-in') between sexes are known. Here, we determine the relationship between 'time-in' of each sex and a known OSR to estimate the ASR of a unique cephalopod breeding aggregation.

The giant Australian cuttlefish *Sepia apama* (Gray) is the largest cuttlefish species in the world, and forms the only known cuttlefish breeding aggregation. From May to August each year, hundreds of thousands of mature individuals converge on a highly localised area of sub-tidal rocky reef (approximately 60 ha) at Point Lowly, northern Spencer Gulf (Fig. 2.1), Australia, to breed. The mean OSR during the breeding season is skewed towards males by 4:1 (although it can reach 11:1 near the beginning of the season; Hall & Hanlon 2002) and the intense competition between males has led to the development of spectacular and complex mating strategies and displays (Norman *et al.* 1999; Hall & Hanlon 2002; Naud *et al.* 2004; Hanlon *et al.* 2005).

Like many other cephalopods, *S. apama* are short-lived (generally 12-24 months; Hall *et al.* 2007) and semelparous, spawning once at the end of their life-cycle. Although processes of sexual selection during the aggregation are well studied in this iconic species (Hall & Hanlon 2002; Naud *et al.* 2004; Hanlon *et al.* 2005), little is known about sex ratios at birth, mortality throughout the life-history, or adult sex ratios. *Sepia apama* therefore provides an excellent model to estimate the ASR using the known OSR and differences in 'time-in' between sexes.

The aggregation site is devoid of cuttlefish outside of the four-month breeding period (late April to early September), and given that all individuals present during the breeding period are reproductively mature, we make the assumption that presence at the site during the breeding season indicates a readiness to mate – 'time-in'. This assumption was made by Hall & Hanlon (2002) when calculating the OSR, so we adopt this definition for consistency (although we acknowledge that debate exists on the most accurate way to measure population OSR; i.e. which animals are actually available to mate vs. already committed to other parental activities). By quantifying residence time as a proxy of breeding durations, we can also obtain a measure of individual transience at the aggregation, and therefore have a greater confidence in estimates of population size. In other words, where mean residence times are significantly shorter than the overall breeding period (i.e. a high degree of transience), density-based abundance estimates will underestimate population size.

Acoustic telemetry is a central tool for the identification of fish home ranges and habitat use (Bellquist *et al.* 2008; Papastamatiou *et al.* 2009), activity patterns (Andrews *et al.* 2009; Blumenthal *et al.* 2009), and habitat connectivity (Pecl *et al.* 2006; Semmens *et al.* 2010). A range of approaches are employed, including the deployment of presence/absence receiver curtains (Pecl *et al.* 2006), high spatial resolution positioning systems (Jorgensen *et al.* 2006; Tolimieri *et al.* 2009), and the emerging accelerometry technique (Tsuda *et al.* 2006; Whitney *et al.* 2007). Several studies have used acoustic telemetry to examine cephalopod behaviour during spawning periods (Sauer *et al.* 1997; Pecl *et al.* 2006; Downey *et al.* 2010), but we are not aware of any published studies that have specifically examined gender differences in breeding durations. Using acoustic telemetry, we tested the hypothesis that individual residence times at the aggregation will be higher for males than females. If the ASR of the population is unbiased (1:1), then the ratio of 'time-in' between males and females should correspond to the OSR of 4:1.

2.3 MATERIALS AND METHODS

Study site

The study was carried out at Point Lowly, northern Spencer Gulf (33°00'S, 137°44'E; Fig. 2.1), South Australia. Spencer Gulf is a relatively shallow (mean depth 22 m) inverse estuary, with salinities ranging from 36 g L^{-1} at its entrance to an annual mean of 45 g L^{-1} at its head (Corlis *et al.* 2003). In northern Spencer Gulf, water temperatures range from 12°C in mid-winter to 28°C in mid-summer (Nunes & Lennon 1986).

Sepia apama aggregate over a highly localised area (approximately 60 ha) of low relief rocky reef along the southern side of the Point Lowly peninsula, up to 150 m from shore. The highest densities of *S. apama* occur along approximately 3 km of coastline between Black Point and Stony Point (Fig. 2.1) and can be as high as one individual per square metre (Hall & Hanlon 2002; Hall & Fowler 2003).

Acoustic receivers

Twelve Vemco (Halifax, Canada) VR2W acoustic receivers were deployed throughout the aggregation site. These single channel (69 kHz) receivers consist of an omni-directional hydrophone that records the time, date and identity of digitally coded transmitters within range of the receiver. The effective detection range of receivers is variable, being influenced by environmental conditions such as wind speed, tidal movement, salinity, substrate type, and biological noise (Voegeli & Pincock 1996; Heupel *et al.* 2006a; Simpfendorfer *et al.* 2008). Range tests were conducted at a variety of locations throughout the reef prior to the study, and indicated that with prevailing wind speeds of 5-10 knots (the seasonal average), detection efficiency for tags (transmitters used were V13-1H coded pingers) was 46.6% (± 15.3 SE) 200 m from receivers, and 19.5% (± 14.7 SE) 300 m from receivers. Nine receivers were moored equidistantly along the reef between Black Point and Stony Point, such that the space between consecutive receivers was 400 m (Fig. 2.1). A further three receivers were positioned on isolated patches of reef to the east of the main aggregation area (Fig. 2.1). Receivers were moored 1.5 m from the seafloor, approximately 150 m from shore, in water

NOTE:
This figure is included on page 23
of the print copy of the thesis held in
the University of Adelaide Library.

Figure 2.1 Giant Australian cuttlefish *Sepia apama* aggregation site at Point Lowly, South Australia. Isolines indicate 47% (200 m; short dash) and 20% (300 m; long dash) detection probabilities around each receiver. The area of highest cuttlefish density is between Black Point and Stony Point. Image source: Google Earth^{TM} mapping service depths ranging from 5-9 m. Given the overlap of detection ranges, and that breeding substrate occurs only to approximately 150 m from shore, we had a high level of confidence in the ability to detect tagged cuttlefish throughout this section of reef. Data were retrieved periodically throughout the monitoring period.

Acoustic transmitters and tagging

The acoustic transmitters used in this study were Vemco V13-1H coded pingers. Each transmitter (6 g in water, 10 mm diameter, 30 mm length) emits a unique sequence of acoustic pings at a frequency of 69 kHz throughout the battery life of the tag (approximately 360 days). This sequence is repeated after a pseudo-random delay of between 60 and 180 seconds, thereby minimizing the probability of signal collision between tags.

Cuttlefish (15 to 33 cm mantle length) were caught via SCUBA and jigging and placed in 100-L holding tubs. Transmitters were secured to the interior of the mantle, ventro-laterally, by a hypodermic needle attached to the transmitter with two-part epoxy glue. The needle was pushed through the mantle and secured externally with a stainless steel crimp. Silicone washers were placed on either side of the mantle to minimise abrasion of the animal. Animals were typically released within 60 s of capture and were observed to jet strongly through the water column. This tagging technique has previously been employed in a suite of cephalopod tagging studies (Aitken *et al.* 2005; Jackson *et al.* 2005; Stark *et al.* 2005; Pecl *et al.* 2006).

Nineteen animals were tagged, six of which were females (females are distinguishable by their shorter arms and distinctive skin patterns and postures). To capture potential differences throughout the breeding period, tagging occurred on two dates; 10 animals (seven males and three females) were tagged on the 22nd of May 2008 (as cuttlefish began to arrive at the aggregation), and nine (six males and three females) on the 15th of July 2008 (during the middle of the season). The initial tagging date of 22nd May is somewhat later than the traditional start of the spawning season (late April; Hall & Fowler 2003), however very few cuttlefish were present at the aggregation in late April/early May 2008, so tagging was delayed to account for this delayed onset of spawning in this year.

Data analyses

The residence time of an individual was defined as the number of days where greater than one detection per day was recorded from its transmitter throughout the array. We also calculated residence period (the number of days between the first and last day that the individual was detected), as a period of absence from the array could have resulted from either 'time-out' (i.e. a brief cessation of mating), or from mating in an area outside the detection range of receivers. Since we could not discriminate between these two models, we report both residence time and residence period. Data up to 12 hours following release were excluded from analyses due to the possible influence of the tagging process on animal behaviour subsequent to release.

Unpaired t-tests were used to test null hypotheses that there were no differences in residence times or residence periods among sexes and tagging dates. Variances remained heterogeneous despite transformation, so heteroscedastic t-tests were performed to decrease the probability of Type I error.

For a meaningful test of whether the estimated 'time-in' ratios between sexes are equivalent to the OSR of 4:1 (and therefore that the ASR is unbiased) requires an estimate of variation in the sample ratios. To achieve this, we used a bootstrap approach, calculating the ratios of 5000 random combinations of male:female residence times (and periods). The resulting positively-skewed distribution was normalised by calculating the means of 500 samples (of $n = 19$) randomly taken from the bootstrap data. We then calculated the 95 and 70% confidence limits of the resulting normal distribution (by transforming the upper and lower boundaries of the standard normal distribution), and used these critical values to test the null hypothesis that the 4:1 OSR is indistinguishable from our generated mean ratios. The 95% limits were chosen for convention, and the 70% limits were used to reduce the probability of false retention of the null hypothesis. If the OSR fell within the range of critical values, then the 'time-in' ratios would be equivalent to the OSR of 4:1, thereby supporting the model that there are an equal number of males and females in the population (the ASR is unbiased).

2.4 RESULTS

Residence times ranged from 1 to 72 days (mean 19.0 ± 4.8 SE), and residence period ranged from 1 to 132 days (mean 30.2 ± 7.8 SE, Fig. 2.2). The majority of cuttlefish were detected consistently throughout their residence period (residence times and periods were generally similar), however there were some exceptions to this trend (Fig. 2.2). Male residence times (mean 24.69 ± 6.5 SE) were significantly longer than females (mean 6.67 ± 1.54 SE; unpaired t-test, $t = 2.70$, $P < 0.05$, Fig. 2.3a), such that the ratio of residence times between sexes was 3.7:1. Males also displayed significantly longer residence periods than females (40.07 ± 9.90 vs. 8.67 ± 2.58, respectively; unpaired t-test, $t = 2.97$, $P < 0.05$, Fig. 2.3b) with a ratio of residence periods between sexes of 4.6:1. Residence times and periods were similar for animals tagged on the 22nd of May and those tagged on the 15th July (unpaired t-tests, $t = 0.60$, $P > 0.05$ for residence time and $t = 1.47$, $P > 0.05$ for residence period).

