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Quantifying ecological aspects of the seasonally abundant box jellyfish *Chironex fleckeri* within coastal and estuarine waters of far north Queensland

Thesis submitted by
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in December 2014

For the degree of Doctor of Philosophy
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Dedication:

To Bob and Julieanne

Acknowledgements

Looking back over the past decade, I realise that this thesis really only exists because of the amazing and inspirational people that I have met along the way, all of whom have been willing to give a lot and expect little in return. Thank you to you all.........

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Chapter Four

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ABSTRACT

The occurrence of *Chironex fleckeri* medusae in the near shore waters of tropical Australia represents a significant safety, financial and medical concern during the warmer months of the year, particularly along vast stretches of unprotected coastline. Although a number of theories relate to the seasonal occurrence of medusae, quantitative data documenting key aspects of *C. fleckeri's* ecology are currently lacking. As a result, management protocols tend to be based on anecdotal / opportunistic evidence or generalisations extrapolated between geographic regions. While considerable improvements have been made in reducing the risk of an envenomation occurring along confined stretches of coastline, such as within stinger nets, the capacity to model the occurrence and distribution of medusae, both spatially and temporally, would allow for more effective management of the broader tropical coastline. This study represents the first contribution to the development of such models by quantifying some of the long held theories relating to the ecology of *C. fleckeri*.

The ability to predict when the medusae season will commence would be of considerable benefit to risk management models. Currently, the timing of stinger net installation is largely based on the generalisation that medusae are common inhabitants of the coastline during the warmer months of the year, their arrival being associated with changes in a suite of physical parameters including water temperature, estuary flows and salinity. In contrast, this study has shown that the shift from the polyp to medusa phase was temporally constrained across six seasons in Weipa, with the onset of medusae production varying by only one week in late August / early September. While variables such as water temperature, rainfall and tidal height were not correlated with the onset of medusae production, photoperiod could provide a higher degree of temporal consistency in the onset of the medusae season. Whether

photoperiod acts as the cue for metamorphosis in this species is worthy of further investigation.

Once within the medusa phase of the lifecycle, juvenile medusae are thought to undertake a downstream migration from the polyp habitat to the coastline where they grow in size rapidly, accumulating as the season progresses. Population structure data collected across seven seasons does not suggest that this is the case for Weipa sites, however. Instead, larger, older and sexually mature medusae were found in abundance within Weipa's estuarine habitats, while a high proportion of juvenile medusae were represented within the coastal habitat. Given that the oldest medusa collected was estimated to be 78 d in age and was from the estuarine habitat, medusae do not appear to accumulate along the coastline as the season progresses either. Not only were medusae growth rates rapid, increasing at up to ~3 mm d^{-1} IPD (inter pedalia distance), tentacles were added more rapidly (up to a maximum of 13 per pedalium) as size increased. At this growth rate, medusae would undergo an ontogenetic shift in their cnidome and venom complement after ~50 - 60 d, at which time they become potentially lethal to humans. Given that sexual maturity would also be realised after ~45 - 50 d, the potential for several generations to occur within a single season has also been raised. Determining whether ecological patterns observed in Weipa are consistent between geographic locations is an aspect of *C. fleckeri* ecology into which future research should extend, allowing predictive models of regional rather than local context to be developed.

Many references are made to the exceptional swimming abilities of *C. fleckeri* medusae, who, unlike other species of cnidaria, possess the ability to actively move from one location to another, presumably in response to the development of unfavourable conditions. Recent advances in acoustic telemetry allowed the movement patterns of 12 adult medusae to be documented for between 10 and 38 h, within both

the coastal and estuarine habitats using manual telemetry. The rates at which medusae travelled varied from approximately 52 ± 26 SE m h^{-1} to 828 ± 306 SE m h^{-1}, with both tidal state and time of day found to influence rate of travel. This effect was habitat specific, however, whereby medusae within the coastal habitat travelled at relatively consistent rates both day and night, while estuarine medusae travelled at significantly faster rates at night. At increased current speeds, estuarine medusae also appeared to travel with the current rather than across or against it, as they did at slower current speeds. With acoustic telemetry now demonstrated to be an effective method by which medusae movement data can be collected, future studies can focus on quantifying how medusae movements are influenced by changes in physical parameters such as wind speed and direction (wave action).

Manual acoustic telemetry possess a number of logistical constraints that limit the timeframe over which movement data can be collected, however. To this end, this study has also demonstrated the novel application of automated acoustic arrays to *C. fleckeri* medusae. Combined with a revised method of tag attachment, the timeframe over which medusae were tracked increased from a mean of 16 ± 8 SE h and maximum of 38 h for manual acoustic telemetry to a mean of 37 ± 16 SE h and maximum of 84 h for acoustic arrays. A trade off in the resolution of position estimates derived from acoustic arrays was evident, however, particularly in those cases where position estimates were based on data from a single receiver (839 ± 89 SE m). Error was significantly less where multiple receivers contributed to a position estimate, with a minimum error of 157 ± 10 SE m observed when position estimates were based on four receivers. This was still significantly greater than the 10 m associated with manual telemetry, however. For two medusae tracked using both manual and array techniques, rate of travel estimates from manual telemetry (170 ± 24 SE m h^{-1}) varied significantly from those calculated from array data (487 ± 55 SE m h^{-1}). Similarly, diurnal and tide related movement patterns were inconsistent between manual telemetry and acoustic array analysis.

When array data was considered in terms of presence / absence, however, repeated longshore movements of individual medusae became evident, the direction of which corresponded with tidally generated currents. Movements on several occasions that were restricted to relatively narrow stretches of beachfront (~200 m) indicate that medusae are not simply passive drifters, however. As such, automated acoustic telemetry provides a method by which either presence / absence data or low resolution position estimates can be collected over extended periods of time.

Collectively then, this study has quantified some fundamental aspects of *C. fleckeri's* ecology, validating some long held theories, while bringing others into question. A contribution to the broader understanding of jellyfish ecology has also been made, with growth and development rates along with population structure and demographics now quantified. The development of a predictive model remains subject to further quantitative comparisons, particularly across geographic locations, however.

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CHAPTER ONE

General Introduction

Jellyfish, or Scyphzoan, Hydrozoan and Cubozoan medusae, increasingly represent a significant commercial and economic consideration, particularly in those cases where they occur unpredictably in large abundances (Rajagopal et al., 1989, Masilamoni et al., 2000, Matsumura et al., 2005) or constitute a serious medical risk (Pearn, 1995, Purcell, 2007, Júnior et al., 2010, Chiaverano et al., 2013, Gershwin et al., 2013). While jellyfish envenomation is a worldwide phenomenon (Pearn, 1995, Gershwin et al., 2009), this appears particularly true for cubomedusae jellyfish, with evidence emerging that highly venomous Chirodropids are present within South Western Pacific, Indonesian, Malaysian (Pearn, 1995), Japanese (Lewis and Bentlage, 2009) and Australian waters (Southcott, 1956). Yet, the ecology of the class Cubozoa is poorly understood and there are few quantitative studies documenting medusae distribution patterns (Kingsford et al., 2012). Within the Australian tropics, it is the seasonal occurrence of two Cubomedusae, the Chirodropids *Chiropsella bronzei* Gershwin and

Chironex fleckeri Southcott, that represent a particular medical and economic consideration. A shared preference for the shallow waters of gently sloping beachfronts, particularly on calm, hot days, has resulted in more than 80 fatal envenomations in the past century, as well as hundreds more non-fatal, but nonetheless painful stings, annually (Gershwin and Dabinett, 2004, Gershwin et al., 2009).

The importance of preventing envenomations from occurring has been recognised since the mid 1990’s (Pearn, 1995), but is becoming an increasingly significant aspect of stinger season management given the continued increase in northern Australia's population combined with the diversity of activities that occur along vast stretches of unprotected coastline. The economic implications of an envenomation cannot be underestimated either with Queensland tourism representing a $6.4 billion industry in 2012 (Deloitte Access Economics 2013), forecast to grow at up to 4% per year to 2022 (Hajkowicz et al., 2013). As Hartwick (1987) proposed, there are several pathways through which overall management of the stinger season can be approached (Figure 1.1). To date, significant advances have been made along the intervention pathways, particularly in terms of the physical prevention and medical treatment of stings.

For instance, stinger nets are deployed at 23 locations along the north Queensland coast between November and May each year (Gershwin et al., 2009) and stinger suits are becoming increasingly widespread in application. Public education is ongoing, as are collaborations between local councils and organisations such as Surf Life Saving. Research into venom constituents (Winter et al., 2010, Saggiomo and Seymour, 2012, Chaousis et al., 2014) and first aid treatment (Pearn, 1995, Seymour, 2002, Little, 2008, Yanagihara and Shohet, 2012) has not only increased the understanding of how best to manage an envenomation, but also identified key areas for future research to focus on.

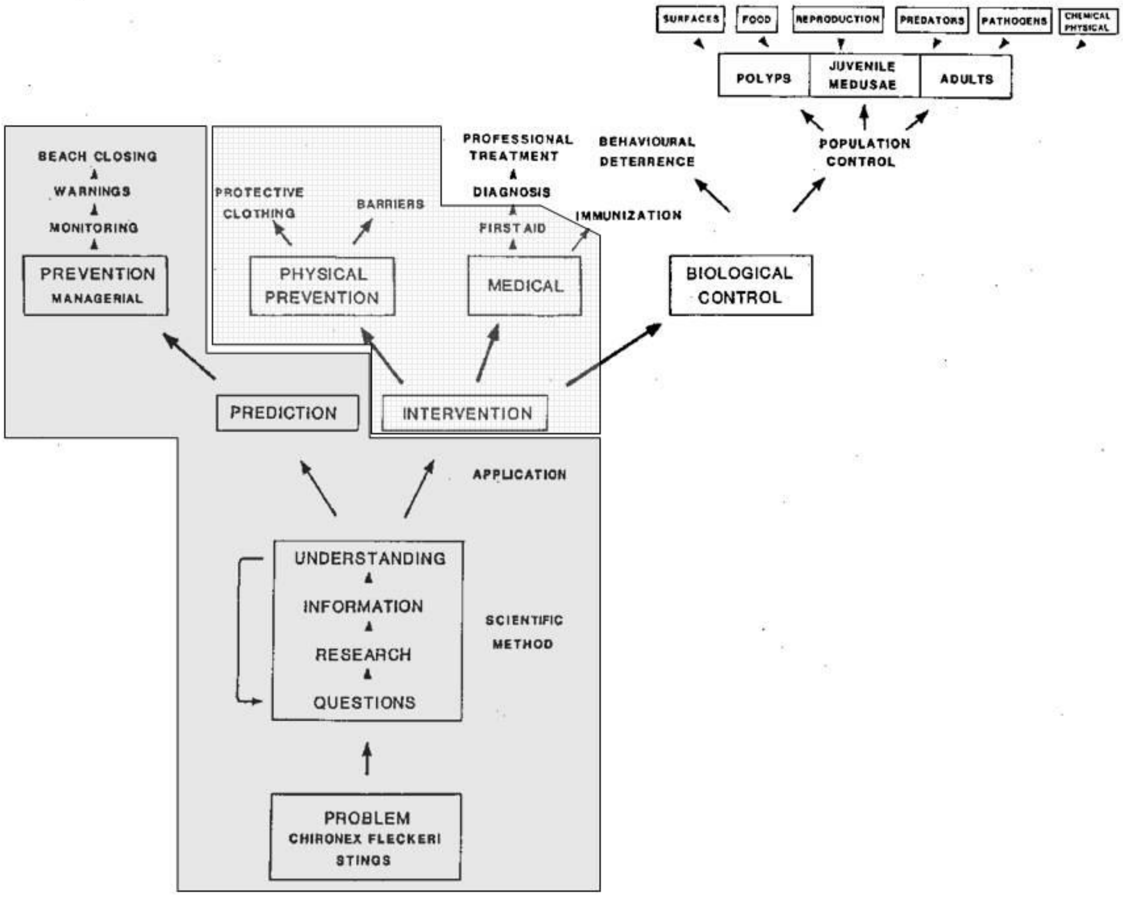

Figure 1.1 A stylised representation of the various pathways through which overall management of the stinger season can be approached (from Hartwick, 1987)

With more than 60 years of largely qualitative research relating to *C. fleckeri* medusae, a unique opportunity currently exists to quantify fundamental ecological aspects of this species, an important first step in the development of models capable of predicting the risk of an envenomation occurring. To this end, this study aims to:

- investigate a number of long held theories relating to specific ecological aspects of *C. fleckeri* medusa
- demonstrate the novel application of acoustic telemetry to *C. fleckeri* medusae as an effective tool by which ecological questions can be addressed
- make an initial contribution to the development of predictive models by providing base line data sets to which future studies can compare, and

- contribute to the overall understanding of a relatively understudied taxon, the cubozoa.

More specifically:

Chapter Three aims to quantify fundamental ecological aspects of *C. fleckeri* medusae including growth and development rates, population structure between estuarine and coastal habitats and the degree to which the onset of the medusae season varies between years. Long held theories to be discussed within this chapter include whether:

- medusae growth and development is rapid
- the onset of the stinger season varies between seasons
- medusae begin their lives within estuaries and undertake a downstream migration, accumulating along the coastline where they mature and grow
- the shift from the polyp to the medusa phase of the lifecycle is influenced by a change in a suite of parameters thought to include rising water temperature or fluctuations in salinity

Chapter Four aims to demonstrate that manual acoustic telemetry techniques are an effective method by which medusae movement patterns can be quantified within both coastal and estuarine habitats. It is the continued miniaturisation of acoustic tags, combined with the development of tag attachment techniques specific to a species with only two cell layers that permits the current research to be undertaken. The long held theories investigated within this chapter include that:

- medusae can move independently of tides / currents
- medusae can move substantial distances over extended periods of time

Chapters Five and Six assess the suitability of automated acoustic telemetry (SUR’s) in documenting medusae movement patterns over longer periods of time, demonstrating the importance of matching the data collection technique to the ecological question being investigated.

This study is the first to approach stinger season management from a quantitative perspective. This provides a platform from which future studies of broader ecological questions such as whether geographic distribution is extending, bloom / sting frequencies are intensifying or season length is increasing can extend.

CHAPTER TWO

Ecology of *Chironex fleckeri* - theories and long held beliefs relating to the occurrence, distribution and movements of medusae

2.1 Cnidarian Taxonomy and the Australian Chirodropid Assemblage

The Cnidaria are one of the oldest extant phyla, having appeared in the fossil record some 600 million years ago, prior to the Cambrian explosion (Coates, 2003). Present in all marine and some freshwater environments, they have derived their name from their unique mechanism of envenomation, the cnidome (Hartwick, 1987). Of the four Cnidarian classes, it is only the Anthozoa (eg. anemones and corals) that do not possess a mobile medusa phase, with an alternation in generations between an asexual polyp phase and a sexual medusa phase typical for the Scyphozoa, Cubozoa and Hydrozoa (Werner et al., 1976, Rifkin, 1996).

Initially grouped within the Scyphozoa, Cubozoans were raised to a new class (Werner et al., 1976) due to the absence of many typical scyphozoan characteristics (Werner et al., 1971) and the presence of a one to one polyp to medusa conversion ratio rather than strobilation of polyps into ephyra (Werner et al., 1971, Werner, 1973, Werner, 1975, Arneson and Cutress, 1976, Werner et al., 1976). The Cubozoa are the smallest class of Cnidarians, with a single order (Cubomedusae), two Families (Carybdeidae and Chirodropidae) and approximately 50 species identified worldwide (Bentlage et al., 2009, Toshino et al., 2013). A highly regular, cube shaped bell is representative of both Carybdeid and Chirodropid medusae, their four sided symmetry emphasised by the pedalia, the fleshy blade like structures located in each lower corner of the bell from which the tentacles arise. Carybdeid medusae are typically smaller in size and possess only one tentacle per pedalium (Hartwick, 1987, Burnett et al., 1996, Rifkin, 1996). Chirodropids not only attain a larger size, but also develop a greater number of tentacles per pedalium (Southcott, 1956, Barnes, 1966, Southcott, 1971, Kinsey, 1986, Hartwick, 1987).

The Australian Chirodropid assemblage is represented by four species: *Chirodectes maculatus* Gershwin, *Chiropsella bart* Gershwin, *Chiropsella bronzei* Gershwin and *Chironex fleckeri* Southcott (Gershwin, 2005; Gershwin, 2006). *Chirodectes maculatus* is unique within this assemblage in that it was collected from an outer edge coral reef ~40 km from the far north Queensland coastline (Cornelius et al., 2005). *Chiropsella bart* is also atypical in that it occurs only within the dry season and is limited in distribution to the Gove region (Gershwin and Alderslade, 2006).

While *C. bronzei* and *C. fleckeri* are considered to typify the Australian Chirodropid assemblage and are the species to which the majority of literature relates, their taxonomy has been subject to considerable confusion since research commenced in the 1950's. Although *C. fleckeri* ultimately achieves a larger size than *C. bronzei*

(Barnes, 1960, Barnes, 1965, Barnes, 1966) and is also the more venomous of the two species (Barnes, 1966, Keen, 1971, Baxter and Marr, 1974), species names were used interchangeably until a review by Barnes (1965) summarised their morphological characteristics. *C. bronzei* itself has only recently been renamed, previously being referred to as *Chiropsalmus quadrigatus* Haeckel until *Chiropsalmus* sp. was used in an attempt to distinguish the Australian species from that found in Burmese waters (Gershwin and Alderslade, 2006). Further clarification of Chirodropid taxonomy would be beneficial to ecological studies, particularly in relation to the possibility that several regional species are currently denoted as a single species (Gershwin and Alderslade, 2006).

2.2 The Geographic Range of Australian Chirodropids

Australian Chirodropids are generally tropical in distribution, being found between Gladstone on the east coast and Exmouth on the west (Figure 2.1). The specific geographic range of each species differs considerably, however. *Chiropsella bart*, for instance, is reported only from the Gove (Nhulunbuy) peninsula in the Northern Territory (Figure 2.1) (Currie et al., 2002, Currie and Jacups, 2005), while *C. bronzei* is reported only from the coastline between Cooktown and Townsville (Figure 2.1) (Barnes, 1966, Brown, 1973, Burnett et al., 1996, Currie et al., 2002). In contrast, reports of *C. fleckeri* medusae can found for much of tropical Australia (Barnes, 1960, Barnes, 1965, Cleland and Southcott, 1965, Brown, 1973, Kinsey, 1986, Hartwick, 1987, Kinsey, 1988, Hartwick, 1991a, Currie, 1994, Hamner and Doubilet, 1994, Sutherland, 1994, Hamner et al., 1995, Currie, 2000, O'Reilly et al., 2001, Currie et al., 2002, Currie, 2003, Currie and Jacups, 2005), suggesting that its geographic range is much wider than that of other chirodropids.

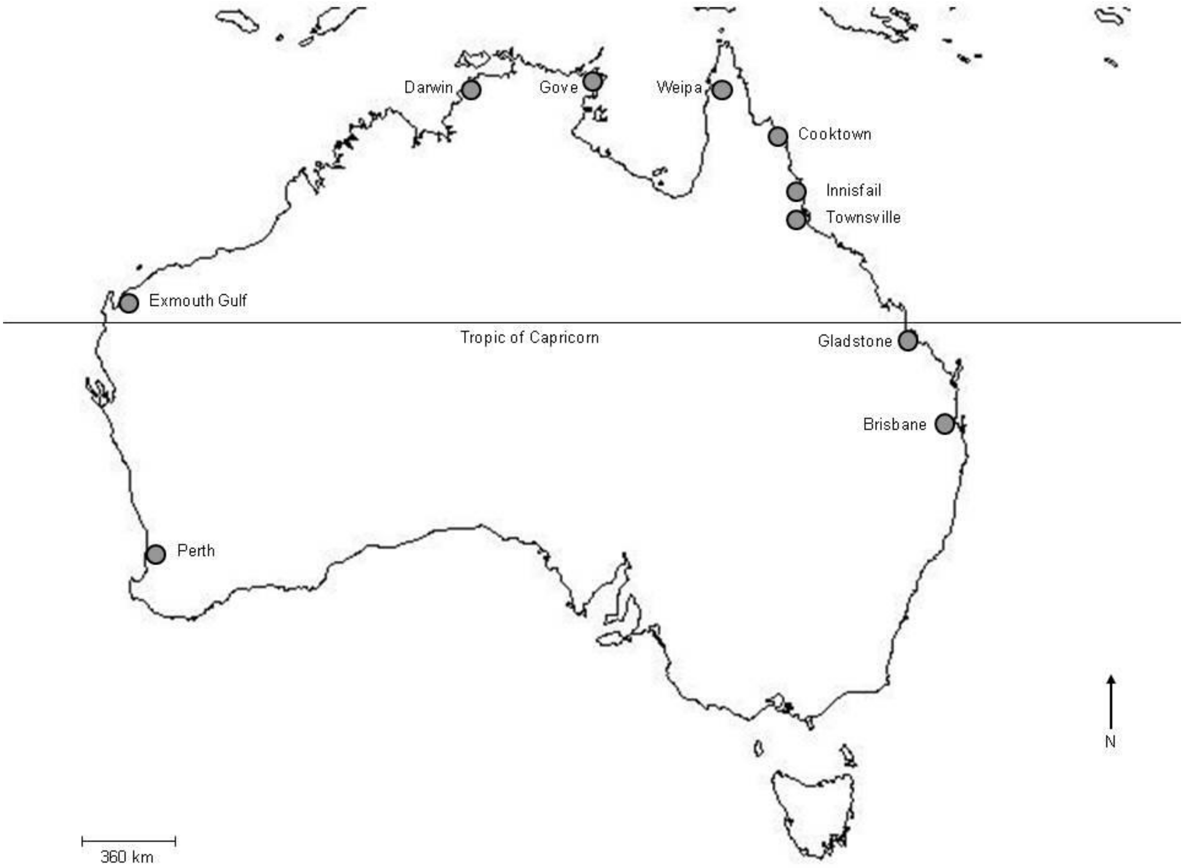

Figure 2.1 Map of Australia showing Tropic of Capricorn and relevant townships marking boundaries to the distribution of Chirodropid medusae within Australian waters

Much of the difficulty in defining the true geographic range of each species can be attributed to the biases associated with using qualitative studies (such as Barnes (1965) and Yamaguchi and Hartwick (1980)) and sting records (such as Southcott (1960), Southcott (1971) and Williamson et al. (1980)) to define a species distribution. Sting records may reflect the absence of bathers from a given location rather than the absence of medusae, especially in remote areas of tropical Australia where vast stretches of coastline are relatively uninhabited.

2.3 The Cubozoan Life Cycle

Cubozoans are known to possess a complex life history (Leonard, 1980) in which an asexually reproducing polyp phase alternates with a sexually reproducing medusa phase (Werner et al., 1971, Werner, 1973, Werner, 1975, Werner et al., 1976, Yamaguchi and Hartwick, 1980, Hartwick, 1987, Hartwick, 1991a, Rifkin, 1996). The mobile medusae phase maintains genetic variability and geographic distribution through migration (Hartwick, 1987, Hartwick, 1991a), spawning (Yamaguchi and Hartwick, 1980, Hartwick, 1987, Hartwick, 1991a) or copulatory processes (Studebaker, 1972, Werner, 1973, Arneson and Cutress, 1976, Hartwick, 1991b, Lewis and Long, 2005). The cryptic, sedentary polyp phase ensures an abundance of juvenile medusae at the onset of the following season by rapidly increasing the polyp population through asexual budding of secondary polyps (Werner et al., 1976). This is a particularly important process given the one to one conversion ratio of polyps into medusae.

2.4 The *Chironex fleckeri* Life Cycle

To date, *C. fleckeri* is the only Australian Chirodropid for which the complete life cycle has been described (see Hartwick, 1987). It has been suggested that *C. fleckeri's* alternation in generations not only occurs on a seasonal basis, but also incorporates a shift between the estuarine and coastal habitats (Figure 2.2) (Hartwick, 1987, Hartwick, 1991a). That is, while an abundance of medusae are found in coastal waters during the warmer months of the year, it is the polyps that inhabit coastal estuaries throughout the winter months.

Mature medusae are thought to migrate up estuary systems towards the end of the season, where a yet to be observed semelparous spawning event occurs (Hartwick, 1991a). Ciliated planulae hatch ~12 h after fertilisation, settle onto a solid substrate within 2-3 d and undergo metamorphosis into motile primary polyps within 3 d of settlement (Yamaguchi and Hartwick, 1980). Following a period of site selection, sedentary secondary polyps of less than 2 mm height develop (Yamaguchi and Hartwick, 1980). Secondary polyps require little energy for survival and can endure several weeks of starvation in an encysted form, yet take immediate advantage of favourable conditions by rapidly increasing the polyp population through asexual reproduction (Hartwick, 1991a). Although asexual budding was observed ~3 wk after fertilisation in laboratory based studies, metamorphosis did not commence until 58 d after spawning (Yamaguchi and Hartwick, 1980).

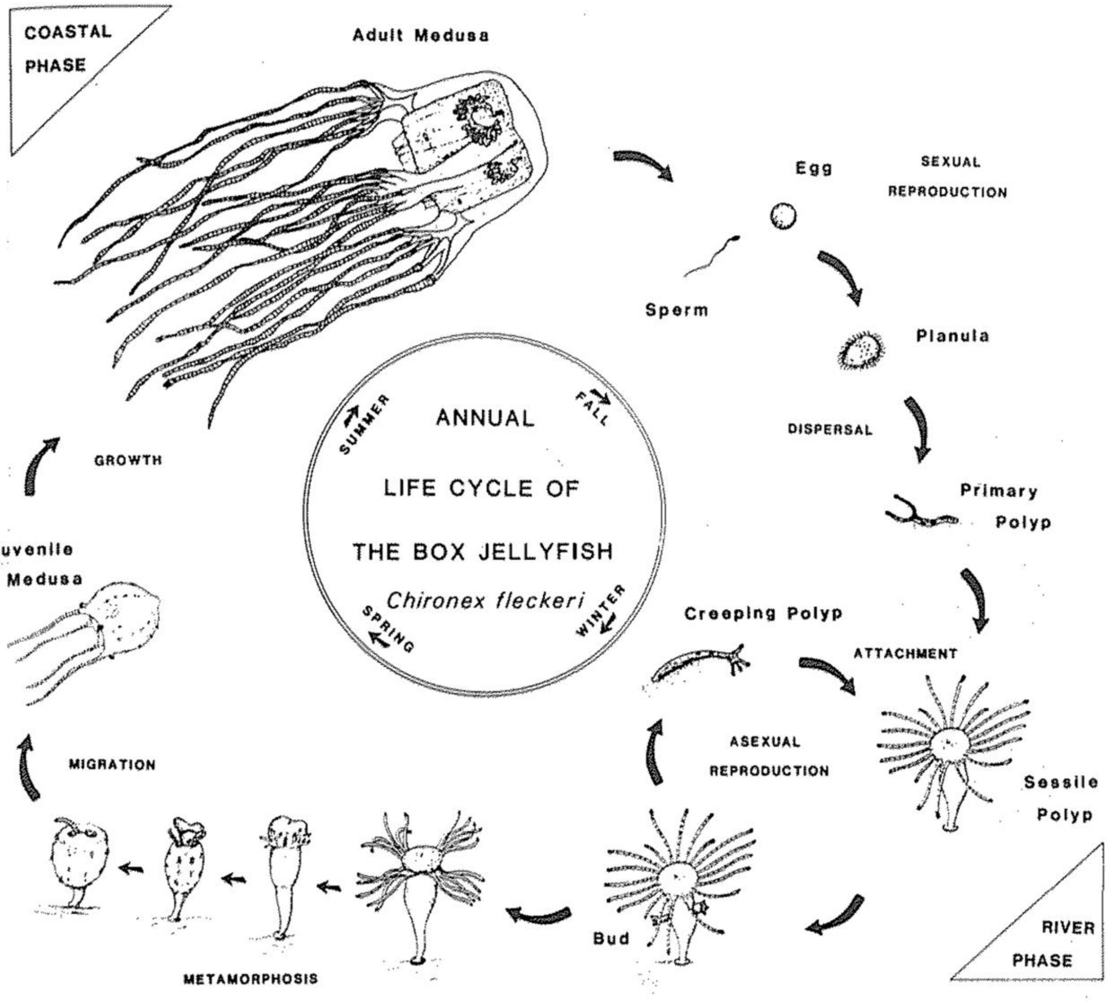

Figure 2.2 The *Chironex fleckeri* life cycle showing an alternation in both generations (sexual medusa phase to asexual polyp phase) as well as between the estuarine and coastal habitats (modified from Hartwick (1987))

In the wild, polyp metamorphosis appears to be delayed for several months, with September being the earliest that juvenile medusae have been collected from coastal estuary systems (Hartwick, 1991a). Juvenile medusae are thought to begin their existence some distance from the coastline, gradually moving out to the beaches where they grow and mature until late in the season (Hartwick, 1987).

Some conjecture exists around the species to which this life cycle refers, however. That is, as seen in Figure 2.2, juvenile medusae possess nematocyst warts on the bell, a characteristic considered typical of Carybdeid, not Chirodropid medusae. The polyps illustrated within Figure 2.2 are also morphologically similar to carybdeid polyps (see Straehler-Pohl and Jarms, 2005; Straehler-Pohl and Jarms, 2011). Fundamental aspects of *C. fleckeri's* life cycle are yet to be confirmed as well. Included here is the progression in population structure from an abundance of small medusae early in the season to fewer but larger, sexually mature adults late in the season that has been used to imply an annual reproductive cycle (Hartwick, 1991a). So too is the upstream migration of mature medusae, their subsequent aggregation and spawning as well as the early season downstream migration of juvenile medusae. That juvenile medusae were only found between 6 and 15 km from estuary mouths in intensive offshore, coastal and estuarine sampling suggests that the polyp habitat is located some distance from the coastline. Yet, the only wild polyp population was discovered just 1 km from the mouth of a creek (Hartwick, 1991a), a population that did not persist beyond a single season, however (Hartwick, 1991a). As such, the location of the polyp habitat also remains elusive despite its relevance to understanding the overall ecology of this species as well as the mechanisms driving the shift from one phase of the lifecycle to the next.

Validation of specific aspects of *C. fleckeri’s* lifecycle and the subsequent description of *C. bronzei* and *Chiropsella bart’s* lifecycles are a critical next step in the development of models predicting medusae occurrence within a season. Links between physical / biological parameters and the progression from one phase of the life cycle to the next or between habitats / locations cannot be investigated quantitatively until the longevity and location of each life cycle phase is understood.

2.5 Current Definition of the Stinger Season

The official stinger season extends from November to May for east coast sites (Hartwick, 1987, Hartwick, 1991a, Williamson et al., 1996a) and October to June for the Northern Territory (Southcott, 1971, Currie et al., 2002, Currie and Jacups, 2005). The onset and duration of each stinger season appears to vary, however, both geographically and temporally. For instance, medusae have appeared along the far north Queensland coastline as early as October in some seasons (Southcott, 1971, Hartwick, 1991a, Fenner and Williamson, 1996), having being present within estuary systems from as early as September (Hartwick, 1991a). While April or May are generally accepted as the end of the stinger season (Southcott, 1971, Hartwick, 1991a, Fenner and Williamson, 1996), *C. fleckeri* medusae have been observed along the coastline as late as June (Hartwick, 1987). Furthermore, as latitude decreases, there is a trend for the stinger season to increase in length (Sutherland, 1983, Fenner and Williamson, 1996, Williamson et al., 1996a), with medusae observed in all months of the year in equatorial regions (Williamson, 1988, Williamson et al., 1996b).

That these definitions of the stinger season are somewhat arbitrary can be attributed to the majority of studies to date having focused on the presence of medusae along the coastline (stinger season) rather than the total time frame over which the medusa phase occurs (medusae season). Further confounding this situation is a general lack of data relating the temporal changes of the polyp habitat to the first occurrence of juvenile medusae. As a result, the mechanisms driving the shift from one phase of the life cycle to the next are poorly understood. It is not until a better understanding of these mechanisms is developed that the seasonal occurrence of medusae can be defined with greater accuracy.

Of particular importance is developing an understanding of which factors are driving the shift from the asexual phase of the life cycle to the sexual phase. Manipulative trials investigating cubopolyp metamorphosis with respect to specific parameters are very limited, however, with it being necessary to source common themes from literature relating to the scyphozoa. Unlike the 1:1 polyp to medusa metamorphosis ratio of cubopolyps, scyphopolyps produce multiple ephyra through the process of strobilation, in which each ephyra gives rise to a medusa (Calder, 1982, Cargo and King, 1990, Brewer and Feingold, 1991, Fitt and Costley, 1998, Purcell, 2005). A number of factors have been identified or implicated as cues for metamorphosis / strobilation, including changes in water temperature, fluctuations in salinity, nutrition levels and polyp starvation, light intensity and photoperiod, the presence of symbiotic organisms, development of polyps to a maximum number of tentacles, oxygen concentration levels and the presence or introduction of chemicals such as thyroxin or iodine (Table 2.1).

For many species of Scyphozoans and Cubozoans, temperature acts as a cue for metamorphosis or strobilation (Table 2.1). Both the mechanism of, and threshold at which temperature becomes significant differs between species, however. For instance, while metamorphosis in *Tripedalia cystophora* (cubozoan) occurs when water temperatures rise from 20 to 24ºC (Laska-Mehnert, 1985), for *Nemopilema nomurai* (scyphozoan), it is a rise from 10 to 20ºC that results in strobilation (Kawahara et al., 2006). Although it is unclear as to how polyps would monitor water temperature, it has been suggested that a positive relationship between metabolic rates and temperature would provide additional energy for the process of strobilation in *Cassiopea* sp. (Rahat and Adar, 1980).

Parameter	Effect	Class	Species	References
Temperature increasing 20 to 24ºC	Metamorphosis	Cubozoa	*T. cystophora*	(Laska-Mehnert, 1985)
Temperature stable @ 28ºC (stable salinity)	Several waves		*C. fleckeri*	(Yamaguchi and Hartwick, 1980)
Temperature @ 20 to 24ºC	Metamorphosis		*C. xamachana*	(Hofmann et al., 1996)
Temperature @ 28 - 29ºC	Metamorphosis		*T. cystophora*	(Werner et al., 1971, Werner, 1975)
Temperature @ 25-28ºC	Metamorphosis		*A. moseri*	(Carrette et al., 2014)
Temperature increasing 15 to 20 to 25ºC	Strobilation	Scyphozoa	*C. andromeda*	(Hofmann et al., 1978)
Temperature increasing 10 to 20ºC	Strobilation		*C. quinquecirrha*	(Cargo and Schultz, 1966, Calder, 1974, Cargo and King, 1990, Purcell et al., 1999)
Temperature stable @ 25ºC	Strobilation		*S. megalaris*	(Calder, 1982)
Temperature stable @ 21ºC	Strobilation		*C. mosaicus*	(Pitt, 2000, Holst et al., 2007)
Temperature increasing from 10 to 20ºC	Strobilation		*R. verilli*	(Calder, 1973)
Temperature increasing to 18 to 27ºC*	Strobilation		*R. esculenta*	(Chen and Ding, 1983)
Temperature decreasing to below 19ºC	Strobilation		*Cyanea sp.*	(Brewer and Feingold, 1991)
Temperature increasing to ~20ºC	Strobilation		*M. papua*	(Sugiura, 1965)
Temperature increasing 13 to 23ºC	Strobilation		*N. nomurai*	(Kawahara et al., 2006)
Temperature decreasing 15 to 10ºC	Strobilation		*R. octopus*	(Holst and Jarms, 2007, Holst et al., 2007)
Temperature rise 5 to 10 or 10 to 15ºC*	Strobilation		*R. octopus*	(Holst and Jarms, 2007, Holst et al., 2007)
Temperature above 25ºC + photoperiod > 5 h	Metamorphosis	Cubozoa	*C. morandinii*	(Straehler-Pohl and Jarms, 2011)
Temperature increasing 20 to 28 º + starvation	Metamorphosis		*C. marsupialis*	(Stangl et al., 2002)
Temperature 23 to 28ºC + increased feeding	Metamorphosis		*C. marsupialis*	(Straehler-Pohl and Jarms, 2005)
Temperature increasing + symbiotic algae	Strobilation	Scyphozoa	*C. andromeda*	(Rahat and Adar, 1980)
Temperature 16 to 24ºC + increasing photoperiod	Strobilation		*P. punctata*	(Rippingale and Kelly, 1995)
Increasing temp, photoperiod + light intensity	Strobilation		*A. labiata*	(Purcell, 2007)
Temperature ~ 22ºC + zooxanthellae*	Strobilation		*M. papua*	(Sugiura, 1965)
Temperature 19 to 25ºC + zooxanthellae	Strobilation		*C. tuberculata*	(Kikinger, 1992)
Temperature increasing + presence of light	Strobilation		*C. quinquecirrha*	(Loeb, 1973)
Decreasing temperature + lower light intensity	Prolific strob.		*A. aurita*	(Custance, 1964, Custance, 1967)
Temperature @ 21 - 24ºC + food twice / week	Continuous		*A. aurita*	(Spangenberg, 1965)
Temperature ~25ºC + higher light intensity	strob.		*A. aurita*	(Liu et al., 2009)

Parameter	Effect	Class	Species	References
Iodine addition of *	Strobilation	Scyphozoa	*Aurelia sp.*	(Spangenberg, 1967, Black and Webb, 1973, Olmon and Webb, 1974)
Iodine addition of	Strobilation		*C. quinquecirrha*	
Thyroxine addition of	Strobilation		*Aurelia sp.*	(Spangenberg, 1971, Spangenberg, 1974)
Introduction of metamorphosing polyps	Metamorphosis	Cubozoa	*C. marsupialis*	(Stangl et al., 2002)
Photoperiod	Strobilation	Scyphozoa	*C. capillata*	(Grondahl and Hernroth, 1987)
Photoperiod	No		*Aurelia sp.*	Kakinuma in (Spangenberg, 1968)
Photoperiod **	Strobilation		*R. esculenta*	(Chen et al., 1984)
Reduced feeding frequency	Metamorphosis	Cubozoa	*A. mordens*	(Courtney and Seymour, 2013)
Salinity decreasing to 5‰	No	Cubozoa	*C. fleckeri*	(Hartwick, 1991a)
Salinity increasing 20 to 35‰	Strobilation	Scyphozoa	*C. quinquecirrha*	(Purcell et al., 1999)
Rainfall runoff - peaks in estuary discharge	Suspected / Supported but not conclusive	Cubozoa	*C. bronzei*	(Gordon, 1998, Gordon et al., 2004)
Development of 16 tentacles			*C. alata*	(Arneson and Cutress, 1976)
Presence of algal photosynthesis			*C. andromeda*	(Hofmann and Kremer, 1981)
Temperature - elevated			*C. xamachana*	(Fitt and Costley, 1998)
Temperature - decreasing		Scyphozoa	*A. aurita*	(Watanabe and Ishii, 2001, Miyake et al., 2002)
Constant temperature but well fed			*R. nomadica*	(Lotan et al., 1992)
Tidal peaks - rhythms			*C. quinquecirrha*	(Calder, 1974)
Light			*A. aurita*	(Henroth and Grondahl, 1985)
Attainment of large size + adequate food*			*Various species*	(Berrill, 1949)
Temperature + light + food			*Various species*	(Lucas, 2001)

* did not result in metamorphosis unless a period of preconditioning at a low temperature has occurred

Table 2.1 Summary of factors that act as cues for metamorphosis in cubozoans or strobilation in scyphozoan, showing threshold levels and for which trials have been undertaken

That a decrease in water temperature can also act as the cue for strobilation in some species of scyphozoa initially appears contradictory (Table 2.1), however, it is suggested that this may be a form of preconditioning at a lower threshold, with strobilation inhibited despite increasing temperatures for those polyps that have not spent time at a lower temperature (Holst and Jarms, 2007, Holst et al., 2007). Indeed, *Chrysaora quinquecirrha* (Loeb, 1972) and *Mastigias papua* (Sugiura, 1965, Hofmann et al., 1978) require approximately six to eight weeks of chilling at low temperatures before a rise in water temperature will induce strobilation (Sugiura, 1965, Spangenberg, 1967, Black and Webb, 1973, Hofmann et al., 1978).