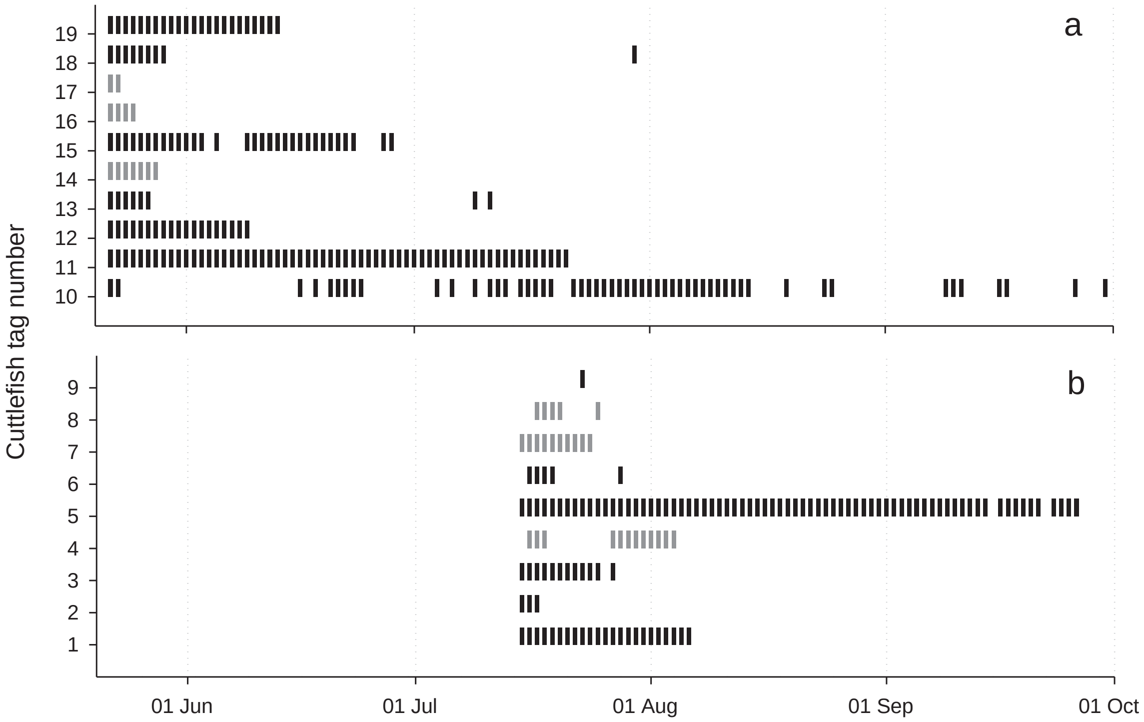


Figure 2.2 Detection summary for *Sepia apama* tagged on a) 22 May and b) 15 July 2008. Individual ticks represent days where each tagged animal was present at the aggregation; females indicated by grey ticks, males indicated by black ticks

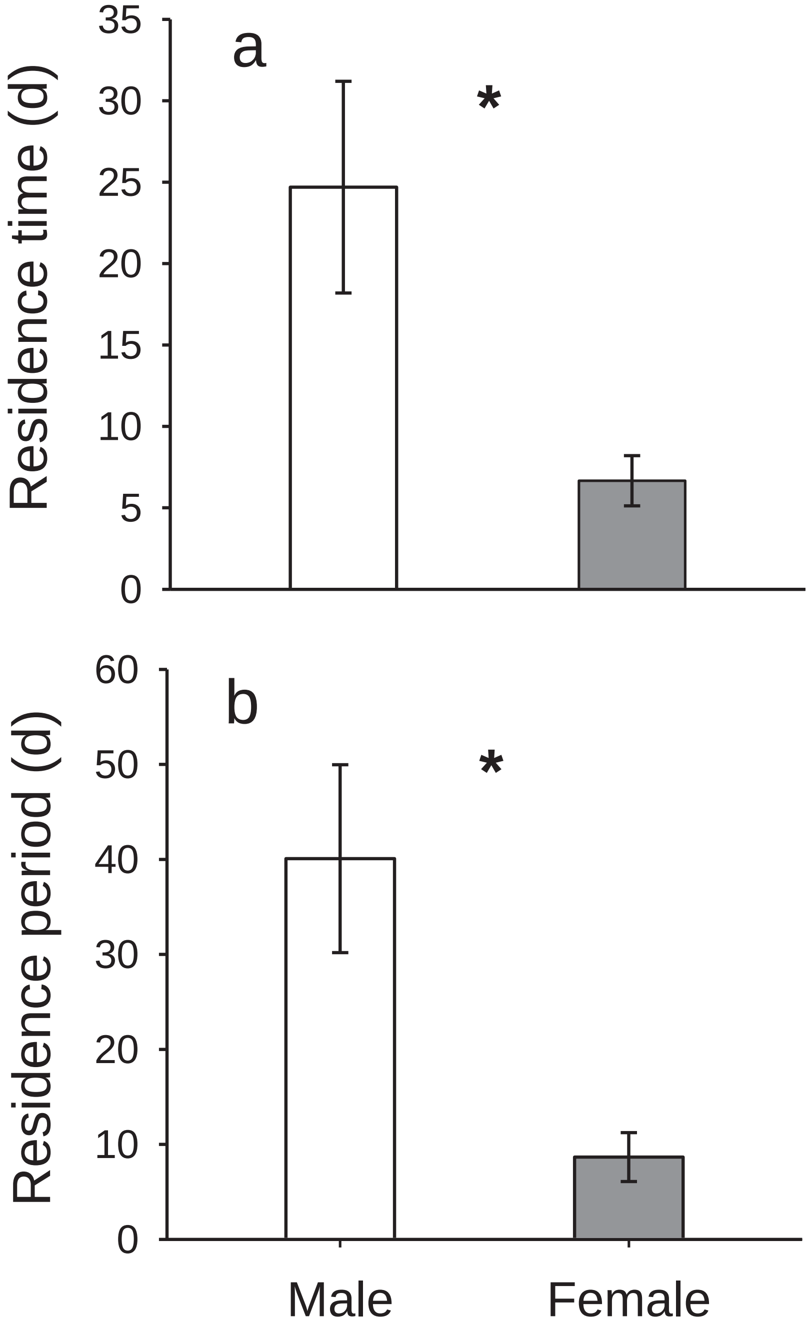


Figure 2.3 Comparison of male (n=13) and female (n=6) (a) residence times and (b) residence periods (mean ± SE) as metrics of ‘time-in’. Heteroscedastic t-tests were performed due to the heterogeneity of variances, and asterisk (*) represents significance at $\alpha = 0.05$. Note the variable scales on the y-axes

Bootstrapped data produced slightly higher mean ‘time-in’ ratios for residence time (5.29 ± 0.07 SE) and period (7.73 ± 0.11 SE). However, at the 95% confidence level, the OSR falls within the confidence limits of the standard normal distribution of estimates for residence time (2.24% to 8.33%) and residence period (3.08% to 12.37%) so we retain the null hypothesis that the OSR is indistinguishable from both ‘time-in’ ratios at this level. At the 70% level, the OSR falls within the confidence limits for residence time (3.67% to 6.9%), but outside for residence period (5.26% to 10.19%). We therefore have no evidence that the OSR is dissimilar to the residence time ratio, and would only reject the null hypothesis for residence period at the 70% confidence level.

2.5 DISCUSSION

In a population with an ASR of 1:1, any bias in the OSR should correspond to a difference in male:female ‘time-in’ of the same magnitude (Cluttonbrock & Parker 1992; Kvarnemo & Ahnesjo 1996; Prohl 2005). Given the striking congruence between the OSR (4:1) and gender differences in both ‘time-in’ metrics (3.7:1 for residence time and 4.6:1 for residence period; both of which were indistinguishable from the OSR at the 95% confidence level) for the *Sepia apama* breeding aggregation, we suggest that the ASR is indeed unbiased, and that the highly skewed OSR is a result of males displaying an extended breeding period relative to females. This is consistent with trawling by-catch data, which suggests the sex ratio of *S. apama* in northern Spencer Gulf outside of the spawning season is close to unity (Hall & Fowler 2003).

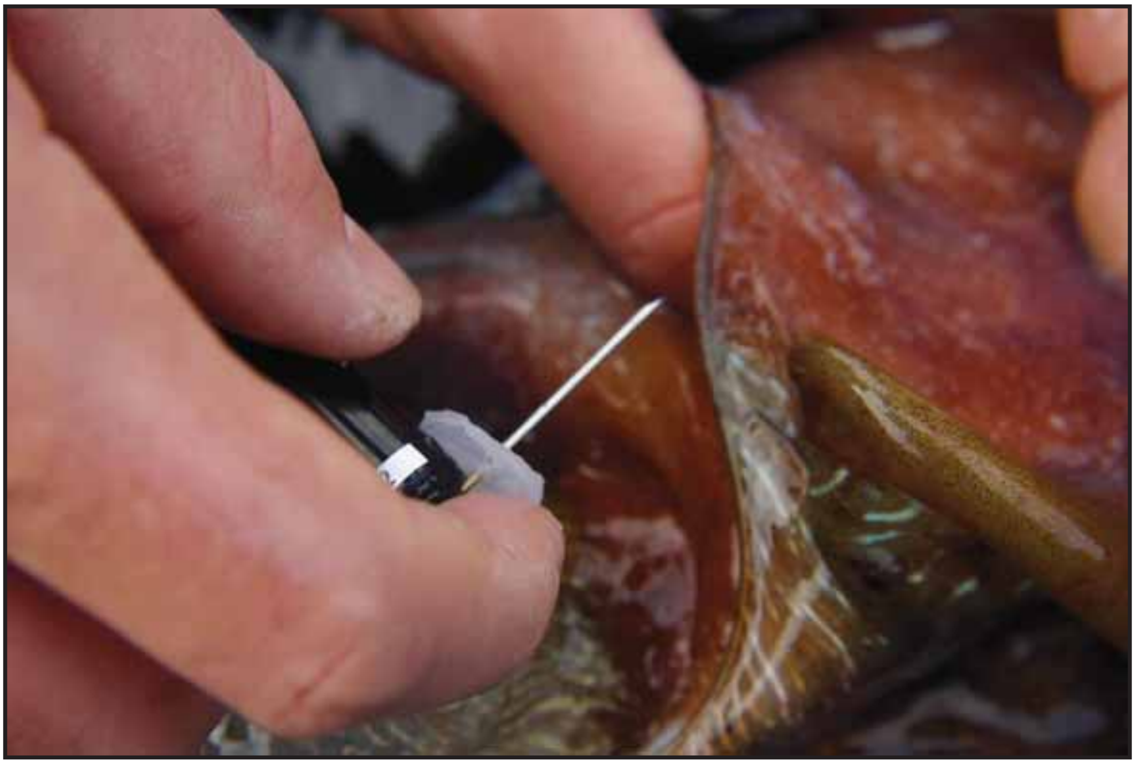
By determining the relationship between the OSR and gender differences in breeding durations, we provide a new method for indirectly estimating the ASR. This method requires the independent calculation of two parameters; 1) the OSR, by estimating the ratio of fertilizable females to sexually active males at the site and time of mating, and 2) the ratio of ‘time-in’ between sexes. Clearly, using the ratio of ‘time-in’ between sexes to estimate the OSR for our method would be inappropriate, as this requires the assumption of an unbiased ASR in the first place.

The mechanisms underlying the differences in ‘time-in’ between sexes were not addressed in this study, but are likely to be a result of differences in reproductive investment. Where there is an unbiased ASR and no post-mating parental care, anisogamy (where females produce larger gametes than males) predicts that males, having a higher potential reproductive rate, will compete for limited females, and a male-biased OSR will result (Cluttonbrock & Vincent 1991; Cluttonbrock & Parker 1992; Kokko & Jennions 2008). This, in turn, leads to increased variance in male mating success, and the selection of male competitive traits; such as the spectacular male displays that have evolved in the *S. apama* breeding aggregation.

Irrespective of sex, *S. apama* displayed lower-than-expected residence times (mean 19.0 ± 4.8 days) given the relatively long breeding period (approximately four months). Obviously individuals were present for an unknown period prior to tagging, however animals of the initial cohort were tagged at the beginning of the season, and those of the second cohort had departed well before the end of the season, suggesting that individuals were indeed resident for only a fraction of the breeding season. Given the apparent transient nature of individuals within this aggregation, our results suggest that previous density-based biomass surveys may have significantly underestimated actual population size. As proposed by Hall and Fowler (2003), an 'area under the curve' approach (English *et al.* 1992; Hilborn *et al.* 1999), which accounts for individual residence times, may provide a more accurate estimate of actual population size.

The conclusions drawn from this study are underpinned by several important, but untested, assumptions, and these must be considered: First, that the relatively small sample sizes (13 males and 6 females) are representative of the exceedingly large (> 200,000) population. The variance of residence times amongst both males and females were significant, and larger sampling effort would provide greater confidence in the accuracy of estimates of breeding durations for this species. Second, that individuals were present for negligible periods of time prior to tagging. Staggering more sampling effort across a greater variety of tagging dates, or tagging animals prior to arriving at the aggregation, would reduce the uncertainty in pre-tagged durations, and these approaches may be feasible in many other (particularly terrestrial) systems. Third, that tagging does not have a sex-specific influence on behaviour. This assumption may be difficult to test for many species, but is likely to be particularly important for those displaying high degrees of sexual dimorphism, where tags are likely to have a disproportionate influence on the physiology of each sex. Fourth, that female behaviour does not reduce the detectability of tags. Where mating behaviour differs between sexes, there must be minimal sexual bias in the method of observing breeding durations (which was acoustic telemetry in this case). Although female *S. apama* are thought to spend more time sheltering in rock crevices than males (Hall & Fowler 2003), our protocol requires the successful decoding of just two detections (out of a mean maximum of 720 per day) to define an individual as resident on any given day. Where the calculation of breeding durations requires a temporal resolution greater than daily, a different observational approach may be required. Lastly, an extrapolation of the unbiased ASR to *S. apama* in greater northern Spencer Gulf would require the assumption that Point Lowly is the only source of recruits for the region. There is some suggestion that the northern Spencer Gulf population is genetically distinct from individuals throughout the rest of its range (and indeed southern Spencer Gulf; B. Gillanders, unpublished data), and trawling by-catch data supports an unbiased ASR throughout northern Spencer Gulf. In the absence of information on recruitment and natal homing however, we restrict our conclusion of an unbiased ASR to those individuals actually involved in the Point Lowly aggregation.