The onset of medusae production in some species requires the interaction between two or more factors. Such is the case for *Chrysaora quinquecirrha* (scyphozoan) which requires light, temperature and food levels to be increasing before strobilation will commence (Purcell et al., 1999). Significantly higher levels of strobilation have also been reported for this species when iodine was added to water that was already rising in temperature (Black and Webb, 1973), while Cargo and King (1990) found that strobilation only commenced when salinity was above 11‰ and water was warmer than 18ºC. An interaction between temperature and light, either in the form of light intensity or photoperiod, has been noted for several species. For example, *Carybdea morandinii* (cubozoan) requires temperatures to rise above 25ºC and more than 5 h d^{-1} of direct sunlight (Straehler-Pohl and Jarms, 2011), while *Aurelia labiata* (scyphozoan) requires increasing temperatures, photoperiod and light intensity for the onset of strobilation (Purcell et al., 2007). That *Cotylorhiza tuberculata* and *Chrysaora quinquecirrha* (scyphozoans) require increasing temperature and the presence of zooxanthellae (Loeb, 1973, Kikinger, 1992) suggests the onset of strobilation for these species is also linked to light (intensity or photoperiod) with zooxanthellae being symbiotic primary producers. The requirement for either an interaction between several factors or a period of preconditioning at a lower / upper threshold could serve as a safety mechanism preventing the progression from one phase of the lifecycle to the next at an unsuitable time.

Given the role that temperature appears to play in regulating the onset of medusae / ephyra production in a diversity of species of both Scyphozoans and Cubozoans, either singularly or as an interaction with other cues, temperature should be strongly considered in future research attempting to identify the cue for polyp metamorphosis in other species.

Manipulative trials quantitatively linking changes in physical parameters (such as temperature) to polyp metamorphosis for *C. fleckeri* are currently lacking, however. Instead, many theories relating to the onset of the *C. fleckeri* medusae season attempt to link the arrival of medusae along the coastline with a generalised suite of environmental parameters occurring at that time. For instance, some authors suggest medusae arrival is closely linked to the first rains of the summer wet season, with the onset of the stinger season delayed if the wet season is late (Barnes, 1966). Some authors discount the relevance of the wet season (Burnett et al., 1996, Jacups, 2010a), however, suggesting that medusae arrival is instead linked with rising water temperatures (Barnes, 1966, Hartwick, 1987, Jacups, 2010a, Jacups, 2010b). Quantitative data linking these two events does not currently exist, however. Furthermore, while laboratory studies on *C. fleckeri* polyps did result in several waves of juvenile medusae, temperature could not be identified as the cue for metamorphosis as all trials were conducted at 28°C (Hartwick, 1991a). Metamorphosis did not appear to be related to a decrease in salinity either, with polyps able to function normally down to 20‰ and recover from an encysted state induced by salinities as low as 13‰ (Hartwick, 1991a). Whether dilution rates used within these trials were rapid enough to induce metamorphosis rather than encystment remains unclear, however. Indeed, this is an area of cubozoan ecology that is poorly studied and worthy of further investigation, with the cue for metamorphosis having been identified for only four cubozoan species.

Whether metamorphosis is a single event or occurs over an extended period of time is also of importance. A size range of *C. fleckeri* medusae at the onset of the season (Brown, 1973) could be attributed to a difference in ages rather than growth rates if metamorphosis was to occur over an extended time frame or on more than one occasion. Not only have successive waves of juvenile medusae been observed in polyp studies (Yamaguchi and Hartwick, 1980), but the presence of juvenile medusae in plankton samples between September and January (Hartwick, 1991a) combined with the presence of smaller medusae in January than in December (Brown, 1973) collectively suggest that metamorphosis is not limited to a single occasion for *C. fleckeri*. Likewise, the observation of four cohorts of *C. bronzei* within the one season (Gordon, 1998, Gordon et al., 2004) suggests that metamorphosis for this closely related species is not limited to a single event either. Since *C. fleckeri* polyps can endure periods of low salinity by undergoing encystment, it is possible that metamorphosis ceases only when salinity reaches a yet to be defined lower lethal limit (Hartwick, 1991a).

2.6 Defining the Typical Chirodropid Medusae Habitat

Australian Chirodropids display a general preference for the shallow waters of estuary mouths, mangrove systems and sandy beachfronts. For instance, large numbers of *C. bart* have been reported from the shallow waters of sandy beaches of Gove (Nhulunbuy, NT) (Currie et al., 2002), as have *C. bronzei* from the shallow waters of gently sloping sandy beaches of far north Queensland (Gordon, 1998, Gordon et al., 2004). Although a generalised affinity for the mouths of rivers and creeks has been indicated for *C. fleckeri* (Kinsey, 1986, Hartwick, 1987), medusae have also been collected regularly from beach systems (Barnes, 1966, Brown, 1973, Kinsey, 1986, Kinsey, 1988, Hartwick, 1991a) and only rarely more than a few kilometres from shore (Kinsey, 1988, Hamner et al., 1995, Burnett et al., 1996). Eighty three percent of stings in the Darwin region occur in water less than 1 m deep (Currie and Jacups, 2005), further supporting the theory that an abundance of medusae are present in shallow coastal waters, an area also frequently utilised by swimmers. With research to date having been concentrated within these shallow, coastal areas rather than the adjoining estuary, island or offshore systems, the potential for such generalisations to be an artefact of disproportionate sampling is considerable. Much of the data found within the literature is also qualitative or anecdotal, based on opportunistic sightings or sting records, with studies documenting medusae abundance between habitats / across shelf limited to Hartwick (1991a) and Kingsford et al. (2012). Further quantitative data is required before a robust definition of *C. fleckeri*'s habitat can be developed.

Reports of both *C. fleckeri* and *C. bronzei* from Magnetic Island, located approximately 2km from the coastline at Townsville, also appear contradictory to the above preferred habitat generalisations. It is worth noting, however, that medusae were typically collected from the shallows of gently sloping beachfronts, particularly the tidal sand flats of a protected bay (Brown, 1973). Furthermore, Magnetic Island is not only large enough to support mangrove communities and small estuary systems, but also possesses numerous sandy beaches separated by rocky headlands. Such features make Magnetic Island similar to the coastal sites from which both *C. fleckeri* and *C. bronzei* have been collected.

2.7 Factors Likely to Influence the Distribution and Abundance of Medusae

The distribution and abundance of medusae is highly variable, both spatially and temporally (Hartwick, 1987, Kingsford et al., 2012). That is, it is not uncommon to find

90% of medusae along 10% of the beachfront (Kinsey, 1986), yet it is not always the same 10% of beachfront along which an abundance of medusae are found. A number of factors potentially influence medusae distribution, with salinity (Barnes, 1960, Barnes, 1966, Hartwick, 1987), the abundance and distribution of prey items (Barnes, 1960, Hartwick, 1991a) and turbidity (Gordon, 1998) thought to play a role. It is unclear, however, whether these factors are themselves driving medusae abundance or simply correlated with increased water turbulence, rainfall runoff or wind energy (Southcott, 1958, Barnes, 1960, Barnes, 1966, Yamaguchi, 1982). While moon phase does not appear to influence medusae movements (Kinsey, 1986), some evidence suggests that time of day and tidal regime do (Southcott, 1959, Kinsey, 1986, Seymour et al., 2004). Many theories relating to the occurrence and distribution of medusae along the coastline are based on anecdotal evidence and qualitative data, however, with a need for quantitative data documenting medusae movement patterns evident.

2.7.1 Influence of Wave Action

Inshore prevalence of *C. fleckeri* medusae is closely related to local weather conditions, with the beachfront approached only during flat, calm conditions (Barnes, 1960, Barnes, 1966). The risk of an encounter is at its maximum when the surface of the water is not riffled (Barnes, 1966, Kinsey, 1986). In the presence of a small swell, medusae are thought to move seaward of the wave break and virtually disappear when rough conditions develop (Barnes, 1960, Barnes, 1966). A return to calm conditions is typically accompanied by the reappearance of medusae within a day or two (Kinsey, 1986).

Since such wave action is often driven by prevailing breezes, wind direction may secondarily influence medusae movement patterns (Hartwick, 1987). Indeed, the presence of large *C. fleckeri* medusae in Cairns is said to be dictated by the wind

(Kinsey, 1986) as is the appearance of *C. fleckeri* on the eastern side of Magnetic Island associated with periods of fresh to strong northerly winds (Brown, 1973). Under these conditions, bays with a northerly aspect become quite choppy, while those on the eastern side of the island remain protected (Appendix 2.1) (Brown, 1973). While Barnes (1966) also noted that medusae were more common along the coastline following winds of a northerly component, it is unclear whether this observation was made in reference to chirodropid or carybdeid medusae.

2.7.2 Influence of Salinity

The occurrence of medusae along a beachfront may be influenced by rainfall, though not dependent upon it (Hartwick, 1987). With few quantitative studies linking medusae abundance to salinity (or rainfall runoff), species specific responses remain difficult to define. For instance, lower abundances of *C. bronzei* medusae during periods of increased rainfall and runoff (Gordon, 1998) suggest a negative relationship between abundance and salinity for this species. Likewise, the absence of medusae from samples following periods of high freshwater outflow (Kingsford et al., 2012) suggests a similar case exists for *C. fleckeri*. Yet, other authors suggest that *C. fleckeri* demonstrates a preference for lower salinity waters, with areas in which *C. fleckeri* and *C. bronzei* coexist being of intermediate salinities (Barnes, 1966).

A preference for higher salinity waters appears evident for other Chirodropid species, however. For instance, *Chiropsella bart* medusae only occur within the winter dry season (Currie et al., 2002) when rainfall is typically low and salinity regimes are more likely to be elevated and stable. Similarly, the swarming of *Chiropsalmus quadrumanus* Agassiz within the estuaries of the Gulf of Mexico has been attributed to the advent of unusually high salinities (Guest, 1959, Phillips and Burke, 1970, Kraeuter and Setzler, 1975). Considered to be an oceanic species that cannot tolerate low salinities for any length of time (Guest, 1959), no further specimens were recorded once estuarine salinities returned to their typical, lower levels (Guest, 1959, Kraeuter and Setzler, 1975, Phillips and Burke, 1970).

2.7.3 Influence of Prey Distribution

C. fleckeri is a large nektonic predator that would expend considerable amounts of energy swimming and feeding (Hartwick, 1991a). As a result, medusae would be dependent upon an adequate supply of prey (Burnett et al., 1996) such as small crustaceans and fish (Halstead, 1965, Barnes, 1966, Southcott, 1971, Endean, 1988, Hartwick, 1991a, Hamner and Doubilet, 1994, Carrette et al., 2002). Given that medusae have been found in large numbers swimming amongst such prey in the shallows (Hartwick, 1991a), there is potential for medusae distribution to reflect that of their prey. In particular, seasonal rains influence the availability of shrimp and fish fry (Kinsey, 1986), with increased rainfall runoff facilitating enhanced primary production along the coastline (Hartwick, 1991a), allowing fish and prawns to aggregate in these areas (Barnes, 1966, Hartwick, 1987). The already patchy and variable distribution and abundance of prawns and fish (Gunn and Milward, 1985, Xiao and Greenwood, 1993, Sheaves, 1996, Hanamura, 1999, Omundsen et al., 2000) is likely to be further compounded by variations in physical parameters. It is in this way that the distribution and occurrence of medusae may be further compounded by variations in their prey items.

2.7.4 Size of Medusae

The response of a medusa to a given abiotic or biotic factor appears to vary with size. For instance, small *C. fleckeri* medusae are reported to “put up with a whole lot more” than large medusae when rough conditions develop along the coastline (Barnes, 1966). Large medusae also usually swim against the current (Kinsey, 1986), while smaller individuals swim in either direction (Rifkin, 1996). It is also only large medusae that move from the northern bays of Magnetic Island to those on the eastern side when northerly winds and increased wave action develop (Brown, 1973). Since large medusae are able to supplement their diet of prawns with fish following an ontogenetic shift in their cnidome (Carrette et al., 2002), potentially it is only large medusae that possess the capacity to undertake such relocations. Alternatively, since larger medusae are able to travel at faster speeds while using a slower pulse rate (Shorten et al., 2005), perhaps it is only larger individuals that possess the ability to travel the distances that separates northern and eastern bays. Both the movement capabilities of medusae and the degree to which movement patterns are size specific remain unclear, however, with terms such as small and large undefined and distances of travel or medusae swim speeds unquantified. The lack of a reliable method by which medusae movement data can be collected has almost certainly prevented a comprehensive understanding of medusae movement patterns from being developed, however.

2.7.5 Time of Day, Moon Phase and Tidal State

There is limited evidence within the literature that medusae movements are not consistent across tidal states, throughout the lunar month or throughout the day. Indeed, *C. fleckeri* medusae have typically been collected on a making or high tide, with few individuals observed on the low tide (Kinsey, 1986). While retrospective analysis of sting data has suggested that *C. fleckeri* stings were most frequent during the moon's last quarter and least frequent during the first quarter (Fenner, 2000), it does not appear that these data were standardised for the number of people present at the beach. As such, these results may reflect beach attendance rather than altered medusae behaviour.

The effect that time of day has on movement patterns is more pronounced, with *C. fleckeri* medusae undertaking a period of inactivity in the mid to late afternoon (Kinsey, 1986, Seymour et al., 2004). In fact, rates of movement during the day (0600 h to 1500 h) were more than 20 times those observed at night (1501 h to 0559 h) (Seymour et al., 2004). Diurnal patterns have also been reported for two other species of cubozoa whereby *Tripedalia cystophora* medusae are active exclusively during the day while *Carybdea civickisi* medusae only become active at night (Garm et al., 2012). Significantly higher rates of cell proliferation have been shown for certain body parts of *Tripedalia cystophora* medusae during these periods of inactivity (Gurska and Garm, 2014), while it has also been suggested that *C. fleckeri* medusae may conserve energy throughout the night when they are unable to feed due to their inability to see (Seymour et al., 2004). Alternatively, these periods of 'sleep' may allow the neural network to recover from the high demands placed on it during daylight hours (Kavanau, 2006). Such theories suggest that medusae possess mechanisms by which external stimuli may can be collected, processed and incorporated into movement patterns. Determining the extent to which cubomedusae are capable of such processes is therefore of importance.

Collectively then, it appears that while a number of biological, physical and chemical factors may influence medusae movement patterns, the extent to which each factor is driving the relocation of medusae from one area to another has yet to be quantified. This is an area of *C. fleckeri's* ecology that future research must extend if medusae occurrence and distribution is to be modelled with any reliability.

2.8 Are Cubomedusae Capable of Coordinated Directional Movements ?

The sensory capabilities, behaviour and propulsion of cubomedusae are considered unique among medusae, with their distinctive swimming traits well suited to their foraging strategies (Colin et al., 2013). Their elaborate behavioural repertoire (Garm et al., 2011), regarded by some to be the most elaborate of all cnidarians (Garm et al., 2007b, Garm et al., 2007c), includes complex behaviours such as obstacle avoidance, light shaft attractance, fast directional swimming and mating (Garm et al., 2007a; Garm et al., 2012). Indeed, cubomedusae are extremely efficient, agile swimmers, capable of changing direction in just a few bell pulses in response to a variety of photic and visual stimuli (Satterlie, 2002). While the level of neuronal processing that cubomedusae are capable of has long been a matter of dispute (Garm and Mori, 2009), there is a growing body of literature that suggests cubomedusae possess the anatomical structures necessary to collect information on their environment, process this sensory input and coordinate directional and deliberate responses, processes normally undertaken by the nervous system of later evolving invertebrates (Satterlie and Nolen, 2001). The cnidarians are the earliest metazoans to possess such a system (Kavanau, 2006, Garm et al., 2007a), with the cubomedusae nervous system comprised of four rhopalia, a nerve ring and the motor nerve net (Coates, 2003). Of these, the rhopalia and nerve ring form the central nervous system (CNS) (Garm et al., 2006, Skogh et al., 2006, Garm et al., 2007a, Garm and Mori, 2009) whose main function is to convert several input channels into an output signal which controls the motor nerve net for movement (Garm et al., 2007a).

2.9 Sensory Input Types and Methods of Collection

It would appear that the rhopalia play a significant role in the collection of sensory input for cubomedusae (Figure 2.3). Situated just above the bell margin, equidistant from each pedalium (Burnett et al., 1996), cubomedusae possess four rhopalia, one of each face of the bell (Coates, 2003, Skogh et al., 2006). Such an arrangement allows information to be collected from all directions simultaneously (Coates, 2003). Each rhopalium is housed within an indented exumbrella cavity called a rhopalial niche

(Satterlie, 2002, Skogh et al., 2006) which is in turn partially covered by a flap of tissue (Satterlie, 2002). It is on the club shaped rhopalium that the statolith, sensory epithelia, complex eyes and up to four pigment cup ocelli are located (Satterlie, 2002).

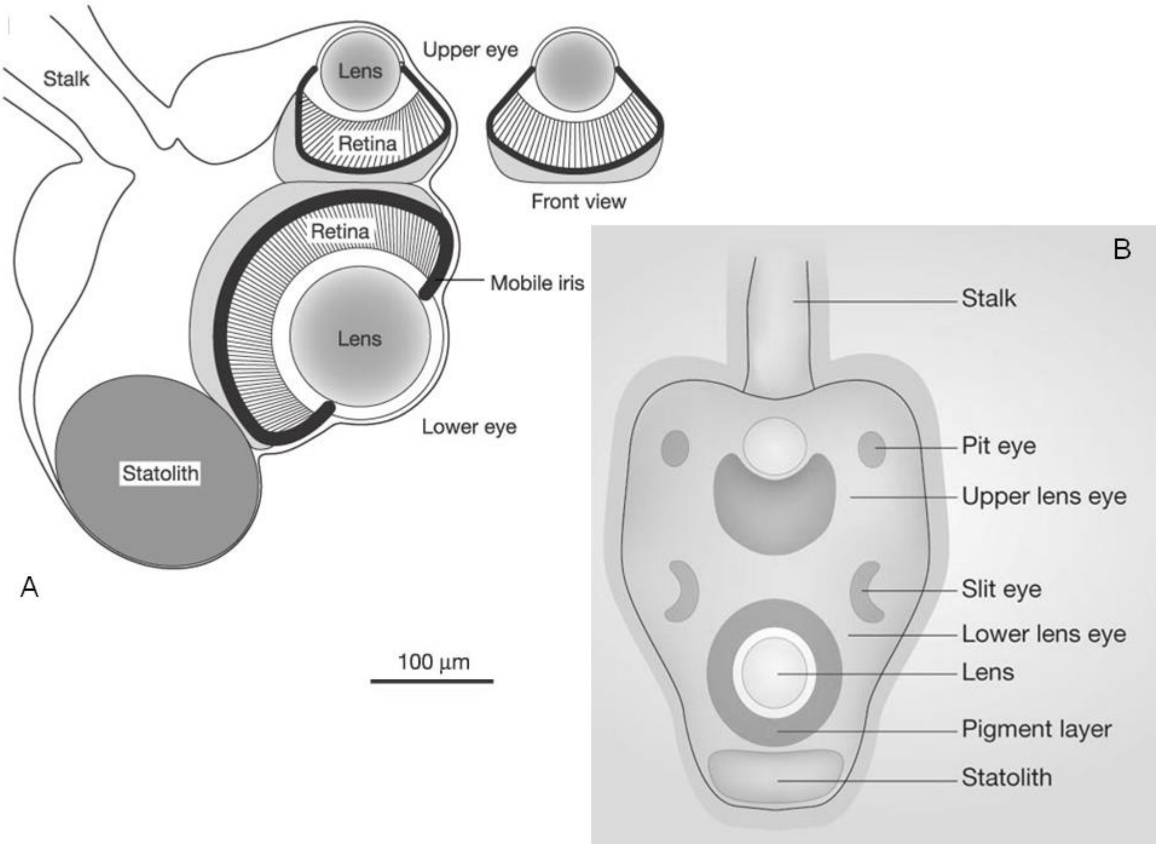

Figure 2.3 Stylised cubozoan rhopalium showing (A) upper and lower lens type eyes and statolith at base of rhopalium (from Nilsson et al. (2005)) and (B) two eye slits, two eye pits and two complex eyes in profile orientation (from Wehner (2005))

The statolith located at the base of each rhopalium acts as a weight, ensuring that the eyes are orientated the same way with respect to gravity no matter the plane in which the medusa is travelling (Garm et al., 2011), an important function given that there is little doubt that the eyes play a major role in controlling the behaviour of medusae (Skogh et al., 2006). Cubomedusae possess a well developed visual system that includes both simple and complex eyes (Pearse and Pearse, 1978, Martin, 2004, Nilsson et al., 2005, Piatigorsky et al., 1989, Piatigorsky and Kozmik, 2004, Wehner, 2005, Skogh et al., 2006). Considered to be the most elaborate of all cnidarians (Skogh et al., 2006, Garm et al., 2007c), the cubomedusae visual system consists of 24 eyes of four morphological types (Coates et al., 2006, Skogh et al., 2006, Garm et al., 2007b, Garm et al., 2007c, Garm and Mori, 2009, O'Connor et al., 2009). All currently known cubozoan species share the same overall features (O'Connor et al., 2009) with two slit eyes, two pit eyes and an upper and lower lens eye found on each rhopalium (Martin, 2002, Coates, 2003, Coates et al., 2006, O'Connor et al., 2009). Cubomedusae are also the only cnidarians known to possess a camera type lens (Nilsson et al., 2005) capable of forming images (Nilsson et al., 2005). Indeed, it is spatial vision such as this that may allow cubomedusae to move freely within their habitats, and thus avoid collision with physical structures (Garm et al., 2012).

C. fleckeri exhibits a number of movement patterns that are likely to be visually guided. For instance, medusae will actively swim away from dark objects such as humans, piers and jetties (Kinsey, 1986, Hamner and Doubilet, 1994). In captive trials, medusae were also found to consistently avoid dark objects by swimming away from or around them, yet no corresponding response was noted for light coloured objects (Hamner et al., 1995). Similarly, a marked positive response for white, red, yellow and orange light has been reported for *C. fleckeri* medusae*,* with what appeared to be feeding behaviours displayed in response to blue coloured light (Gershwin and Dawes, 2008). Barnes also noted that avoidance behaviours were most evident when objects were 15 to 45° from a medusa’s line of travel, being absent if objects were either directly in front of or behind a medusa (Kinsey, 1988). Vision is also likely to play a role in the coordinated predatory behaviours exhibited by *C. fleckeri* medusae in which pairs of medusae herd prawns through the systematic circling of prey items (Kinsey, 1986). What an individual 'see's' is not only dependent upon their eyes, but also on the nervous system that is processing this information, however (Nilsson, 1989). The mechanisms by which cubomedusae process visual information are currently being debated, as medusae do not possess a centralised brain (Wehner, 2005).

2.10 Coordinating a directional response

Jellyfish swim by contracting the circular (and sometimes radial) muscles that line the subumbrella of the bell (Satterlie, 2002, Garm and Mori, 2009). Swim speed is determined by the rate and strength of bell contractions (Gladfelter, 1973; Shorten et al., 2005). A swim pacemaker initiates each contraction of the bell (Satterlie, 2002), with electrophysiological studies demonstrating that pacemaker signals arise from the rhopalium itself (Garm et al., 2006, Garm and Mori, 2009). While the rate at which individual rhopalia emit a pacemaker signal is variable (Satterlie, 2002, Garm and Mori, 2009), effective and directional movement requires the signals from each rhopalium to be coordinated. Here, the activity of the fastest pacemaker resets that of all others (Horridge, 1959, Satterlie, 2002). Furthermore, the agile swimming behaviours and rapid directional changes reported for cubomedusae are thought to be facilitated by asymmetrical stimulation of the motor nerve net (Satterlie and Nolen, 2001, Coates, 2003, Skogh et al., 2006). Such rapid transmission of impulses between rhopalia is facilitated by the nerve ring (Satterlie, 2002), with medusae unable to swim when all four of their rhopalia are removed or if the nerve ring is severed (Satterlie and Spencer, 1979).

Collectively then, it would appear that medusae not only possess the capacity to assess their surrounding environment, but also to respond to changes in that environment via coordinated responses such as directional swimming. Although a number of parameters can influence medusae distribution, a lack of data quantifying medusae movement patterns limits the ability to distinguish between those factors driving movement patterns and those merely correlated with them, an aspect of *C. fleckeri's* ecology into which future research should extend.

2.11 Conclusions and Future Research Directions

With over 60 years of research providing a solid knowledge base upon which future research can build, there currently exists a unique opportunity for further research into the ecology of the Australian Chirodropid assemblage to contribute not only to the management of a commercially and socially significant issue within the Australian tropics, but also to the wider understanding of the understudied field of jellyfish ecology. Several aspects of Chirodropid ecology that warrant further study have been identified within this review. In particular, it is the quantification of medusae growth and development parameters, the identification of the cue(s) regulating both the onset and duration of the medusae season and the quantification of medusae movement patterns that will benefit both Chirodropid medusae management practices as well as provide a data set to which Scyphozoan and Hydrozoan ecological studies can be compared. With the continued growth in human coastal populations, the ability to make such comparisons is becoming increasingly relevant, particularly in relation to claims of increased bloom frequency and intensity, increased season lengths and expansion of the geographic range of various species. Given the general lack of quantitative data documenting such aspects of Scyphozoan, Cubozoan and Hydrozoan ecology, future investigations into the Australian Chirodropid assemblage must take a multidisciplinary approach while remaining focused on the traditional goal of increasing safety to users of the tropical coastline (Barnes, 1966).

CHAPTER THREE

Growth, development and temporal variation in the onset of six *Chironex fleckeri* medusae seasons: a contribution to understanding jellyfish ecology

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3.1 Abstract

Despite the worldwide distribution, toxicity and commercial, industrial and medical impacts jellyfish present, many aspects of their ecology remain poorly understood. Quantified here are important ecological parameters of *C. fleckeri* medusae, contributing not only to the understanding of an understudied taxon, the cubozoa, but also to the broader understanding of jellyfish ecology. *C. fleckeri* medusae were collected across seven seasons (1999, 2000, 2003, 2005-07 and 2010), with growth rates, temporal variation in the medusae season onset and differences in population structure between estuarine and coastal habitats quantified. With a mean of 2nd September ± 2 d (mean ± 95% confidence limits), the earliest date of metamorphosis was temporally constrained between seasons, varying by only 7 d (30th August to 5th September). Juvenile medusae appeared to be added over an extended period, suggesting polyp metamorphosis was an ongoing process once it commenced. At a maximum of 3 ± 0.2 mm d^{-1} IPD, medusae growth to an asymptotic size of ~190 mm IPD was rapid, yet, with the oldest medusae estimated to be ~78 d in age, medusae did not appear to accumulate along the coastline. Furthermore, a greater proportion of juveniles were observed along the coastline, with estuarine sites typified by larger medusae. With key aspects of *C. fleckeri*'s ecology now quantified, medusae season management protocols can be further developed.

3.2 Introduction

The occurrence of jellyfish, particularly in blooms, negatively affects a range of recreational, industrial and commercial activities. For instance, while some commercially important fisheries have been unable to function when jellyfish have clogged fishing nets (Graham et al., 2003, Purcell, 2007), in other fisheries, jellyfish have become predators of and competitors to those species being targeted (Purcell and Arai, 2001, Graham et al., 2003). Serious industrial issues have also been attributed to increased jellyfish abundances, with power station shut down necessary when water intake pipes have become clogged with medusae (Rajagopal et al., 1989, Masilamoni et al., 2000, Matsumura et al., 2005). For other regions, it is the medical liability that jellyfish represent that continues to adversely affect the tourism industry which is often integral to local and regional economies (Burnett, 2001). Such is the case for the Australian tropics where the seasonal occurrence of the cubomedusae *C. fleckeri* significantly impacts the way in which coastal areas are utilised. Despite significant issues relating to the currently sporadic and unpredictable occurrence of *C. fleckeri* medusae, quantitative data documenting key aspects of this cubozoans ecology are, in general, lacking, with many of the currently favoured theories relating to the temporal variation in medusae occurrence, medusae growth and development as well as population structure based on sting records or qualitative data. As a result, claims of increased season length or intensity and frequency of blooms are also difficult to validate.

The generalisation that *C. fleckeri's* life cycle is seasonal is based on numerous reports of medusae and stings from the warmer months of the year (Southcott, 1956, Barnes, 1965, Barnes, 1966, Southcott, 1971, Keen, 1971, Brown, 1973, Hartwick, 1987, Hartwick, 1991a, Hamner and Doubilet, 1994, Burnett et al., 1996, Fenner and Williamson, 1996) contrasted with a lack of such reports from the winter months. Although it is widely accepted that the asexually reproducing polyp phase alternates with a sexually reproducing medusae phase on a seasonal basis (Yamaguchi and Hartwick, 1980, Hartwick, 1987, Hartwick, 1991a), the timing of the shift from the polyp to medusa phase appears to vary both spatially and temporally (Barnes, 1966, Southcott, 1971, Hartwick, 1987, Hartwick, 1991a, Fenner and Williamson, 1996). For instance, while *C. fleckeri* medusae typically appear along the far north Queensland coastline in December, they have been reported as early as October in some seasons (Southcott, 1971, Hartwick, 1991a, Fenner and Williamson, 1996). There is also a suggestion that the season commences earliest on the west coast, shortly after the first rains of the wet season, with the onset of the stinger season delayed if the wet season is late (Barnes, 1966, Kinsey, 1986). Other authors discount the relevance of the wet season (Burnett et al., 1996, Jacups, 2010a), however, suggesting that medusae arrival is associated with rising water temperature (Barnes, 1966, Hartwick, 1987, Jacups, 2010a, Jacups, 2010b). With the polyp habitat thought to be located some distance from the coastline within estuary systems (Hartwick, 1987), the onset of the stinger season is unlikely to accurately reflect the timing of polyp metamorphosis given that the colloquial term 'stinger season' typically refers to the arrival of medusae along the coastline rather than the timing of polyp metamorphosis. Many of these theories are yet to be validated, however, given a general lack of quantitative data documenting fluctuations in environmental parameters at the beginning of the season as well as the timeframe over which medusae production occurs.

Few extrapolations can be drawn from other cubozoan species either, with studies identifying a species cue for metamorphosis limited to a handful of species. Here, increasing water temperature (Laska-Mehnert, 1985), an interaction between increasing water temperature and food (Stangl et al., 2002, Straehler-Pohl and Jarms, 2005) or increasing water temperature and photoperiod (Straehler-Pohl and Jarms, 2011) have been identified as cues. While the development to a specific number of tentacles (Arneson and Cutress, 1976) and the presence of photosymbiotic algae

(Hofmann and Kremer, 1981) have also been suggested as potential cues for metamorphosis, these links have not been validated quantitatively. Similarly, while the correlation between rainfall events and successive pulses of juvenile cubomedusae (Gordon, 1998, Gordon et al., 2004) may implicate salinity as a cue for metamorphosis, quantitative data demonstrating this link is lacking. Given this paucity in data, a need therefore exists for research distinguishing between those mechanisms driving polyp metamorphosis and those merely correlated with this process.

At metamorphosis, juvenile *C. fleckeri* medusae are reported to measure approximately 1.2 to 1.4 mm in size (Yamaguchi and Hartwick, 1980). The presence of cnidocyte batteries on the bell and an overall morphology not unlike that of juvenile carybdeid medusae raise the possibility that these observations relate to a species of cubozoa other than *C. fleckeri*, however. Nonetheless, medusae reportedly increase in size rapidly as the season progresses (Hartwick, 1987, Hartwick, 1991a, Hamner and Doubilet, 1994), reaching sexual maturity late in the season (Barnes, 1966, Hartwick, 1991a). While such growth patterns are reported for a number of scyphozoan species (Arai, 1997), for cubomedusae, it is only for *C. bronzei* that growth parameters have been quantified (Gordon, 1998, Gordon et al., 2004). This paucity in data is largely due to the lack of a reliable method by which medusae can be aged. For medusae, size is an unreliable indicator given that degrowth of the bell can occur in cases where feeding regimes are inadequate (for example Hamner et al. (1995)). While some authors have used tentacle number as an alternative indicator of development (Kinsey, 1986), tentacles are added across a size range, and hence, may still be somewhat dependent upon feeding regime. More recently, the statoliths contained within the statocysts of each rhopalium (eye bearing sensory structures) have been shown to contain fine growth rings that are added on a daily basis (Ueno et al., 1995, Ueno et al., 1997, Kawamura et al., 2003, Ueno et al., 2003, Gordon et al., 2004).

Insights into the ecology of a species can also be gained from population structure data. For example, several cohorts of *C. bronzei* medusae occurring within a single season, as well as a correlation between cohort appearance and significant rainfall events, were elucidated from population structure data (Gordon, 1998, Gordon et al., 2004). Comparable data for *C. fleckeri* is, however, currently limited to the generalisation that an abundance of small individuals early in the season progresses to fewer but larger medusae late in the season (Hartwick, 1991a). Given that the shift from estuarine to coastal habitats is thought to accompany the shift from polyp to medusa phases of the life cycle (Hartwick, 1987) whereby medusae are washed from within estuaries at the onset of the season (Cropp and Cropp, 1984, Hartwick, 1991a), coastal populations are likely to be dominated by larger and older medusae, while smaller and younger medusae would typify estuarine populations. Yet, it would appear that large medusae can be present even in early season samples (Barnes, 1966, Brown, 1973) and "no specific rule can be given to the range of sizes encountered in a given area at a specific time" (Barnes, 1966). Whether the presence of only large medusae at a given location and small medusae in another indicates the presence of a nursery location (Brown, 1973), or perhaps the polyp habitat itself, is yet to be demonstrated. A need therefore exists to quantify aspects of population structure if the overall ecology of *C. fleckeri* is to be better understood.

The aims of the current chapter are to quantify some of the long held theories relating to the ecology of *C. fleckeri* medusae. Key aspects investigated include the:

- relationship between statolith size and growth rings
- temporal variability in the onset of medusae production (metamorphosis)
- growth and development of *C. fleckeri* medusae
- population structure of coastal and estuarine medusae populations.

3.3 Methods

A total of 484 medusae were collected from seven sites at Weipa during the 1999, 2000, 2003, 2005, 2006, 2007 and 2010 stinger seasons. While not all sites were visited on each occasion or within each season (Appendix 4.1), Landfall Point (12º 34' 53" S, 141º 39' 50" E), Andoomajetti Point (12º 36' 20" S, 141º 49' 21" E), Rocky Point (23º 37' 10" S, 141º 52' 38" E), Jessica Point (12º 40' 05" S, 141º 51' 42" E), Hey Point(12º 44' 23" S, 141º 53' 35" E), Wooldrum Point Beach (12º 42' 19" S, 141º 48' 03" E) and Westminster South (12º 50' 11" S 141º 44' 56" E) represented Weipa (Figure 3.1).

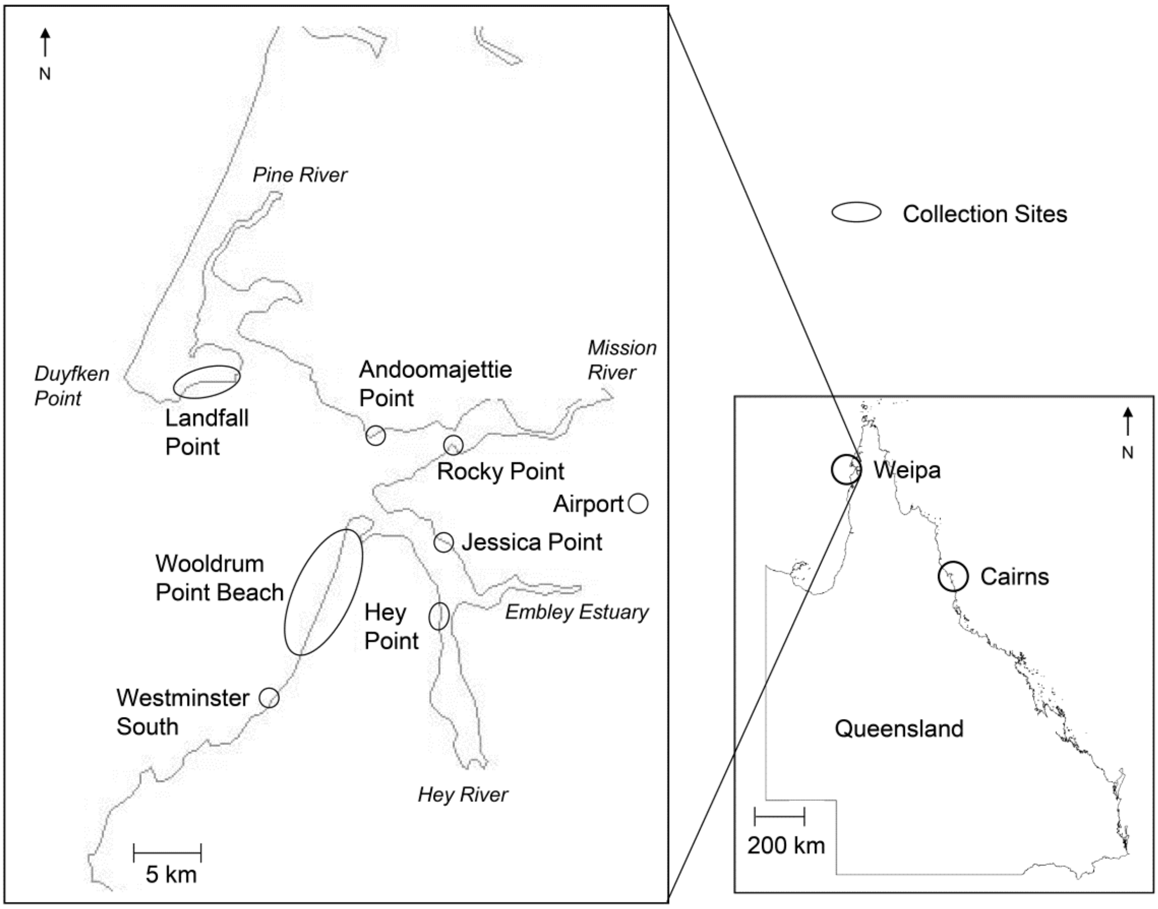

Figure 3.1 Geographic location of Weipa medusae collection sites, western Cape York, north Queensland, Australia

Weipa sites were classified as either coastal or estuarine sites. Coastal sites were typified by a gently sloping sand / mud beachfront that occurred along the embayment of Albatross Bay, such as Wooldrum Point Beach. Estuarine sites, such as Andoomajetti Point, were those that either occurred wholly within an estuary system or at the intersection of an estuary system and the coastal embayment of Albatross Bay, typified by a sand / mud substrate and nearby mangrove stands.

A further 46 medusae were collected during the 2005, 2006 and 2007 seasons from 12 east coast sites between Cairns and Townsville, which included Mission Beach, Gin Camp, Yorkeys Knob, Port Douglas, Cardwell, Palm Cove, Buchan Point, Lugger Bay, Townsville and the Tully, Murray and Hull Rivers. Given the low numbers collected on each occasion, east coast sites were excluded from most analyses.

3.3.1 Statolith Technique

Medusae were collected by hand and their inter pedalia distance (IPD), the distance between the mid line of alternate pedalia along the line passing through the rhopalia, was measured to the nearest mm. Each of the four rhopalial niches and a gonad sample (if gonads were evident) were removed and preserved in 98% ethanol.

For 71 medusae, the statoliths of two rhopalia were dissected from the base of their eye set. Undamaged and unshattered statoliths were cleaned of any cellular material before being embedded in resin in the profile plane (kidney shape was evident). The top 50% of each statolith was ground using 1200 gauge wet and dry sand paper, polished with Brasso, rinsed and then polished with tooth paste. The number of rings present in each statolith was counted under oil immersion using x 400 magnification on a light microscope (Figure 3.2). Each dark band with a light band either side was considered to be one growth ring (Figure 3.2). The length of each statolith was measured using a calibrated stereo dissector microscope, with length taken to be the longest distance between curved apical ends of a statolith. Both the average number of rings and average length of each statolith pair were then calculated, with the relationship between the number of growth rings and statolith length investigated using regression analysis.

Two statoliths from a further 437 medusae were dissected and their length measured under a calibrated stereo dissector microscope. The average length of each statolith pair was calculated, from which, the number of rings was estimated using the relationship between statolith length and number of rings.

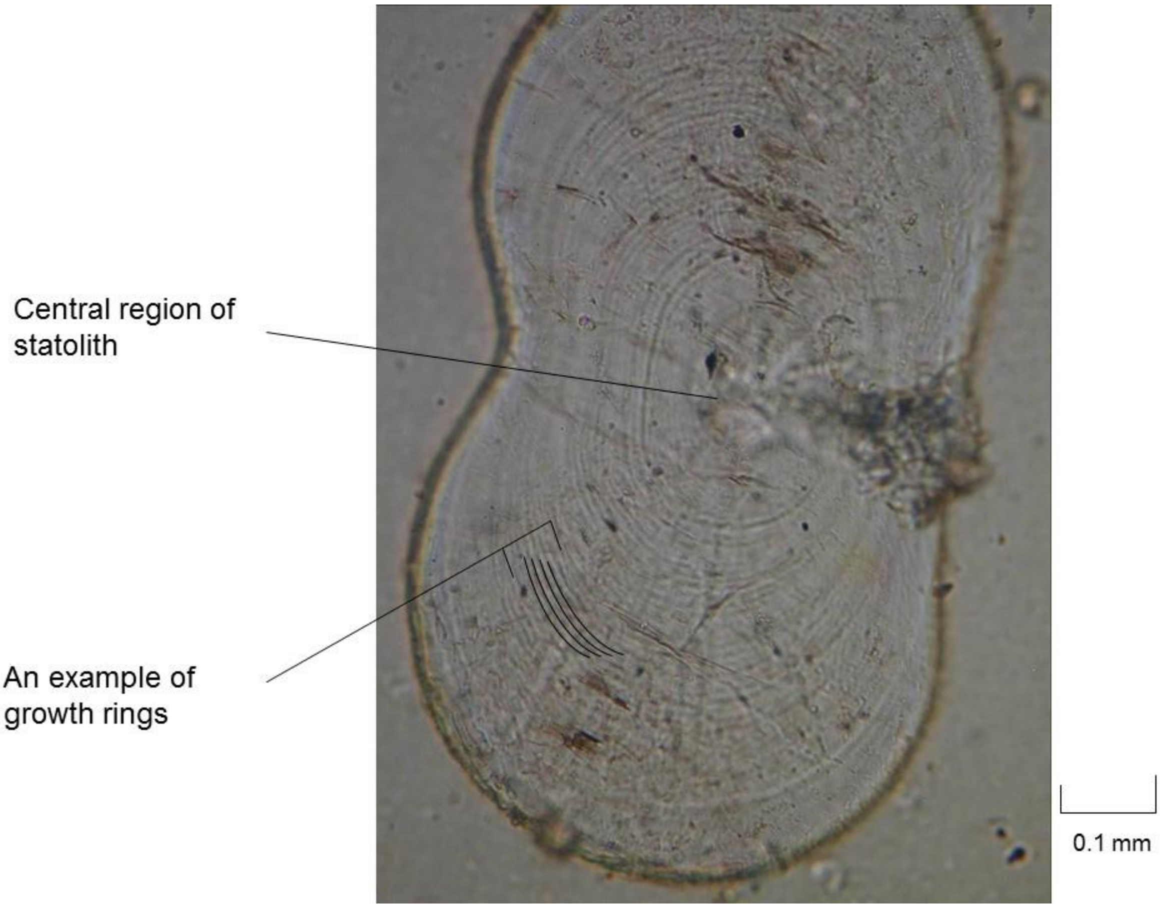

Figure 3.2 *Chironex fleckeri* statolith in profile plane showing alternating light and dark bands representing growth rings

3.3.2 Quantifying the Time Frame of Statolith Growth Rings

Establishing a significant relationship between medusae size (mm IPD) and age (number of rings in statolith) was a two stage process. Firstly, regression analysis was used to determine whether medusae size (IPD mm) could be predicted from tentacle number, and secondly, whether tentacle number was correlated with ring number. Given that medusae can undergo both growth and degrowth, tentacle number was considered a more reliable indicator of medusa development as tentacles are not lost once added.

To be able to age medusae, however, it was necessary to quantify the unit of time represented by successive growth rings. While tetracycline in solution has been used since the early 1980’s for growth ring time frame validation in fish otoliths (Schmitt, 1984), several attempts at applying this technique to *C. fleckeri* medusae were unsuccessful despite various concentrations of tetracycline being trialled and medusae being housed in large, custom made cylindrical tanks. While this has limited the methods by which the interval between successive rings can be quantified, several pieces of evidence suggest that rings are added on a daily basis. Firstly, if growth rings were added at hourly or weekly intervals, not only are these arbitrary units of time, but they would also make medusae within this study less than four days old (hourly) or 1.5 years (weekly). Monthly or annual units of time are also unrealistic given that the oldest medusae would have been 7 or 80 yrs of age respectively. Rather, if successive rings were added on a daily basis, medusae collected within this study would have ranged between one and three months of age. Not only are these realistic age estimates, given that medusae are unlikely to survive between seasons, but growth rings are added on a daily basis in three other species of cubozoa, *Carybdea rastoni* Haacke*,* *Chiropsalmus quadrigatus* Haeckel and *C. bronzei* (Ueno et al., 1995, Ueno et al., 1997, Kawamura et al., 2003, Ueno et al., 2003, Gordon et al., 2004).

In this scenario, alternating dark and light bands would reflect the diurnal behaviours of medusae (Kinsey, 1986, Seymour et al., 2004). That is, throughout the day, *C. fleckeri* medusae expend considerable amounts of energy swimming and feeding (Hartwick, 1991a), with less energy available for growth. In contrast, reduced activity levels at night (Seymour et al., 2004) would allow relatively more energy to be devoted to growth. Such diurnal patterns of growth have been demonstrated for certain body parts of *Tripedalia cystophora* medusae, whereby cell proliferation rates were significantly greater at night (Gurska and Garm, 2014). Under this scenario, alternating light and dark bands would arise from the variation in statolith density associated with differential growth between day and night. Collectively then, the most plausible unit of time between consecutive growth rings is daily, as has been shown in other cubozoans (Ueno et al., 1995, Ueno et al., 1997, Kawamura et al., 2003, Ueno et al., 2003, Gordon et al., 2004).

3.3.3 Calculating Date of Metamorphosis

Only medusae collected from Weipa sites were included in these analyses, as small sample sizes from east coast sites made analysis unreliable. The age of 461 medusae was taken to be either:

a) the number of rings observed within their statoliths (64 medusae) or
b) the number of rings estimated from the relationship between statolith length and ring number (397 medusae).

The metamorphosis date of each individual was calculated by subtracting an individual's age from its date of capture. The percentage of each sample that underwent metamorphosis on a given day was calculated and plotted against season number. Season number was used in preference to year number as it allowed successive samples within a season to be plotted together. That is, a sample collected in January 2007 was denoted a season number of 2006 as was a sample collected in November 2006.

Temporal variation in the onset of the 2000, 2003, 2005, 2006, 2007 and 2010 seasons was quantified by calculating the average earliest metamorphosis date of these six seasons and the associated 95% confidence limits.

A number of environmental parameters are potentially relevant to the shift from the polyp to medusa phase of the life cycle, with the following parameters quantified for the five weeks prior to the onset of metamorphosis in each season:

- the daily sea surface temperature at midday for bearing 16.59 S, 141.56 E (Albatross Bay) was obtained from the Integrated Marine Observing System at www.marine.csiro.au/remotesensing/imos;
- daily rainfall totals were obtained from the Bureau of Meteorology for Weipa Eastern Avenue (location 27042);
- photoperiod, or the total number of hours of daylight per day, was calculated from sunrise and sunset times for Weipa obtained from Geoscience Australia at http://www.ga.gov.au/geodesy/astro/sunrise.jsp;
- tidal amplitude was calculated from hourly tide height (m) data for Humbug Wharf, obtained from MSQ at http://www.msq.qld.gov.au/Home/Tides.

Data were assigned to a week category (1 to 5) in which week category represented the number of weeks prior to the onset of metamorphosis for that season. The weekly variation between years in each of these parameters was investigated using two way Analysis of Variance. The date of the full moon within the five weeks prior to the onset of metamorphosis in each season was obtained from Geoscience Australia at http://www.ga.gov.au/earth-monitoring/geodesy.

3.3.5 Growth Curve Calculations

Gordon et al. (2004) established that a Gompertz growth equation most accurately described the growth parameters of a closely related cubozoan, *C. bronzei*, using the criteria described by Kaufmann (1981). Parameters for a four criteria Gompertz growth curve were estimated in Sigma Plot 10 using size at age data for 461 medusae. Size was taken to be IPD at time of capture and age was either

a) the number of rings present in an individual's statolith (64 medusae) or
b) an estimate using the relationship between statolith size and ring number (397 medusae).

The maximum daily growth rate (mm d^{-1}) and 95% confidence limits were estimated by regression analysis of the linear component of the growth curve, which occurred between 40 and 70 d. The time to the onset of sexual maturity was also estimated using the resulting growth equation.

3.3.6 Investigation of Population Structure

Since each site was not visited on each occasion, data for medusae collected in Weipa were pooled for all seasons and sites within habitat type. East coast sites were not analysed here due to small sample sizes. Those medusae in which gonads had not developed were denoted as immature juveniles. For mature individuals, sex was determined by examining gonad samples under a stereo dissecting microscope. Individuals in which ova were visible were denoted as females, while those in which sheets of convoluted filamentous tissue were evident were denoted as males. In those cases where it was unclear whether tissue was that of a small male or an immature specimen, individuals were classified as undistinguishable (~60 individuals) and grouped with immature specimens. A chi squared homogeneity test was used to determine whether any significant difference between the proportion of males, females and juveniles existed between the estuarine and coastal habitats.

3.4 Results

A significant and positive relationship between statolith length (mm) and number of rings was established ($F_{1,\ 69} = 151.243$, $n = 71$, $P < 0.001$), whereby the number of rings present within a statolith increased as did statolith length. Within the size range of statoliths sampled, a linear relationship (Figure 3.3) provided a better fit than curvilinear equations, with this linear relationship best described by the equation:

$$\text{number of rings} = (78.213 \times \text{statolith length}) - 0.088$$

where statolith length is in mm.

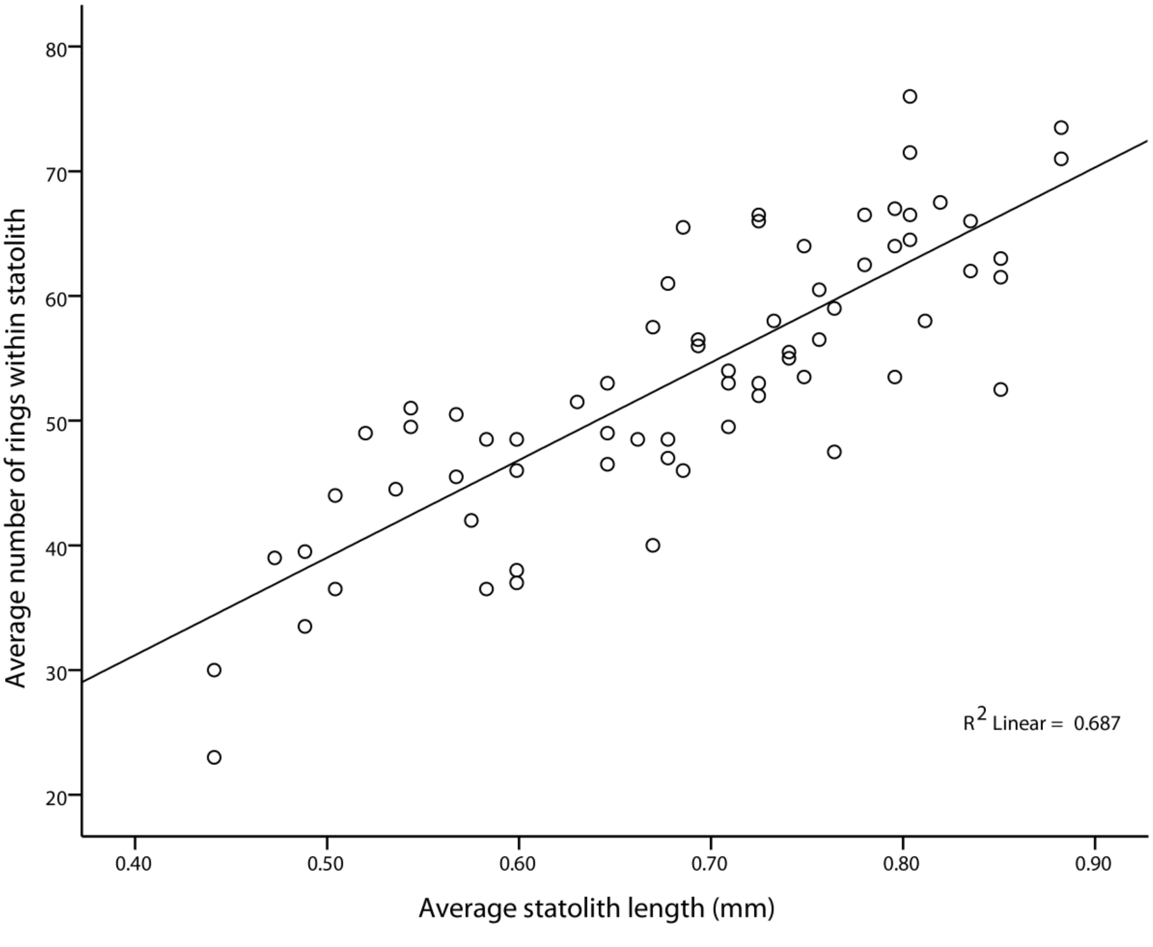

Figure 3.3 Positive linear relationship between statolith length (mm) and number of rings within a *Chironex fleckeri* statolith (average / statolith pair)

A significant and positive relationship was established between the number of tentacles per pedalium and inter pedalia distance (mm) ($F_{1,\ 420}$ = 1201.176, n = 422, $P < 0.001$) whereby medusae size (IPD in mm) increased as did the number of tentacles per pedalium (Figure 3.4). A power curve described this relationship most appropriately, with inter pedalia distance increasing at a faster rate as more tentacles were added to each pedalium. This curve provided a minimum medusa size at the one tentacle stage (i.e. following metamorphosis) of ~1.8 mm and is best described by the equation:

$$\text{Inter Pedalia Distance} = 1.846 \times \text{tentacle number}^{1.628}$$

where inter pedalia distance is in mm.

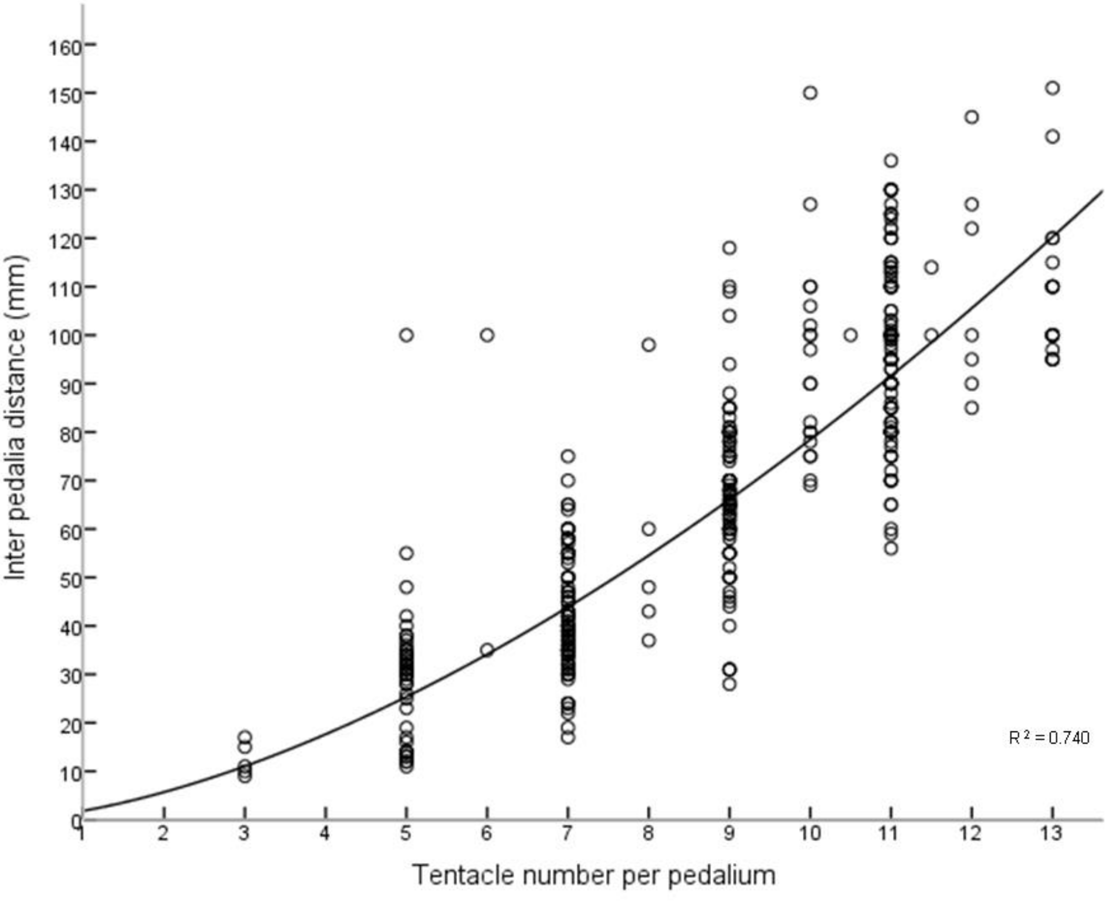

Figure 3.4 Positive curvilinear relationship between number of tentacles per pedalium and Inter Pedalia Distance (mm) for *Chironex fleckeri* medusae

Medusae appeared to add tentacles in pairs, with only ~8% of the 422 medusae for which tentacle number was collected possessing an even number of tentacles per pedalium. A significant and positive relationship was identified between the number of rings within a statolith and the number of tentacles suspended from each pedalium ($F_{1, 410} = 639.733$, $n = 412$, $P < 0.001$). Within the age range of medusae sampled, a linear relationship in which tentacle number increased as did ring number (Figure 3.5) provided a better fit than did curvilinear equations and is best described by the equation:

Tentacle number = (0.189 x number of rings) – 0.899

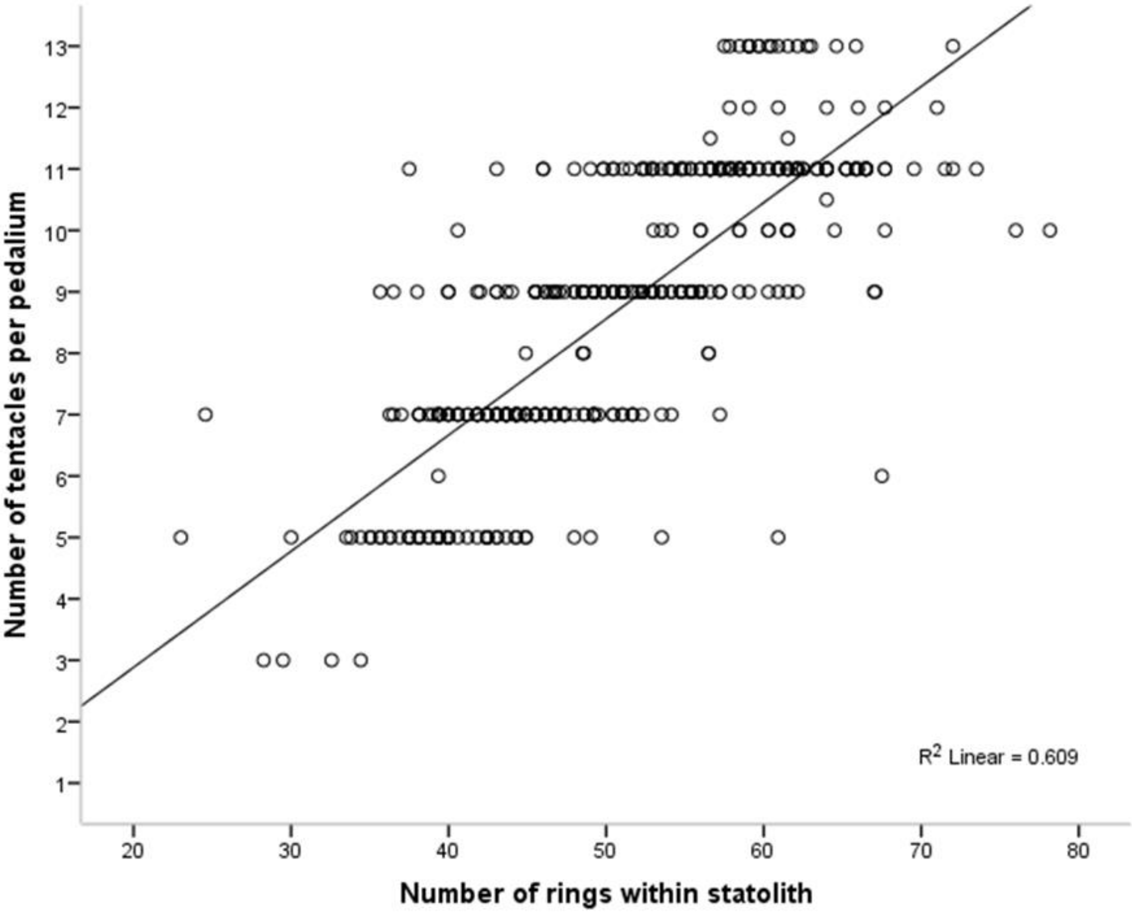

Figure 3.5 Linear positive relationship between number of rings (average for statolith pair) and number of tentacles per pedalium for *Chironex fleckeri* medusae

The earliest date of metamorphosis was the 30th August and occurred in the 2007 season (Figure 3.6). Despite the earliest date of specimen collection varying by 33 d, the earliest date of metamorphosis did not vary by more than 7 d. That is, while medusae were first collected on the 18th October in the 2000 season in which the earliest date of metamorphosis was the 31st August, in the 2010 season for which the earliest metamorphosis date was the 5th September, medusae were not collected until the 20th November (Figure 3.6). The mean earliest date of metamorphosis was the 2nd September ± 2 d (mean ± 95% confidence limits).

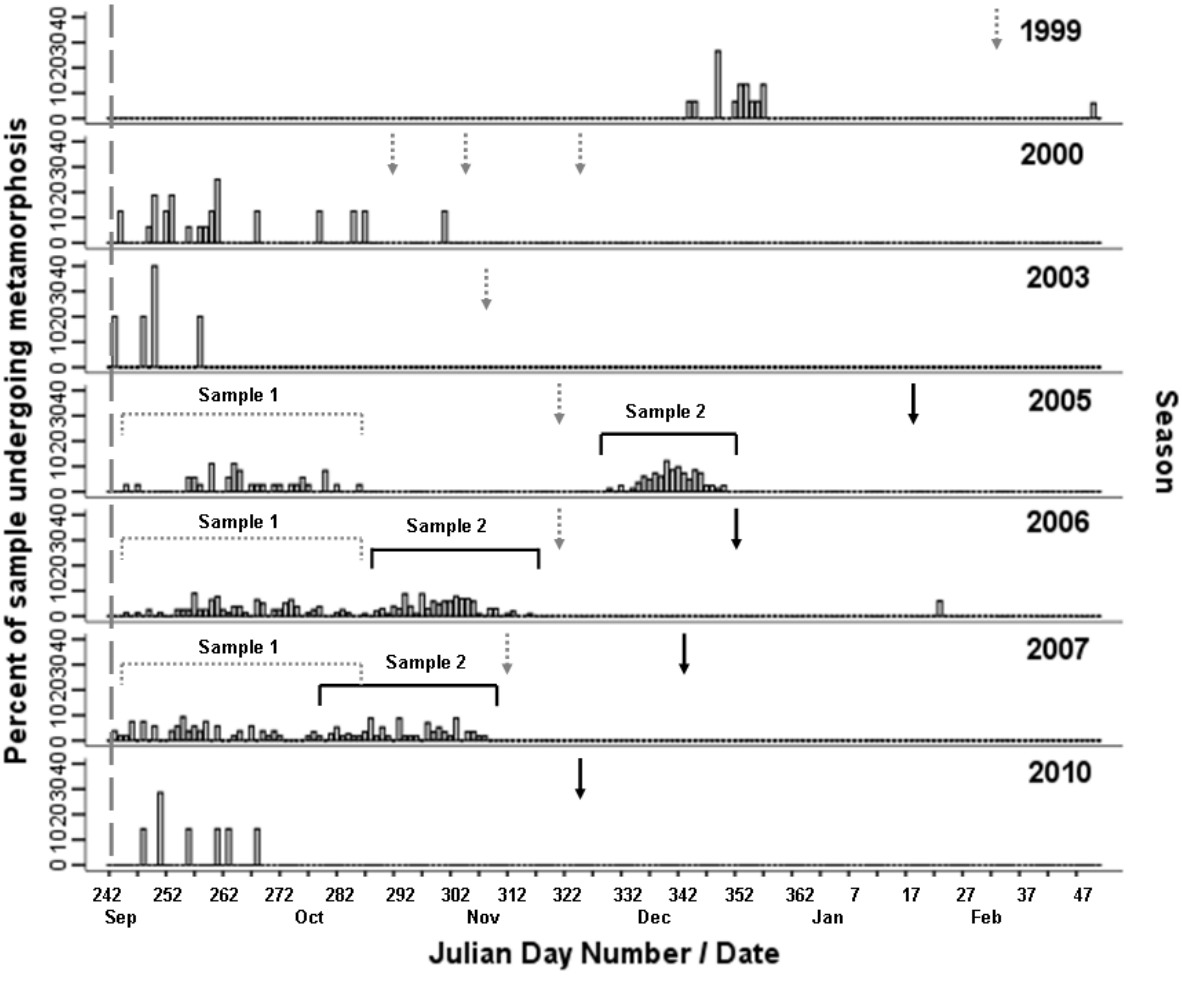

Figure 3.6 Frequency of *Chironex fleckeri* medusae that metamorphosed per day where frequency is percentage within a sample and date is Julian day number or month the first sample date within a season is denoated by ⇣ while ↓ denotes the second sample date; the reference line (— —) delineates 30th August

Within the 2005, 2006 and 2007 seasons, medusae were added to the population on an almost daily basis, with each date of metamorphosis represented by ~2%, with no one date accounting for more than 10% of a sample (Figure 3.6). Metamorphosis also appeared to be an ongoing process once it commenced. That is, in those seasons where the interval between successive samples (between ⇣ and ↓) was approximately one month (2006 and 2007 seasons), metamorphosis dates were either continuous (2006 season) or overlapped slightly (2007 season). In the 2005 season, however, successive samples were collected ~60 days apart (November 2005 and January 2006), with the gap in metamorphosis corresponding to the length of time between sampling occasions.

Water temperature (^{0}C), daily rainfall (mm) and tidal amplitude (m) each showed significant weekly variation between years in the five weeks prior to the earliest date of metamorphosis within each season (Table 3.1). Photoperiod (h daylight d^{-1}), however, did not vary significantly by week between years. The dates of the full moon in the five weeks preceding the onset of metamorphosis differed by 19 days across the six seasons studied here, ranging from the 9th August in 2006 to the 28th August in 2008.

Table 3.1 ANOVA results for the variation in water temperature (^{0}C), total daily rainfall (mm), tidal amplitude (m) and photoperiod (h of daylight d^{-1}) in the five weeks prior to the onset of metamorphosis in each season

Parameter	F	df	*P*
Water Temperature (^{0}C)	2.031	20 x 192	0.008
Total daily rainfall (mm)	1.814	20 x 210	0.022
Tidal amplitude (m)	1.696	10 x 697	0.030
Photoperiod (h daylight d^{-1})	0.088	20 x 210	1.000

The degree to which each environmental parameter varied in the five weeks prior to the onset of metamorphosis was parameter specific. With a $\log_{10}$ transformed CV value of ~1, daily rainfall total (mm) showed the greatest degree of variation, while photoperiod showed the least amount of variation with a $\log_{10}$ transformed CV value of ~-3 (Figure 3.7). The variation in both water temperature (ºC) and tidal amplitude (m) was also considerably greater than photoperiod, ranging from ~0 (tidal amplitude in m) to ~-1.5 for water temperature (ºC) (Figure 3.7).

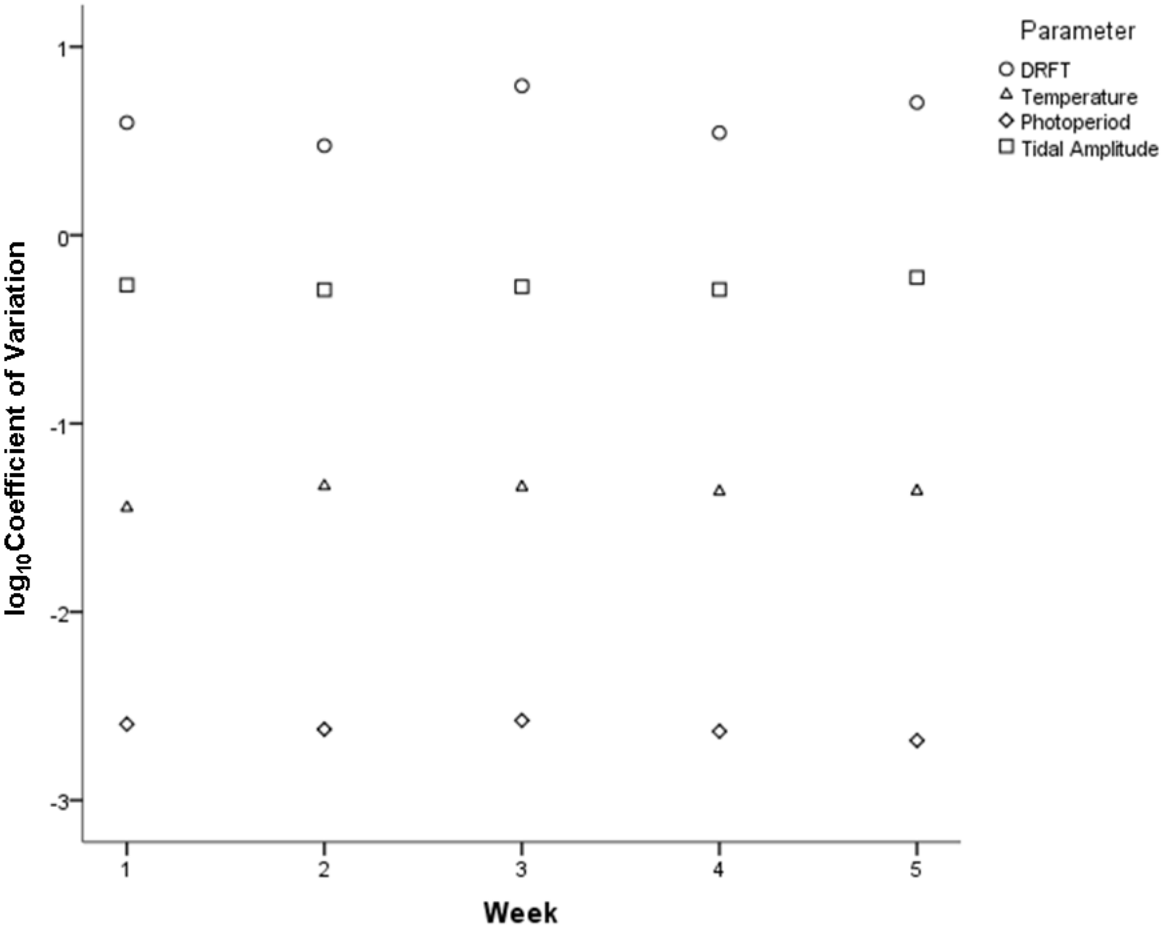

Figure 3.7 Coefficient of variation ($\log_{10}$ transformed) for daily rainfall total (mm), tidal amplitude (m), water temperature (^{0}C) and photoperiod (h daylight d^{-1}) for each of the five weeks preceding the earliest date of metamorphosis in each season

A significant and positive relationship between IPD (mm) and age (days) of medusae ($F_{3,457}$ = 423.3479, n = 461, $P < 0.0001$) was identified whereby medusae increased in size with age towards an asymptotic size of ~190 mm IPD (Figure 3.8). The Gompertz growth equation had the following format:

$$\text{IPD} = 1.5 + 186.5617 \times \exp(-\exp(-((\text{age} - 51.8408) / 21.9887)))$$

where IPD in mm and age is in days

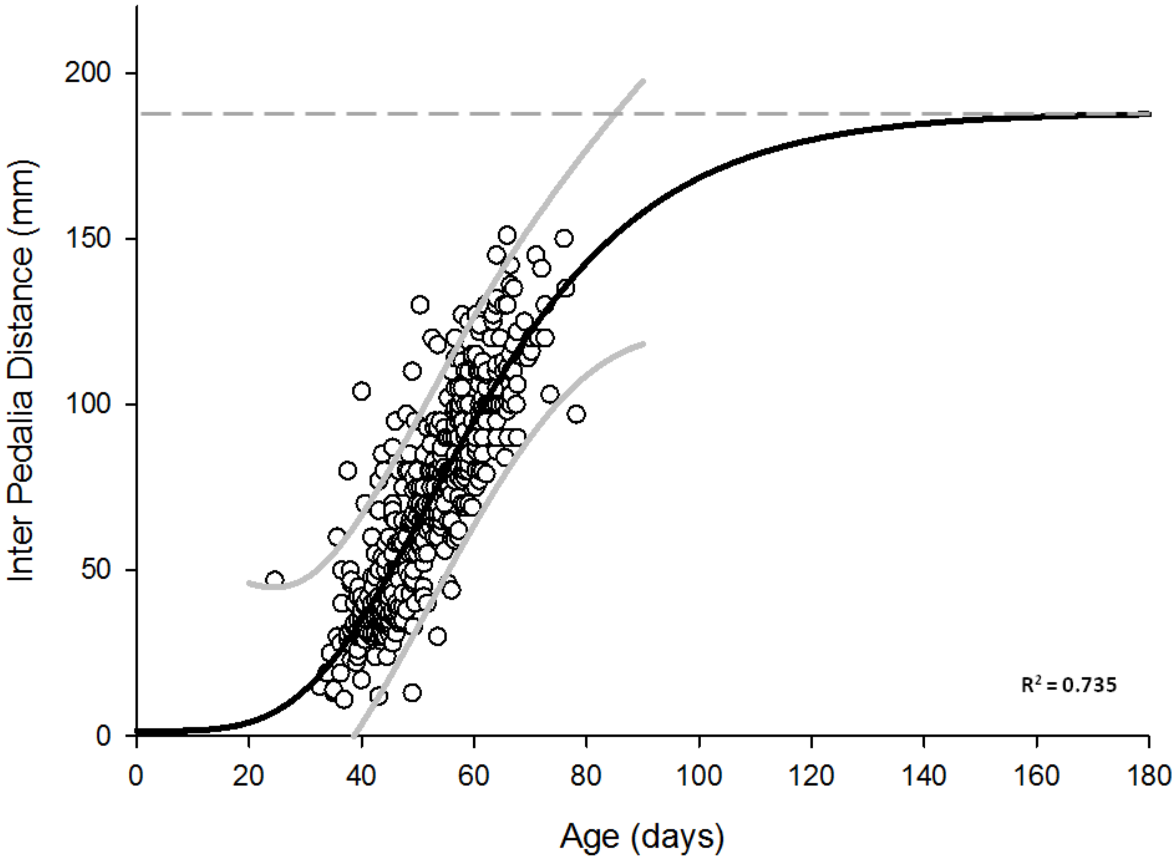

Figure 3.8 Gompertz growth curve for significant, positive relationship between age (d) and IPD of *Chironex fleckeri* medusae with 95% prediction limits (grey solid line)

A maximum growth rate of ~3 mm d^{-1} (± 0.2 mm d^{-1}) was determined from regression analysis of the linear component of this relationship (from 40 to 70 d). The minimum size (IPD in mm) at which males and females could be reliably distinguished was 46 mm IPD and 50 mm IPD respectively, which would take medusae ~45 d to reach (from the above growth equation). At an average 96 mm IPD for male medusae and 97 mm IPD for females, the mean size of male and female medusae did not vary significantly ($F_{1, 240}$ = 0.25, n = 242, P = 0.621).