With the current reliance on PVA for predicting extinction risk, methods to increase the accuracy of estimating ASRs and population size are crucial. This study provides a new approach to indirectly estimating the population ASR, and highlights the importance of incorporating temporal information into estimates of population size.



Attaching a position-only acoustic transmitter. Photo credit: Martin Bower.

CHAPTER 3

ACCELEROMETRY ESTIMATES FIELD METABOLIC RATE IN GIANT AUSTRALIAN CUTTLEFISH SEPIA APAMA *DURING BREEDING*

CHAPTER 3 PREAMBLE

This chapter is a co-authored paper *in press* in the *Journal of Animal Ecology*, and as such, is written in plural throughout. It is included with permission from John Wiley & Sons (see Appendix A), and can be cited as:

Payne NL, Gillanders BM, Seymour RS, Webber DM, Snelling EP & Semmens JM (2011) Accelerometry estimates field metabolic rate in giant Australian cuttlefish *Sepia apama* during breeding, **in press**.

In this paper, Bronwyn Gillanders and Jayson Semmens supplied funding, assisted with study design and intellectual development. Roger Seymour and Dale Webber provided equipment and assistance with study design, and Edward Snelling assisted with intellectual development and data collection. I conducted the study, collected and analysed the data, and wrote the paper.

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Jayson M. Semmens

ACCELEROMETRY ESTIMATES FIELD METABOLIC RATE IN GIANT AUSTRALIAN CUTTLEFISH *SEPIA APAMA* DURING BREEDING

3.1 ABSTRACT

Estimating the metabolic rate of animals in nature is central to understanding the physiological, behavioural and evolutionary ecology of animals. Doubly labelled water and heart-rate methods are the most commonly used approaches, but both have limitations that preclude their field application to some systems. Accelerometry has emerged as a powerful tool for estimating energy expenditure in a range of animals, but is yet to be used to estimate field metabolic rate in aquatic taxa. We combined two-dimensional accelerometry and swim-tunnel respirometry to estimate patterns of energy expenditure in giant Australian cuttlefish *Sepia apama* during breeding. Both oxygen consumption rate ($\dot{V}O_2$) and swimming speed showed strong positive associations with body acceleration, with coefficients of determination comparable to those using similar accelerometers on terrestrial vertebrates. Despite increased activity during the day, field metabolic rate rarely approached $\dot{V}O_{2max,}$ and night-time $\dot{V}O_2$ was similar to that at rest. These results are consistent with the life-history strategy of this species, which has a poor capacity to exercise anaerobically, and a mating strategy that is visually-based. With the logistical difficulties associated with observation in aquatic environments, accelerometry is likely to prove a valuable tool for estimating energy expenditure in aquatic animals.

3.2 INTRODUCTION

Understanding the energetic status of unrestrained organisms in their natural environment is fundamental to basic and applied ecology (Cooke *et al.* 2004) as the energetic costs associated with different activities will heavily influence life-history strategies of animals. Although measuring the metabolic rate of animals in the field is central to the work of ecologists on a broad range of taxa, the two main methods for determining field metabolic rate (doubly-labelled water and heart-rate methods) have major limitations that preclude their application to some studies (Butler *et al.* 2004). For example, the doubly-labelled water method generally provides limited temporal resolution, and the heart-rate method is often complicated by the need to surgically implant electrodes and telemetry devices or data loggers that must be retrieved. Accelerometry has emerged as an effective alternative, which relies on the relationship between body acceleration and energy expenditure (Yoda *et al.* 2001; Wilson *et al.* 2006). During activity, animals expend energy to contract muscles, which leads to body or limb acceleration that can be correlated with energy expenditure (Wilson *et al.* 2006; Halsey *et al.* 2009a). Whilst accelerometry is now considered a reliable method of quantifying energy expenditure via the measurement of body acceleration, the majority of studies have focussed on humans or birds (Eston *et al.* 1998; Yoda *et al.* 2001; Mathie *et al.* 2004; Johansson *et al.* 2006; Wilson *et al.* 2006; Green *et al.* 2009; Halsey *et al.* 2009a; Halsey *et al.* 2009b). Two recent studies have evaluated its use with captive sharks (Whitney *et al.* 2007; Gleiss *et al.* 2010) but the technique is yet to be used to estimate field metabolic rate for aquatic taxa. Accelerometry is a potentially powerful tool for marine and freshwater ecologists, particularly given the logistical complications associated with doubly-labelled water and heart-rate methods in aquatic environments (Butler *et al.* 2004).

The physiology of cephalopods has enabled the use of telemetered jet pressure as a proxy for metabolic rate in this group (Webber & O'Dor 1986; O'Dor *et al.* 1993; O'Dor *et al.* 1994; Webber *et al.* 2000; Aitken *et al.* 2005), but accelerometry is potentially a more adaptable technology, and will likely provide information over a wider range of species, and over time-scales that will have greater ecological significance. Cephalopods are present in all of the world's oceans (Boyle 1983) and are renowned for their extremely rapid growth rates, high metabolic rates and short life spans (O'Dor & Webber 1986; Webber & O'Dor 1986). Their anaerobic capabilities are poor compared to most aquatic vertebrates (Storey & Storey 1979), and when food is lacking, they catabolise their own body proteins (O'Dor *et al.* 1984; Jackson *et al.* 2004), as they have a limited capacity to store and oxidise lipids (Ballantyne *et al.* 1981; O'Dor & Webber 1986). This protein catabolism is used to fuel reproduction in many species (Fields 1965; O'Dor & Wells 1978; Arkhipkin & Bjorke 1999; Gabr *et al.* 1999; Jackson *et al.* 2004), and although multiple spawning events occur in some squid species (Moltschaniwskyj 1995; Maxwell & Hanlon 2000; Pecl 2001), the partitioning of energy derived from somatic tissue is often followed by a single terminal spawning event. Quantifying the rate of energy expenditure in cephalopods during reproductive periods is therefore critical for understanding patterns of growth, reproduction and the evolution of life-history strategies.

The only known breeding aggregation of *Sepia apama* occurs from May to August each year in northern Spencer Gulf, South Australia, where more than 200,000 mature animals converge on a highly localised area (60 ha) of rocky reef to breed. Individuals of this species can exceed 10 kg in weight, have a life span of just 12-18 months, and undergo a single terminal spawning event (Hall & Hanlon 2002). A strongly male-biased operational sex ratio (4:1) has led to the development of spectacular, visually-based male mating displays (Norman *et al.* 1999; Hanlon *et al.* 2005; Payne *et al.* 2011a), and whilst processes of sexual selection in this aggregation are well studied, energetics of this species remain poorly understood. Stomach content analysis suggests fasting during the breeding season, and somatic condition decreases as the season progresses (Hall & Fowler 2003). Reproductive periods are the most energy-consuming stages of life for many animals, and intense competition between male *S. apama* occurs during daylight hours (Norman *et al.* 1999; Hall & Hanlon 2002; Naud *et al.* 2004). Given the limited anaerobic capacity of cephalopods in general (Storey & Storey 1979) and the visually-based mating strategy of *S. apama* in particular, it would be expected that these cuttlefish spend very little time exercising near the upper limit of their aerobic range, and display greater metabolic rates during daylight hours, when reproductive displays are most effective. We combined swim-tunnel respirometry with accelerometry to describe patterns in *S. apama* metabolic rate at night and day during their breeding season, and to test whether reproductive metabolic rates approach the aerobic maximum for this species.

3.3 MATERIALS AND METHODS

Study site, species and tagging technique

All *Sepia apama* individuals were collected via SCUBA from breeding grounds at Point Lowly, South Australia (33°00’S, 137°44’E) during July/August 2009. Bi-axial accelerometer acoustic transmitters (Vemco, Halifax, Nova Scotia. Model V9AP-2L, 69 kHz, 3.3 g in water, 66 mm length) were attached to the interior, ventro-lateral surface of the mantle with two fine hypodermic needles (one at each end of the tag) that passed through the mantle, and were secured externally with a silicone washer and crimp (after Webber & O'Dor 1986). A silicone washer was also placed between the tag and the mantle to minimise skin abrasion. Transmitters were secured with the length of the transmitter (the longest dimension of the cylindrical transmitter) parallel to animal length, and they recorded acceleration in the X (side to side) and Z (up and down) axes. In this way, acceleration in these two axes recorded expansion and contraction of the mantle (thereby a proxy of ventilation), not movement of the animal forward or backwards. The V9AP transmitted acceleration data on a pseudorandom schedule every 60-180 s (mean 120 s). Acceleration (± 29.4 m s^{-2} range) was sampled at 10 Hz for 17-33 s every second transmission cycle (24% duty cycle). Acceleration was calculated as an average root mean square (RMS) value for both axes (acceleration = $[X^2 + Z^2]^{0.5}$) each 17-33 s period and transmitted as an 8 bit digital value. The static contribution to overall g was filtered out prior to RMS calculation. RMS acceleration resolution was 0.0191 m s^{-2}.

Laboratory acceleration calibration

Seven mature males (16-19 cm mantle length, 420-590 g wet weight) were transported to a controlled temperature room at the University of Adelaide. Animals were fasted for 24 h prior to swim trials to reduce the influence of digestion on respiration. RMS acceleration values were correlated with swimming speed and oxygen consumption rate ($\dot{V}O_2$) by placing tagged cuttlefish in an 80 L, sealed, recirculating Brett-type swim tunnel with a swim chamber 750 mm long, 200 mm wide and 107 mm deep (Seymour *et al.* 2004). Water temperature was maintained at 14.0 ± 1ºC (equivalent to that at the aggregation site near Point Lowly in July-August), salinity at 38 ± 1g L^{-1}, and oxygen concentration at > 80% for the duration of all experiments. To encourage consistent swimming, the ‘upstream’ half of the swim chamber was covered with opaque plastic, with the downstream section left open to ambient light. This proved a very successful means of motivating cuttlefish to swim at pre-selected constant speeds.

Cuttlefish were acclimated for 45 min prior to measurements. All animals were subsequently swum at increasing speeds of 7, 11 and 15 cm s^{-1} for 15 min at each speed, with a recovery period of 20 min between stages to reduce any accrued oxygen debt. Oxygen consumption was measured with a polarographic probe (YSI, Yellow Springs, Ohio. Model 58 meter and 5739 electrode) that was ventilated at a constant rate, independent of flume velocity, with an external pump in a separate circuit. Oxygen uptake was calculated from the decline in oxygen concentration, assuming 5.89 mL O_2 L^{-1} in saturated saltwater (38 g L^{-1}) at 14 ºC (Riley & Chester 1971). Although expressing $\dot{V}O_2$ as a mass-specific value rarely eliminates the effect of body mass on $\dot{V}O_2$ (Packard & Boardman 1999), proper mass-independent values require knowledge of the scaling of metabolic rate, which is not known for this species because the range of body mass was relatively small in our study. We chose to present mass-specific values in an attempt to correct for the potential influence of body mass on $\dot{V}O_2$ in view of the known metabolic rate scaling exponent of 0.92 in Loliginidae (Seibel 2007). This is so close to 1.0 that simply dividing by body mass within a limited range should introduce little error. Blank runs (without cuttlefish) were carried out at the beginning and end of each day to check probe drift and microbial uptake, both of which were negligible.