The age structure of the overall population differed significantly between the estuarine and coastal habitats (χ^2 = 49.477, df = 2, n = 281, P < 0.001) (Figure 3.9).

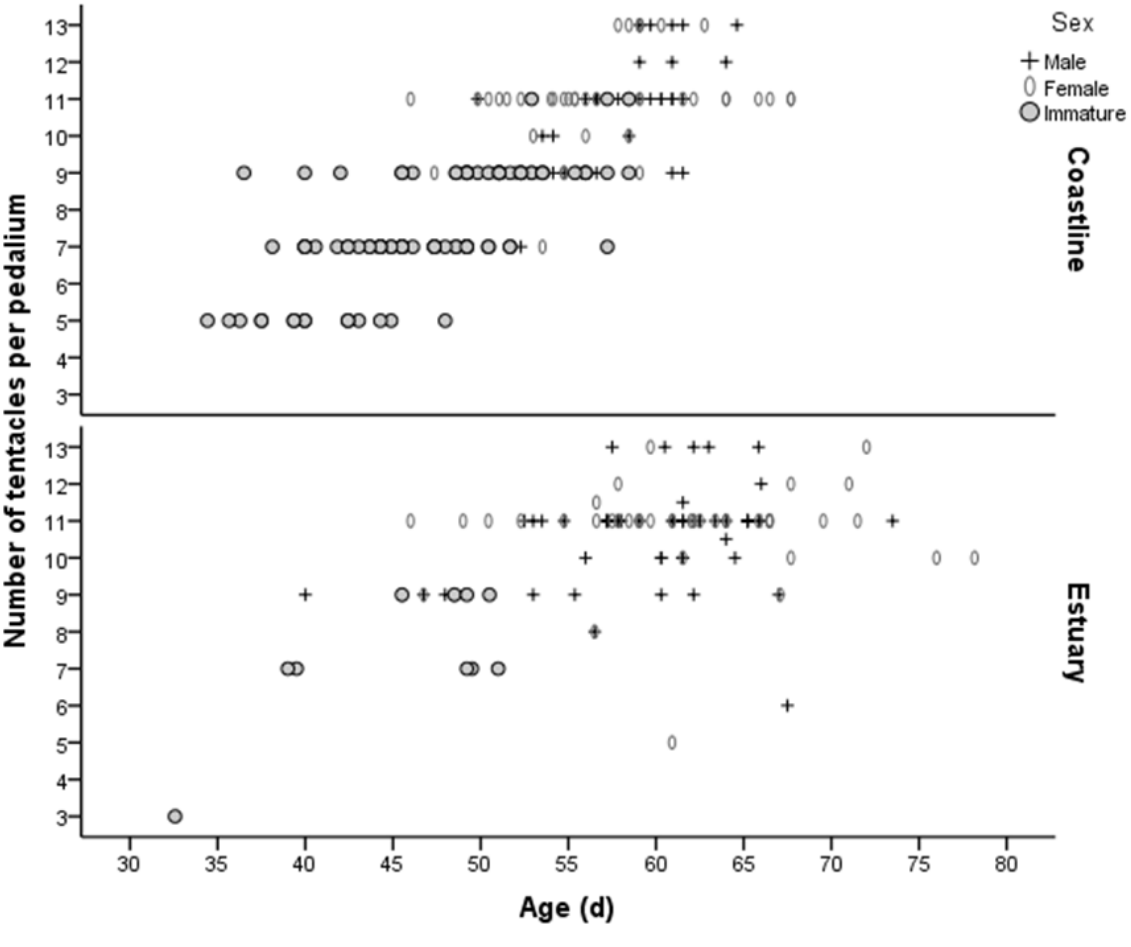

Figure 3.9 Age of male, female and immature *Chironex fleckeri* medusae in respect to tentacle number within the coastal and estuarine habitats of Weipa

Younger medusae were not as well represented within the estuarine habitat, with a predominance of larger and older individuals evident instead (Figure 3.9). Along the coastline, however, a greater spread of males, females and immature medusae was observed, ranging in age from 30 - 70 d and from five to 13 tentacles per pedalium. Furthermore, the oldest medusae within the estuarine habitat were older than those along the coastline. Although medusae added new tentacles over an age range, it was typically possible to determine the sex of an individual providing it possessed more than nine tentacles. This was consistent between the estuarine and coastal sites for all but three medusae within the coastal habitat who were classed as immature at the 11 tentacle stage.

3.5 Discussion

One area of jellyfish ecology that has received increasing attention of late is the shift from the polyp to the medusa phase, and the factors associated with, or acting as cues for this shift. Not only was the onset of medusae production temporally constrained between seasons, but it also commenced earlier than expected. That is, the earliest metamorphosis date was the 30th August (2007 season) and varied by only ~7 d across six seasons. Not only is September also the earliest month in which juvenile medusae are reported in plankton tows from east coast studies (Hartwick, 1991a), but medusae of 120 mm collected in December on Magnetic Island (Brown, 1973) would be ~70 d of age (from the above growth curve), also giving them a metamorphosis date in September. This result is particularly significant in terms of modelling the overall medusae season in that the onset of each season can now be defined with greater accuracy.

Quantifying when polyp metamorphosis commenced also allows the factors associated with the shift from the polyp to medusa phase to be identified. It is these factors that polyp based studies should include when quantitatively identifying the cue for metamorphosis. For instance, the significant between year variation in weekly mean water temperature suggests that water temperature is unlikely to have provided the temporal periodicity observed in the onset of metamorphosis within this study. A similar case exists for both tidal amplitude and rainfall. That is, while unusually high amplitude tides could result in salinity changes at the polyp habitat (currently favoured to be located within coastal estuaries) by pushing higher salinity waters further into estuary systems or allowing fresh waters to drain further down estuaries, the timing of any tidally driven salinity fluctuations would have varied between years. Likewise, significant between year variation in total daily rainfall (mm) suggests that rainfall (or rainfall driven salinity changes) was unlikely to have acted as a cue for the onset of polyp metamorphosis in the seasons investigated within this study. Indeed, the climate of Weipa is dominated by strong seasonal patterns (Cyrus and Blaber, 1992, Vance et al., 1996b, Vance et al., 1998), with low rainfall and elevated, stable salinity typical for late August / early September (Vance et al., 1998). Salinity did not appear to be related to the metamorphosis of *C. fleckeri* polyps in laboratory based trials either (Yamaguchi and Hartwick, 1980, Hartwick, 1991a), although dilution rates may have induced encystment rather than metamorphosis. The influence of temperature is also unclear from polyp based studies, with all trials conducted at 28^0C (Yamaguchi and Hartwick, 1980, Hartwick, 1991a). While the results of this study suggest that salinity, temperature, tidal amplitude or moon phase are unlikely to provide the temporal consistency in the onset of metamorphosis as was observed within this study, their role as an interacting variables cannot yet be disregarded.

One parameter that could provide a higher degree of temporal consistency in the onset of metamorphosis is photoperiod, with the average hours of daylight consistent between seasons in the five weeks prior to the earliest date of metamorphosis within each season. The influence of photoperiod on polyp metamorphosis remains largely untested, however, with *Carybdea morandinii* the only cubozoan for which a link between photoperiod and metamorphosis has been established (Straehler-Pohl and Jarms, 2011). Not only has light been positively correlated with asexual reproduction for some Scyphozoans (Purcell, 2007, Lieu et al., 2009), but Purcell (2007) suggests that melatonin, a light sensitive hormone, may also play an important role in coordinating the strobilation of the Scyphozoan *Aurelia labiata*. Given a similar mechanism may also play a role in coordinating polyp metamorphosis in *C. fleckeri*, future polyp based research should quantify the significance of photoperiod on polyp metamorphosis.

It has previously been suggested that the polyp and medusae phases of the *C. fleckeri* lifecycle alternate on a seasonal basis (Hartwick, 1991a). Metamorphosis did not appear to be a single or pulse event for Weipa populations of *C. fleckeri*, however, but rather an ongoing process whereby low numbers of medusae were produced on an almost daily basis (between 2 and 10% d^{-1}). The collection of a medusa in March 2000 (metamorphosis date of the 19th February) and another in March 2007 (metamorphosis date of the 23rd January) further suggests that medusae production continued over an extended time frame. Reports of 0.6 to 1.8 mm juvenile *C. fleckeri* medusae in estuarine plankton samples between September and January (Hartwick, 1991a), the presence of both adult and small medusae in the first arrivals along the coastline (Barnes, 1966, Brown, 1973), as well as the collection of 6 mm medusae in January and February when 120 mm medusae were collected in December further suggest that metamorphosis occurs over an extended timeframe. Reports of juvenile medusae occurring in successive waves in the only laboratory based study conducted on *C. fleckeri* polyps (Yamaguchi and Hartwick, 1980) initially appear contradictory to the results of this study, however, without the timeframe between successive waves quantified, the potential for pulses to have occurred on a daily basis, as observed within this study, cannot be disregarded. Collectively then, the alternate phases of *C. fleckeri*'s lifecycle appear to overlap rather than alternate on a seasonal basis.

Once within the sexual phase of the life cycle, medusae growth was rapid at up to 3 ± 0.2 mm d^{-1}, which is up to three times that established for a closely related and often co-occurring species, *C. bronzei* (Gordon, 1998, Gordon et al., 2004). Although growth rates are likely to vary between individuals due to prey availability (Hartwick, 1991a, Burnett et al., 1996), medusae would typically reach their estimated asymptotic size of ~190 mm (IPD in mm) after ~140 days. That *C. bronzei* has an estimated asymptotic size of 71 mm IPD (Gordon, 1998, Gordon et al., 2004) and *Chiropsalmus quadrumanus* is reported to reach 110 mm (Guest, 1959) suggests that *C. fleckeri* medusae attain a larger size than do other Chirodropid species**.** On an applied level, Hartwick (1987) has previously suggested that medusae reach a size dangerous to humans within approximately two to three months. Indeed, *C. fleckeri* medusae undergo an ontogenetic shift in their cnidome (and diet) that potentially explains the lethality of larger medusae to humans (Carrette et al., 2002) at ~60 - 100 mm IPD (Carrette et al., 2002), a size they would reach after ~50 - 65 d. This is an important parameter to consider in the further development of management protocols in that the time at which medusae are likely to become lethal to humans can now be defined with greater reliability.

That the onset of sexual maturity occurred at approximately ~50 mm IPD compares favourably to estimates provided by (Barnes, 1966) who noted that the development of a very large area of gonad material commenced at the eight tentacle stage (~60 mm IPD based on the regression equation developed here) (Kinsey, 1986). At the rapid rate of growth quantified here, medusae would become sexually mature after ~45 - 50 d, which is considerably less time than the typical length of a season (~180 d). Given that the oldest medusa was ~78 d, it does not appear that medusae accumulated as the season progressed (Hartwick, 1991a). Potentially, medusae relocated from within the estuarine and coastal areas sampled here, with the infrequent collection of medusae up to several km from shore (Kinsey, 1988, Hamner et al., 1995, Burnett et al., 1996) suggesting that some form of emigration could take place. Alternatively, medusae that underwent metamorphosis in early September would have had several months of stable conditions and an abundant food supply prior to the onset of the wet season in which to grow and mature. That is, September to December falls within Weipa's dry / pre wet season (Vance et al., 1996b, Vance et al., 1998) when salinity regimes are typically stable and elevated (Vance et al., 1996b), water temperatures are typically increasing (Cyrus and Blaber, 1992, Vance et al., 1996b, Vance et al., 1998) and an abundance of post larval prawns occurs within the Embley Estuary (Vance et al., 1996b, Vance et al., 1998). Whether several generations of medusae occur within a single season is an aspect of *C. fleckeri*'s ecology that future research should examine.

Medusae development can also be considered with respect to tentacle number, with some authors using tentacle number rather than size when discussing the development of medusae (eg Kinsey (1986)). The maximum number of tentacles per pedalium observed within this study was 13, which compares favourably to maximums of 12 (Southcott, 1956, Barnes, 1966) and 13 (Kinsey, 1986), but suggests that 15 tentacles may be limited to those individuals of ~300 mm IPD which are rarely observed (Barnes, 1960, Southcott, 1971, Burnett et al., 1996). Given that newly metamorphosed medusae possess one tentacle per pedalium (Yamaguchi and Hartwick, 1980) and only 8.3% of medusae within this study possessed an even number of tentacles, *C. fleckeri* would appear to add tentacles in pairs. With 12 medusae possessing an odd number of tentacles and 10 possessing an even number of tentacles, samples in Kinsey (1986) suggest that tentacles are added singularly, however this may be an artefact of a small sample size. *C. bronzei* also appears to add tentacles in pairs, with 92% of the 1652 medusae collected possessing an odd number of tentacles (Gordon, 1998). On a more applied level, tentacle number may provide a more standardised method by which groups such as Surf Life Saving can provide consistent estimates of medusa size and age, given the significant relationships that exist between these variables.

A difference in the population structure of coastal and estuarine habitats would be expected if a seasonal alternation in generations and habitats (Hartwick, 1987) is occurring for *C. fleckeri*. That is, juvenile medusae would be representative of estuarine populations while coastal populations would be typified by both a greater size range of medusae as well as an accumulation of larger individuals as the season progressed. Data collected within this study do not support this theory, however. Not only were medusae from coastal sites typically smaller (fewer tentacles) and younger than those found within the estuarine habitat, but the oldest medusa (~78 d) was also collected from within the estuarine habitat. While these results appear contradictory to expected patterns, juvenile medusae reported by Hartwick (1991a) were as small as 0.6 mm, at which size, identification to the species level is considered unreliable. Given that juvenile medusae were collected using plankton tows (Hartwick, 1987, Hartwick, 1991a), the visually based collection techniques used within the current study may also have possessed an inherent bias against such small individuals. Further research employing non visually based collection techniques (eg plankton tows) are therefore required to fully validate the observed population structure trends. Differences in population structure may also reflect the suitability and availability of prey within the estuarine and coastal habitats. For instance, mangrove stands within the Embley Estuary (Weipa) have been shown to be important nursery areas for numerous species of prawns and fish (Vance et al., 1996a), while greater abundances of ~60 species of fish (Blaber et al., 1989) and post larval / juvenile prawns have been reported for areas adjacent to mangroves stands or within creeks adjoining estuaries (Vance et al., 1990). In contrast, both species diversity and overall abundance of fish was lower along the coastline (Blaber et al., 1995). Further data quantifying medusae abundance, gastrovascular cavity content and prey abundance is required to further validate these claims.

Collectively, the results of this study are relevant in both an applied and an ecological context. By quantifying growth and development rates as well as the temporal variation in the onset of polyp metamorphosis between seasons for *C. fleckeri* medusae, this study has contributed to the understanding of the ecology of an understudied taxon, the Cubozoa, as well as to the broader understanding of jellyfish ecology. On an applied level, this study has also presented quantitative data upon which models predicting the relative risk of an envenomation event occurring can now be based. For example, estimates of when medusae are likely represent a considerable risk to humans following an ontogenetic shift in their cnidome and venom complement can now be made with greater accuracy, with a temporally constrained onset to the medusae season demonstrated and rapid growth and development rates quantified. Such models are of particular relevance given the way in which the seasonal occurrence of *C. fleckeri* impacts the way in which the tropical Australian coastline is utilised throughout the warmer months of the year. However, it is only when a complete understanding of the medusae phases ecological relationships are developed that the occurrence and distribution of *C. fleckeri* can be modelled with accuracy and reliability. This study represents the first attempt at quantifying such parameters, however, further long term studies are required if management practices are to be optimised and broader ecological questions regarding season length or the intensity and frequency of jellyfish blooms are to be validated.

CHAPTER FOUR

Quantifying movement of the tropical Australian Chirodropid *Chironex fleckeri* using manual acoustic telemetry

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4.1 Abstract

Cubomedusae are considered to have superior swimming abilities compared to other pelagic cnidarians, yet many of the theories describing such behaviours are based on anecdotal evidence, sting records or opportunistic sightings, rather than quantitative data. Acoustic telemetry was used to document the movements of twelve adult *C. fleckeri* medusae for periods of between 10 and 38 h within both coastal and estuarine habitats. The rate at which tagged medusae moved was influenced by an interaction between time period (day or night) and habitat (coastal or estuarine), with rates of travel being relatively similar during the day (135 ± 31 SE m h^{-1}) and night (88 ± 22 SE m h^{-1}) within the coastal habitat, but significantly greater at night (467 ± 65 SE m h-1) than during the day (245 ± 42 SE m h^{-1}) within the estuarine habitat. Medusae in coastal habitats travelled at similar rates throughout all tidal states (from 52 ± 26 SE m h^{-1} to 187 ± 53 SE m h^{-1}) while estuarine medusae travelled at significantly faster rates towards the middle of the tide (828 ± 306 SE m h^{-1}) than at the low (323 ± 77 SE m h^{-1}) and high (from 110 ± 53 SE m h^{-1} to 169 ± 27 SE m h^{-1}) ebbs. While such movements occurred both with and across / against tidally generated currents, at increased current speeds, medusae tended to travel with the current. Data are also presented that show that large medusae may move from coastal to estuarine habitats.

4.2 Introduction

A range of strategies are used by species of cnidaria to regulate the distance and direction in which they travel, and as a result, some are considered to be more active than others. Hydromedusae and scyphomedusae for instance, are often considered to be passive in their movements (Shorten et al., 2005). Although they may actively move vertically between currents of differing speed and direction (Rifkin, 1996), movement in a horizontal plane is often an indirect result of their vertical movement. In contrast, the swimming behaviours of cubomedusae appear to surpass all other jellyfish in terms of power and complexity (Gladfelter, 1973), enabling them to move independently of currents and local weather conditions (Barnes, 1966, Kinsey, 1986, Hartwick, 1987). *C. fleckeri* is a large cubozoan found in Australia's tropical waters and can attain an unassisted speed of 414 m h^{-1} using a pulse-coast style of jet propulsion (Shorten et al., 2005). Anecdotal reports of *C. fleckeri* medusae swimming at "3 to 4 knots all day" (Kinsey, 1986) suggest that elevated levels of activity can be sustained for extended periods of time. The extent to which speed in this species is influenced by physical and biological factors remains unclear with many currently favoured theories based on qualitative data such as sting records, opportunistic sightings and anecdotal evidence, rather than quantitative data.

Physical factors that may directly or indirectly influence *C. fleckeri* medusae movements include tidal state, water currents (Kinsey, 1986), time of day (Kinsey, 1986, Seymour et al., 2004) and exposure to and changes in local weather conditions (Southcott, 1956, Barnes, 1960, Barnes, 1965, Barnes, 1966, Brown, 1973, Yamaguchi, 1982, Kinsey, 1986, Marsh et al., 1986, Hartwick, 1987). Wind speed for instance, may indirectly influence movement patterns by increasing wave action, as *C. fleckeri* medusae appear to be particularly sensitive to turbulence (Barnes, 1966, Kinsey, 1986). It remains unclear, however, whether the absence of medusae during rough conditions is due to their relocation to less turbulent areas such as sheltered bays (Brown, 1973), estuary systems (Kinsey, 1986) or offshore waters (Barnes, 1966, Kinsey, 1986), or is just sampling bias. The influence of tidal state is also unknown, although anecdotal evidence suggests that medusae are never found at low tide and collections are more successful on a making tide (Kinsey, 1986). Tidal currents can also potentially influence cubomedusae movements in that travel can be directional (with or against the current) or random (Yamaguchi, 1985, Kinsey, 1986). Movement patterns in *C. fleckeri* are further complicated by changes in activity levels, with a resting behaviour reported for medusae in the mid to late afternoon (Kinsey, 1986, Seymour et al., 2004). During this time, medusae lie motionless on the substrate with tentacles either outstretched along the seafloor (Seymour et al., 2004) or retracted to or inside the bell (Kinsey, 1986). Thus, any study on movement patterns in *C. fleckeri* needs to incorporate time of day into the study.

Biological factors may also influence movement patterns of *C. fleckeri* medusae. For example, the abundance and distribution of prey, such as fish and prawns, may vary spatially and temporally. Given that large aggregations of prey provide *C. fleckeri* medusae with particularly favourable feeding conditions (Southcott, 1971, Kinsey, 1986, Hartwick, 1991a, Rifkin, 1996) and these medusae have the potential to actively locomote to different areas, medusae movements may then be influenced by the abundance of their prey.

If an understanding of the links between physical and biological factors and movement in *C. fleckeri* are sought, a more quantitative approach is required. Acoustic telemetry is widely used to gather data from a diverse range of marine species, although the majority of animals are large in size or have suitable sites for internal or external tag attachment (Egli and Babcock, 2004, Atkinson et al., 2005, Heupel and Simpfendorfer, 2005, Jackson et al., 2005, Kerwath et al., 2005, Heupel et al., 2006b, Willis and Hobday, 2007, Wingate and Secor, 2007). Although continued miniaturisation has seen acoustic tags attached to increasingly smaller animals (Jackson et al., 2005), cubomedusae present a number of unique issues that have limited the effectiveness of acoustic telemetry as a tool to study their movement patterns. In particular, cubomedusae are often small in size and their entire body is used in locomotion, making tag attachment difficult. However, a new technique for tag attachment has been developed (Seymour et al., 2004) allowing movement of these animals to be investigated using manual acoustic tracking. The aim of this study was to use acoustic telemetry to quantify the influence of time of day, tidal state, tidal current and habitat on the movements of *C. fleckeri* medusae.

4.4 Materials and Methods

4.4.1 Sample Sites

Medusae movements were documented at five sites within three locations in north Queensland, Australia (Figure 4.1):

- Location 1 – Townsville

  The Australian Institute of Marine Science (AIMS) site was a shallow, mangrove lined embayment into which a permanently flowing estuary drained. A degree of protection was provided by a headland and rocky / coral reef to the north.

- Location 2 - Mission Beach region

  The coastline of the Mission Beach sites was typified by long stretches of gently sloping sand / mud beachfronts separated by several rocky headlands. Three mangrove lined estuary systems, the Tully, Hull and Murray Rivers, flow directly onto this stretch of coastline. Two sites represented the Mission Beach region:

  1. Tully, which was located between the Hull and Tully rivers and included the Tully River itself, and
  2. Mission Beach, which was located approximately 8 km north of the Hull River

- Location 3 – Weipa

  Situated on the eastern boundary of the shallow (7 to 10 m) embayment of Albatross Bay into which three major estuary systems flow (the Mission, Embley and Pine rivers), Weipa was represented by two sites:

  1. Wooldrum Point is an uninterrupted stretch of gently sloping sand / mud beachfront that extends approximately 9 km south of the Embley Estuary mouth, protected in the north by Duyfken Point.
  2. Hey Point is situated at the intersection of the Hey River and Embley Estuary, at an interface between a mangrove forest and a stretch of muddy intertidal beachfront. The Embley Estuary is a 32 km long system into which the Hey River flows. Both systems are lined with extensive mangrove forests, seagrass beds and intertidal flats, with depth typically increasing rapidly from a shallow (~2 m) narrow (~100 m) intertidal shelf to a maximum of 21 m.

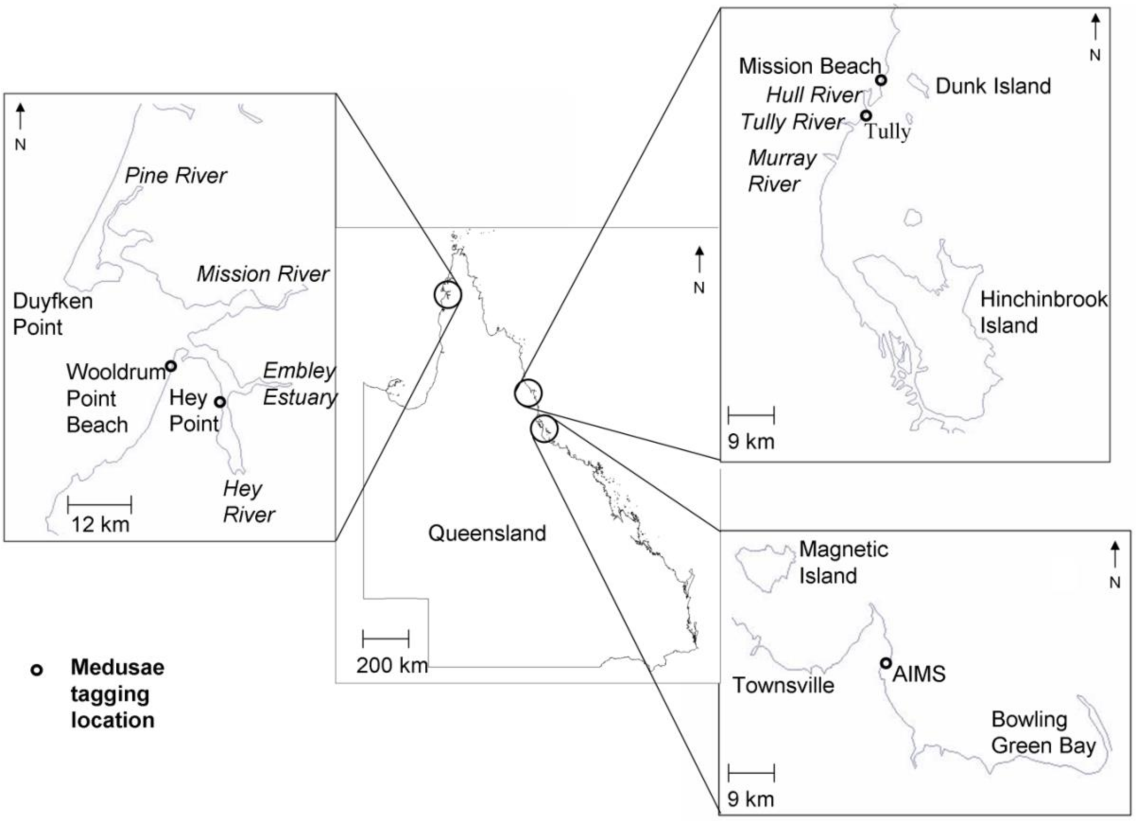


Figure 4.1 Geographic location of sites at which acoustic tracking studies were conducted showing medusae tagging locations: AIMS, Mission Beach, Tully, Wooldrum Point and Hey Point in north Queensland, Australia

The AIMS, Mission Beach, Wooldrum Point and Tully sites were classed as coastal sites while Hey Point and Tully River were denoted as estuary sites.

4.4.2 Acoustic Tracking

Twelve *C. fleckeri* medusae were manually tracked: two at AIMS, two at Mission Beach, one at Tully, two at Wooldrum Point and five at Hey Point. Medusae selected for tracking had a minimum inter pedalia distance (i.e. distance between the mid-line of two pedalia along the line passing through the rhopalial niche (IPD)) of 80 mm to reduce potential confounding effects due to tag weight. No tag used was more than 0.2% of the medusa's wet weight (where wet weight was calculated using regression equations for a morphometrically similar species, *C. bronzei* (Gordon, 1998)). All medusae used had no deformation or damage to the bell and no damaged or missing pedalia. Specimens were collected by hand and their IPD was measured to the nearest mm. An acoustic tag was glued to an external face of the bell with Histoacryl, a non-toxic surgical glue that is used for closing incisions and sets in seconds. Tags were positioned half way between the top of the bell and the velarium, along the fold line that forms between the rounded shoulder that gives rise to the pedalia and the more flattened interpedalial face (upon which the rhopalia are located) (Figure 4.2). Three types of coded Sonotronics transmitters were used within this study (Pico, IBT 96-2, IBDT 97-2), varying in length and weight from 19 mm and 1 g to 49 mm and 3 g.

The capture and release point of each medusa was determined by a Garmin GPS 12 hand held GPS and the location classified as either estuarine (within an estuary) or coastal (outside an estuary). Individual medusa were actively tracked for between 10 and 38 h (Table 4.1) using a Sonotronics USR 96 receiver and unidirectional hydrophone. Individuals were relocated between fixes by returning to the last known location and estimating the distance and direction to the new location using signal strength. The tracking vessel was then moved in a straight line along the axis of maximum signal strength until the source of the signal was passed, at which point, a decreasing spiral search pattern allowed the animal's location to be determined. Several visual contacts during daylight hours confirmed an accuracy of approximately 10 m to the animal's true location. The time of successive readings was determined by the Garmin GPS 12 clock, and the location was again classified as either estuarine or coastal. Animals were grouped according to the habitats in which their movement patterns were documented, with three habitat categories identified as:

1) coastal - individuals that remained wholly within the coastal habitat
2) estuarine - individuals that remained wholly within the estuarine habitat
3) combination - individuals that moved between habitats

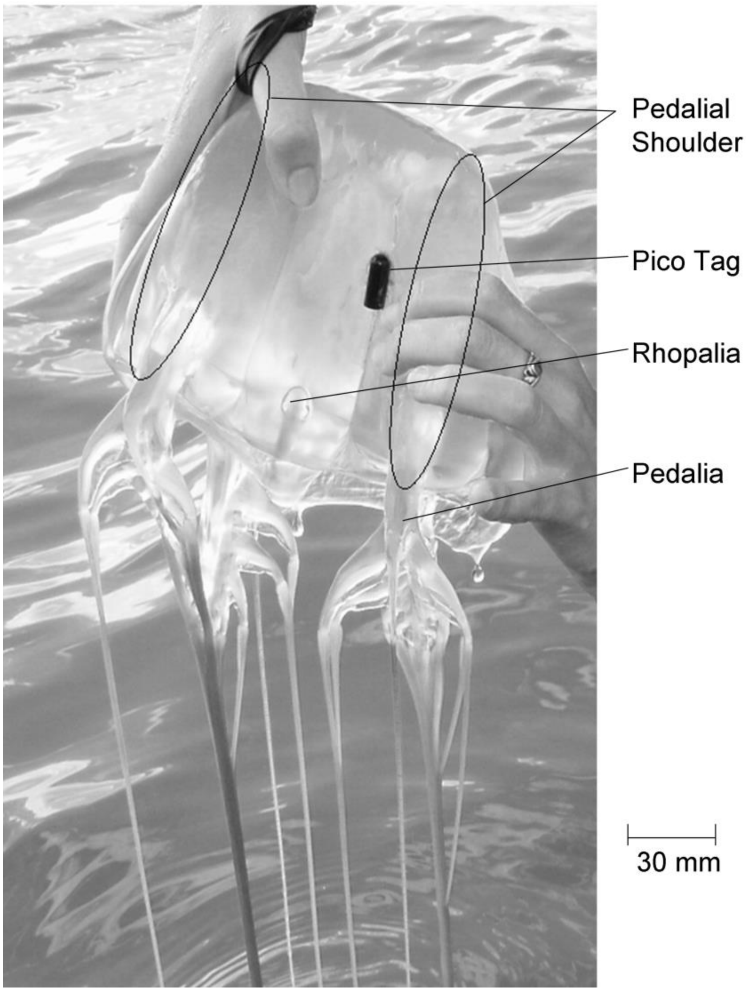


Figure 4.2 Site of tag attachment on *Chironex fleckeri* medusae – half way between the top of the bell and the velarium, along the fold line of the rounded shoulder giving rise to the pedalium, and the interpedalial face

Details of each medusa's size (IPD in mm), the habitat and site at which it was tracked, the date and time (h) at which it was fitted with an acoustic tag, the total duration over which it was tracked (h) and tag type used are summarised in Table 4.1.

Table 4.1 Details of each medusa's size (IPD in mm), the habitat and site at which it was tracked, the date and time (h) at which it was fitted with an acoustic tag, the total duration over which it was tracked (h) and the tag type used

ID	IPD (mm)	Habitat	Site	Date	Time Tagged (h)	Duration (h)	Tag Type
24	140	Coastal	AIMS	13/01/03	2100	11	IBDT 97-2
25	145	Coastal	AIMS	17/01/03	2100	12	IBDT 97-2
22	150	Coastal	Mission Beach	09/04/03	0930	11	IBDT 97-2
23	175	Coastal	Mission Beach	10/04/03	0645	12	IBDT 97-2
16	90	Coastal	Wooldrum Point	17/12/06	0925	38	Pico
19	105	Coastal	Wooldrum Point	17/12/06	1344	10	IBT 96-2
21	180	Combination	Tully	23/03/05	1000	26	IBDT 97-2
6	110	Estuarine	Hey Point	17/11/05	1100	17	Pico
7	115	Estuarine	Hey Point	17/11/05	1115	10	Pico
26	96	Estuarine	Hey Point	09/11/07	0848	15	Pico
27	100	Estuarine	Hey Point	09/11/07	0903	16	Pico
28	100	Estuarine	Hey Point	09/11/07	0914	11	Pico

4.4.3 Calculation of Distance Estimates

The distance travelled by each medusa was measured as the distance between two successive GPS location readings. This estimate represented the minimum distance travelled as it did not allow for any deviation from travel in a straight line. Since the time between successive readings was not consistently 1 h, the rate of travel (m h^{-1}) for each estimate was calculated by dividing the minimum distance travelled (m) by the time (h) between readings.

4.4.4 Environmental Factors

To investigate diurnal patterns, rates of travel during the day and night were estimated separately. Given that medusae are more active between 0600 and 1500 h than between 1500 and 0600 h (Seymour et al., 2004), an artificial definition of day (0600 – 1500 h) and night (1501 – 0559 h) was used.

The magnitude and duration of individual tides varied between sites (Table 4.2), with proportion of tidal state rather than absolute tide height used to investigate the effect of tidal state on rates of travel.

Table 4.2 Tidal characteristics experienced at each location during tracking of medusae showing minimum and maximum tidal height (m), minimum and maximum tidal amplitude (m) plus minimum and maximum tide duration (min)

Location	Minimum Tide Height (m)	Maximum Tide Height (m)	Minimum Tidal Amplitude (m)	Maximum Tidal Amplitude (m)	Minimum Tide Duration (min)	Maximum Tide Duration (min)
Townsville	0.58	3.48	1.40	2.90	384	403
Mission Beach	1.36	2.49	0.19	2.07	237	520
Weipa	0.46	2.81	0.79	2.32	364	951

A complete tidal cycle was defined as two successive high tides separated by a single low tide (Figure 4.3). Each complete tidal cycle was divided into its flood and ebb components, which were further divided into six equal tidal classes, each representing 1/6 of an individual tide (Figure 4.3A). Each tidal class was assigned a tidal proportion class (1-10), according to whether it occurred on a flood or ebb tide (Figure 4.3A).

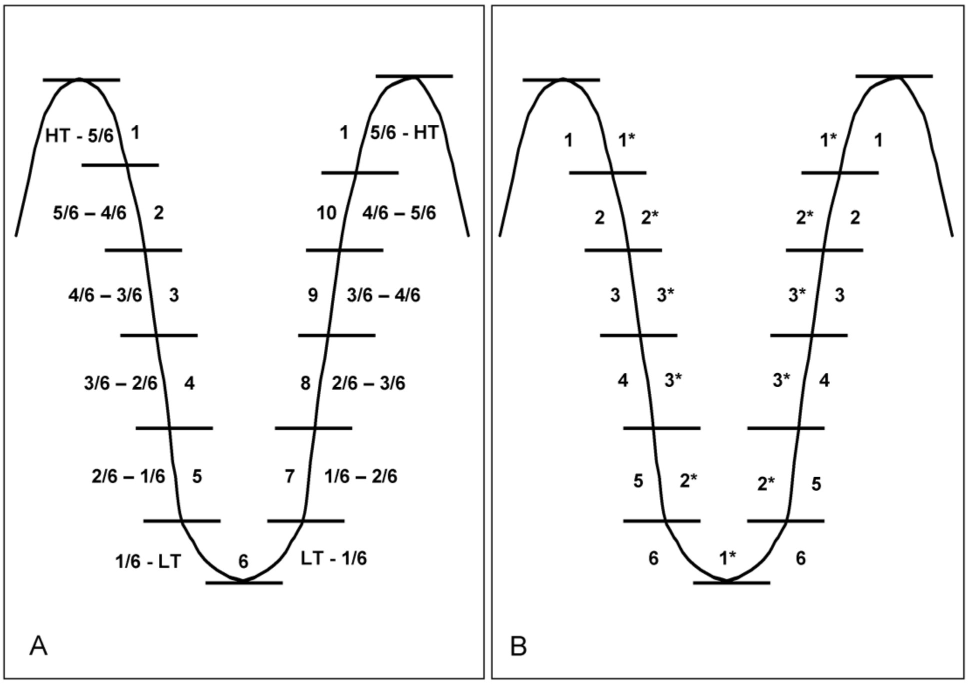


Figure 4.3 One complete tidal cycle showing tidal classes (1/6's) with corresponding tidal proportion classes (1-10) (A) and tide portion classes (1-6) with corresponding current numbers (1-3 with *) (B)

Tidal proportion values for each location reading were calculated as follows:

$$P = \frac{D}{T}$$

where P is the proportion of the individual tide at which a reading was taken, D is the time interval (min) between when a reading was taken and high tide and T is the total duration of the individual tide (min). For instance, a location reading taken 300 min from high tide (D) on a tide with a total duration (T) of 400 min would have a tidal proportion value (P) of 0.75. The corresponding tidal proportion class for this reading would be 5 if it was taken on an ebb tide but 7 if it was recorded on a flood tide. For ebb tides, D was taken to be the time (min) between when a location reading was taken and the previous high tide while for flood tides, D was calculated as the time (min) between a location reading and the approaching high tide.

Due to the small number of medusae tracked in each habitat (five estuarine, six coastal) it was necessary to use sequential rate of travel (m h^{-1}) estimates as replicates to allow statistical analysis to be performed. As such, sequential rate of travel (m h^{-1}) estimates for each individual were not truly independent and a repeated measures analysis was conducted to determine this effect of dependency. No significant effect was found ($F_{2, 30} = 0.075$, $P = 0.92$) and as such analysis was conducted assuming the replicates were independent. A three way Analysis of Variance was used to compare rates of travel with respect to habitat (estuarine or coastal), time of day (day or night) and tidal state (tidal proportion class). Least Significant Difference *post hoc* analysis was carried out on tidal state to determine which states were statistically different. These analyses were performed only on medusae that spent their entire time in either the coastal or the estuarine habitat.

To investigate the effect of current speed and direction on medusae movements, a flow index for each rate of travel estimate was calculated. Analysis was limited to data collected from the Weipa sites of Wooldrum Point and Hey Point, as these trials were conducted during the late dry season (as defined by Cyrus and Blaber (1992)) when currents are tidally driven (due to a lack of rainfall runoff). Such parameters have not been defined for east coast sites, especially during the wet season, when trials were conducted at these sites. Individual flood and ebb tides were divided into six equal tide portion classes as shown in Figure 4B. Current numbers (C) were assigned to each tide portion class according to the generalisation that the greatest amount of tide induced water movement would occur towards the middle of a tide, tapering off towards the top and bottom of the tide. Tide portion classes 1 and 6 were assigned a current number of 1 as they represented the top and bottom of the tide, 3 and 4 were assigned a current number of 3, representing the middle portions of the tide, and the intermediate tidal portions of 2 and 5 were assigned a current number of 2 (Figure 4.3B). The flow index (F) for each rate of travel estimate was calculated as:

$$F = C \times A$$

where F is the flow index (m), C is the current number and A is the tidal amplitude (m).

Given that each rate of travel estimate arose from two successive location readings, the direction of medusa travel in relation to the direction of tidal current could be established. This was defined as either having been with the current or across / against the current. Significant relationships between flow index and rates of travel were investigated using linear and curve estimation regression analysis for travel both with and across / against the current.

4.5 Results

No significant interaction between habitat, tidal state and time of day was found, with medusae in both habitats travelling at similar rates throughout the tidal cycle both day and night ($F_{4, 83} = 1.836$, $P = 0.130$).

Medusae movements were, however, significantly influenced by an interaction between time of day and habitat ($F_{1, 83} = 4.729$, $P = 0.033$). Medusae within the coastal habitat travelled at similar rates throughout the day (135 ± 31 SE m h^{-1}) and night (88 ± 22 SE m h^{-1}), but estuarine medusae travelled significantly faster at night (467 ± 65 SE m h^{-1}) than during the day (245 ± 42 SE m h^{-1}) (Figure 4.4).

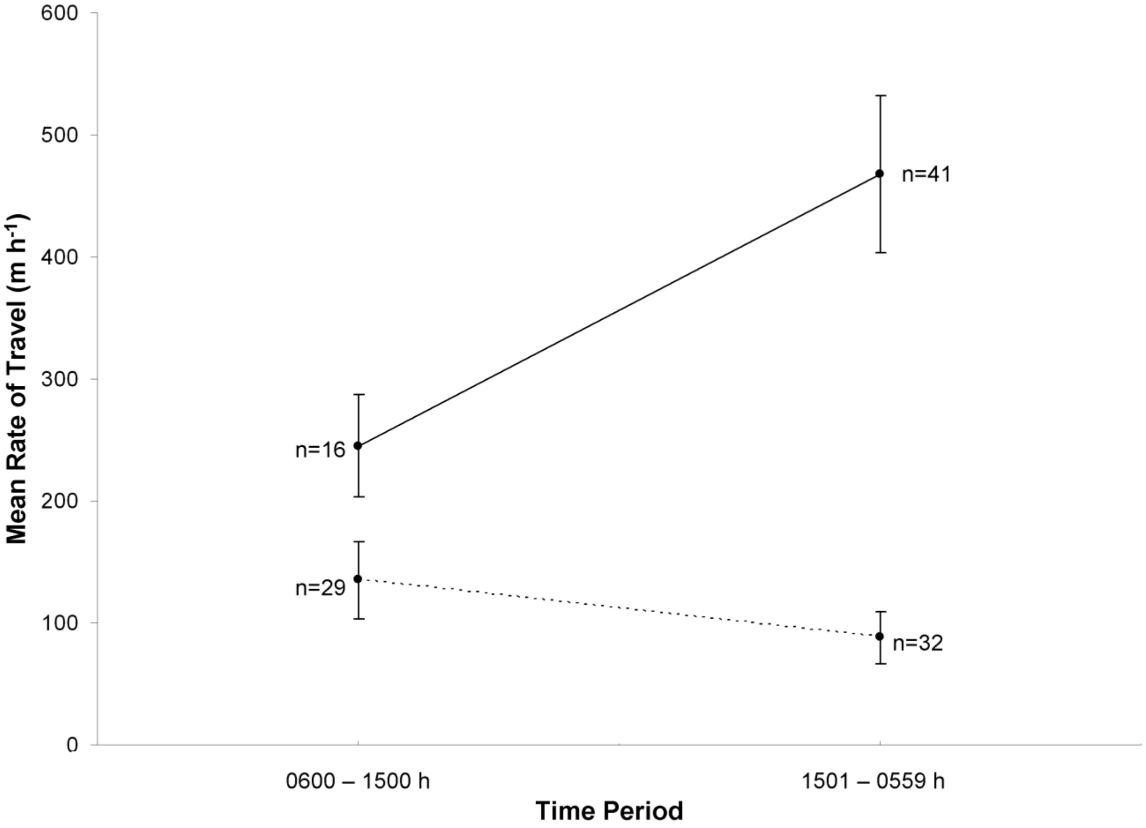


Figure 4.4 Mean rate of travel (m h^{-1}) ± 1 SE for medusae within the coastal (·········) and estuarine (——) habitats during the day (0600 – 1500 h) and night (1501 – 0559 h)

Rates of travel were also significantly influenced by an interaction between tidal state and habitat ($F_{9, 83}$ = 2.063, *P* = 0.042). While coastal individuals travelled at similar rates throughout all tidal states (from 52 ± 26 SE m h^{-1} to 187 ± 53 SE m h^{-1}), rates of travel for estuarine medusae varied substantially with tidal state. Rates of travel during slack water at the top of the tide (from 110 ± 53 SE m h^{-1} to 169 ± 27 SE m h^{-1}) were similar to those observed within the coastal habitat, but increased throughout the tidal cycle, peaking at 828 ± 306 SE m h^{-1} and 568 ± 241 SE m h^{-1} for the ebb and flood tides respectively, before decreasing again to 323 ± 77 SE m h^{-1} at low tide (Figure

4.5). Rates of travel within each tidal state were relatively consistent between day and night however, with no significant interaction between tidal state and time of day established ($F_{9,83}$ = 1.096, P = 0.375).

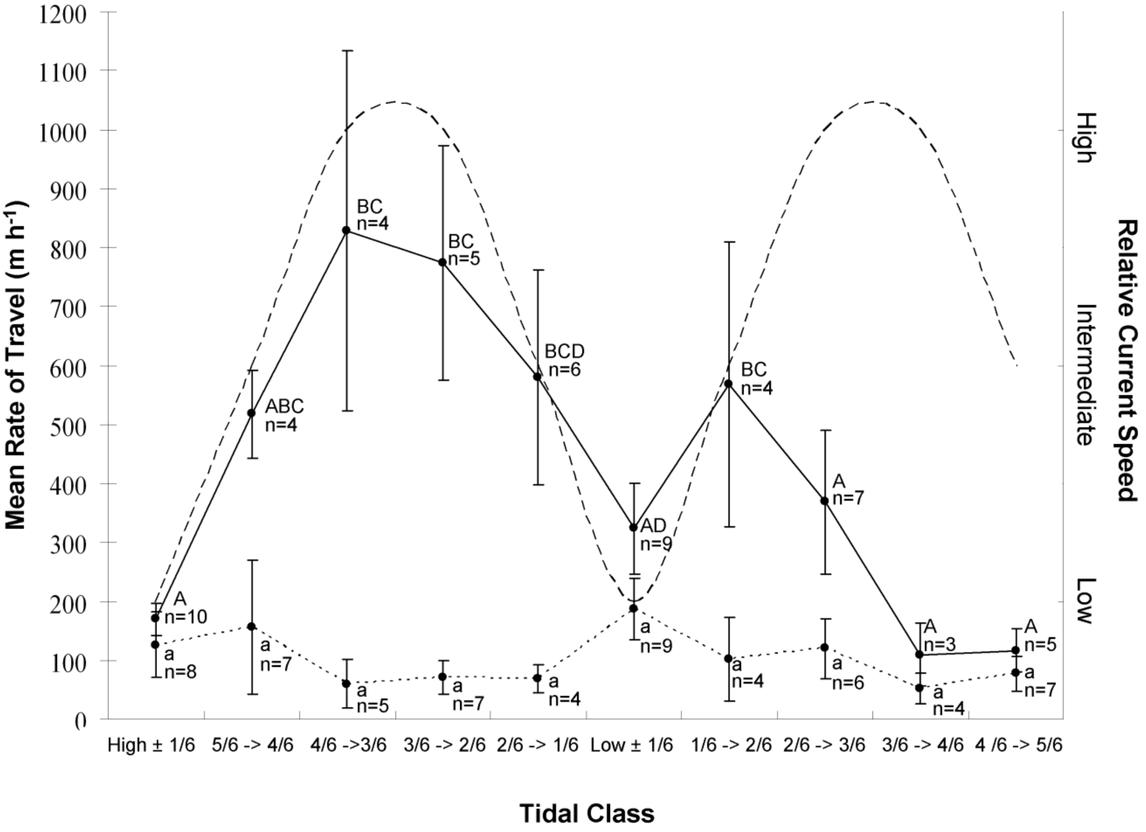


Figure 4.5 Mean rate of travel (m h^{-1}) ± 1 SE for medusae within the coastal (···········) and estuarine (———) habitats with respect to relative current (------) at each tidal class with means followed by the same letter not significantly different at the 95% level

While medusae were found to move both with and across / against the current, they typically travelled with the current at higher flow indexes (Figure 4.6). No significant relationship between rate of travel and flow index was established for travel across / against of the current ($F_{2,18}$ = 2.126, P = 0.148), however, a significant relationship did exist between rate of travel and flow index for movements with the current ($F_{2,55}$ =

11.715, $P < 0.001$). The rate at which medusae travelled increased with flow index, with this relationship best described by

$$R = 146.023 + (54.478 \times F) + (8.381 \times F^2)$$

where R is the rate of travel (m h^{-1}) and F is flow index (m).

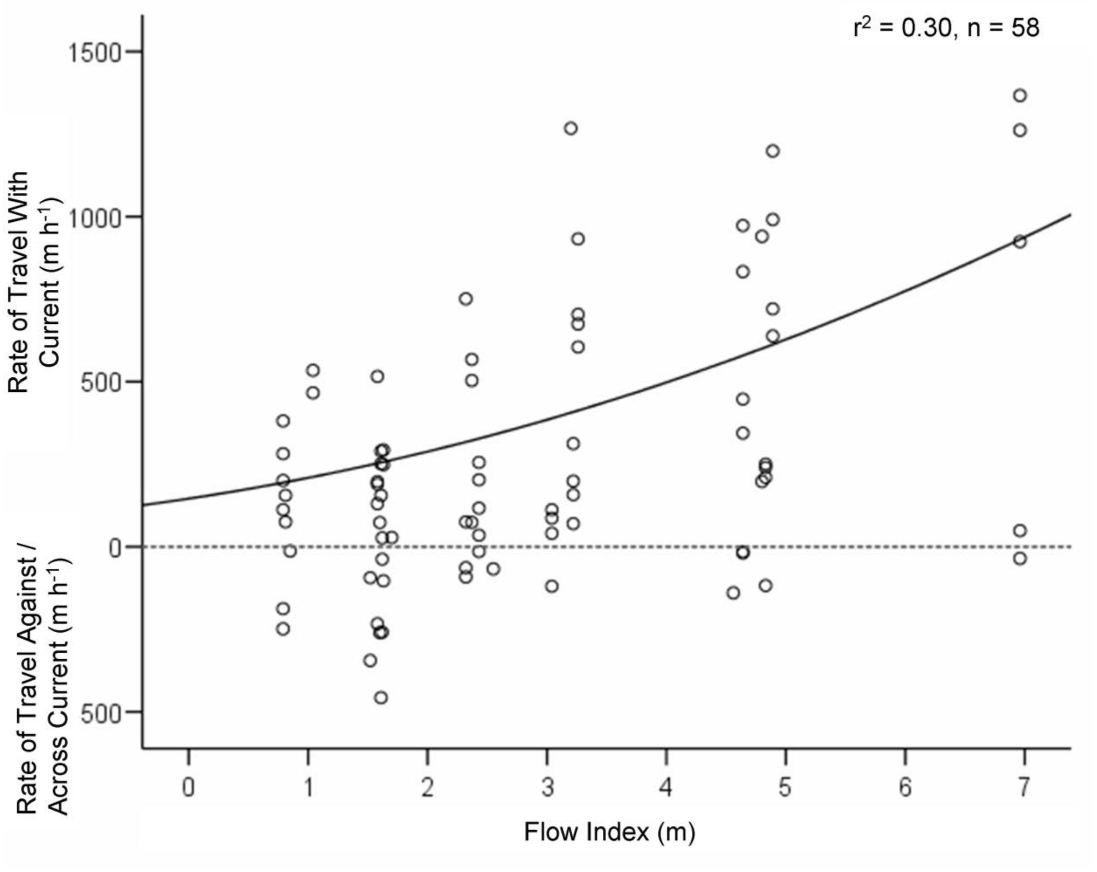


Figure 4.6 Rates of travel (m h^{-1}) with and against / across the current flow in relation to flow index (m) showing regression line (———) and zero reference line (------)

While movement patterns were relatively consistent between the majority of individuals tracked, those of a large adult medusa (180 mm IPD) tagged at the Tully site on the 23rd March 2005 (Figure 4.7) differed in a number of key aspects. This was the only individual that used both coastal and estuarine habitats within 24 h and it did not display the diurnal behaviours observed for other individuals. The medusa was tagged at the mouth of the Hull River at 1000 h and moved south at an average speed of 269 ±

81 SE m h^{-1} to be located adjacent to the Tully River mouth at 2100 h. From 2100 h to 0200 h, the medusa returned north along the beachfront at an average speed of 383 ± 105 SE m h^{-1}. By 0600 h, it had again returned south to the Tully River mouth at an average speed of 714 ± 186 SE m h^{-1}. During the daylight hours of the 24th March (0600 to 1200 h), the medusa moved from the coastal to the estuarine habitat, into the Tully River (Figure 4.7). Although water current speeds were not collected while this animal was tracked, the first 3 h of movement up the Tully River (average speed 503 ± 85 SE m h^{-1}) corresponded with an incoming tide while the second 3 h down the river (average speed 99 ± 15 SE m h^{-1}) occurred during an outgoing tide (Figure 4.7). While this medusa exhibited a similar trend to coastal medusae whereby rates of travel during the day (average speed 364 ± 110 SE m hr^{-1}) were similar to those travelled at night (average speed 403 ± 86 SE m h^{-1}), the actual rates of travel were of a similar scale to that of estuarine medusae.

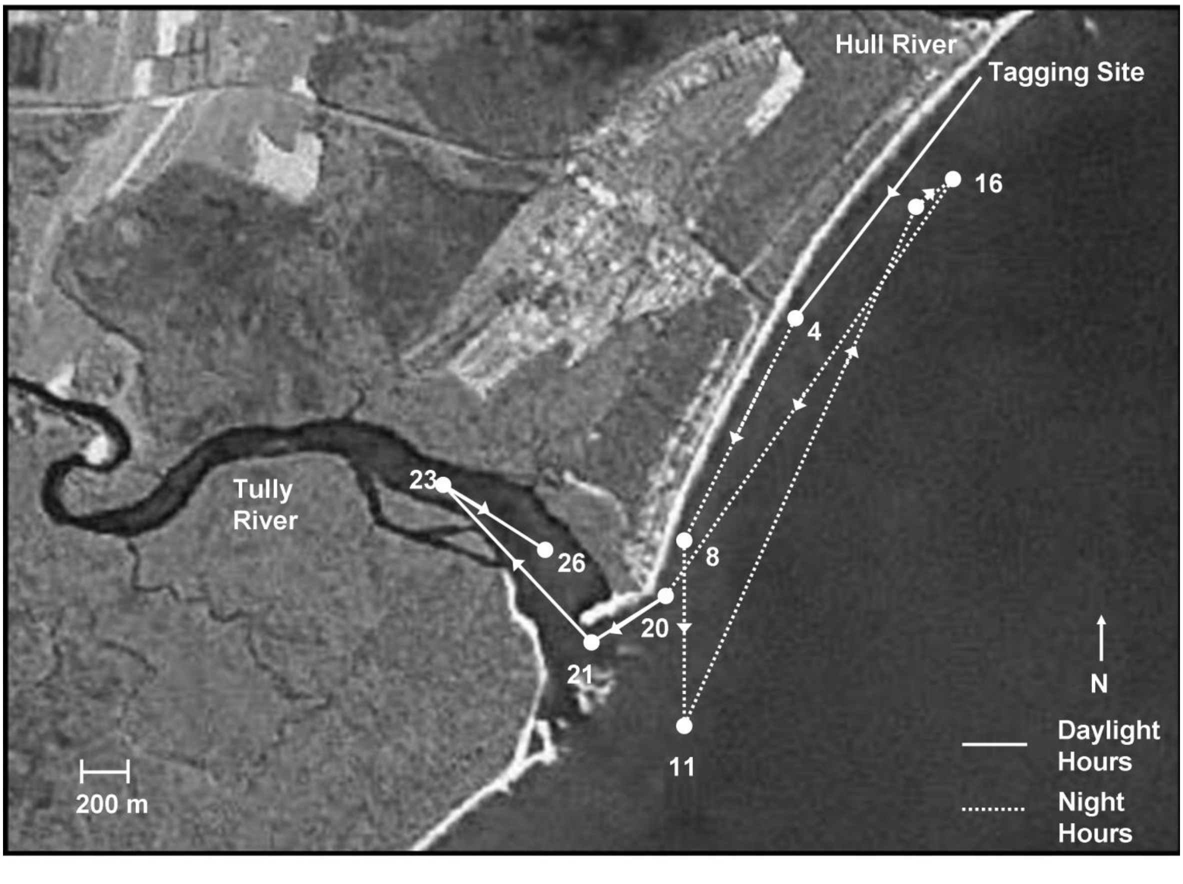


Figure 4.7 Movements of Medusa 9 (Table 5.1) over a 26 h period at the Tully site in March 2005 showing time (h) since tagging

4.6 Discussion

Previous research has reported diurnal (Seymour et al., 2004) and afternoon resting behaviours (Kinsey, 1986) for *C. fleckeri* whereby medusae are active between 0600 and 1500 h but have extended periods of relative inactivity from 1501 to 00559 h (Seymour et al., 2004). Such diurnal patterns were less pronounced in the current study and were not consistent between habitats. During the day (0600 – 1500 h), coastal medusae travelled at an average rate of 135 ± 31 SE m h-1, which is slower than the 212 m h-1 reported by Seymour et al. (2004). A mean rate of travel of 88 ± 22 SE m h^{-1} at night (1501 - 0559 h) was, however, considerably more than the 10 m h^{-1} previously reported (Seymour et al., 2004). Diurnal patterns were reversed for estuarine medusae that travelled at an average rate of 467 ± 65 SE m h^{-1} at night but only 245 ± 42 SE m h^{-1} during the day. The rate travelled at night was also more than 40 times the rate previously established for coastal medusae at night. It remains unclear whether biological or physical mechanisms drove these observations.

The relatively slow rates of travel by medusae during the day, within both the estuarine and coastal habitats, could represent localised hunting and feeding behaviours. When hunting, *C. fleckeri* swim at or near the surface with tentacles outstretched, sampling deeper waters only periodically by suspending bell activity and passively sinking (Barnes, 1966, Kinsey, 1986). Following prey capture, bell activity is typically suspended until prey have been removed from the tentacles (Barnes, 1966), further decreasing the distances medusae are likely to travel while actively feeding. Since prey such as prawns and small fish are characterised by spatial and temporal variability (Blaber et al., 1989, Vance and Staples, 1992, Xiao and Greenwood, 1993, Blaber et al., 1995, Omundsen et al., 2000), it may be energetically beneficial for medusae to remain searching within an area of high prey concentration rather than leave the area in search of other prey.

Visually guided behaviours, such as feeding and copulation, have been reported for a number of species of cubozoa (Buskey, 2003; Garm et al., 2007; Garm et al., 2011; Garm et al., 2012). The ability of medusae to successfully capture prey or avoid underwater obstacles would be greatly reduced at low light intensities (e.g. night), at which time it may become energetically beneficial for medusae to remain motionless on the substrate. The reduced rates of travel of coastal medusae at night may have arisen from the short periods of activity medusae demonstrate when disturbed from their otherwise motionless, energy conserving state (Kinsey, 1986, Seymour et al., 2004). Estuarine medusae, however, may have been unable to sink to the substrate and become inactive due to the relatively strong currents that are likely to persist within estuaries. The greater rates of travel exhibited by estuarine medusae during the night, therefore, may be attributed to medusae moving with the tidal current until sufficient light returned for feeding activities to resume.

Alternatively, tidal state could indirectly influence the rates at which medusae travel by limiting access to either prey or the habitats in which high abundances of prey occur. For instance, greater densities of potential prey occur on the flood rather than the ebb tide within the Embley Estuary (Xiao and Greenwood, 1992), particularly towards the high tide (Vance and Staples, 1992, Vance et al., 1994). In contrast, the majority of fish species found in the near shore coastal habitat of Weipa are not influenced by tidal state (Blaber et al., 1995). As such, the relatively slow and consistent rates of travel observed for coastal medusae may have arisen from a more reliable access to prey, a situation that does not exist for estuarine individuals, whose movements varied considerably throughout each tidal cycle.

The duration for which abundant prey are accessible to medusae is also likely to vary between habitats. For example, in tropical estuaries, mangrove communities that act as nursery areas (Blaber and Blaber, 1980, Robertson and Duke, 1987, Staples and

Vance, 1987, Blaber et al., 1989, Robertson and Duke, 1990, Blaber et al., 1995, Vance et al., 1996a) and intertidal mudflats adjacent to mangroves which support a greater species composition and biomass of fish (Blaber et al., 1989) would have only been accessible to medusae at higher tidal states. Medusae would have most likely fed, and hence travelled shorter distances, at these times. If medusae were no longer able to feed at intermediate tidal states, however, it may have become energetically efficient to move with the current rather than maintain a position against the current. In contrast, a more uniform topography may have provided coastal medusae with more consistent access to favourable feeding areas, with the more uniform rates of travel arising from their ability to feed throughout a greater proportion of the tidal cycle. This assumes a close association between medusae and their food supply. Given the rapid digestion rates of *C. fleckeri* (Hamner and Doubilet, 1994, Hamner et al., 1995), the high metabolic rates (Gordon, 1998), fast growth rates (Gordon et al., 2004) and rapid digestion rates (Yamaguchi, 1985) of closely related species, this appears to be a reasonable assumption.

Movement patterns of medusae were also influenced by tidally generated currents. At slower flow indexes, medusae moved both with, and independently of, the current. When flow index (i.e. current) increased, medusae were not only more likely to move with the current, but the rates at which they travelled also increased. Collectively, these data suggest that while medusae possess the ability to move independently of water currents, they do so only until the current reaches a critical velocity. Above this velocity, medusae appeared unable to move against the current, or the energetic costs required to move against the current outweighed the benefits. While this critical flow level cannot be quantified here, future studies should focus on this aspect of medusae movement.

The potential for medusae movement patterns to vary between individuals was highlighted by the movements of a large medusa (180 mm IPD) over 26 h at the Tully site in March 2005. This medusa lacked the diurnal behaviours reported elsewhere (Seymour et al., 2004) and the rates at which it travelled were more consistent with those of an estuarine individual, despite remaining within the coastal habitat for all but the final six hours of tracking. It remains unclear whether such movements were typical of large coastal individuals or occurred because this individual utilised both the estuarine and coastal habitats. Alternatively, medusae are thought to take part in an upstream semelparous spawning event towards the end of the season (Hartwick, 1987, Hartwick, 1991a). Whether the movement patterns displayed by this individual represent some form of estuary selection process, the first stages of an upstream migration or were predominantly driven by tidally generated currents is something that future research should attempt to resolve.

Overall, this study has demonstrated the significant relationships that exist between medusae movements and time of day, tidal state, tidal current and habitat. The movement of a large medusa between the coastal and estuarine habitats was also documented. These accounts represent a base line to which future studies can compare movement patterns under a range of conditions. It is only when clear links are established between medusae movement patterns and factors such as prey availability, local weather conditions, time of season or medusae size (in particular, large individuals between habitats) that accurate predictions of medusae distribution and occurrence can be made.

CHAPTER FIVE

Evaluating the performance of five types of coded acoustic tags with automated receivers

5.1 Abstract

A base line data set for the use of five types of Sonotronics coded ultrasonic tags (CHP, CT, IBT 96-9, IBT 96-2 & Pico) with automated receivers (SUR-1's) was quantified within this study. In the absence of acoustic tags, SUR's recorded intervals from 300 to 745 ms, with a modal interval of ~500 ms when in the absence of depth sounder noise, but ~600 ms when an active depth sounder was within close proximity. When in the presence of acoustic tags, not only were ~98 % of detections within ±5 ms of a tags true interval, but ~76 % occurred at the tags true interval (ms). Based on these observations, data downloads were filtered for detections within ±5 ms of a tags interval. The mean maximum range of each tag was consistent within tag type, but at 395 m (Pico), 276 m (IBT 96-2), 321 m (IBT 96-9), 642 m (CT) and 664 m (CHP), varied significantly between tag types. That no false detections were being included in analysis was verified by placing tags both within and outside SUR range and demonstrating that no detections within ±5 ms were recorded by SUR's outside of a tags maximum range. The distance over which tags could be detected decreased from 100% at a gain setting of 10 to approximately 60 % at a gain setting of 1. A significant and negative relationship between detection probability and distance between a tag and receiver was also established for all five tag types. Collectively, this study has shown that the performance of Sonotronics SUR's and coded acoustic tags was consistent between units, has defined a process by which raw data downloads can be filtered for only true tag detections and provided a method by which the distance between a tag and receiver can be estimated.

5.2 Introduction

Compared to terrestrial species, the current understanding of movement patterns and behaviours of marine species is limited, largely due to the difficulties associated with monitoring animals in the marine environment (Klimley et al., 2001). Increasingly, however, passive automated acoustic receivers have become popular research tools for studying teleosts, crustaceans, cephalopods and sharks (Heupel et al. 2006b) in both near shore and continental shelf ecosystems (Heupel et al., 2006b, Semmens et al., 2007, Simpfendorfer et al., 2008). What was once considered novel technology has rapidly developed into a powerful tool for recording the presence of animals fitted with progressively smaller acoustic transmitters, allowing a diverse range of scientific and management questions to be addressed (Heupel et al., 2006b, Semmens et al., 2007).

Automated receivers possess a number of inherent advantages over more traditional manual acoustic techniques. For instance, since their use is not constrained by the logistics of human resources and adverse weather conditions, automated receivers allow the presence of tagged individuals to be recorded over extended periods of time (Heupel & Hueter 2001; Simpfendorfer et al. 2002). A far greater number of tagged individuals can also be tracked simultaneously. Since the hydrophone of an automated receiver is omnidirectional, it is only the presence, not direction, of a tagged individual that can be recorded, however. A resulting trade off in the resolution (accuracy) of position estimates has also been reported for automated arrays (Simpfendorfer et al. 2002; Simpfendorfer et al. 2008), while the overall total area within which animal movements can be documented is fixed, cannot be easily altered and is determined by the array in which receivers are initially deployed. There is also a common assumption between studies that automated receivers are equally efficient at detecting an acoustic tags signal and that all tags have consistent transmitting properties (Clements et al., 2005).

The ability of a receiver to distinguish an acoustic signal from background noise is, however, dependent upon the strength of the signal relative to background noise at the receiver's hydrophone, or its SNR (signal to noise ratio) (Cote et al., 1998). Inherently, SNR is a function of the initial power of the acoustic transmission minus the deleterious effects of signal spreading and attenuation (Cote et al., 1998). Given that there are a number of biotic and abiotic factors that can potentially reduce the SNR at a receiver, quantifying receiver performance within the habitat in which they are to be deployed is a fundamental process all studies should incorporate into the overall research plan.

Biological and artificial noise occurring at the same frequency to which a receiver is listening can reduce receiver efficiency (Simpfendorfer et al., 2002, Heupel et al., 2006b, Simpfendorfer et al., 2008), as can wind, wave action and heavy rain (Klimley and Holloway, 1999, Simpfendorfer et al., 2002). Turbidity (Simpfendorfer et al., 2008), dissolved air bubbles (Thorstad et al., 2000) and boat traffic (Thorstad et al., 2000, Simpfendorfer et al., 2002, Heupel et al., 2006b, Simpfendorfer et al., 2008) can also reduce signal quality. Since the speed at which sound travels through water can be affected by salinity, temperature and water depth (Cote et al., 1998), heterogeneity of the environment between tag and hydrophone can also corrupt an acoustic signal (Simpfendorfer et al., 2002, Simpfendorfer et al., 2008). In some instances, strong boundary layers such as thermoclines reduced tag range by ~75% (Singh et al., 2009). Alternatively, code collision can occur in those cases where large numbers of tags are simultaneously within the range of a receiver, whereby the signal of one tag becomes overlaid with that of another (Cote et al., 1998, Voegeli et al., 2001, Simpfendorfer et al., 2002, Simpfendorfer et al., 2008). In each case, a detection will not be recorded due to the reduced signal quality at the receivers hydrophone.

In some instances, a receivers hydrophone may be completely blocked from a tags signal. Substrate topography, submerged vegetation, frames used for receiver mounting (Clements et al., 2005, Simpfendorfer et al., 2008) or the orientation of a receiver within the water column (Voegeli et al., 1998, Clements et al., 2005) may physically prevent signals from arriving at the receivers hydrophone. Similarly, the behaviour of the tagged individual can affect receiver performance, depending on whether the species is fast moving (may cause variations in transmission quality) or cryptic (may block part or all of a transmission) (Skajaa et al., 1998, Simpfendorfer et al., 2008).

While the effects of such variables on SNR are often considered when interpreting automated receiver data, two fundamentally important considerations are often overlooked, despite the effect they could have on data analysis and subsequent interpretation. Firstly, whether transmitting capabilities are consistent between tags, and secondly, whether all receivers log detections with equal efficiency, and hence, all individuals have the same chance of being detected (Clements et al., 2005). Indeed, both tags and receivers may have inherently different levels of power and sensitivity that could influence the ability to emit or detect a transmission (Simpfendorfer et al., 2002). The aims of this chapter were to:

- define a protocol to distinguish valid data (for analysis) from spurious data
- determine whether the performance of Sonotronics SUR-1 automated receivers and coded acoustic tags was consistent between units
- examine the effect of gain on tag range, and
- calculate equations by which the distance between an acoustic tag and a receiver can be estimated.

5.4 Materials and Methods

5.4.1 Study Sites

Calibration trials were undertaken at three sites in far north Queensland, Trinity Inlet and Yarrabah in Cairns and Wooldrum Point in Weipa (Figure 5.1):

- Trinity Inlet is a mangrove lined, tidally influenced estuary which suffers minimal background noise in terms of recreational or commercial traffic. All trials at this site were conducted along a 3.5 km straight section of the south arm of the estuary in depths of ~3 - 4 m;
- the Yarrabah site was located ~8 km from Trinity Inlet and was sheltered from prevailing SE breezes by False Cape headland. The substrate was predominantly sand / mud, with trials conducted at depths of ~2 - 3 m;
- Wooldrum Point is a gently sloping sand / mud beachfront uninterrupted by underwater obstructions and sheltered from prevailing SE breezes by the mainland. Trials were conducted ~1 km south of the Embley Estuary mouth at depths of ~3 - 4 m.

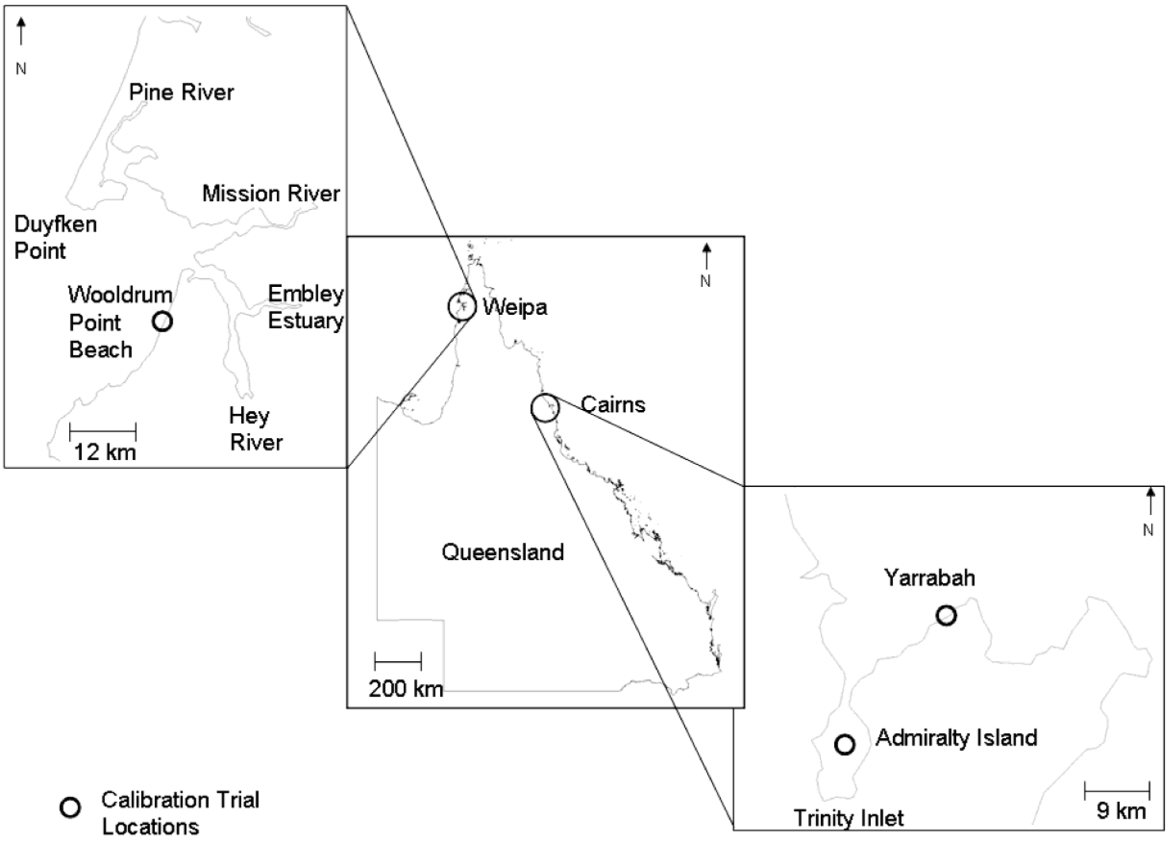


Figure 5.1 Geographic location of calibration trial sites: Yarrabah, Admiralty Island and Wooldrum Point, north Queensland, Australia

All trials were conducted using Sonotronics (Tucson, USA) coded acoustic tags and SUR-1 (Submersible Ultrasonic Receivers) automated receivers. All GPS references were taken using a Garmin 12 hand held GPS. Data arising from each trial were downloaded from SUR's using either an Optima Centoris C500 lap top running SURSoft version 4.1 - 4.8 software, or to a Sonotronics Palm Pilot (HP iPAQ rx3000 series Mobile Media Companion) running SURSoft 2.1 for Palm Pilots.

5.4.2 Sonotronics Tags

A total of five different types of coded acoustic tags were investigated in this study (Table 5.1), ranging from small, light tags with reduced range and limited battery life (Pico and IBT) to larger, more powerful tags with increased range (CT and CHP).

Table 5.1 Properties of coded acoustic tags used within trials, including length (mm), diameter (mm), weight (g) and battery life (d), with manufacturers claims of range for both manual and automated detection systems

Tag Type	Length (mm)	Diameter (mm)	Weight (g)	Battery Life (d)	Range (m) (manual)	Range (m) (SUR)
PicoTag	16	9	1.0	21	up to 1000	~ 250
IBT 96-2	28	9.5	2.5	60	up to 1000	~ 250
IBT 96-9	42	10.5	4.0	180	up to 1000	~ 250
CT 82-2	53	16	19.0	420	up to 1500	~ 350
CHP 87-S	65	18	20.0	210	up to 3000	~ 400

All tags used within this study were coded tags, in which, successive pulses of sound were arranged into a predetermined pulse sequence. The pulse interval, or time between successive pulses within a pulse sequence, was of a fixed length (ms), with successive pulse sequences separated by a rest pulse interval which is twice the length of a pulse interval. Both pulse sequence and frequency are used to identify individual tags in manual telemetry, while automated receivers use a combination of frequency and pulse interval to distinguish tags. Sonotronics tags transmit a constant signal strength until battery level becomes too low to generate a transmission, at which point, tag shutdown occurs. As such, signal strength does not vary with battery life and would not affect tag performance.

5.4.3 Automated Receivers

Submersible Ultrasonic Receivers (SUR’s) are standalone automated detection devices produced by Sonotronics (USA) that log the date, time, frequency and interval of acoustic signals detected by an omnidirectional hydrophone atop each unit. Several parameters within an SUR can be varied by the end user, including the frequencies to be searched for (frequency map), length of the rest period between listening for each frequency (blanking time), time between successive cycles through the frequency map and finally, the gain setting (or sensitivity).

5.4.4 SUR Programming and Deployment

Prior to each sampling occasion, the frequency of each tag used within that trial was programmed into the frequency map of each SUR, the between channel and between cycle blanking was set to 0 ms and the gain set to 10 (in all trials other than effect of gain trials). SUR batteries were replaced every three months.