Since some animals occupied greater than 10% of the cross-sectional area of the swim chamber, a blocking correction was applied to all swimming speeds after Bell and Terhune (1970) to account for the increased water speed caused by the profile of the animal in the respirometry chamber:

$$U_F = U_T(1 + \varepsilon_S),$$

where U_F is the corrected flow speed and U_T is the speed in the flume without a cuttlefish in the swim chamber. The fractional error due to solid blocking (ε_S) was calculated for each cuttlefish as:

$$\varepsilon_S = 0.8\lambda(A_O/A_T)^{0.5},$$

where λ is a shape-based constant for the cuttlefish (= 0.5 × body length/body thickness for streamlined objects such as fish and cuttlefish), A_O is the maximum cross-sectional area of the cuttlefish, and A_T is the cross-sectional area of the swim chamber (Bell & Terhune 1970; Korsmeyer *et al.* 2002; Cannas *et al.* 2006). All cuttlefish were allowed to choose their preferred swimming orientation, and the swim chamber was sufficiently wide for them to change orientation during swimming.

While $\dot{V}O_2$ measurements were being made, mean acceleration values were telemetered every 30 s from the V9AP-2L tags to a VR100 hydrophone and receiver unit (Vemco, Halifax, Nova Scotia) that was fixed externally to the swim tunnel. Those few periods of erratic behaviour (i.e. changing orientation in the chamber) that were likely to produce acceleration values not representative of steady-state swimming were excluded from analysis. Mean acceleration values were then calculated for each animal at each swimming speed. Generalized linear mixed models (GLMMs) were used to estimate the relationships between swimming speed, acceleration, and $\dot{V}O_{2,}$ with subject ID included as a random factor in each model (to account for the non-independence of data). Akaike's Information Criterion (AIC) were generated as a measure of goodness of fit (Bolker *et al.* 2009) for each model with and without including ID as a random factor, and the model with the lowest AIC (and therefore the most parsimonious) was chosen.

To further evaluate the aerobic range of this species, 25 non-tagged cuttlefish were collected and introduced to the swim tunnel (using the procedure mentioned above), and swum at one of four speeds (7, 11, 15 or 25 cm s^{-1}) for at least 5 min. Five animals were swum at each speed, and 5 were used as controls (resting $\dot{V}O_2$ only). $\dot{V}O_2$ was measured throughout each trial, and compared between speeds with ANOVA and SNK post-hoc tests. Homogeneity of variances was verified by Cochran's *C*-test ($P > 0.05$). To test whether tagging had an influence on $\dot{V}O_2$, we compared $\dot{V}O_2$ for tagged and non-tagged cuttlefish at 0, 7, 11 and 15 cm s^{-1} using unpaired t-tests.

Field acceleration measurements and estimated $\dot{V}O_2$

To estimate field $\dot{V}O_2$, ten male cuttlefish (16-27 cm mantle length, 384-1964 g wet weight) were fitted with the V9AP-2L accelerometer transmitters (using the technique mentioned above) at the Point Lowly breeding grounds in July 2009, and subsequently monitored with an array of ten 69 kHz VR2W acoustic receivers (200 m apart, mean depth 8 m, approximately 150 m from shore). We exclusively selected males, because females are present at the aggregation for significantly shorter durations (Payne *et al.* 2011a). Cuttlefish were held in 100 L tubs during tagging, typically returned to the water within 2 min of capture, and were observed to jet strongly through the water column.

For analysis, each acceleration value was assigned to either dawn (600 to 0900 h), daylight (0900 to 1600 h), dusk (1600 to 1900 h) or night-time (1900 to 0600 h) periods, and the mean value was calculated for each period for each animal over the entire tracking period. Acceleration values were compared between diel periods for each individual using one-way ANOVA and SNK post-hoc tests. Data were log-transformed, and where variances remained homogenous (Levene's test, $P < 0.05$), alpha was adjusted to 0.01 to reduce the probability of Type 1 error (Underwood 1997). Mean acceleration values per diel period were calculated for each individual, and the grand mean values from all individuals ($n = 7$) were transformed to $\dot{V}O_2$ and swimming speed using the relationships derived in the calibration experiments. Standard errors of $\dot{V}O_2$ and swimming speed estimates (SEE) were calculated, and estimates were compared among diel periods using *Z*-tests following the conventions of Zar (1999, p. 339), which have been used as a basis for deriving SEE in various heart-rate and accelerometry studies (Green *et al.* 2001; Froget *et al.* 2004; Green *et al.* 2009). Alpha was set at 0.05 for these comparisons (i.e. $Z > 1.96$).

As a proxy of the proportion of time cuttlefish swam at various speeds, we assigned each transformed swimming speed value to one of four speed ranges (<7, 7-11, 11-15 and >15 cm

s^{-1}). We stratified these data by night and day, and compared the percentage of detections within each speed range between night and day using paired t-tests ($n = 7$ cuttlefish).

Mean total daily energy expenditure (TDE) was calculated as

$$\text{TDE (kJ kg}^{-1}) = \dot{V}O_2 \times 20 \times 24 / 1000,$$

where $\dot{V}O_2$ is the grand mean hourly mass-specific oxygen consumption rate from 7 individuals (ml $kg^{-1}h^{-1}$ ± SEE), and 20 is the Joule equivalent of 1 ml of O_2. We acknowledge that converting $\dot{V}O_2$ to energy expenditure requires assumptions about the metabolic substrate used, so have used an intermediate value of energy density between protein and carbohydrate (21.1 and 18.8 J ml^{-1} O_2, respectively; Schmidt-Nielsen 1997). We converted the TDE estimate to percentage body mass equivalent to estimate the amount of tissue catabolism required to sustain breeding (assuming these cuttlefish are in fact fasting for the duration of the reproductive season; Hall & Fowler 2003). No estimates of energy density are available for *S. apama*, so we assumed an intermediate value for *Sepia esculenta* and *Sepia pharaonis* (3.43 kJ g^{-1}; derived from O'Dor & Wells 1987).

3.4 RESULTS

Laboratory acceleration calibration

Cuttlefish had little difficulty maintaining steady-state swimming at each speed, and swam with their head orientated downstream for the vast majority of trials. A combination of lateral finning and propulsive jetting were used for locomotion, with the contribution of jetting negligible at speeds below 15 cm s^{-1}, but increasing significantly at 25 cm s^{-1}. Cuttlefish displayed a linear increase in acceleration values with increasing swimming speed (Fig. 3.1a,) and quadratic models best described the increase of $\dot{V}O_2$ with swimming speed (Fig. 3.1b) and acceleration (Fig. 3.1c). AIC's were lower when ID was included as a random factor for each GLMM (209.1 vs. 208.5, 228.7 vs. 216.9 and 208.4 vs. 202.1 for swimming speed vs. acceleration, swimming speed vs. $\dot{V}O_{2,}$ and acceleration vs. $\dot{V}O_{2,}$ respectively), so we chose parameter estimates from the models including ID in each case. An almost exponential increase in $\dot{V}O_2$ from resting to 15 cm s^{-1} occurred in tagged (Fig. 3.1b) and non-tagged cuttlefish (Fig. 3.2), and for non-tagged cuttlefish, $\dot{V}O_{2max}$ (the maximum rate of oxygen consumption) was reached at 15 cm s^{-1} ($\dot{V}O_2$ at 15 and 25 cm s^{-1} were not significantly different, Fig. 3.2). Maximum $\dot{V}O_2$ was considered to be reached at 15 cm s^{-1} for cuttlefish in the field. No significant differences in $\dot{V}O_2$ were detected between tagged and non-tagged cuttlefish for resting, 7, 11 or 15 cm s^{-1} ($P > 0.05$ for all comparisons).

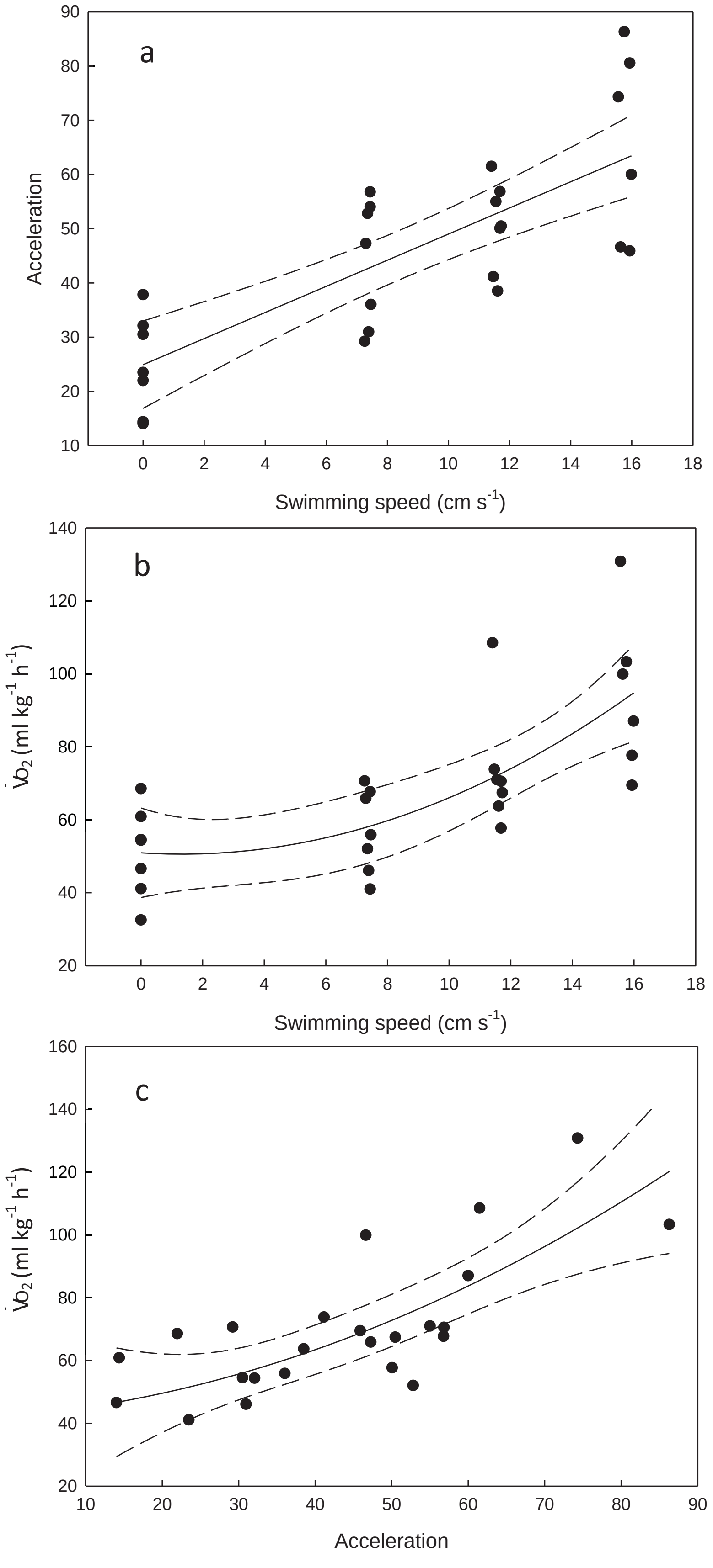

Figure 3.1 Relationships between mean acceleration, swimming speed (adjusted for solid blocking effects) and $\dot{V}O_2$ for *Sepia apama* (n = 7) in swim-tunnel calibration experiments. The models chosen were: a) acceleration = 2.409[swimming speed] + 24.93, R^2 = 0.62; b) $\dot{V}O_2$ = 0.2057[swimming speed]^2 - 0.548[swimming speed] + 50.95, R^2 = 0.54; c) $\dot{V}O_2$= 0.0081[acceleration]^2 + 0.209[acceleration] + 42.18, R^2 = 0.55. Dashed lines represent 95% confidence intervals of the model.