A deployment technique that allowed receivers to be launched and retrieved quickly and reliably such that the hydrophone of each SUR remained unobstructed was developed. Since SUR’s are air filled, they will naturally orientate with the hydrophone in the vertical plane whereby the zone of detection remains at the optimal 180° to the substrate. Each SUR was attached to a cement cinder block of ~8 kg by 4 mm polyester rope in such a way that it remained ~0.5 m above the block. A ~6 m length of 12 mm polyester rope was attached to the opposite end of the block with a 300 mm polystyrene float used to mark the location of each SUR station. SUR stations were deployed in water ~3 - 4 m deep (as determined by a depth sounder), with flattened areas of substrate preferable to areas of sloping topography. Care was taken to avoid underwater obstructions such as fallen trees or sand gutters. A GPS reference was taken for each SUR station.

5.4.5 Determining Whether All Download Data Should Be Retained For Analysis

Quantifying what SUR's detect in the absence of tags

SUR's were deployed as above with the time of deployment and removal noted along with the time of tag deployment and removal. SUR download files were filtered for those periods in which SUR's were deployed but tags were not. Each detection was denoted as having occurred in either the presence or absence of a depth sounder signal. The effect that a depth sounder signal has on the distribution of intervals detected by an SUR was investigated by chi square analysis. Here, the overall maximum and minimum interval recorded by SUR's within this trial was identified and divided in two, with the frequency of detections above and below this midpoint compared using a chi square homogeneity test.

Quantifying what SUR's detect in the presence of tags

Nine SUR’s were deployed in three clusters such that each SUR within a cluster was ~15 m from the next and SUR clusters were ~700 m apart. Seven acoustic tags were suspended ~1 m below the surface on monofilament line from a 300 mm polystyrene buoy. Five low power tags (Pico, IBT 96-2 and IBT 96-9) and two high power tags (CT and CHP) were placed at known locations within the grid where they remained (stationary) for 2 h. Given that the true interval (ms) for each tag was known, the distance to true interval (in ms) of each detection was calculated by subtracting a tags true interval from the interval reported in the SUR output file. The distribution of time to true interval (ms) calculations was plotted in order to determine which data should be kept for analysis and that which can be excluded.

5.4.6 Quantifying the Performance of SUR units

Nine SUR's were deployed in three straight line arrays (three SUR's per row) such that the distance between SUR's within a row was ~10 m and the distance between successive rows was ~350 m. Five coded tags were deployed in a straight line array at SUR row one, each suspended at a depth of ~1 m from a marker buoy. In this configuration, tags were simultaneously 0 m from SUR row one, 350 m from SUR row two and 700 m from SUR row three. Tags remained at 0 / 350 / 700 m for 1 h before being relocated 50 m to a distance of 50 / 400 / 750 m, where they remained for 1 h. This process was repeated at 50 m intervals out to a distance of 300 / 650 / 1000m, with the time of tag deployment and recovery noted in each case. Whether detection performance was consistent between SUR's was determined by comparing the total number of detections each SUR recorded for each tag type using a three way ANOVA, with SUR, tag type and distance between tag and receiver used as fixed factors.

5.4.7 Quantifying the Performance of Acoustic Tags

Nine SUR’s were deployed in a straight line array perpendicular to the estuary bank whereby each SUR was 15 m from the next. A GPS reference for the middle of the SUR array was recorded, considered to represent 0 m between the SUR array and acoustic tags. All subsequent measures of distance between SUR array and tags were taken in reference to this GPS mark. Five tags were suspended on monofilament line from the bow of the research vessel, with any one frequency represented by only a single tag. All tags were between ~1 - 2 m below the surface and were not removed from the water at any time throughout the trial.

All trials commenced at the SUR array (taken to be 0 m), where the research vessel remained stationary for 2 min. The tags were then moved out to 50 m, where they again remained stationary for 2 min. This process was repeated every 50 m to a maximum distance of 900 m from the SUR array in all trials except one, in which the maximum distance was extended to 1500 m. The arrival and departure times of each 50 m interval were recorded such that distance from SUR array was known throughout the trial. This process was repeated on the return towards the SUR array, giving a second estimate of maximum distance. This process was repeated on the opposing side of the SUR array, giving a total of four estimates of maximum distance from each trial for each tag. For each tag type, the effect of individual tag on the mean maximum distance over which tags were detected was compared using a one way ANOVA. The effect of tag type on the maximum distance over which tags were detected was investigated using a two way ANOVA, with Least Significant Difference *post hoc* analysis identifying means that were not statistically different at the 95% level.

5.4.8 Validating That All Retained Detections Are Valid

Nine SUR's were deployed in three clusters such that each SUR within a cluster was ~15 m from the next. SUR clusters were spaced at ~700 m intervals, giving a total grid length of ~1400 m. Seven acoustic tags were suspended ~1 m below the surface at known locations within and adjacent to the grid (Figure 5.2). Each tag was suspended on monofilament line from a 300 mm polystyrene buoy anchored to a cinder block by

12 mm polyester anchor rope. A total of five low power tags (Pico, IBT 96-2 and IBT 96-9) and two high power tags (CT and CHP) were placed at known distances from SUR clusters: one high and one low power tag at either end of the grid (~1600 m apart), two low power tags half way between SUR clusters (~350 m from SUR clusters) and one low power tag towards the middle of the grid (0 and ~700 m from SUR's) (Figure 5.2).

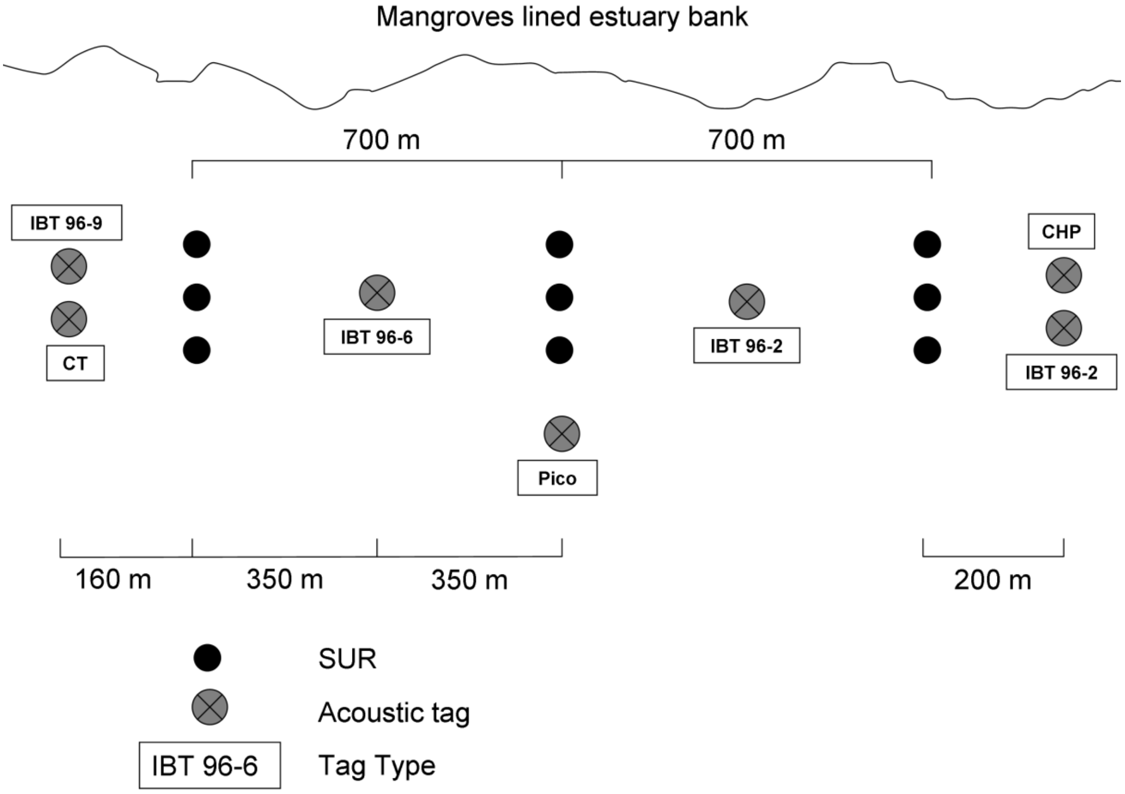


Figure 5.2 Array of SUR's and acoustic tags for trials validating true from false detections of five types of Sonotronics coded acoustic tags

The times of SUR and tag deployment and retrieval were noted, with receivers remaining in the presence of tags for at least 2 h. Each detection recorded by an SUR was identified as a true or a false hit, with a true detection being one that was recorded by an SUR within the overall maximum range for that tag type. A false hit was considered to be one recorded by an SUR that was positioned at a greater distance from a tag than that tags overall maximum range. For instance, if a PicoTag, whose overall maximum distance was 850 m, was 600 m from an SUR, then any detection recorded by that SUR for that PicoTag was considered true.

In order to determine the dispersion of detection intervals logged by SUR's in relation to the true interval of each tag, the difference between each logged detection and the true interval of the corresponding tag was calculated. The frequency (percentage of the total true data set) of each interval was plotted, from which, protocols for determining what data should be retained for further analysis were developed.

5.4.9 Effect of Gain on Range Over Which Tags Are Detected

Gain, or receiver sensitivity, ranges from a minimum of 1 (least sensitive) to a maximum of 10 (most sensitive). To test the effect of gain on maximum detectable range, seven SUR stations were deployed in a straight line array perpendicular to the estuary bank with ~15 m between stations. Three SUR’s were set to a gain setting of 10, two to a gain setting of seven and two to a gain setting of one. Four estimates of the maximum detection distance for each tag type were obtained using the methods outlined in section 5.4.7 (page 86) where trials were conducted to a distance of 900 m.

5.4.10 Probability of Tag Detection at Known Distances From an SUR

SUR's are programmed to wait for a signal at a given frequency within the frequency map for 2500 ms before moving on to the next if nothing is detected. The current firmware also includes a 2500 ms test for a Ping and Response transmission that is used to interrogate the SUR for the presence or absence of data in its memory (not used within this study). As such, it would take a total of 15 s for an SUR with five channels programmed into its frequency map to cycle through the frequency map but not log any detections.

In order to log a valid detection, however, an SUR must detect four pulses at a frequency listed within the frequency map, for which the pulse intervals are of equal length (ms). In some cases, however, the captured intervals may include a rest pulse interval. As such, there are two scenario's whereby an SUR can log a valid detection, with the time taken to do so ranging between:

1. 3 x pulse interval (ms) in those cases where no pulse rest interval is included in the captured sequence; or
2. 4 x pulse interval (ms) in those cases where a pulse interval is included in the capture sequence ie. two pulse intervals of equal length and a pulse sequence interval twice the length of the other two pulse intervals.

Given that it is possible to determine what proportion of valid detections would contain a pulse sequence interval and what proportion would not, it is possible to calculate the mean tag handling time for each tag and hence, the maximum number of detections possible within a given time frame. As an example, for a tag with a pulse sequence of 3, 6, 5 and a pulse interval of 965 ms, there were five ways a valid detection based on three pulse intervals could occur (┆┈┈┈┈┈┆) and nine ways a validation detection could be made that would include a pulse rest interval (└ – – – – – ┘) (Figure 5.3).

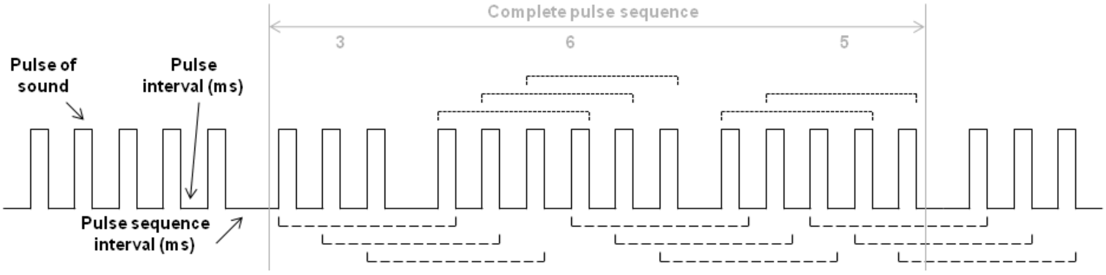


Figure 5.3 Stylised pulse sequence of a coded acoustic tag showing the number of permutations that a valid detection could be made, both including and excluding a pulse sequence interval for a tag with a 3, 6, 5 pulse sequence

The chance of a detection including a pulse sequence interval would be 9/14 while the chance of this detection not including a pulse sequence interval would be 5/14. With a default setting of a 100 ms ‘rest period’ after each valid detection included, the overall mean tag handling time in this example would be:

- 9/14 of the time, it would take (3 x 965) + 100 ms to log a valid detection
- 5/14 of the time, it would take (4 x 965) +100 ms to log a valid detection

The mean tag handling time required to log a validation detection is:

(5/14 x ((3 x 965) + 100)) + (9/14 x ((4 x 965) + 100)) ms = 3615 ms.

Given that both the pulse sequence and pulse interval (ms) varied between tags, the mean tag handling time for each tag was calculated individually. The average time that it would take an SUR to cycle through its frequency map for each permutation of tags heard / not heard could then be calculated. That is, if five tags were used in a trial, between zero and five tags could be heard in any one cycle through the frequency map of the SUR. Pascals triangle was used to calculate the number of permutations at each level of tag number eg. if four tags were heard, there would be 10 different ways that this could occur (Appendix 5.1). That is, tags 1 - 4 could be heard, but not 5, or tags 1, 2, 4 and 5 but not 3 could be heard, and so on, with each sequence giving four valid detections. The time to cycle through the frequency map under each scenario was calculated and averaged. This provided a mean frequency map cycle time (Appendix 5.2) and assumed that each permutation of which tags would be heard in each cycle was equal. While it was not possible to validate whether this was a reasonable assumption, it is unclear how future studies could achieve this either. The mean time to cycle (ms) was calculated to be:

(∑ mean tag handling times + 2500) / # of permutations

The theoretical maximum number of detections within a trial period could then be calculated by dividing the total trial time by the overall mean cycle time for that trial.

The detection probability then became a function of the number of detections logged as a function of the theoretical maximum number of detections possible.

Quantifying detection probability over distance

Three rows of three SUR's were deployed in straight line arrays such that distance between SUR's within a row was ~10 m and distance between rows was ~350 m. Coded tags were suspended ~1 m from 300 mm polystyrene buoys and deployed in a straight line array at row one of SUR's. As such, tags were simultaneously 0 m from SUR row one, 350 m from SUR row two and 700 m from SUR row three. Tags remained at 0 / 350 / 700 m for 1 h, before being relocated 50 m to a distance of 50 / 400 / 750 m for a further 1 h. This process was repeated at 50 m intervals out to a distance of 1150 / 1500 m from SUR rows, with the time of tag deployment and recovery noted in each case.

The time between the deployment of the last tag and retrieval of the first tag in each trial was taken to be the total time over which an individual trial ran. The number of valid detections h^{-1} could then be quantified for each SUR and each tag allowing the detection probability to be calculated for each tag using the following equation:

$$P = D / TD$$

where P is the probability of a detection occurring, D is the actual number of detections and TD is the theoretical number of detections (as calculated above).

Given that the distance between the tags and the SUR’s was known in each case, the relationship between distance of a tag from an SUR and the detection probability for each of the five tag types could be investigated using linear regression analysis. Equations for predicting tag distance from SUR were developed in those cases where a significant relationship between distance and detection probability could be established.

5.5 Results

When in the absence of acoustics tags, intervals (ms) recorded by SUR's at each of the frequencies (kHz) programmed into the frequency map ranged from 300 ms to 745 ms (Figure 5.4). Two peaks in the frequency of intervals were noted, one at ~500 ms and the other at ~600 ms (Figure 5.4). A significant effect of depth sounder noise was established ($F_{1,}$ = 105.736, df = 1, *P* < 0.001) whereby a significantly greater proportion of detections were logged at ~600 ms when SUR's were in the presence of depth sounder noise, while a greater proportion of detections were recorded at ~500 ms when SUR's were operating in the absence of a depth sounder (Figure 5.4).

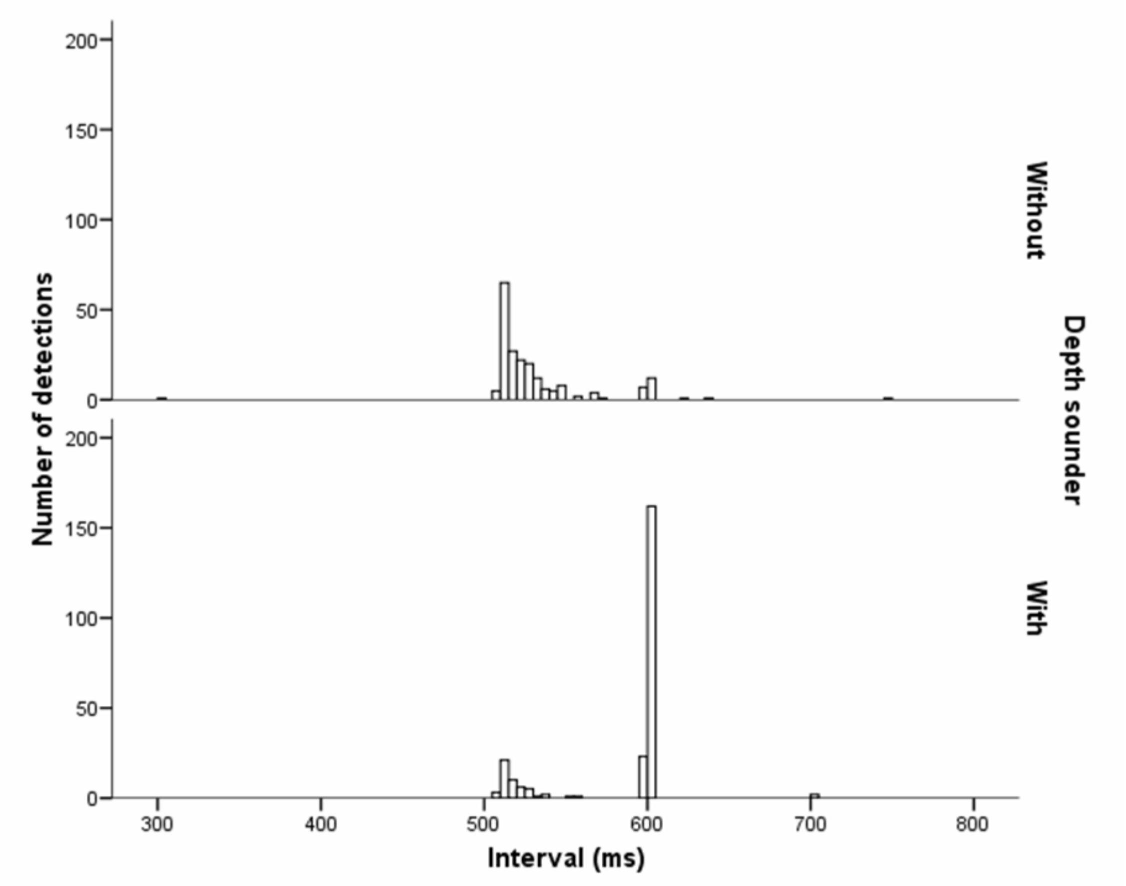


Figure 5.4 Frequency histogram of intervals recorded by SUR’s in the absence of tags when in the presence or in the absence of a depth sounder

When in the presence of acoustic tags, detections logged by SUR's for each of the frequencies (kHz) programmed into the frequency map ranged from 174 ms below a tags true interval to 998 ms above (Figure 5.5A). Not only were more than ~ 98 % of detections within ±5 ms of a tags true interval, but ~ 76% of detections were at a tags true interval (Figure 5.5B). Of the remaining detections, approximately 6% were above a tags true interval, while ~ 16 % were below (Figure 5.5B).

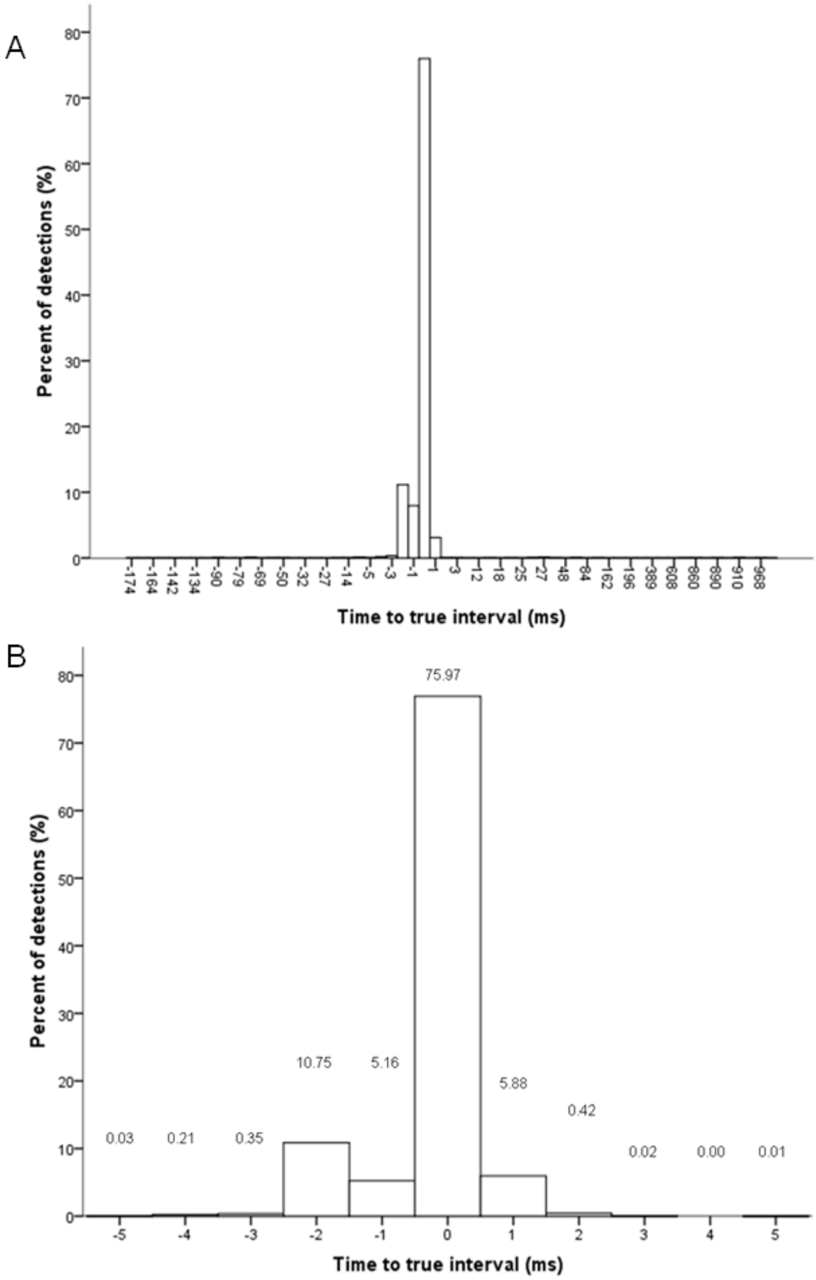


Figure 5.5 Time to true interval for all detections above 800 ms logged within maximum distance trials, showing percentage of detections that each interval (ms) accounts for (A) over all detections recorded throughout trials and (B) for detections that were within ± 5 ms of a tags true interval (ms)

The performance of individual SUR units was found to be consistent at each distance between SUR and tag ($F_{37,\ 19}$ = 0.146, *P* = 1.00). That is, the number of detections recorded for each tag type h^{-1} did not vary significantly between SUR's with distance (m between SUR array and tags).

No significant difference in the mean maximum range (m) could be established for tags of the same type (Table 5.2). As such, maximum range estimates for tags of the same type were pooled.

Table 5.2 Effect of individual tag on mean maximum range (m) over which tags were detected for Pico, IBT 96-2, IBT 96-9, CT & CHP tags

Tag Type	F	df	P
Pico	0.001	1 x 16	0.989
IBT 96-2	2.447	1 x 16	0.137
IBT 96-9	1.488	1 x 16	0.240
CT	0.995	1 x 11	0.340
CHP	0.001	1 x 13	0.979

Tag type was found to have a significant effect on the range over which tags were detected ($F_{4,\ 77}$ = 75.103, *P* < 0.001). Here, CT and CHP tags had a significantly greater range (664 & 642 m respectively) than Pico (395 m), IBT 96-2 or IBT 96-9 tags (276 & 321 m respectively) (Figure 5.6). The overall maximum range of tags was much greater than the distance over which they were typically detected (average distance m) and compared favourably to the ~250 – 400 m indicated by the manufacturers specifications: 700 m for IBT 96-2 tags, 600 m for IBT 96-9 tags, 850 m for Pico tags, 900 m for CT tags and 850 m for CHP tags.

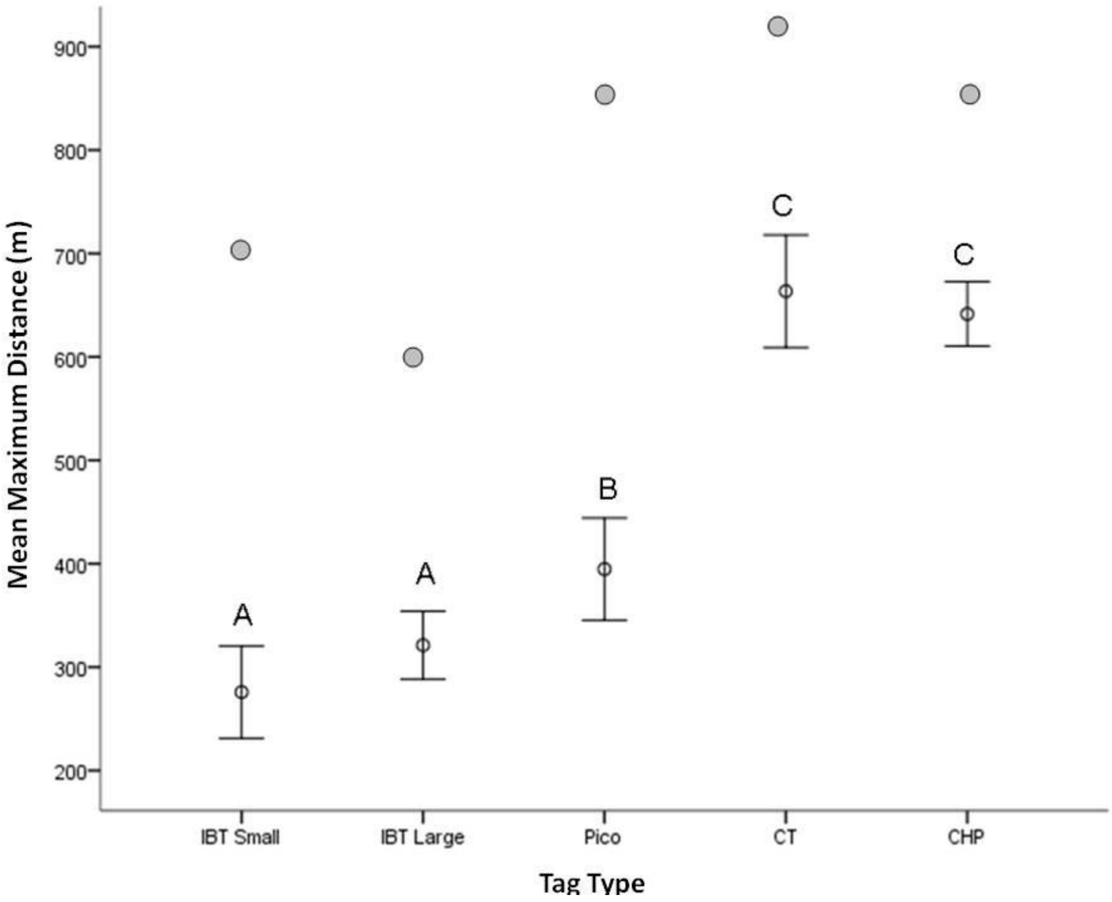


Figure 5.6 Average maximum range (m) that each tag type was detected at by Submersible Ultrasonic Receivers and the overall maximum distance at which each tag type of was detected (●)

SUR's did not record any false detections when in the presence of acoustic tags, based on the overall maximum tag type ranges established above. That is, true detections, or detections for tags within SUR range, accounted for 99 % of all detections (Figure 5.7). Of the remaining 1 % of detections recorded for tags outside SUR range (false detections), none were recorded above 805 ms, which was 146 ms below the lowest tag interval. That is, no false intervals were recorded within ±5 ms of a tags true interval.

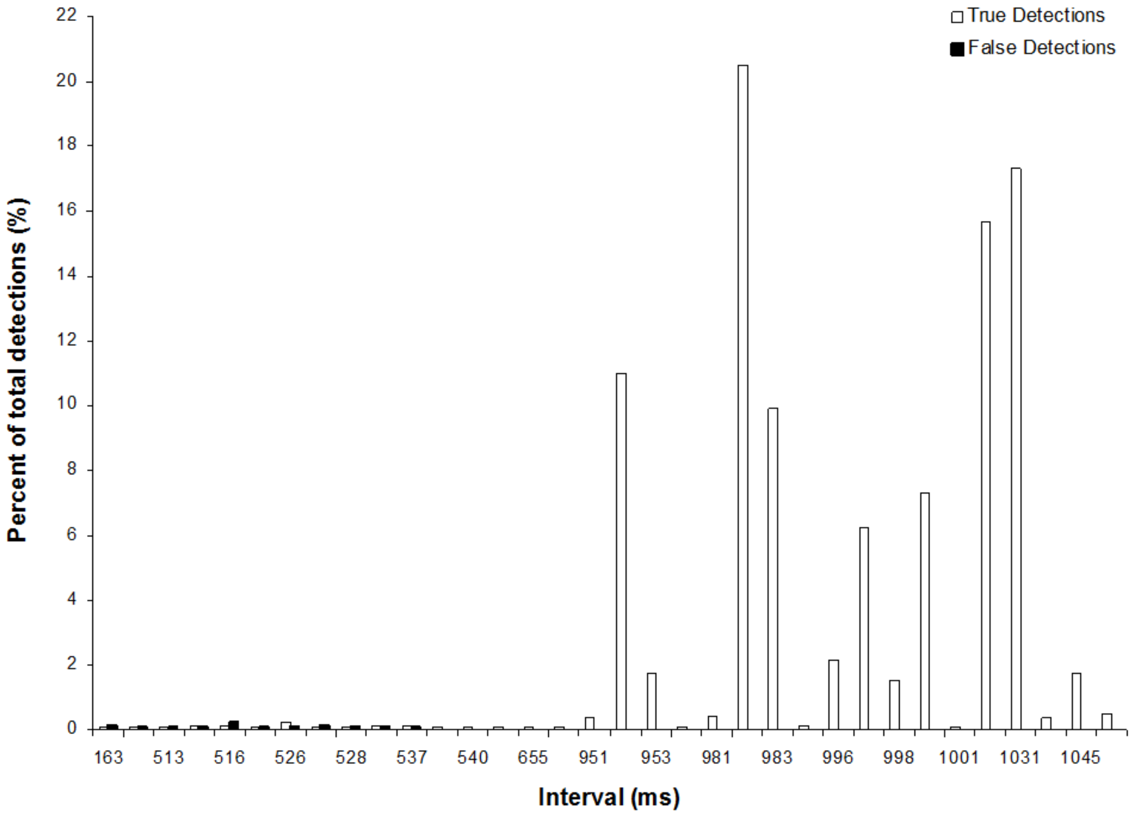


Figure 5.7 Percent frequency that each interval (ms) accounted for within True / False trials showing both true detections (tags were within range of a given SUR) and false detections (tags were not within range of a given SUR)

Gain setting was found to have a significant effect on the range over which tags were detected ($F_{2,\ 118} = 16.365$, $P < 0.001$). This effect was consistent between *post hoc* groups as determined in mean maximum range trials (IBT 96-2 & 9, Pico, CT & CHP) ($F_{4,\ 118} = 0.865$, $P = 0.488$). Here, the range over which tags were detected at a gain setting of 10 was approximately 100% of their mean maximum distance, while at a gain setting of 7, the range had reduced to around 80%. At a gain setting of 1, tag detection range had decreased to approximately 65% of their mean maximum range (Figure 5.8).

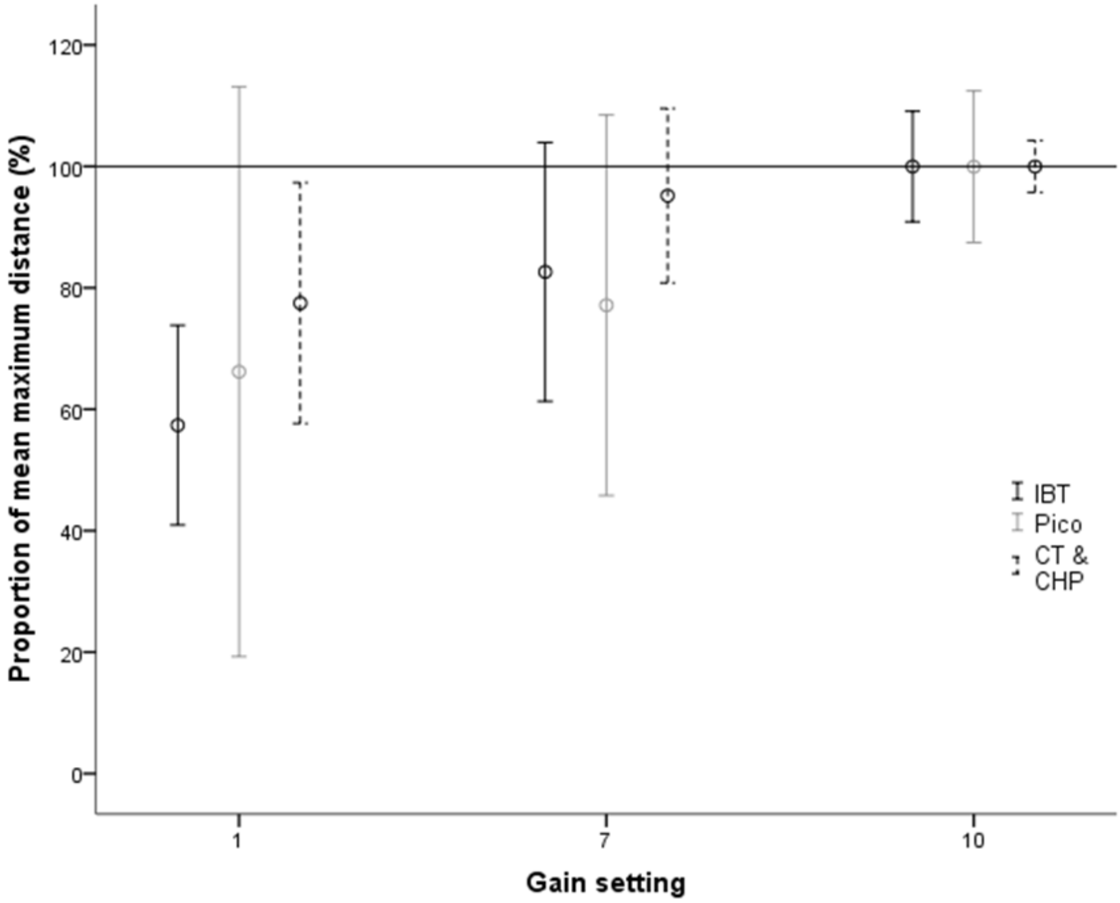


Figure 5.8 Percent of mean maximum distance (m) over which tags were detected at each of three gain settings showing 95% confidence limits showing 100% reference line

All five tag types displayed a significant and negative relationship between the probability of tag detection and increasing distance between tag and SUR (Figure 5.9 & Table 5.3). Distance was expressed as a percentage of the overall maximum detection range to allow for comparison between tag types. Maximum probabilities of detection ranged from ~0.30 for IBT 96-9 / CT tag types to ~0.65 for Pico tags (Figure 5.9).