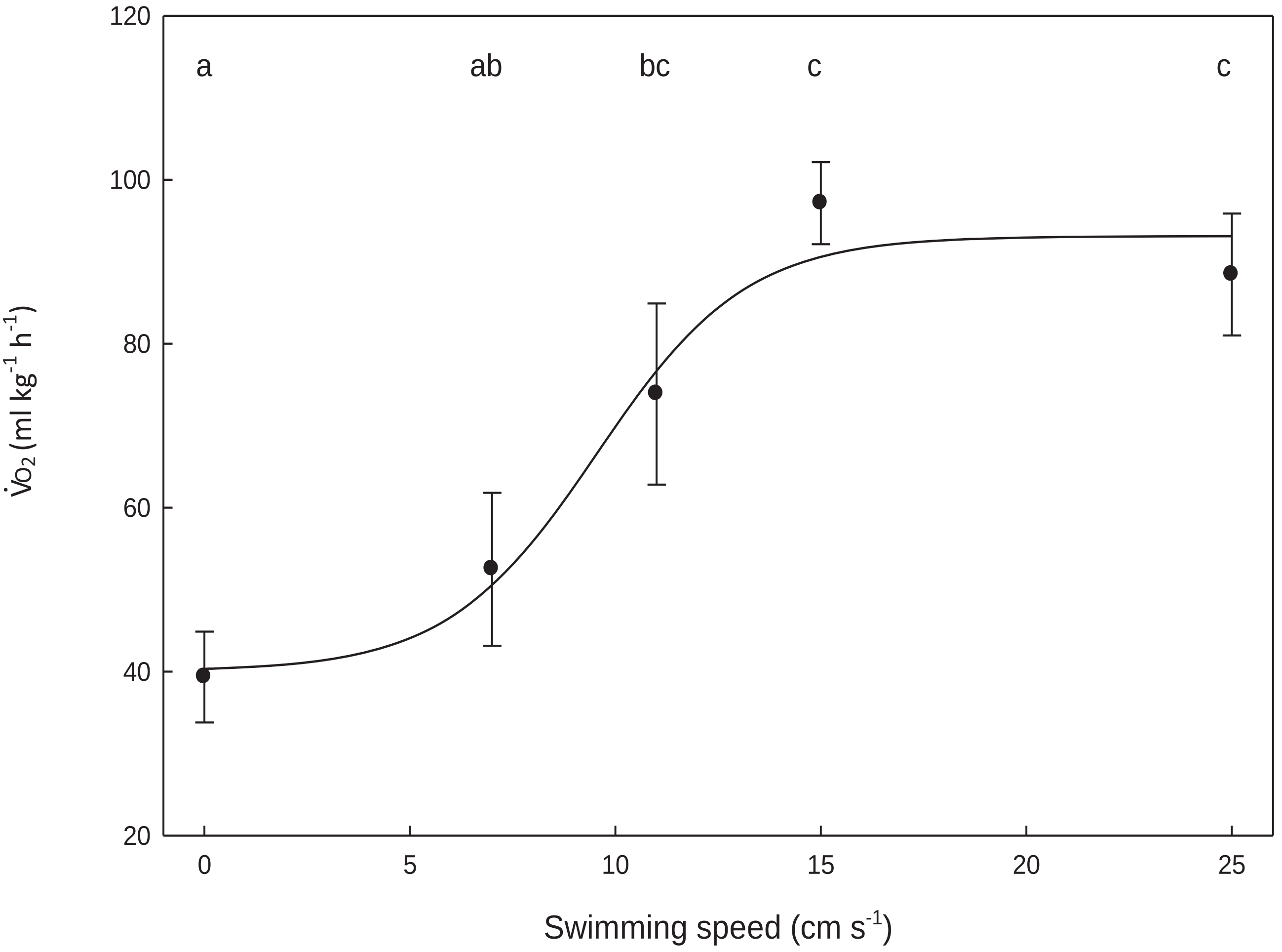

Figure 3.2 Mean (± SEM) mass-specific oxygen consumption rates for non-tagged *Sepia apama* (*n* = 5 individuals per speed; 297-995 g wet weight) at 14ºC, determined by swim-tunnel respirometry. Letters indicate significant differences in $\dot{V}O_2$ between speeds (SNK $P < 0.05$).

Field acceleration measurements and estimated $\dot{V}O_2$

Acceleration values were received frequently from seven of the ten tagged cuttlefish (mean detections per day: 143.4 ± 25.7; mean detection period: 11.1 ± 1.7 days, Table 3.1), with the remaining three leaving the detection range of receivers within 48 h of release. Data from these cuttlefish were excluded from analyses. Acceleration values during the night were significantly lower than during the day for all individuals (Table 3.1). Only cuttlefish number 2 displayed similar acceleration values between day, dawn and dusk, with the remainder displaying varying differences among these three periods (Table 3.1). Swimming speed and $\dot{V}O_2$ estimates increased and subsequently decreased at sunrise and sunset, respectively, but estimated $\dot{V}O_2$ in each hour remained well below $\dot{V}O_{2max}$ (94.7 ml $kg^{-1}h^{-1}$), and swimming speed remained well below the speed that produced $\dot{V}O_{2max}$ (15 cm s^{-1}; Fig. 3.3, 3.4 and 3.5). Estimated $\dot{V}O_2$ was approximately 20% lower during the night than during other periods (Fig. 3.5a), however this difference was not statistically significant (Z-test, $P > 0.05$). Estimated swimming speed was significantly lower during the night than during the day (Z-test, $P < 0.05$; Fig. 3.5b).

During daylight hours, 59.3% of swimming speed values (as a proxy for proportion of time) were less than 7 cm s^{-1}; significantly less than at night (83.7%, $t_{12} = -4.6$, $P < 0.05$, Fig. 3.6). Significantly more time was spent in the three higher speed ranges (7-11, 11-15 and >15 cm s^{-1}) during the day than at night (t_{12} = 3.2, 4.7 and 5.1 respectively, $P < 0.05$ for each comparison, Fig. 3.6), with only a small percentage of time spent swimming above the speed at which $\dot{V}O_{2max}$ is reached (15 cm s^{-1}; 14.3 % during the day and 4.7% during the night, Fig. 3.6).

TDE was 27.84 ± 3.25 kJ kg^{-1} d^{-1} (estimate ± SEE) and the body mass loss equivalent was 8.12 ± 0.95 g kg^{-1}. This equates to a daily percentage body mass loss of 0.81 ± 0.09% per day.

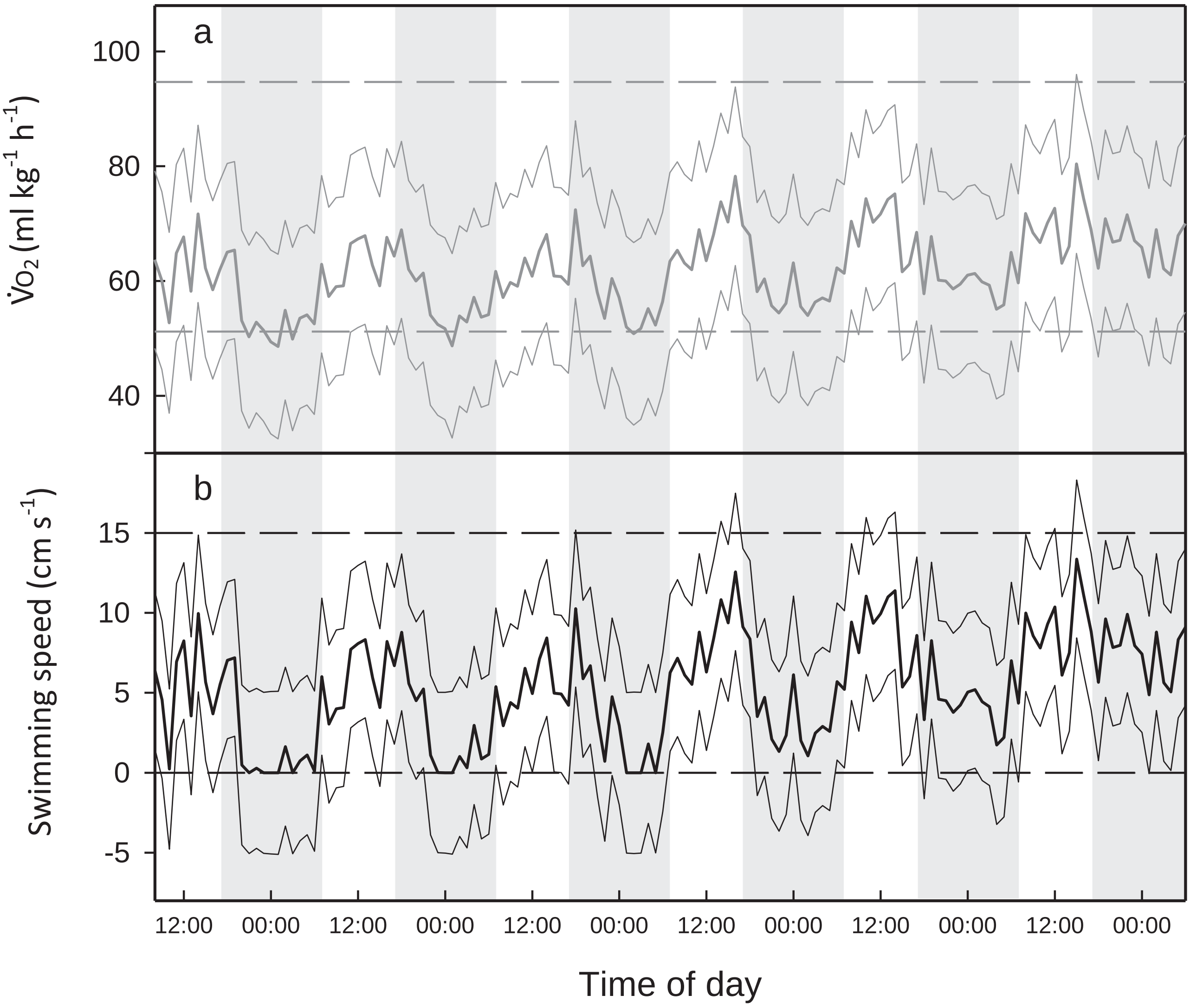

Figure 3.3 Mean acceleration values per hour transformed to a) $\dot{V}O_2$ ± SEE and b) swimming speed ± SEE for a tagged male *S. apama* (16 cm ML, 384 g) over a six-day tracking period. Shading represents night-time, and dashed horizontal lines indicate resting (0 cm s^{-1} and 51.2 ml kg^{-1} h^{-1}) and maximum (15 cm s^{-1} and 94.7 ml kg^{-1} h^{-1}) $\dot{V}O_2$ for the species. We considered acceleration values that resulted in $\dot{V}O_2$ exceeding 100 ml kg^{-1}h^{-1} as erroneous (as this exceeds $\dot{V}O_{2max}$ from swim-tunnel respirometry), so excluded them (approximately 4% of values) from this analysis.

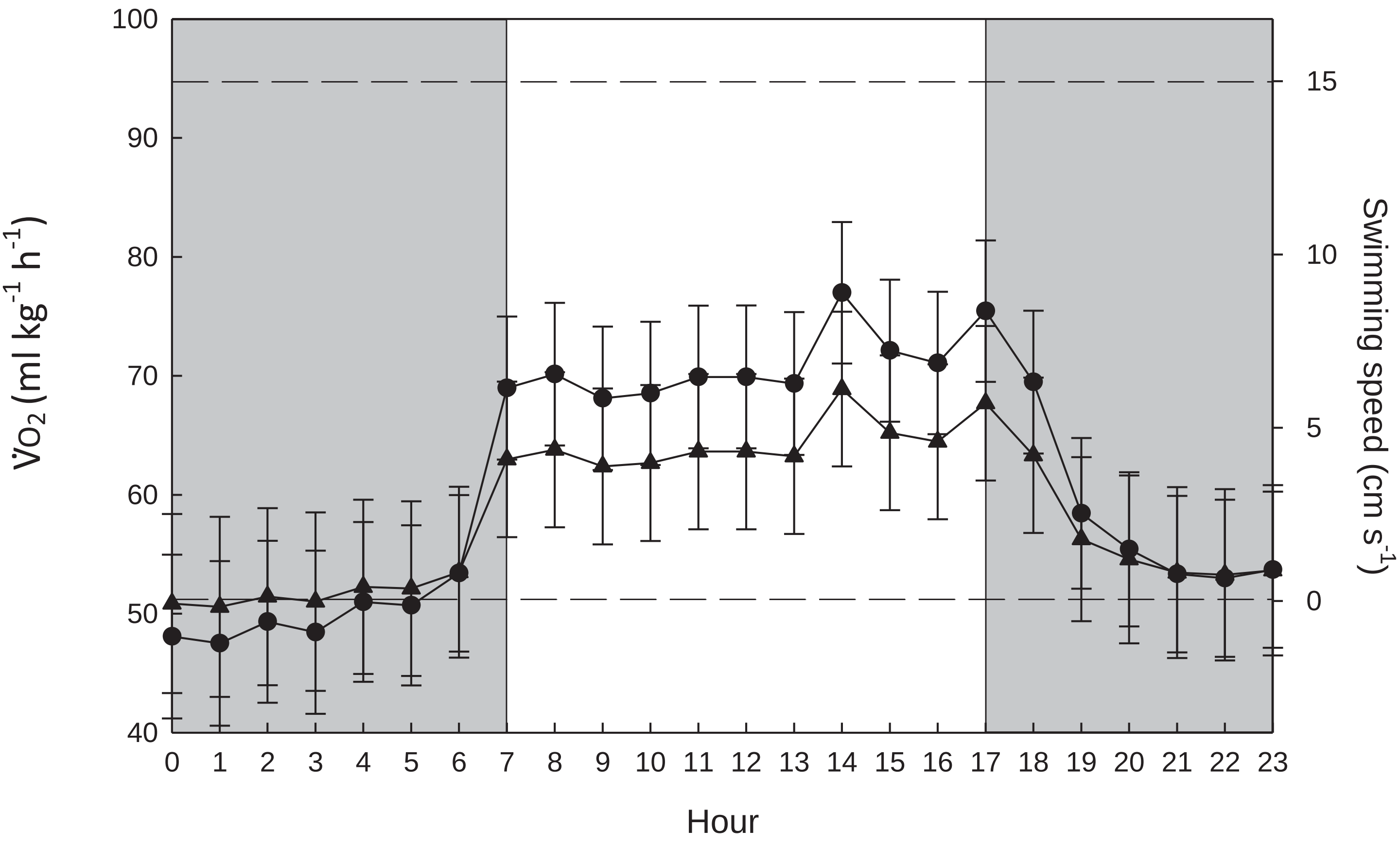

Figure 3.4 Diel pattern in field estimates of $\dot{V}O_2$ (triangles) and swimming speed (circles) per hourly bin for *S. apama* (*n* = 7) on Point Lowly reef during August 2009. Error bars indicate SEE, shading represents night-time, and dashed horizontal lines indicate resting (0 cm s^{-1} and 51.2 ml kg^{-1} h^{-1}) and maximum (15 cm s^{-1} and 94.7 ml kg^{-1} h^{-1}) $\dot{V}O_2$ for the species.

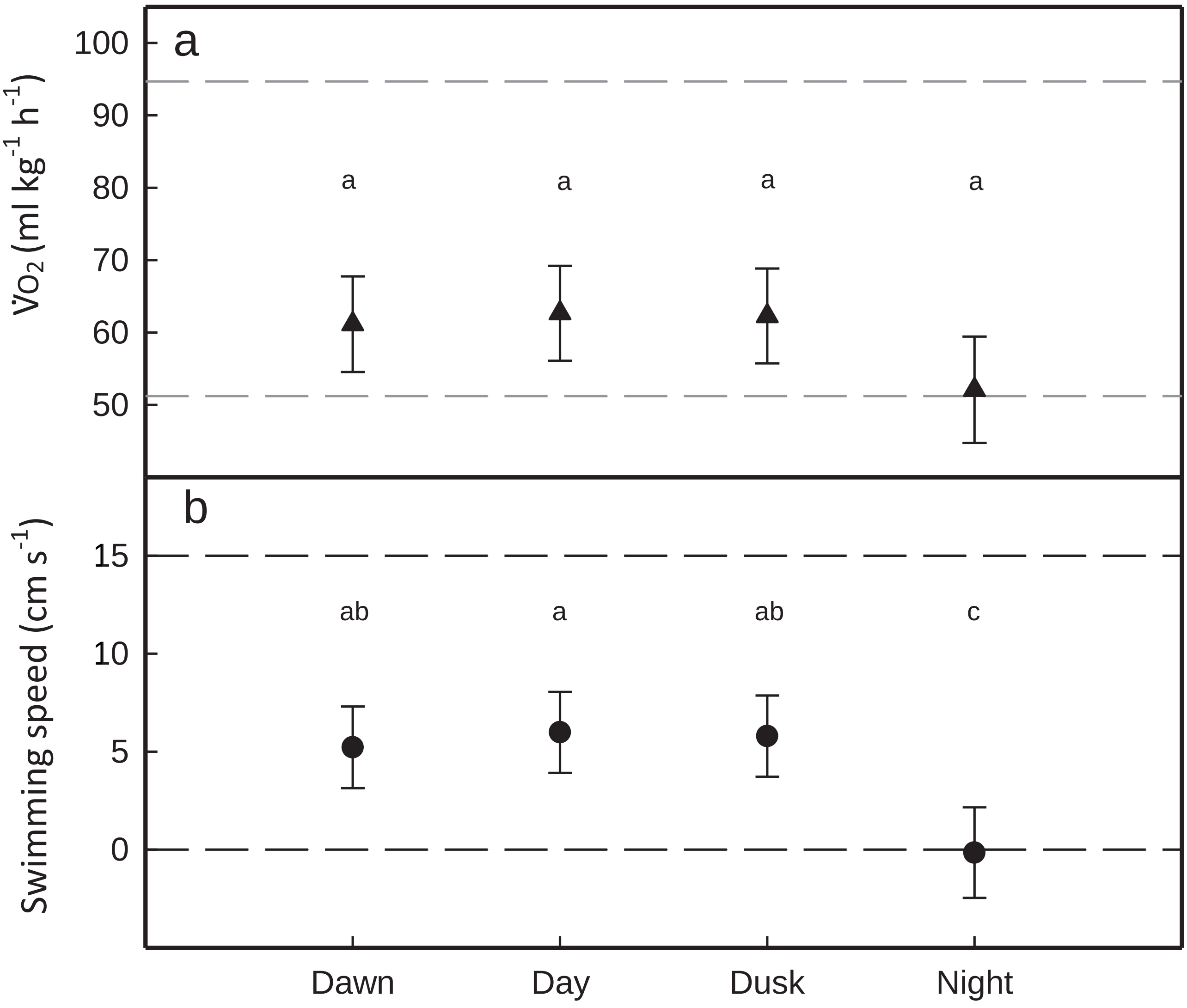

Figure 3.5 Comparison among diel periods for field a) $\dot{V}O_2$ and b) swimming speed from *n* = 7 cuttlefish on Point Lowly reef during August 2009. Error bars represent the SEE, and letters represent significant differences between diel periods (*Z*-test, $P < 0.05$). Dashed horizontal lines indicate resting (0 cm s^{-1} and 51.2 ml kg^{-1} h^{-1}) and maximum (15 cm s^{-1} and 94.7 ml kg^{-1} h^{-1}) $\dot{V}O_2$ for the species.

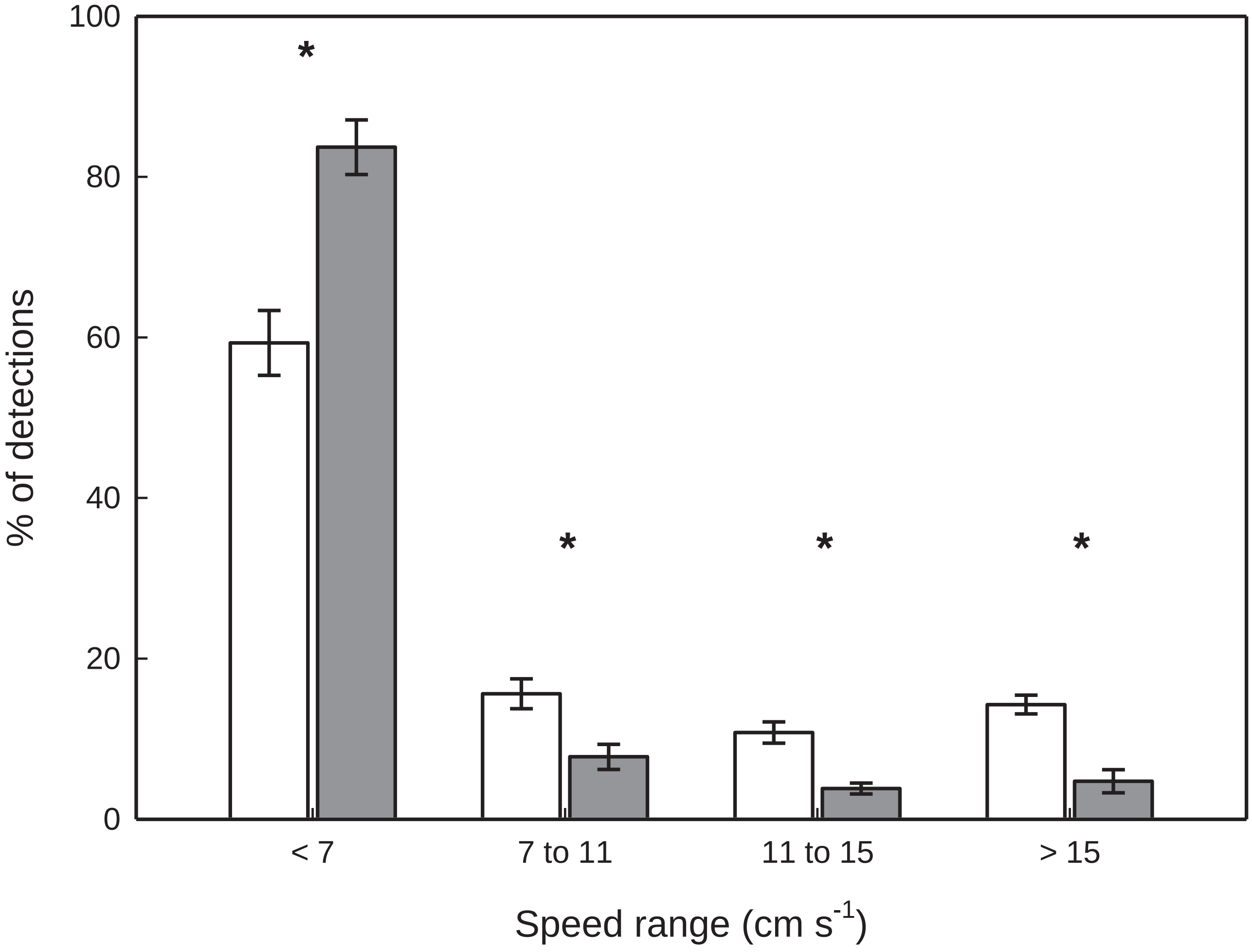

Figure 3.6 Proportion of acceleration values (mean ± SEM) within each swimming speed range at night (dark shading) and day for tagged *S. apama* ($n = 7$). An asterisk represents significant differences between night and day for each speed range (paired *t*-test, $P < 0.05$).

3.5 DISCUSSION

Although the metabolic capabilities of some cephalopod species are well understood under experimental conditions (O'Dor & Wells 1987), and through studies of telemetered jet pressure (Webber & O'Dor 1986; O'Dor 2002), difficulties associated with field observation have led to a paucity of information on the energetic status of these animals (and marine taxa in general) in nature. A combination of accelerometry and swim-tunnel respirometry in this study has revealed that *S. apama* in nature rarely approach $\dot{V}O_{2max}$ throughout any period of the day, and that metabolic rate during night-time hours is similar to that at rest (Figs. 3.4 & 3.5). These animals have a relatively modest factorial aerobic scope (the ratio of maximum: resting metabolic rate was approximately 1.8; Fig. 3.2), and despite increased swimming speeds during the day, cuttlefish appeared to have little difficulty meeting reproductive energy requirements aerobically. This is consistent with the general consensus that cuttlefish have small glycogen stores and that these are reserved for short periods of burst swimming (Storey & Storey 1979; O'Dor *et al.* 1984; Wells & Clarke 1996). By swimming at speeds well below those at which $\dot{V}O_{2max}$ is reached, *S. apama* would minimise the depletion of anaerobic energy stores (i.e. glycogen), which could then be available for burst swimming to avoid predators or capture prey.