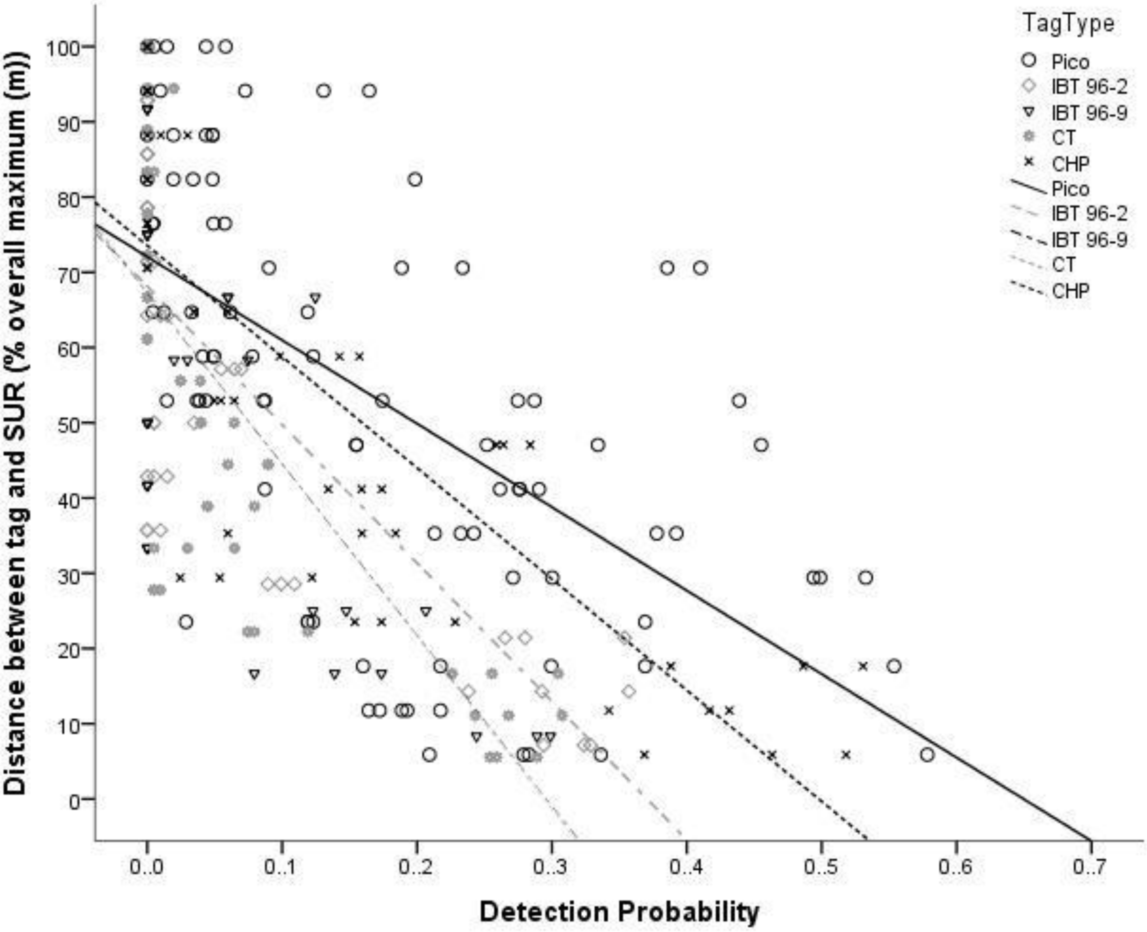


Figure 5.9 Linear relationships between detection probability and distance between SUR and tag where distance is percent of the overall maximum distance for that tag type (% m) (IBT 96-9 & CT regression lines appear to be overlayed)

Table 5.3 Significant negative linear relationships between detection probability and distance between SUR and tag where x is detection probability and y is the distance between SUR and tag (% overall maximum distance (m))

Tag Type	F	df	*P*	R^2	Regression Line Equation
Pico	48.903	1 x 88	< 0.001	0.36	y = 72.602 – 110.937 x
IBT 96-2	61.410	1 x 40	< 0.001	0.61	y = 68.125 – 183.886 x
IBT 96-9	31.558	1 x 34	<0.001	0.48	y = 67.215– 227.106 x
CT	73.865	1 x 52	< 0.001	0.59	y = 67.506 – 229.667 x
CHP	104.758	1 x 49	< 0.001	0.68	y = 73.553 – 147.781 x

5.6 Discussion

An important fundamental consideration regarding the analysis of raw data output files downloaded from automated receivers is whether all data within that file represents true tag detections and should therefore be retained for analysis, or whether some data is spurious and should be excluded from analysis. That SUR's logged intervals within the 500 to 600 ms range in the absence of tags suggests that a method of filtering is required for Sonotronics equipment. With ~98 % of detections occurring within ±5 ms of a tags true interval, and no detections having occurred within this range when SUR's were in the absence of tags, it is suggested that raw data files should be filtered for those detections occurring within ±5 ms of a tags true interval. Validation of these filtering criteria is found in the fact that no false detections were made by SUR's within the true / false trials. That is, SUR's did not log a detection within ±5 ms for a tag that either (a) was not present in the water, or (b) was present, but out of maximum range of the SUR. These data not only demonstrate that SUR output files should be filtered for only those data occurring only within ±5 ms of a tags true interval, but that confidence can be placed in even a single detection, a situation that does not appear to be true for other systems (Clements et al., 2005).

It is noteworthy that the presence of a depth sounder had an effect on the intervals recorded by an SUR. That is, when in the presence of a depth sounder transmission, not only were a disproportionately large number of detections recorded at a higher interval (~600 ms v ~500 ms), but a detection was recorded each time a channel was listened for. This was not the case for background noise, with variable time intervals between successive records. Since this was not a factor of SUR performance intended for investigation within this study, it is unclear whether the presence of depth sounder noise would affect the performance of SUR's when in the presence of tags. This is an aspect of SUR / tag interaction that future research should attempt to quantify, as, if a significant effect is established, both the way in which SUR downloads are filtered and the subsequent data analysis is undertaken will require modification. This is particularly true for those cases in which animal tracking is taking place in an area where there is either heavy or intermittent boating traffic.

An inherent assumption of any tracking study is that both automated receivers and acoustic tag performance is consistent between units. That is, that the presence of a tagged individual is as likely to be detected with reliability no matter which tag / receiver combination is used. This appeared to be the case for Sonotronics coded tags when used in conjunction with SUR’s. Here, the rate at which detections were recorded was consistent between SUR units, both for each tag type and with increasing distance between tag and SUR. Of particular importance here is that this result demonstrates that receiver performance is consistent between units even at the limits of a tags detection range. Tag performance was also found to be consistent between tags of the same type. In terms of overall range, three groupings of tags were evident: those with a range of ~650 m, those with a range of ~400 m and those whose range was ~300 m. These estimates compare favourably with a number of estimates for a number of manufacturers tags (Heupel and Hueter, 2001, Simpfendorfer et al., 2002, Heupel et al., 2006a, Taquet et al., 2007, Singh et al., 2009, Reyier et al., 2011). Collectively then, these results suggest that the performance of Sonotronics SUR's and acoustic tags was be consistent between units within this study.

It is also worth noting that maximum range trials within this study were undertaken in near shore or estuarine habitats during the tropical dry season when wave break was negligible, salinity, turbidity and temperature were stable and topography was uniform. Given that changes in the range of a tag is the variable that most researchers are likely to deal with across trials, between environments and throughout the duration of a study (Heupel et al., 2006b), future maximum range trials investigating tag performance under a range of conditions, such as increased wave action, background noise or environmental heterogeneity, are of particular importance. Also of particular interest for Sonotronics products is the degree to which adjusting the gain setting (receiver sensitivity) effects tag performance, given that the influence of factors such as background noise occurring within the active frequency range (kHz) could be reduced to some extent by reducing receiver gain.

Within this study, the effect of reduced gain setting on maximum tag range was consistent across all tag types in that as gain setting decreased, the range over which a given tag type could be detected also decreased. That is, a reduction in gain setting from 10 to 7 was accompanied by a 15-20 % reduction in overall tag range. At a gain setting of 1, the overall tag range reduced a further 15 to 20 % to ~ 60% of their maximum range. The degree to which gain reduces tag detection range therefore becomes an important consideration when planning the logistics of a tracking project. That is, while reducing gain may be an appealing concept when working in a biologically noisy environment, such as a coral reef for instance, there must be a corresponding increase in the number of SUR units within the array to compensate for the decreased range of each receiver. As to whether a reduction in gain accompanied by an increase in receiver number would alleviate the issue of a tag not being detected despite being within the empirically defined range of a receiver, as is the case for some structurally complex coral reef environment studies (Claisse et al., 2011), is an aspect of receiver performance that is worthy of further investigation.

The probability of a tag transmission being detected and logged decreased as distance between tag and receiver increased. While the maximum detection probability of all tag types was ~0.65 for Pico tags, this parameter ranged between ~0.35 and ~0.6 for the remainder of tag types. These results compare favourably with a mean code detection probability of ~0.4 for equivalent Vemco VR2 receivers (Simpfendorfer et al., 2008). Furthermore, receiver efficiency increased from a mean code detection probability of

~0.35 to ~0.5 as distance from the mouth of an estuary increased from 0 km to ~25 km, with the suggestion being that receiver efficiency improved as noise levels decreased as the estuary mouth was moved away from (Simpfendorfer et al., 2008). Establishing whether a similar relationship between receiver efficiency and ambient noise levels exists for Sonotronics equipment should be a focus of future research, especially given the influence that gain setting has on tag detection range. It is also worth noting that data for 0 m distance (tag to receiver) were removed from analysis as in many cases, very few or no detections were logged at 0 m despite receivers performing well throughout the remainder of the trial. This trend arises as when a tag is so close to a receiver, the vibrations of the piezo electric transducer are heard as a constant hum and are therefore not logged as valid transmissions (pers. Comm. M Gregor, Sonotronics).

Collectively then, this study represents the first quantitative investigation into how effectively Sonotronics coded acoustic tags and SUR's work together. Performance has been shown to be consistent between units for both SUR's and acoustic tags, while a number of performance characteristics that can be incorporated into the logistics and planning phase of a project have been quantified. For instance, overall tag range has now been estimated in real world conditions, as has the effect that receiver sensitivity (gain) has on overall detection range. Finally, a method by which raw data files downloaded from SUR's can be filtered for true detections has been outlined and validated. While these are fundamental aspects of tag / receiver performance, this study also represents a baseline data set to which further studies can determine the extent to which system performance varies under a range of environmental, physical and biological conditions. Given that automated receivers such as SUR’s provide a low cost method of detecting tagged animals without the logistical constraints associated with manual acoustic methods, their application to documenting marine animal movement patterns is only likely to increase. As such, demonstrating their reliable performance under a range of conditions or their ability to provide accurate location estimates should be a priority of future research.

CHAPTER SIX

Automated acoustic arrays and their capacity to accurately, reliably and repeatedly estimate the location of tagged *Chironex fleckeri* medusae within a near shore coastal habitat

6.1 Abstract

Compared to manual telemetry, automated acoustic arrays allow the movements of a greater number of individuals to be documented over longer periods of time. A trade off in the resolution of tag location estimates is typically associated with acoustic arrays, however. Such was the case within this study. Although tracking duration of *C. fleckeri* medusae increased from a maximum of 38 h (manual telemetry) to 84 h (acoustic arrays), error around a position estimate derived from an acoustic array (distance between a manual position estimate and an array position estimate) ranged from 136 m to 1260 m. Error was significantly less in those instances when two or more receivers contributed to a position estimate, however, never as low as the 10 m associated with manual telemetry. The rate at which medusae travelled was considerably overestimated by acoustic arrays as well. For instance, two medusae were estimated to travel at an average of ~170 m h^{-1} using manual telemetry but 487 m h^{-1} based on acoustic array data for the same time period. Similarly, diurnal and tide related rates of travel were between three and five times that estimated from manual telemetry. Receiver data did reveal patterns in the gross scale movement of medusae, however, with longshore movements that were repeated over successive days evident, as were periods of activity where simultaneously tracked individuals operated independently of each other. Collectively then, the results of this chapter demonstrate that the method of documenting movement data must be matched to the research question being posed. That is, if lower resolution data over longer periods of time is required, acoustic arrays may be the more suitable option, especially if logistical constraints limit the use of manual telemetry, which can provide higher resolution data, but requires researchers to remain in the field with tagged individuals.

6.2 Introduction

C. fleckeri medusae are not considered to be an open water species (Hartwick, 1987, Hartwick, 1991a), with their occurrence instead restricted to near-shore waters, estuarine areas and mainland beaches (Kingsford et al., 2012). The most at risk beaches appear to be those that occur within close proximity to estuary systems, possess a gently sloping beachfront that is free from coral and marine vegetation and are afforded an added degree of shelter by protective headlands (Southcott, 1963, Cleland and Southcott, 1965, Barnes, 1966, Brown, 1973, Kinsey, 1986, Hartwick, 1987, Fenner, 1991, Hartwick, 1991a, Coleman, 1993, Hamner et al., 1995). This is particularly true during flat, calm conditions, when medusae appear in high abundance within only a few feet of the beachfront (Barnes, 1960). However, such claims are largely based on anecdotal evidence rather than data documenting location over time, and as such, are of little use in the development of predictive models.

Acoustic telemetry has, however, become a popular research tool by which quantitative data documenting the movements of a diverse range of marine species can be investigated. Two distinct methods by which data can be collected are:

1. Manual acoustic telemetry - typically comprised of a vessel mounted directional hydrophone, a receiver and headphone (as per Chapter Four)
2. Automated acoustic telemetry – automated, stand alone, independent omnidirectional receivers that are increasingly recognised as a low cost, powerful research tool (Simpfendorfer et al. 2002; Heupel et al. 2006b)

Each method has an inherent set of strengths and limitations, however.

Manual acoustic telemetry, for instance, allows detailed, accurate (high resolution) information about the position of tagged animals over variable geographic ranges to be documented. The need to frequently relocate tagged individuals combined with logistical considerations such as high human resource demands, disruptions to sampling with adverse weather and the inability to track large numbers of individuals simultaneously (Baras, 1998, Klimley et al., 1998, Heupel and Hueter, 2001, Collins and Jensen, 2002) limit the use of manual telemetry, particularly over extended periods of time.

In contrast, automated acoustic receivers fitted with an omnidirectional hydrophone allow the presence of a tagged individual, but not its direction, to be documented over extended periods of time (Heupel and Hueter, 2001, Simpfendorfer et al., 2002). Automated receivers have evolved into powerful tools for observing marine animals in their natural habitat (Heupel et al., 2006b) mainly because they are relatively low cost (Simpfendorfer et al. 2002) and allow the presence of a number of tagged individuals to be documented simultaneously over months to years. The total area over which animal movements can be monitored is fixed, however, ultimately determined by the layout in which the receivers are deployed. Grids or arrays of receivers are typically deployed over smaller spatial scales and allow more detailed movement data of tagged individuals within a predefined area, such as a bay or inlet, to be collected (eg Heupel and Hueter, 2001). Linear acoustic arrays (line, gate or curtain systems) operate over large spatial scales and consist of straight lines of receivers set up across or along a path or migration route a species is likely to take (Heupel et al., 2006b). Cluster systems require receivers to be placed at specific locations along a species pathway, such as sea mounts, that a tagged individual is likely to pass during its migration (eg Willis and Hobday, 2007). As such, automated receivers are limited in their capacity to continuously document the movement patterns of species that operate over considerable distances or whose pathways of movement are unpredictable.

A method of calculating a position location that equates to a short term centre of activity (STCOA) has been developed (Simpfendorfer et al., 2002, Simpfendorfer et al., 2008), and, as such, provides an alternative to labour intensive manual telemetry

(Simpfendorfer et al., 2002). Here, position estimates are based on the negative relationship between the number of detections an automated receiver will document per unit time and increased distance between tag and receiver (Simpfendorfer et al., 2002). In theory, if the detection zones of receivers within a gridded array overlap, it would be possible to not only maintain continuous contact with tagged individuals (Heupel et al., 2006b), but to also provide some indication of directional movement within the array (Lacroix et al., 2005). While the STCOA method in one study underestimated the distances travelled between successive 'relocations' by between 28% and 42% (as compared to manual tracking estimates), a total of six sharks were monitored for more than 100 d (Simpfendorfer et al., 2002), a task simply not possible with manual acoustic telemetry where logistical trade offs of resources, personnel and weather often reduce the overall tracking timeframe (Baras, 1998, Klimley et al., 1998, Heupel et al., 2006b).

Quantifying array performance with respect to manual acoustic telemetry (as per Simpfendorfer et al. (2002)) becomes an essential step in the overall data analysis process for location data generated from automated acoustic arrays. Caution must also be exercised in making inferences about behaviour from acoustic data. For instance, a lack of, or reduction in, tag detection rates may be due to the absence of a tagged individual from an array. However, such patterns could also be driven by the negative effects a suite of environmental variables can have on signal quality and hence tag detection rates (Payne et al., 2010, How and de Lestang, 2012). As such, concluding that absences of tag detections equate to behavioural patterns is only valid when adequate controls over the integrity of acoustic data have been made, such as the use of fixed location control tags (Payne et al., 2010).

Sharks were amongst the first marine animals to which acoustic telemetry systems were applied, due partly to the need that existed to better understand their interactions with humans (Voegeli et al., 2001). Driven by the same motive, given the significant impact that the presence of *C. fleckeri* medusae within the near shore waters of tropical Australia has, this study aims to demonstrate the novel application of automated acoustic arrays to tracking *C. fleckeri* medusae within near shore coastal systems and assess their suitability as a method by which location data can be collected for medusae. To this end, the aims of this chapter are to:

- evaluate whether overall tracking timeframes are equivalent to or greater than those achieved with manual acoustic tracking,
- validate array performance with respect to manual telemetry
- describe medusae movement patterns along ~2 km sections of beachfront.

6.3 Methods

6.3.1 Sample Site

Automated acoustic arrays were deployed at the northern end of Wooldrum Point Beach, Weipa, western Cape, far north Queensland (12.69222°S, 141.80345°E) (Figure 6.1). Protected in the north by Duyfken Point and a rocky / rubble reef to the west, Wooldrum Point Beach is an uninterrupted stretch of gently sloping coastline that has a predominantly sand / mud substrate.

6.3.2 Medusae Collection and Tagging Techniques

Eighteen *C. fleckeri* medusae were collected by hand following visual spotting from a research vessel driven approximately 2 m from the shoreline. The IPD (Inter Pedalia Distance) of each medusa was measured to the nearest mm, with acoustic tags only fitted to medusae that were larger than 80 mm IPD, in good condition and had no damage to the bell or missing pedalia.

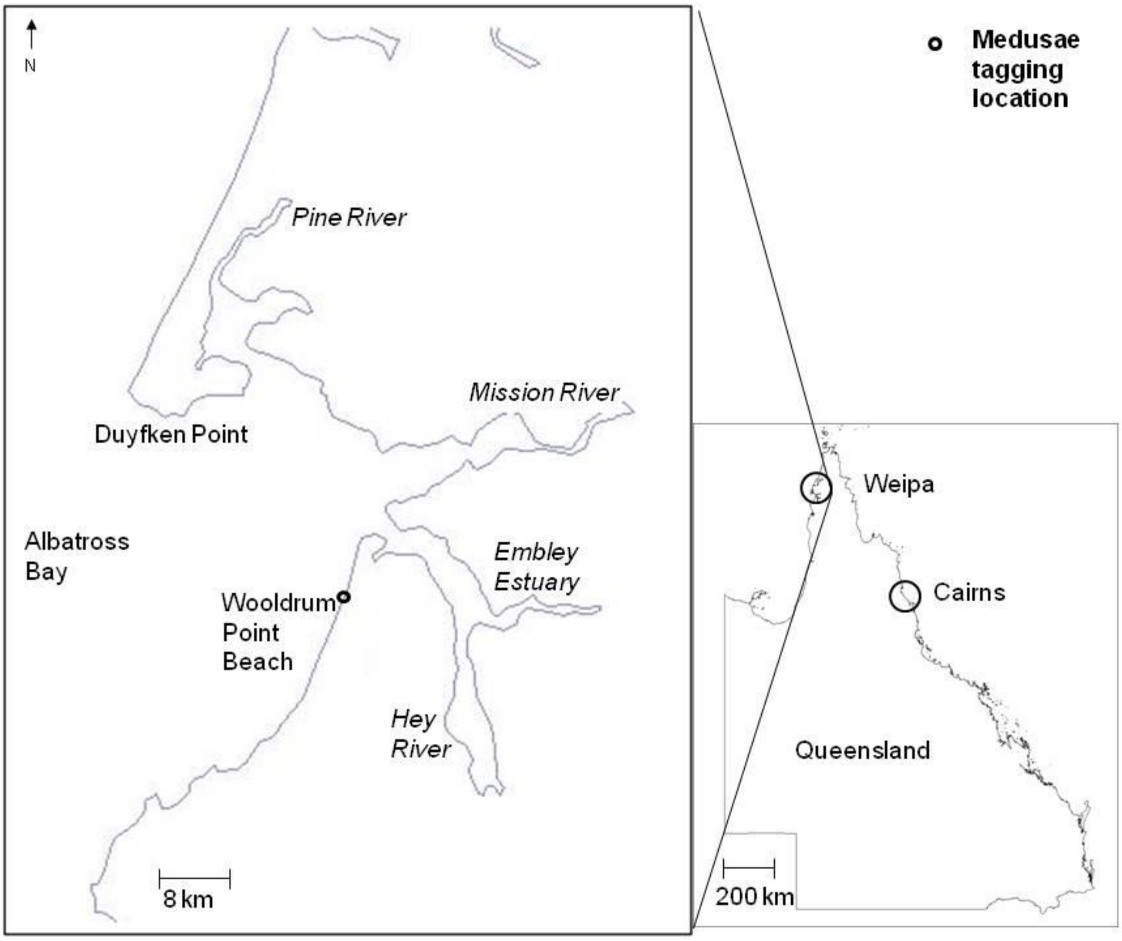


Figure 6.1 Geographic location of Wooldrum Point Beach, along which automated acoustic arrays were deployed

Acoustic tags were glued to five medusae as per the methods outlined in Chapter 4 (page 64) (single attachment point), while a further 13 medusae were tagged using a more robust method which incorporated two attachment points. Here, a Hangman's Knot was tied in one end of a 10 cm length of hat elastic (LE1, Figure 6.2), with a second tied ~15 mm from the first (LE2). The acoustic tag placed within the loop of one knot (LE1), which was then pulled tight around the tag and glued with Histoacryl. The free end of elastic (A, Figure 6.2) was passed behind the pedalium at the elbow where the pedalium joined the bell, then through the second Hangman's Knot (LE2, Figure 6.2). The Hangman's Knot was fastened tight so that the elastic could not slide undone, but in such a way that the loop was not tight around the pedalium. The free hanging tag was then glued to the fold between the pedalial shoulder and the more flattened interpedalial face with Histoacryl (Figure 6.2).

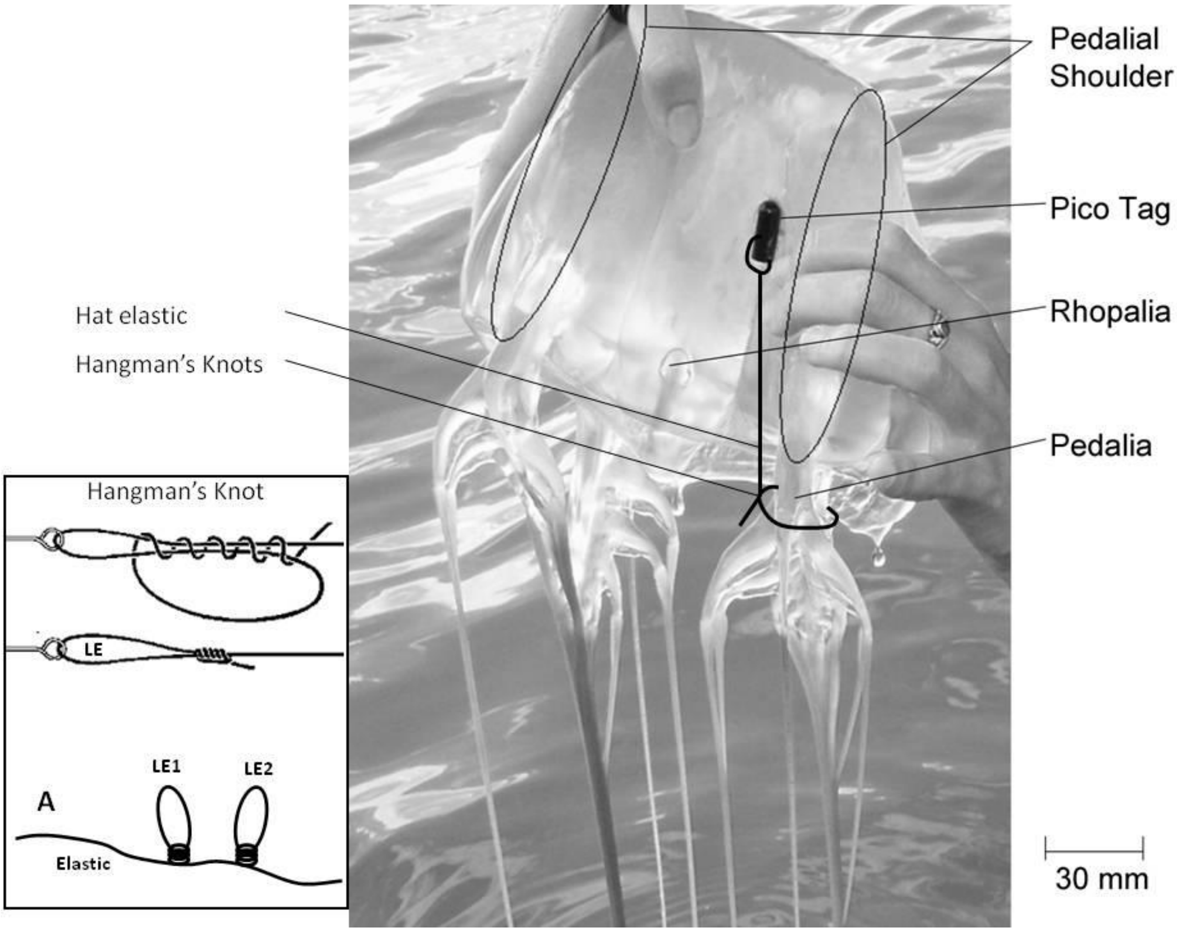


Figure 6.2 Alternate method of acoustic tag attachment to *Chironex fleckeri* medusae using two Hangman's Knots (LE1, LE2) in hat elastic around the pedalium and Histoacryl glue along the fold line of the rounded shoulder giving rise to the pedalium, and the interpedalial face

Three types of Sonotronics coded acoustic tags were used:

1. PicoTag – 16 x 9 mm and 1 g
2. IBT 96-2 – 28 x 9.5 mm and 2.5 g
3. IBT 96-6 – 42 x 10.5 mm and 4.0 g

No tag comprised more than 0.2% of a medusa's body weight (as estimated from Gordon (1998)) to reduce confounding effects of tag weight.

The time and release point of each medusa was determined by a Garmin GPS 12 hand held GPS (Table 6.1). Details of medusa size (IPD mm), tracking duration (h), tagging method (SAP = single attachment point, DAP = double attachment point), tracking method (M = manual, A = array) and the tag type used are summarised in Table 6.1.

Table 6.1 Details of medusa size (mm), tracking duration (h), tagging method (SAP = single point of attachment, DAP = double point of attachment), tracking method (M = manual, A = array) and tag type used

ID	IPD (mm)	Date	Time Tagged (h)	Tracking Duration (h)	Tagging Method	Tracking Method	Tag Type
1	150	16/11/05	1010	53	SAP	A	Pico
2	80	17/11/05	0855	31	SAP	A	Pico
3	80	19/11/05	1245	6	SAP	A	Pico
4	80	19/11/05	1320	3.5	SAP	A	Pico
5	80	19/11/05	1350	4.5	SAP	A	Pico
8	110	14/11/06	1110	1.5	DAP	A	Pico
9	110	15/11/06	0943	25.5	DAP	A	Pico
10	120	15/11/06	1056	55	DAP	A	Pico
11	90	20/11/06	1620	84	DAP	A	Pico
12	110	21/11/06	0725	39	DAP	A	Pico
13	140	21/11/06	0740	40.5	DAP	A	Pico
14	100	21/11/06	0805	35.5	DAP	A	Pico
15	100	16/12/06	0846	12	DAP	A	Pico
16	90	16/12/06	0947	70	DAP	A / M	Pico
17	90	16/12/06	1045	10.5	DAP	A	IBT 96-2
18	130	16/12/06	1056	9.5	DAP	A	IBT 96-2
19	105	17/12/06	1344	10.5	DAP	A / M	IBT 96-6
20	95	20/12/06	0819	8	DAP	A	IBT 96-9

6.3.3 Manual Acoustic Position Estimates

Position estimates for two medusae (ID 16 and 19) were also obtained using the manual acoustic methods as outlined in Chapter Four (page 64).

The total tracking duration of 10 medusae (ID’s 6, 7, 21 – 28) which were tracked using manual acoustic telemetry as per data presented in Chapter Four (page 64) were included in analysis which determined whether tag attachment technique had a significant effect on the total timeframe over which medusae were tracked.

6.3.4 Automated Acoustic Array Position Estimates

Acoustic array layouts

Receiver grids of Sonotronics SUR Submersible Ultrasonic Receivers (SUR's) were deployed parallel to the beachfront in either a single or double line array (Appendix 6.1). Individual SUR’s were attached to a ~8 kg cement cinder block with ~0.3 m of 8 mm poly rope, with their natural upright orientation (due to being air filled), maintaining the omnidirectional hydrophone atop of each unit ~1 m above the substrate. The location of each SUR was marked by a 300 mm polyethylene float for which a GPS location reading was recorded. The first SUR within an array was deployed 200 m directly offshore from the tag and release point of the first medusa within a trial. SUR's were subsequently placed either side such that the original SUR became the midpoint of the grid.

Data management

A text based data file was downloaded from individual SUR's upon completion of each trial using either an Optima Centoris 500 lap top running Sonotronics SUR Soft (versions 4.0 to 4.8) or a HP Palm Pilot running Sonotronics PPT (version 2.1). For each valid detection, five pieces of information are recorded: a unique SUR identifier, the time and date of the detection as well as the frequency (kHz) and interval (ms) of the tag detected. Data management was via a custom made Python based data processing program developed by Andrew Gray-Spence (James Cook University).

Data for all SUR's within a trial were pooled and filtered for detections that did not fall within ±5 ms of a tags true interval (as per Chapter Five). For each 30 min timestamp within a trial, the number of detections that each SUR recorded was quantified. Given that the pulse sequence and interval (ms) of each tag within a trial was known, the theoretical maximum number of detections that could have occurred within a 30 min timestamp could be calculated (as per Chapter Five, page 89). Detection probability became a function of the number of detections logged divided by the theoretical maximum number of detections possible and was used to estimate of distance (m) between a tag (animal) and an SUR using the regression equations from Chapter Five (page 99). Each distance estimate represented the radius of a circle upon the perimeter of which the tag was located.

Data visualisation

The GPS position of each SUR within an array was plotted in Arc GIS version 11 (Figure 6.3A). A circle with a radius of the distance between SUR and tag (as calculated above) was added to each SUR that recorded detections within a given 30 min timestamp (Figure 6.3B-D).

Where tag detections were only recorded by one SUR within a 30 min timestamp, actual tag position could be anywhere on the circumference of that circle (Figure 7.3B). A more accurate estimate of tag location was attempted by looking at where the tag was located in several timestamps prior to and after any timestamp in which only one

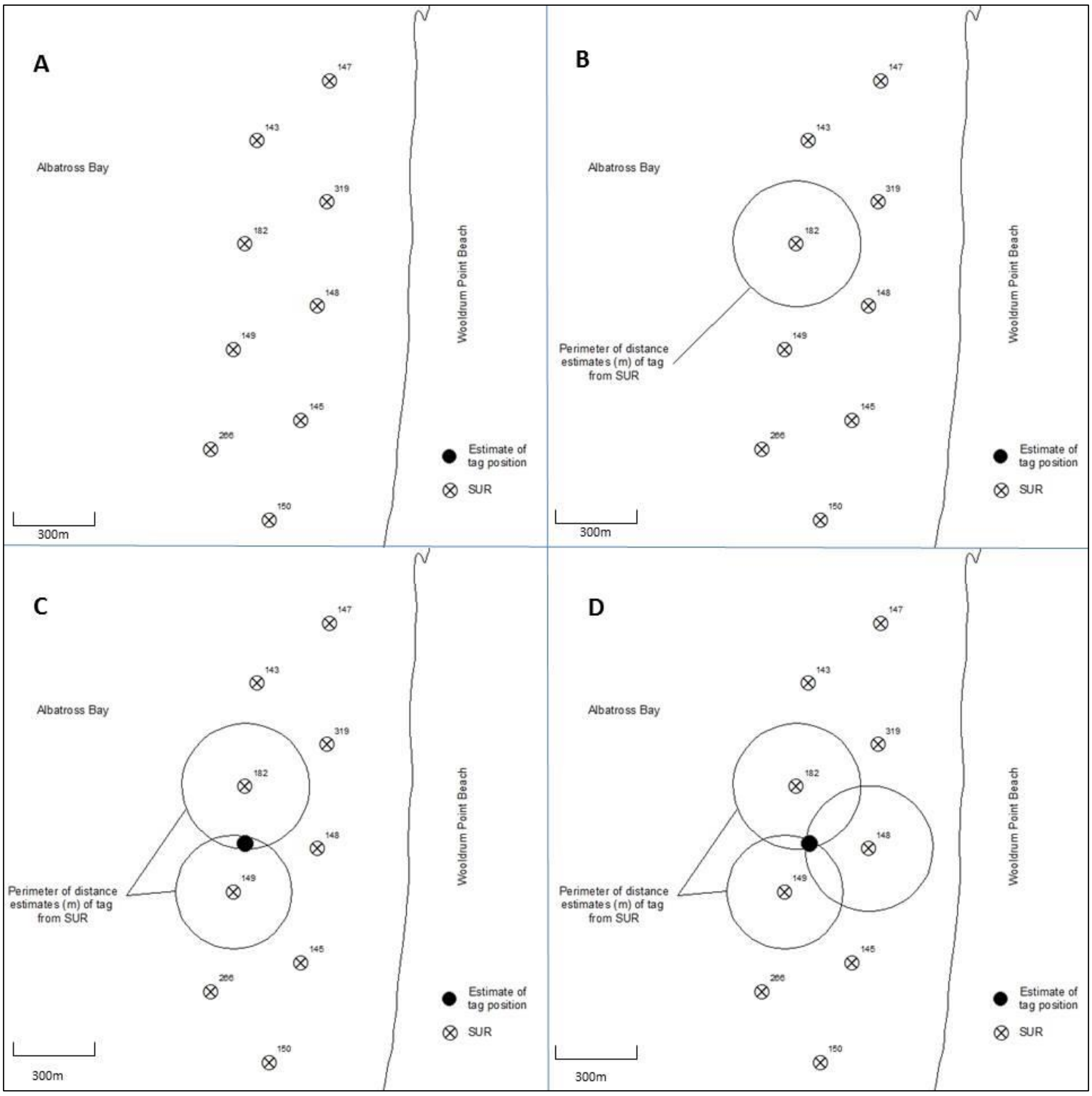


Figure 6.3 SUR grid format (A), one SUR within grid logging valid detections within a timestamp (B), two SUR's logging valid detections within a timestamp showing tag location estimate (C) and three SUR's logging valid detections within a timestamp showing tag location estimate (D)

SUR recorded valid detections. That is, if it could be seen from previous timestamps that the medusa moved down along the coast, the medusa position was placed on the northern portion of the circle perimeter. Alternatively, if the medusa had been undetected for a number of timestamps but then was detected by only an SUR that was not located at either end of the grid, it was assumed that the medusa had been offshore of the grid and was making an onshore movement. Tag location was placed on the circle perimeter directly offshore in this scenario.

Where two or more SUR's recorded valid detection(s) within a timestamp, the position of an acoustic tag was taken to be the point at which the perimeter of successive circles intersected (Figure 6.3C-D). For those timestamps in which a location estimate could have been either onshore of the receiver line or offshore, the onshore position was selected for timestamps that occurred during the day (0600 - 1500 h) and the offshore position selected for timestamps that occurred at night (1501 - 0559 h) as medusae are thought to approach the shore during the day to feed, yet undertake periods of inactivity further from the shoreline at night (Kinsey, 1986, Seymour et al., 2004).

6.3.5 Quantifying the Resolution of Acoustic Array Position Estimates

The accuracy of tag position estimates arising from acoustic arrays (SUR's) was quantified in two ways. Firstly, grid position estimates obtained from acoustic arrays were compared with those obtained using manual telemetry methods. Secondly, variation in successive estimates of the distance between a stationary tag and an SUR calculated for successive 30 min timestamps over a 63 h period were quantified.

Grid position estimates

If an array position estimate fell within ±20 min of a manual position estimate, the straight line distance (m) between these two position estimates was calculated. The number of SUR's that contributed to the position estimate was also noted. A one way ANOVA was used to determine whether resolution (distance in m) varies significantly with the number of SUR's contributing to the position estimate. Least Significant

Difference *post hoc* analysis was carried out on the number of SUR's that contributed to a position estimate to determine which distance estimates were significantly different at the 95% level.

Repeated position estimates of a stationary tag

Within the Weipa November 2006 Trial 1, it would appear that tag frequency 74 kHz from Animal 10 became detached on the 17th November. Evidence supporting the loss of the tag includes:

- 2151 detections logged by 7 of the 10 SUR's within the array showed movements within the grid from 1056 h on the 15th November to prior to 1755 h on the 17th November, yet all 416 detections to the end of the trial were logged by a single SUR (148)
- medusae movements within the array between early morning and afternoon of the 17th November suggest a northerly track for tag 74 kHz - SUR 148 was the second last receiver at the northern end of a single line array
- manual position estimates on both the 18th November and 20th November were within ~300 m of SUR 148 and only ~20 m from each other.

Measures of distance between tag and SUR were calculated for 63 30 min timestamps over a 63 h time interval between 1755 h on the 17th November 2006 and 0725 h on the 20th November 2006. The distance estimate between tag and SUR was plotted for each 30 min timestamp, with tide height (cm) overlaid to identify any patterns in detection consistency or distance estimates.

6.3.6 Calculation of Distance Estimates

The distance travelled by a medusa between 30 min timestamps was considered to be the straight line distance between two successive location estimates derived from ARC

GIS plots. Given that the time between successive location estimates was known, the rate of travel (m h^{-1}) could be calculated for each time stamp. Diurnal patterns in the rate at which medusae travelled during the day (0600 - 1500 h) and night (1501 - 0559 h) were investigated using the methods as per Chapter Four (page 66). Likewise, the effect of tidal state on medusae movement was also investigated using the methods outlined in Chapter Four (page 67). The results of these statistical analyses were then compared to those obtained in Chapter Four to further demonstrate the degree to which acoustic arrays provide accurate and repeatable results.

6.3.7 Time Weighted Average Positions

Time weighted average positions were calculated for five medusae (Animals 10, 11, 12, 13 and 14) using Java code developed by Rhondda Jones of the College of Marine and Tropical Biology at James Cook University. Here, the number of valid detections (as per Chapter Five) was quantified for 5 min timestamps. Where more than one receiver recorded detections for a same timestamp, a tag was considered to be located part way between the receivers, proportionally closer to the receiver that had detected a greater number of detections.

6.4 Results

The total timeframe over which medusae were tracked within acoustic arrays ranged from 1.5 h to 84 h, but did not vary significantly between tracking methods ($F_{2, 27}$ = 1.861, n = 30, P = 0.175) (Figure 6.4). A trend of increasing overall time frame was noted, however, with location data typically collected for 24 h or less using manual telemetry, and never for more than two days (Figure 6.4). Total tracking duration of three or more days were only observed for acoustic arrays, particularly when a second tag attachment point was added to the tagging technique (Figure 6.4). The maximum number of medusae tracked within a trial using manual telemetry was 3 (mode = 1), which increased to 5 (mode = 3) for acoustic arrays.