During dusk, there is a shift in behaviour of this species from conspicuous sexual signalling to a more sessile, camouflaged state which continues until approximately one hour after dawn (Hanlon *et al.* 2007). This observation closely matches the marked decrease in activity during the night in the present study (Figs. 3.4 & 3.5). Given that telemetered jet pressure revealed non-mating individuals of this species in nearby Boston Bay spend more than 95% of the day resting (Aitken *et al.* 2005), the behaviours associated with mating seem responsible for increased activity of individuals at Point Lowly.

Cuttlefish in this breeding aggregation feed very little, and mantle tissue condition decreases significantly as the season progresses (Hall & Fowler 2003), suggesting protein catabolism. The daily energy expenditure of animals determined in the current study equates to the catabolism of approximately 0.81% of their own tissue per day, and assuming they can re-mobilise a maximum of 50% of their body mass (as is the case for *Octopus vulgaris*; O'Dor & Webber 1986), individuals could survive at the breeding aggregation for almost 60 days without feeding. However, male breeding durations for *S. apama* are closer to 40 days (Payne *et al.* 2011a) which suggests that they either sustain lower levels of

Table 3.1. Summary of accelerometry tracking on Point Lowly reef during August 2009. Mean acceleration values were calculated for each animal over the entire monitoring period, and superscripted letters indicate significant differences among diel periods.

n	ML	Mass	Tracking	Detections	Mean acceleration value (± SEM)				*P*
	(cm)	(g)	period (days)	per day	Night	Dawn	Day	Dusk	
1	26	1964	11.8	91	18.5 ± 0.6^{a}	27.7 ± 2.2^{b}	33.4 ± 1.1^{c}	33.3 ± 1.6^{c}	< 0.01
2	17	471	14.4	88	23.4 ± 0.7^{a}	45.5 ± 2.7^{b}	44.5 ± 1.0^{b}	38.6 ± 1.5^{b}	< 0.01
3	22	1099	8.6	175	20.8 ± 0.5^{a}	31.8 ± 1.4^{b}	38.4 ± 0.9^{c}	40.5 ± 1.6^{c}	< 0.01
4	25	1384	8.3	81	29.7 ± 0.8^{a}	48.3 ± 2.1^{b}	39.6 ± 1.3^{c}	42.4 ± 1.9^{c}	< 0.01
5	16	384	16.9	105	20.7 ± 0.5^{a}	29.0 ± 1.1^{b}	35.4 ± 0.9^{c}	28.9 ± 1.2^{b}	< 0.01
6	27	1829	14.2	229	34.7 ± 0.5^{a}	40.9 ± 0.9^{b}	46.5 ± 0.6^{c}	45.9 ± 1.0^{c}	< 0.01
7	26	1739	3.9	234	23.0 ± 0.7^{a}	37.7 ± 1.3^{b}	35.8 ± 0.8^{c}	40.9 ± 1.6^{c}	< 0.01

tissue catabolism than *O. vulgaris*, or that the onset of this catabolism occurs prior to arriving at the breeding grounds. It is thought that these cuttlefish undergo significant northerly migrations (one individual was shown to travel at least 65 km to reach the breeding grounds; Hall & Fowler 2003) prior to breeding, so energy stores may indeed be partially depleted by the time they arrive. Non-breeding, captive European cuttlefish *Sepia officinalis* survive for 54 to 68 days following starvation (Castro *et al.* 1992), so the actual breeding durations of male *S. apama* (40 days) are consistent with the higher metabolic rates of reproductive activities compared to metabolic rates of non-breeding *S. officinalis* in captivity. It is worth noting that males were studied exclusively in the present study, and that the added metabolic burden of producing eggs will likely result in different patterns in energy expenditure for females. Indeed, female *S. apama* do display significantly shorter breeding durations than males (Payne *et al.* 2011a), a likely result of this increased metabolic burden.

Utility of accelerometry for Sepia apama

Acceleration was a marginally better predictor of swimming speed than $\dot{V}O_2$ (R^2 of 0.62 versus 0.55; Fig. 3.1), with coefficients of determination for both relationships being lower than those reported by studies using tri-axial accelerometers (Wilson *et al.* 2006; Halsey *et al.* 2009c), but similar to several studies measuring acceleration in one or two dimensions (Campbell *et al.* 2002). Tri-axial accelerometers are thought to provide the best correlation with $\dot{V}O_2$ (Wilson *et al.* 2006), however this generality may not hold in some aquatic environments, where laterally-moving currents and swell surges could cause acceleration in space that are not related to muscle contraction (and therefore energy expenditure). An advantage of the accelerometry data loggers typically used to date for estimating energy expenditure is that instantaneous raw acceleration values from each axis can be examined to provide the best estimates of partial or overall dynamic body acceleration (PDBA or ODBA respectively; Green *et al.* 2009; Halsey *et al.* 2009a; Halsey *et al.* 2009c). These raw values are not accessible where RMS values are telemetered from acoustic transmitters (as in the present study), but the trade-off is that transmitters do not need to be retrieved post-tagging. This attribute is likely to significantly broaden the applicability of field accelerometry studies in aquatic systems. Another complication exists for cephalopods, which contract their mantle musculature for ventilation as well as locomotion. In resting *Sepia*, ventilation results from movement of the collar flaps and the radial components of the mantle musculature, whilst circular mantle muscles remain inactive (Bone *et al.* 1994). At increased swimming speeds however, circular mantle muscles are involved in expiration, and their contraction contributes to propulsive locomotion. This pattern is seen in *S. apama*, where the relative contribution of jetting for locomotion increases exponentially with swimming speed (Payne unpublished data). The implication of this gait transition is that as swimming speed increases, the relative contribution of jetting to locomotion increases, and so does the proportion of acceleration values that record propulsive muscle contractions rather than those used for ventilation. The influence of this gait transition on the predictive accuracy of accelerometry requires investigation, as similar transitions are known to influence the prediction of $\dot{V}O_2$ for humans (Herren *et al.* 1999; Brage *et al.* 2003; Halsey *et al.* 2008).

Although field acceleration values were significantly higher during the day than at night for all individuals (Table 3.1), the increase seen in swimming speed estimates were not matched by a statistically significant increase in $\dot{V}O_2$ estimates during daylight hours (Fig. 3.5a). This is perhaps unsurprising, given the higher R^2 value during calibrations for swimming speed compared to $\dot{V}O_2$, and that the Residual Mean Square of calibration regressions (together with the number of field measurements) is a major determinant of the size of SEEs (Zar 1999; Green *et al.* 2001). Such a result highlights the importance of maximising the number of measurements taken both during calibration experiments, and in the field (Zar 1999; Green *et al.* 2001; Green *et al.* 2009), to improve the ability to detect significant differences between field estimates for a given effect size.

Conclusions

Despite some potential complexities in its application to cephalopods, accelerometry provided valuable insights into patterns of *Sepia apama* energy expenditure during breeding. Increased activity during the day is consistent with the visual mating strategy of the species, daytime $\dot{V}O_2$ rarely approached $\dot{V}O_{2max}$ (unsurprising for a group considered as having a poor anaerobic capacity), and estimated levels of tissue re-mobilisation correlate well with previously observed breeding durations and starvation experiments for the genus.

While the predictive accuracy of bi-axial accelerometry in cuttlefish is somewhat lower than for some tri-axial accelerometry studies on humans (Halsey *et al.* 2008) and birds (Wilson *et al.* 2006; Green *et al.* 2009), accelerometry is likely to become a powerful tool for physiological and behavioural ecologists working with aquatic animals.

Measuring resting metabolic rate in the swim tunnel respirometer. Photo credit: Edward Snelling.

CHAPTER 4

INTERPRETING DIEL ACTIVITY PATTERNS FROM ACOUSTIC TELEMETRY: THE NEED FOR CONTROLS

CHAPTER 4 PREAMBLE

This chapter is a co-authored paper published in the journal *Marine Ecology Progress Series*, and as such, is written in plural throughout. It is included with permission from Inter-Research (see Appendix A), and can be cited as:

Payne NL, Gillanders BM, Webber DM & Semmens JM (2010) Interpreting diel activity patterns from acoustic telemetry: the need for controls. *Marine Ecology Progress Series*, **419**, 295-301.

In this paper, Bronwyn Gillanders and Jayson Semmens supplied funding and assisted with intellectual development, and Dale Webber assisted with intellectual development. I conducted the study, collected and analysed the data, and wrote the paper.

Signatures of co-authors:

Bronwyn M. Gillanders

Dale M. Webber

Jayson M. Semmens

Payne, N.L., Gillanders, B.M., Webber, D.M. and Semmens, J.M. (2010) Interpreting diel activity patterns from acoustic telemetry: the need for controls
Marine Ecology Progress Series, v.419, pp. 295–301, 2010

Mooring a Vemco^{TM} VR2W acoustic receiver. Photo credit: Simon Bryars.

CHAPTER 5

ELEMENTAL UPTAKE VIA IMMERSION: A MASS-MARKING TECHNIQUE FOR THE EARLY LIFE-HISTORY STAGES OF CEPHALOPODS

CHAPTER 5 PREAMBLE

At the time of printing, this chapter was under peer-review in the journal *Marine Ecology Progress Series*, with Bronwyn Gillanders and Jayson Semmens as co-authors. As such, it is written in plural throughout.

In this paper, Bronwyn Gillanders supplied funding, assisted with study design, egg collection and intellectual development, and Jayson Semmens assisted with intellectual development. I conducted the study, collected and analysed the data, and wrote the paper.

Payne, N.L., Semmens, J.M. and Gillanders, B.M., (2011) Elemental uptake via immersion: a mass-marking technique for the early life-history stages of cephalopods. *Marine Ecology Progress Series, v.436, pp. 169-176, 2011*

CHAPTER 6

GENERAL DISCUSSION

GENERAL DISCUSSION

Difficulties associated with direct field observation have led to a lack of understanding of population dynamics, energetics, and juvenile dispersal of the *Sepia apama* breeding aggregation in northern Spencer Gulf. Despite the scientific and ecotourism value of the aggregation, and overall declines in biomass seen in recent years (Hall 2008), effective management of this population has been hindered by a lack of information on these critical parameters. Throughout this thesis, a combination of approaches were used to address several significant gaps in understanding of the *Sepia apama* breeding aggregation. Estimating breeding durations for each sex revealed that the skewed operational sex ratio is a result of extended male breeding durations compared to females, and that the adult sex ratio is likely unbiased (Chapter 2). Irrespective of sex, relatively short breeding durations suggest that actual population size may be significantly underestimated by density-based surveys (Chapter 2). A likely cause of the brevity of breeding durations was identified by estimating field metabolic rate (at least for males; Chapter 3), with estimated levels of tissue catabolism correlating well with estimated breeding durations (Chapter 2). Levels of activity were significantly higher during daylight hours than at night (Chapter 3), although the influence of external factors on the frequency of acoustic detections suggests that caution must be used when interpreting animal behaviour from detection frequency data (Chapter 4). Finally, a step toward understanding juvenile dispersal and population connectivity was made by the successful development of a mass-marking technique for the early life-history stage of *S. apama* (Chapter 5). This chapter provides a discussion of these key points, and suggests directions for future research.

Population dynamics of Sepia apama

An assumption of sex-role evolution theory is that the operational sex ratio (OSR) predicts which sex will compete more strongly for mates (Emlen & Oring 1977; Cluttonbrock & Parker 1992; Kokko & Jennions 2008), and this assumption appears to hold for the *S. apama* breeding aggregation, where males have developed spectacular competitive displays and tactics (Norman *et al.* 1999; Naud *et al.* 2004; Hanlon *et al.* 2005). It is perhaps this property of the OSR that has historically awarded it far greater focus in biological studies than the adult sex ratio (ASR; Kokko & Jennions 2008), however the significance of the ASR for population and genetic viability as well as for sex role evolution is now recognised (Trivers 1972; Cluttonbrock & Parker 1992; Vargas *et al.* 2007; Kokko & Jennions 2008).

There is a paucity of information on sex ratios at birth for cephalopods globally (probably as sex determination is extremely difficult for immature stages of the majority of species), and although trawl by-catch data suggests the ASR of *S. apama* in greater northern Spencer Gulf is close to unity (Hall & Fowler 2003), the degree of connectivity between individuals from the aggregation and those of greater Spencer Gulf is unknown. Given the highly skewed OSR of the breeding aggregation (mean 4:1; Hall & Hanlon 2002), and that biased ASRs are common in nature (Kruuk *et al.* 1999), it may seem plausible that the OSR skew is contributed to by a skew in the population ASR. By estimating gender differences in 'time-in', our study (Chapter 2) suggests that this is not the case, and that the ASR is indeed unbiased. Anisogamy predicts that, for a population with an unbiased ASR, the gender with a higher potential reproductive rate will compete for the limiting sex, and that this will produce a skewed OSR (Cluttonbrock & Vincent 1991; Kokko & Jennions 2008). Breeding *Sepia apama* catabolise their own body proteins, and given the relatively large, energetically expensive eggs produced by females (Hall & Hanlon 2002; Naud *et al.* 2004), the potential reproductive rate of males (who generally produce small, comparatively cheap sperm; Kokko & Jennions 2008) would likely be higher than females, and this would result in the disparate breeding durations seen throughout the aggregation (Chapter 2).

The skewed *S. apama* operational sex ratio is one of the highest known for any cephalopod. Little is known about other *Sepia* populations, but spawning aggregations of *Loligo* typically consist of OSRs in the range of 1-2:1 (M:F; Hanlon *et al.* 2002; Hanlon *et al.* 2004). Interestingly, research in recent decades suggests many loliginid squid spawn intermittently, and do not display the semelparous, terminal spawning events like that of *S. apama* (Hanlon & Messenger 1996; Melo & Sauer 1999; Laptikhovsky *et al.* 2002; Hanlon *et al.* 2004). Where severe tissue catabolism and subsequent mortality does not occur, perhaps the disparity in potential reproductive rates between sexes is smaller, and if the ASR is unbiased, this would produce less biased OSRs, like those seen for loliginids. The use of acoustic telemetry for comparing breeding durations in loliginids would potentially provide an understanding of whether potential reproductive rates are similar between sexes, or identify any potential bias in the ASR for these populations.

Regardless of gender, individual residence times were significantly shorter than the four-month breeding period (Chapter 2), suggesting that density-based population surveys are likely to underestimate population size. The 'area under the curve' method (English *et al.* 1992) includes measures of individual residence time in order to estimate abundance, and much attention has been paid to improving the accuracy of this approach with respect to salmon abundance in recent years (e.g. English *et al.* 1992; Lady & Skalski 1998; Manske & Schwarz 2000; Shardlow *et al.* 2007; Baker & Schindler 2009). Given the highly transient nature of individuals from the *S. apama* breeding aggregation, a similar approach that accounts for the brevity of breeding durations would be required to accurately estimate population size. The timing of peaks in cuttlefish abundance varies among years (Hall & Fowler 2003), such that inter-annual variability in biomass detected by surveys conducted at the same time each year may be an artefact of the differential timing of peak abundance, rather than actual changes in population size.

We acknowledge that our technique of measuring breeding durations is likely to underestimate the parameter to some extent, since individuals were present at the aggregation site for an unknown period prior to tagging (Chapter 2). However, this uncertainty is likely to be minimal for individuals tagged at the very beginning of the season (22 May), and breeding durations for those individuals were not significantly different from those of the second tagged cohort (tagged 15 July). Furthermore, relatively brief breeding durations would be expected when consideration is given to the fasting, protein-catabolising reproductive strategy of this species (Hall & Fowler 2003), and the rates of energy expended during breeding (Chapter 3).

Energy expenditure and activity patterns

Male *S. apama* expended the equivalent of approximately 0.8% of their body tissue per day (Chapter 3), and for a 40 d residence period (Chapter 2), this represents a 32% reduction in body mass for the duration of breeding. Like many other cephalopods (e.g. *Octopus vulgaris*; O'Dor & Wells 1978; *Gonatus fabricii*; Arkhipkin & Bjorke 1999; *Sepia dollfusi*; Gabr *et al.* 1999; *Moroteuthis ingens*; Jackson *et al.* 2004) *S. apama* are terminal spawners, catabolising large amounts of body proteins to fuel reproduction, with death following soon after (Hall & Fowler 2003). The relatively sedentary *Octopus vulgaris* can sustain no more than a 50% reduction in body mass (O'Dor & Wells 1978), so it seems plausible that *S. apama*, which actively swims throughout the breeding period (at least during the day; Chapter 3), may indeed sustain maximum levels of catabolism closer to our estimates (32%) than the 50% of *O. vulgaris*. Furthermore, given that non-breeding, captive *S. officinalis* survive for 54-68 days following fasting (Castro *et al.* 1992), the higher metabolic costs of reproduction in *S. apama* are consistent with the somewhat lower breeding duration estimates (i.e. 40 days). Whilst field metabolic rate was not estimated for females, the higher costs of breeding (as mentioned with respect to anisogamy above) would likely result in more rapid protein catabolism than for males, as seen for the terminally spawning *Moroteuthis ingens* (Jackson *et al.* 2004). Taken together, estimates of energy expenditure are consistent with estimates of breeding durations for *S. apama*, and it seems unlikely that individuals remain breeding at the aggregation for longer than 1-2 months. For other terminally spawning cephalopod populations, estimating energy expenditure and associated levels of protein catabolism may serve as a useful proxy for estimating breeding duration or residence times, and this will assist in population size estimation when using ‘area under the curve’ approaches.

A feature of cephalopods is their generally poor anaerobic capacity, with limited glycogen stores reserved for brief periods of burst swimming (Storey & Storey 1979; O'Dor *et al.* 1984; Wells & Clarke 1996). When short periods of anaerobic metabolism do occur, accumulation of octopine (the glycolytic end product) has been shown to cause significant intracellular acidosis in several squid species (Portner *et al.* 1991; Portner 2002). Although *S. apama* were shown to have a relatively modest aerobic scope (1.8; Chapter 3), and that reproductive periods are often the most energetically demanding stages of an animal’s life cycle, metabolic rate rarely approached $\dot{V}O_{2max}$ during any period of the day; a finding consistent with an animal exercising below the critical (anaerobic) threshold. Although metabolic rate remained below $\dot{V}O_{2max}$, activity (measured by acceleration values; Chapter 3) was higher during the day than at night for all individuals tagged. This was not unexpected, given the visual mating strategy of the species, and the observation that conspicuous sexual signalling occurs during the day, and sessile, camouflaged behaviour dominates at night (Hanlon *et al.* 2007). Interestingly, the diel pattern in detection frequency from tagged cuttlefish (Chapter 4) closely matched the pattern observed from accelerometry tags (a dramatic increase and subsequent decrease at dawn and dusk, respectively), however the increased frequency of detections during the day was a result of external environmental factors (most likely biological noise), not cuttlefish behaviour. This result may have profound consequences for acoustic telemetry studies examining the rhythmicity of animal behaviour, as although a leading approach is to examine the relative frequency of detections at various temporal scales (e.g. Heupel *et al.* 2006b; Collins *et al.* 2007; Collins *et al.* 2008; Kawabata *et al.* 2008), no published studies to date have employed controls that separate the influences of animal behaviour from those of other factors. At present, the cost of accelerometry tags is approximately twice that of similarly sized position-only transmitters, and battery life is somewhat lower. However, the same acoustic arrays can be used to detect both tag types, and the size and method of attachment are similar. Budget permitting, researchers interested in activity patterns over shorter (2-4 months) rather than longer (2-4 years) time scales may be wise to employ accelerometry techniques rather than examining the frequency of acoustic detections, as the latter will require vigorously controlling for the influence of environmental factors, and will likely require relatively complex corrections (Chapter 4).

Mass-marking the early life history stages of Sepia apama

Whilst acoustic telemetry and accelerometry can provide valuable insights into the dynamics and behaviour of mature individuals (e.g. Chapters 2, 3 & 4), these techniques are not suitable for the early life history stages of *S. apama*, and measuring dispersal of these stages is recognised as one of the main challenges for marine ecologists (Jones *et al.* 2005; Sale *et al.* 2005). For *S. apama*, understanding degrees of natal homing and the level of recruitment from non-aggregation areas is critical for effective management of the Point Lowly aggregation. The fishing closure implemented in 2004 includes essentially all 60 ha of the spawning habitat, and a significant area to the west (but not south) of Point Lowly. However, no data exists to suggest that this area is used during any life-history stage of *S. apama* outside of the 4-month breeding and egg development period. Indeed, hydrodynamic modelling suggests that passive, bottom-dwelling particles released at the aggregation site in October generally display a southerly trajectory (Kaempf *et al.* 2011), and if cuttlefish hatchlings (which have a poor sustained swimming capacity; N. Payne, unpublished data) exhibit a positive association with this direction of flow, they would quickly leave the protection afforded by the year-round spatial fishing closure. Given that non-breeding periods represent approximately 80% of the *S. apama* life-cycle (assuming breeding represents 2 months of a 12-month life-cycle), a lack of information on pre-aggregation movement presents a significant barrier to management.

The successful development of an enriched isotope mass-marking technique for *S. apama* (Chapter 5) represents the first step towards better understanding dispersal, degrees of natal homing and home range size for this species in northern Spencer Gulf. For fishes, advances in the development of chemical mass-marking techniques (e.g. Ennevor 1994; Brown & Harris 1995; Thorrold *et al.* 2006; Munro *et al.* 2008; Munro *et al.* 2009) have let to the field application of these approaches (e.g. Jones *et al.* 2005; Almany *et al.* 2007), but application to the *S. apama* breeding aggregation has been precluded by a lack of development of such methods for cephalopods. Our study found that various combinations of ^{137}Ba concentration and immersion time can produce unequivocal marks in the statoliths of juvenile cephalopods, and that uptake generally occurs only when spiking occurs in the second half of the development period (i.e. when most cephalopod embryos have undergone organogenesis). The importance of timing for mark success has been illustrated for fish (Thorrold *et al.* 2006; Munro *et al.* 2009), and this will be a critical factor determining the success of a marking regime for cephalopods. Applying the elemental spike after organogenesis will likely improve the success rate of marking, however the timing of organogenesis for cephalopods is highly species- and temperature-dependent (Boletzky 1983), so prior understanding of the species' embryonic development is essential.

Conclusions

Throughout this thesis, a combination of approaches were used to address fundamental inadequacies in our understanding of *S. apama* population dynamics and behaviour. The use of acoustic telemetry to estimate breeding durations between sexes provided evidence that the adult sex ratio of this population is unbiased, and that the aggregation consists of far more individuals than originally thought. Currently of concern is the decline in biomass observed over the past decade, however an 'area under the curve' approach is required to accurately estimate population size, and therefore interpret the significance of this apparent decline. Whilst our estimates of breeding durations provide evidence that individuals are indeed highly transient, greater sampling effort is needed to more accurately estimate mean residence times and the associated variation for each gender, as these are central to the accuracy of the 'area under the curve' approach (English *et al.* 1992; Hilborn *et al.* 1999; Manske & Schwarz 2000).

The accelerometry technique estimated *S. apama* field metabolic rate with high temporal resolution, revealing a strong diel pattern in activity, and rates of energy expenditure consistent with estimates of male breeding durations. This emerging technology has strong potential for a range of behavioural, physiological and ecological applications, and I urge marine researchers to consider this technique in favour of examining the relative frequency of acoustic detections, as the latter approach is highly susceptible to misinterpretation where external factors are not accounted for.

The field application of our isotope mass-marking technique to *S. apama* will potentially provide much-needed insights into the connectivity of aggregation individuals to greater northern Spencer Gulf. In light of recent threats presented by industrial developments proposed for the Point Lowly peninsula, understanding the extent of juvenile dispersal and the contribution of recruits from sources other than the aggregation has never been greater.

Difficulties associated with direct field observation have traditionally created barriers to understanding the population dynamics and behaviour of aquatic animals. Here we give an example of how complementary approaches can be used to provide fundamental insights at the individual and population level.

Ready to begin one of many trips back to Adelaide with trusty “RJ”. Photo credit: Sarah Hone.

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