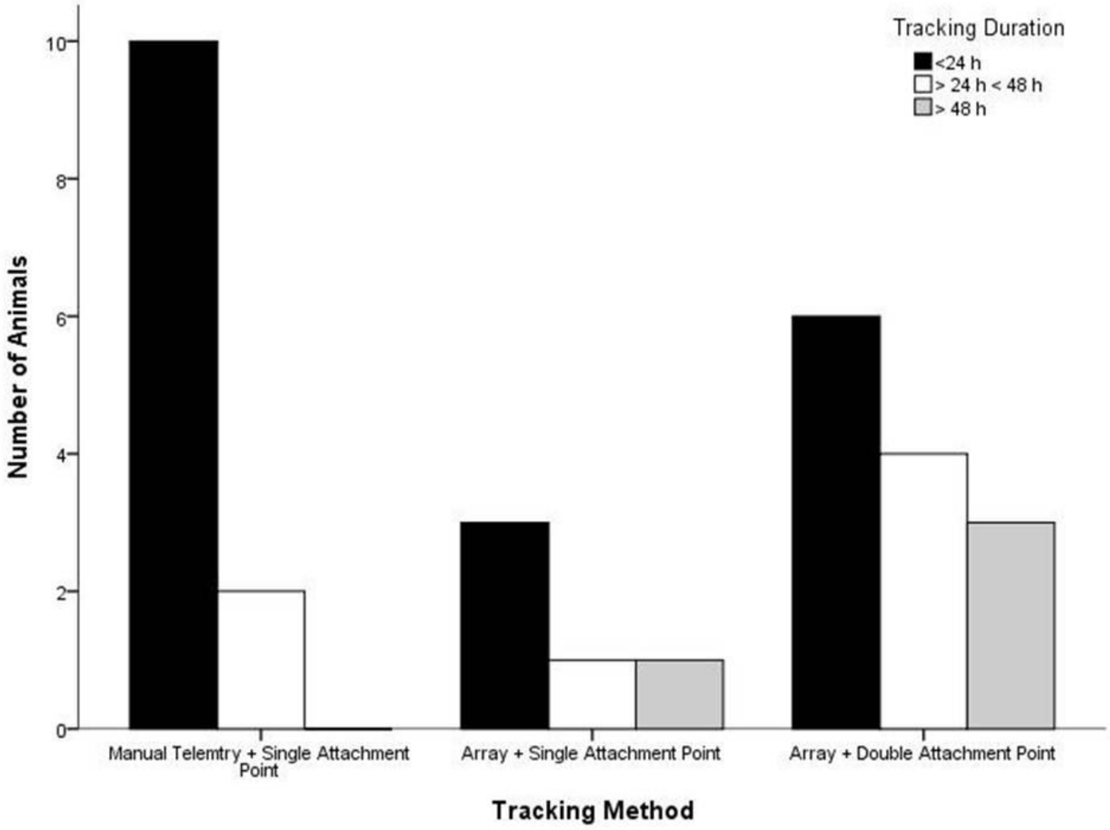


Figure 6.4 Number of medusae tracked for up to 24 h, 24 to 48 h and greater than 48 h using (1) manual telemetry + tags attached at a single point (2) an acoustic array + tags attached by a single point and (3) an acoustic array + tags attached via two points

A total of 27 manual position estimates occurred within ± 20 min of an array position estimate. The distance between manual and array position estimates was significantly affected by the number of SUR’s that contributed to the array position estimate ($F_{3,27}$ = 8.104, n = 27, *P* = 0.001). Here, the distance between manual and acoustic position estimates varied from 258 m to 1260 m when only a single SUR contributed to array position estimates (mean of 823 ± 89 SE m), but was significantly less where two (200 ± 35 SE m), three (453 ± 112 SE m) or four (157 ± 10 SE m) SUR's contributed to position estimates.

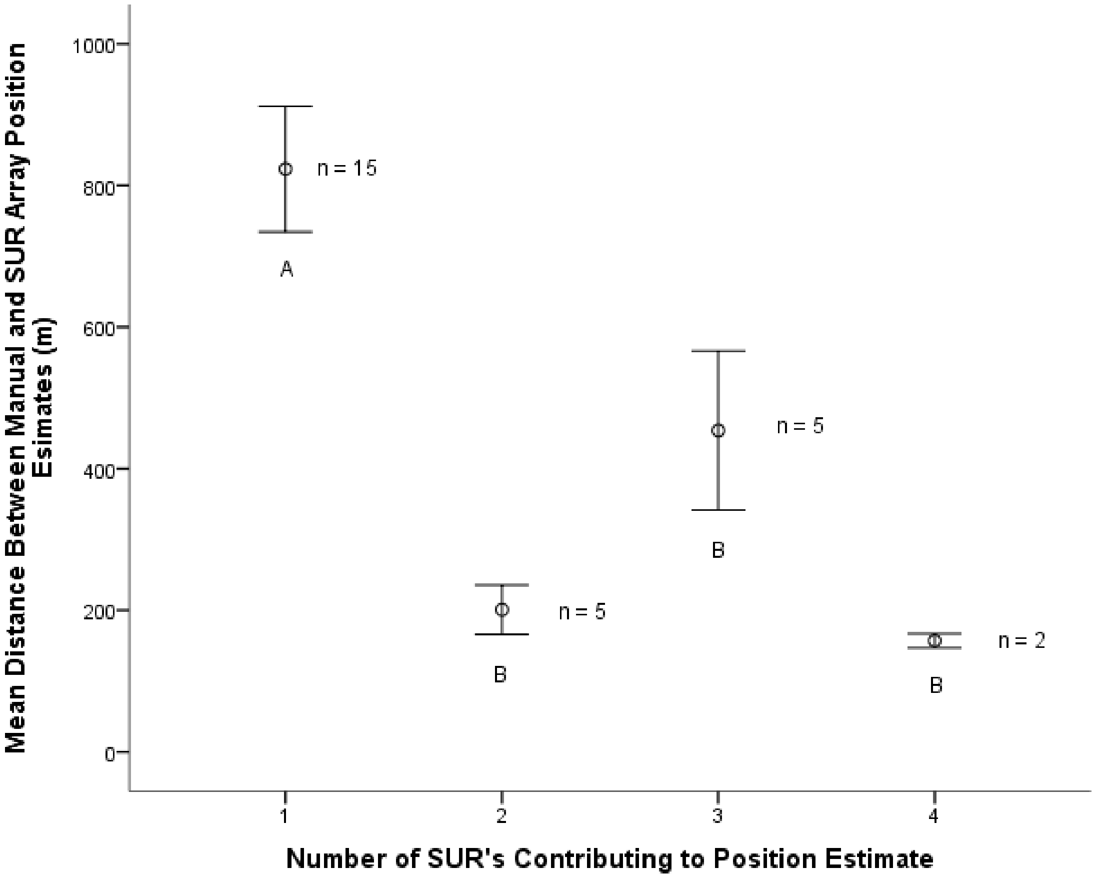


Figure 6.5 Mean distance ± SE (m) between manual telemetry position estimates SUR array position estimates, with means followed by the same letter not significantly different at the 95% level

Variation in repeated estimates of distance between a stationary tag (74 kHz) and SUR 148 (n = 63) ranged from 130 m to 592 m, with an overall mean of 480 ± 13 SE m (Figure 6.6). Of these distance estimates, 62% varied by no more than 100 m, 86% by 200 m or less and only 6% by more than 300 m. A further 63 timestamps did not include any detections, however. While the majority of these were confined to four time periods within the trials (a) 2155 h on the 17th November to 0125 h on the 18th November (n = 7) towards a low tide, (b) 0725 h to 2125 h on the 18th November (n = 28) across varying tide heights, (c) 0755 h to 1155 h on the 19th November (n = 8) and (d) 2325 h on the 19th November and 0355 h on the 20th November (n = 9) towards the low ebb / early making tide, the remaining 11 time stamps occurred sporadically throughout the dataset.

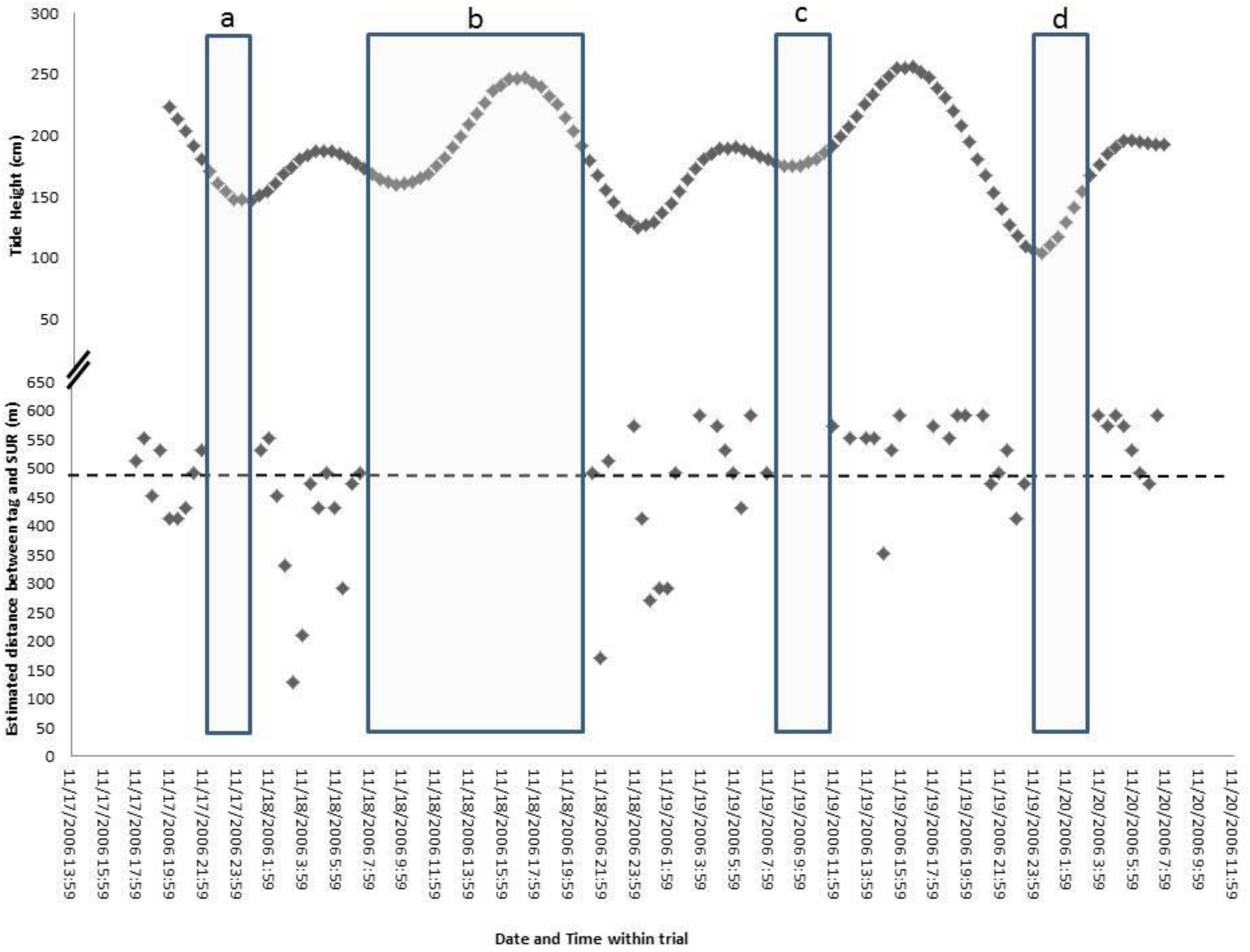


Figure 6.6 Estimated distance (m) between a stationary (detached) tag (74 kHz) and an acoustic receiver (SUR 148) for 63 h showing mean distance estimate (dashed line), time periods during which detections were not recorded (grey boxed areas) and tide height (cm)

Estimates of the rate at which medusae 16 and 19 travelled varied significantly with the type of acoustic telemetry used to determine medusae location ($F_{1, 102}$ = 8.793, P = 0.004). Not only were rates of travel estimated from acoustic arrays (487 ± 55 SE m h^1) nearly three times those estimated using manual telemetry methods (170 ± 24 SE m h^1), estimates also varied to a greater extent (Figure 6.7).

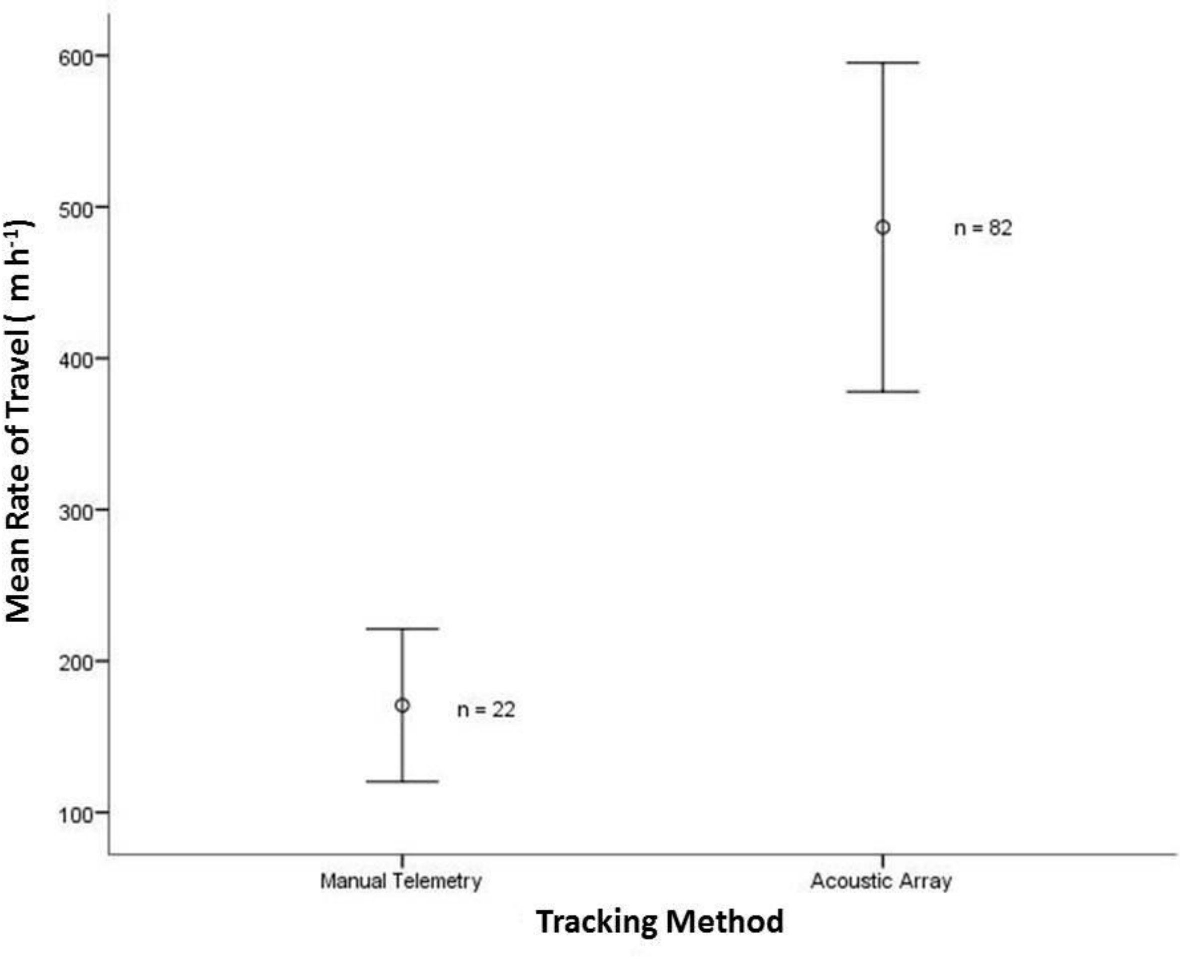


Figure 6.7 Estimates of mean rate of travel (m h^{-1}) ± 95% confidence limits for Animals 16 and 19 where position estimates had been derived using manual telemetry and acoustic arrays

The rate at which medusae travelled varied significantly between day (0600 - 1500 h) and night (1501 - 0559 h) ($F_{1, 560}$ = 8.035, P = 0.005). That is, while medusae travelled at a mean rate of 365 ± 25 SE m h^{-1} between 0600 and 1500 h, average rates of travel increased to 457 ± 23 SE m h^{-1} between 1501 and 0599 h (Figure 6.8).

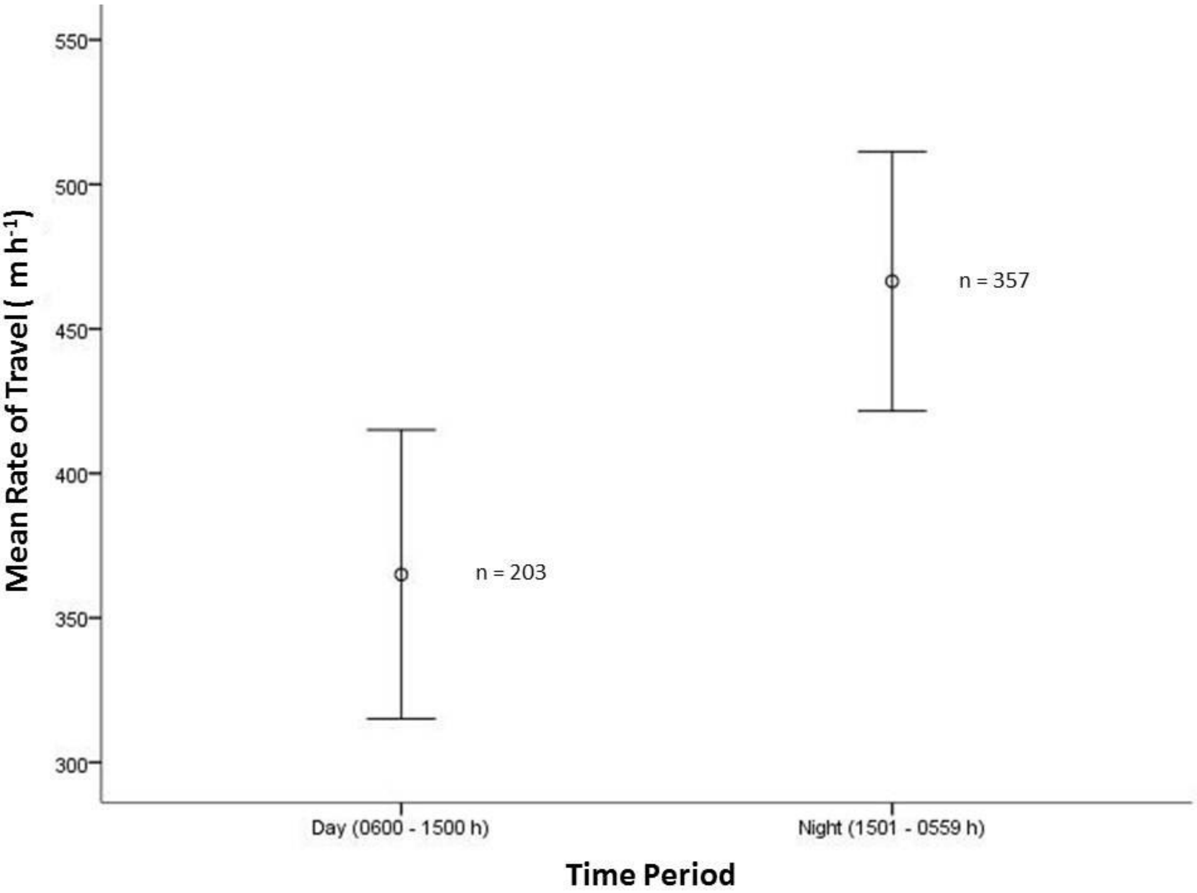


Figure 6.8 Mean rate of travel (m h^{-1}) ± 95% confidence limits for medusae during the day (0600 - 1500 h) and night (1501 - 0559 h)

The rate at which medusae travelled did not vary significantly between tidal class ($F_{1, 560}$ = 1.152; *P* = 0.324). Estimates of the mean rate of travel ranged from 357 ± 46 SE m h^{-1} to 524 ± 65 SE m h^{-1} with an overall average of 430 ± 17 SE m h^{-1} (Figure 6.9).

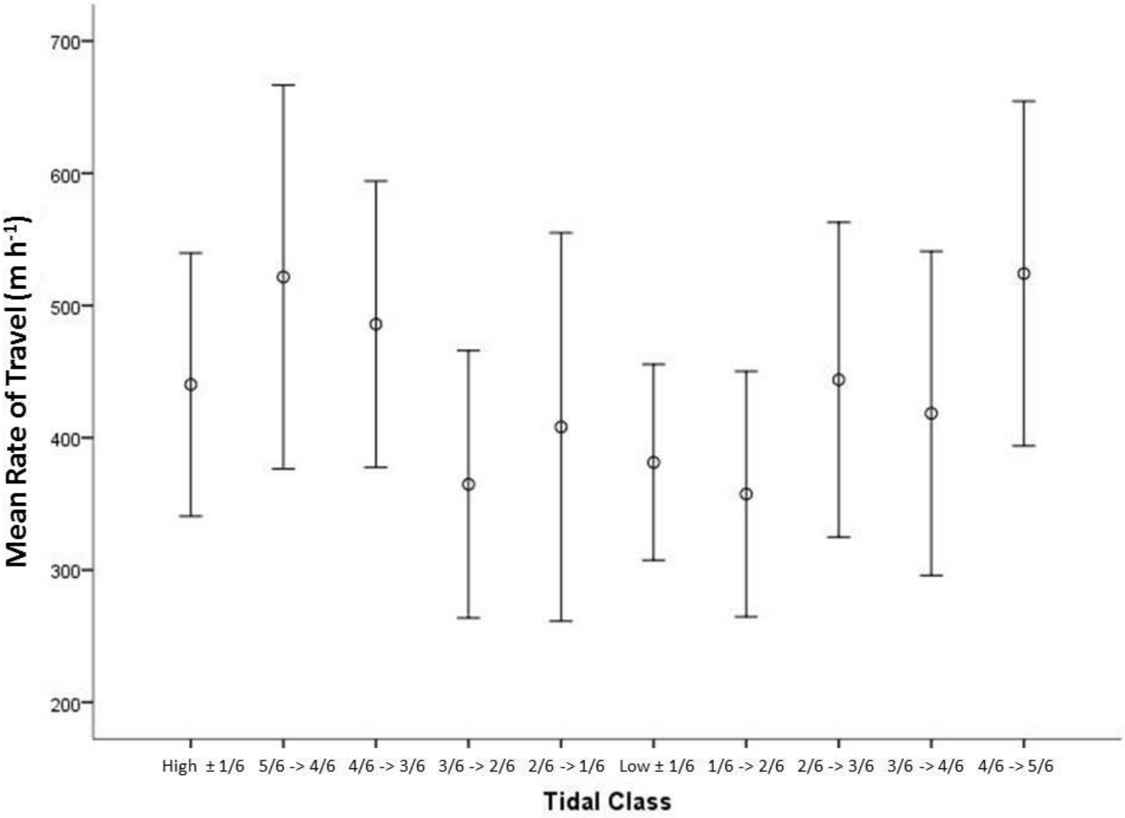


Figure 6.9 Mean rate of travel (m h^{-1}) ± 95% confidence limits for medusae at each of 10 tidal classes

The position of Animal 10 within an acoustic array (deployed on the 14th November 2006) varied over ~1.6 km within the 52 h for which detections were recorded by SUR's (Figure 6.10). In total, the medusa made four repeated movements along the SUR array, with a fifth commencing on the morning of the 17th November. These coincided with the tidal regime, which, at Wooldrum Point, generates longshore currents (as per Chapter Four, page 67). At the peak of both the spring and neap tides, the medusa was located near SUR 2, yet at low ebb of the neap tide, located at receiver 5 and 7 for low ebb of the spring tide. At other times, medusae activity was confined to a narrow stretch of beachfront or was independent of tidal regime, for example, mid morning on both the 16th and 17th November.

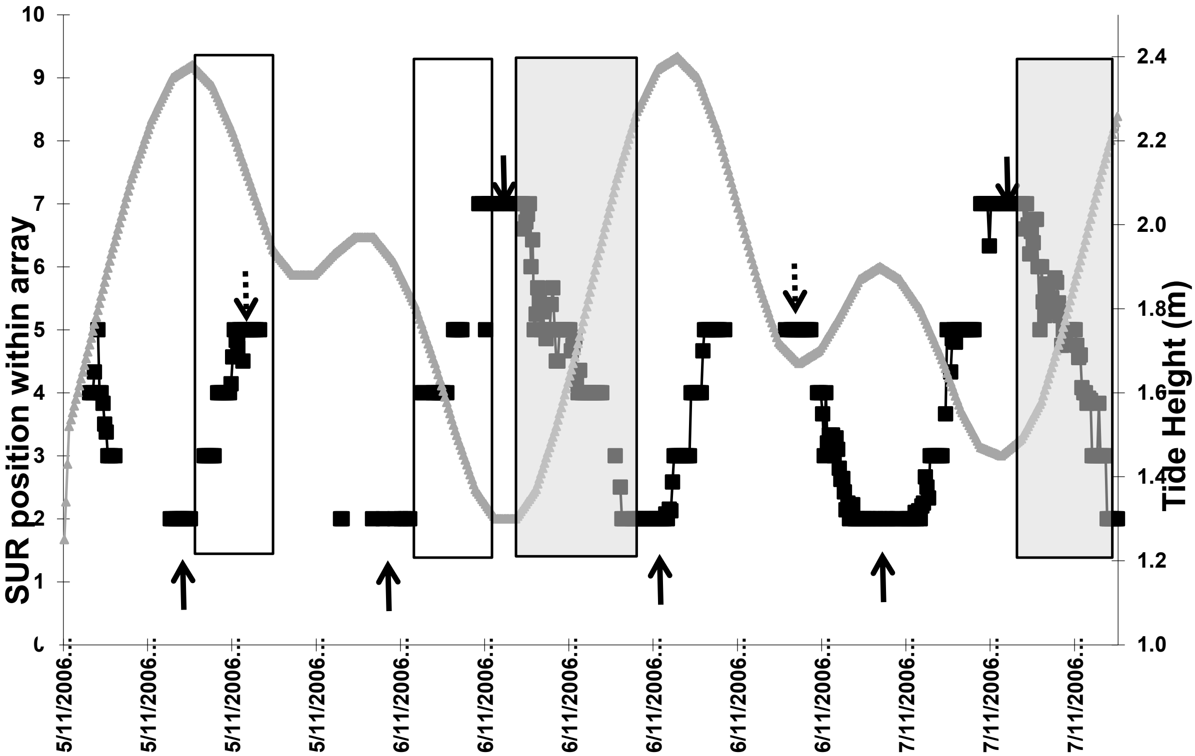


Figure 6.10 Time weighted position of Animal 10 over 52 h within acoustic array at Wooldrum Point Beach November 2006, showing location throughout incoming tide (grey shaded boxes), outgoing tide (black framed box) and at high tide (black and black dashed arrow) and low tide (grey arrows)

Three repeated longshore movements were also evident for a second medusa (Animal 11) along ~1.8 km of beachfront within an 84 h period commencing on the 20th November 2006 (Figure 6.11). While longshore movements typically coincided with tidal regime, this was not the case between sunrise on the 21st November and 2100 h on the 22nd November. Furthermore, localised activity between 2000 h and 2400 h on the 21st November occurred at the opposite end of the grid to which the medusa was located at the low ebb of the three other spring tides for which medusa location was recorded.

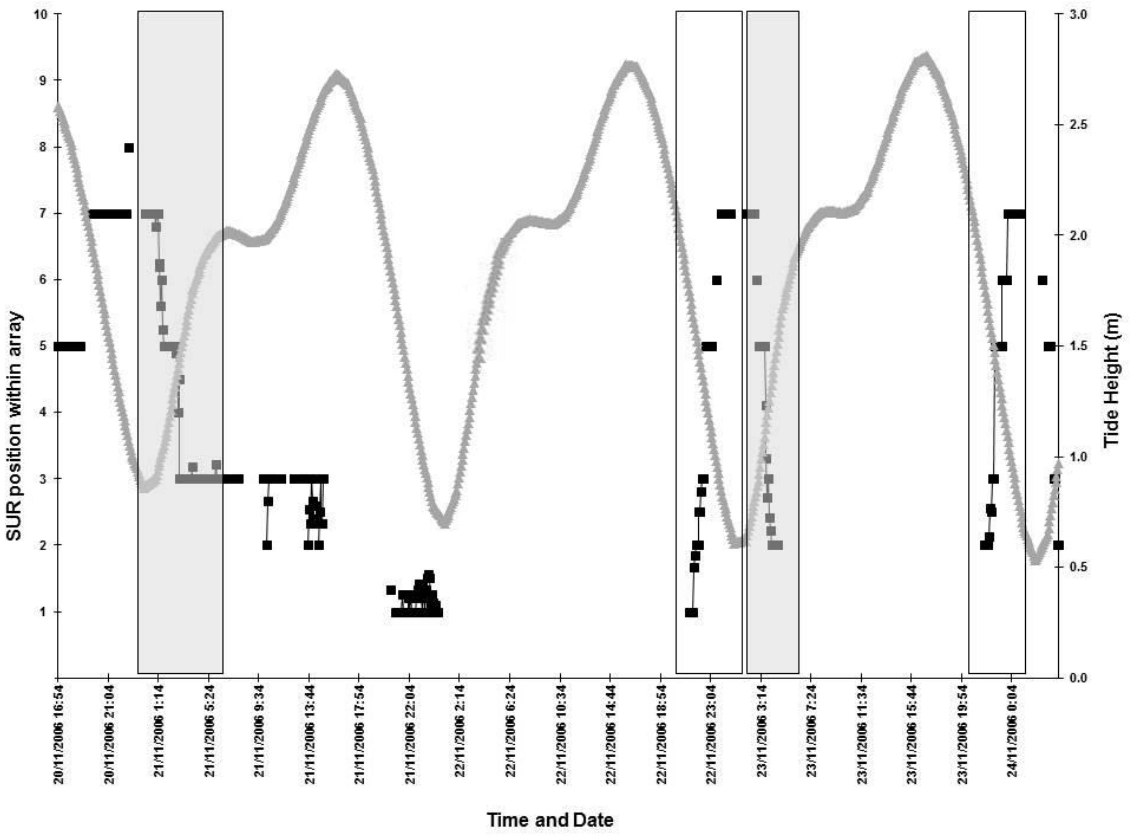


Figure 6.11 Time weighted position of Animal 11 over 84 h within acoustic array at Wooldrum Point Beach November 2006, showing location throughout incoming tide (grey shaded boxes) and outgoing tide (black framed box)

Further evidence that medusae did not simply move with tidally generated currents or shared the same movement patterns was evident in the locations of four medusae that were tracked simultaneously in November 2006 (Figure 6.12). For instance, Animal 11 made several longshore passes through the array, a pattern that was not evident for Animals 12, 13 and 14. Medusae were also situated at three different locations within the grid (SUR receivers 2, 5 and 7) at 1300 h on the 21st November and again at 0200 h on the 22nd November. Similarly, at 0300 h on the 23rd November, medusae were detected at receivers 2 and 7. Medusae also remained within the acoustic array for different lengths of time, with Animals 12, 13 and 14 being tagged after Animal 11, but leaving the grid 40 h earlier.

6.5 Discussion

The timeframe over which a tagged individual is monitored must be relative to the behaviour or pattern under investigation. For instance, if diurnal or tide related patterns are to be identified, animal movement should be document over at least several days. Total tracking time in previous *C. fleckeri* movement studies has been limited not only by the logistics of conducting manual telemetry in the tropical environment, but also by limited tag retention time, with data documenting movements over two days collected on only limited occasions. An increase in total tracking time as well as the number of animals tracked was associated with the combination of acoustic arrays and a more robust tagging technique. While the addition of a second point of attachment (a tether around the pedalial structure) is likely to have contributed to increased tag retention times, at least one tag was recovered that had broken free of the tether. Given that medusae remain inactive on the seafloor for periods of time throughout the night (Seymour et al., 2004), friction created from the tag being rubbed against the sea floor may have resulted in release from the tether. As such, future studies should

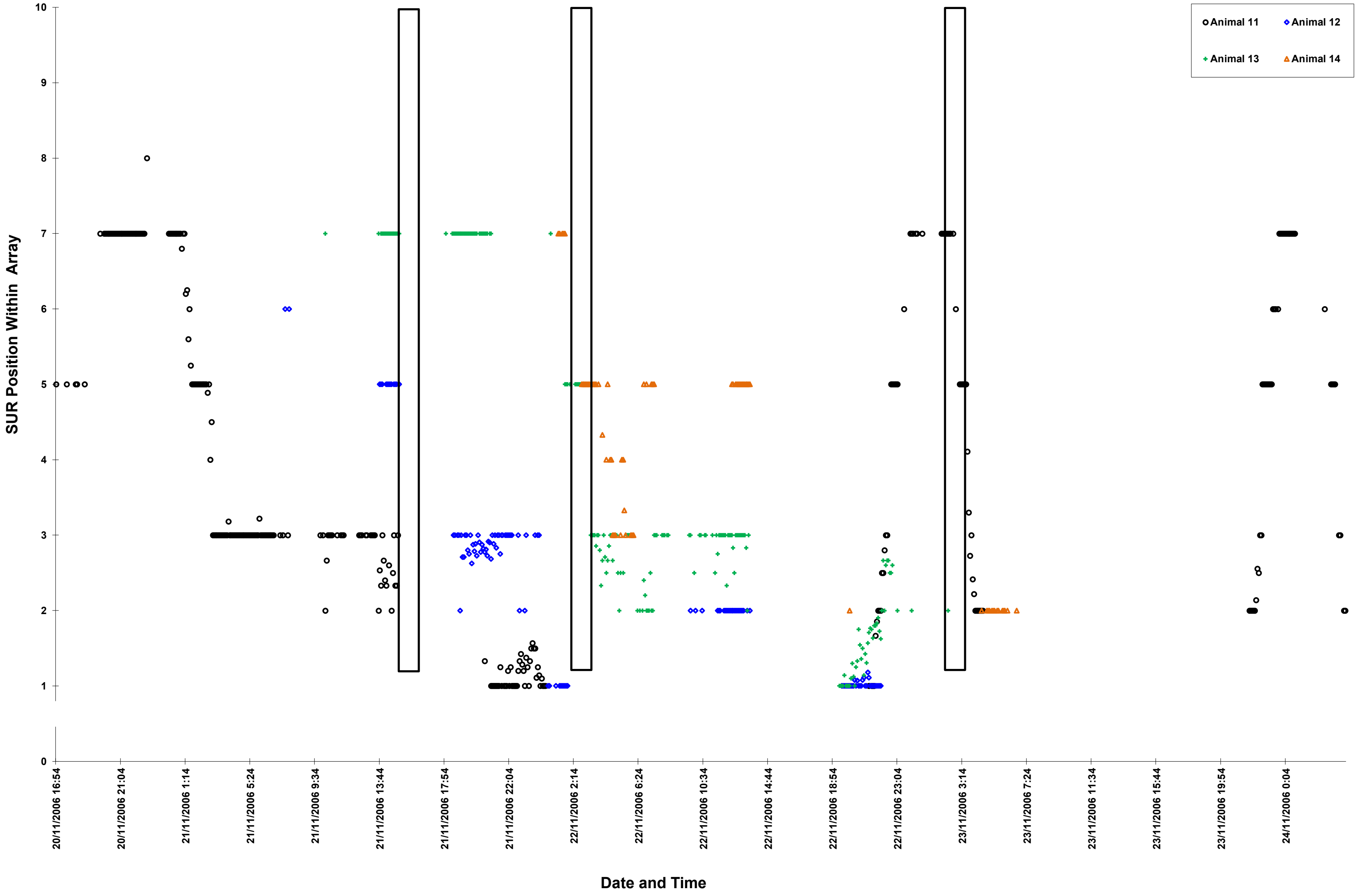


Figure 6.12 Time weighted position estimates of Animals 11, 12, 13 and 14 within acoustic array at Wooldrum Point Beach November 2006; black framed box highlights time periods during which medusae were at different locations

consider a tag attachment technique whereby tags are glued within the bell rather than to an external face, in an attempt to increase tag retention times further.

Compared to manual telemetry, acoustic arrays do not typically provide a high degree of resolution, unless they are of the Radio-Acoustic Positioning Telemetry type (for example Rigby et al. (2005)). Indeed, array position estimates tend to describe a short term centre of activity (Simpfendorfer et al., 2002) rather than a precise location. Such was the case within this study. On average, the distance between a position estimate derived automated array data and a relocation point derived from manual acoustic telemetry ranged from ~157 ± 10 SE m (estimated using detections at four SUR's) to ~823 ± 89 SE m (estimated using detections from a single SUR), with any single estimate varying by up to 1260 m from its corresponding manual relocation estimate. This is compared to ~10 m associated with manual telemetry (Chapter Four). Given that error decreased significantly when multiple receivers contributed to a position estimate, increasing the number of receivers within an array, reducing the distance between receivers to less than their average detection range and using multiple rows of receivers are aspects of array design that future medusae tracking studies should consider.

Inaccurate position estimates derived from automated arrays also led to a nearly three fold overestimation of the rates at which medusae travelled: 170 ± 24 SE m h^{-1} from manual telemetry versus 487 ± 55 SE m h^{-1} for acoustic array data. Furthermore, while no diurnal behaviours could be identified from manual telemetry movement data, which estimated medusae travelled at ~100 – 200 m h^{-1} both day (0600 - 1500 h) and night (1501 - 0559 h), acoustic array data not only indicated a significant diurnal effect, but again grossly overestimated medusae rates of travel at 365 ± 25 SE m h^{-1} during the day and 457 ± 23 SE m h^{-1} at night. While tidal cycle was not found to have a significant effect on rate of travel using either manual telemetry (Chapter Four) or acoustic array data, rate of travel estimates for acoustic arrays (from 357 ± 46 SE m h^{-1} to 524 ± 65 SE m h^{-1}) were again significantly greater than for manual telemetry calculated in Chapter Four (from 52 ± 26 SE m h^{-1} to 187 ± 53 SE m h^{-1}). Collectively, these results suggest that acoustic arrays are not suitable for the collection of rate of travel data for *C. fleckeri* medusae.

Care must also be taken when inferring behaviours from acoustic telemetry data (Payne et al., 2010, How and de Lestang, 2012). For instance, Payne et al. (2010) demonstrated that a reduction in the detection frequency of tagged cuttlefish was not related to animal movement, but to other factor(s) that caused a decrease in detection rates, both for tags fitted to cuttlefish as well sentinel tags. Concluding that an absence of tag detections equated to an absence of cuttlefish would have led to an incorrect interpretation of animal behaviour. Evidence for a similar case within this study exists. That is, a tag that became dislodged from a medusa was within range of an SUR for 63 h, yet within this timeframe, 63 time stamps did not document the presence of the tag. Given the riffled nature of the substrate along Wooldrum Point Beach, a physical barrier between tag and receiver may have significantly reduced tag detection frequencies. Of the 63 timestamps that did not log detections, 16 of these occurred at or around low tide, at which time the hydrophone may have been too close to the surface, been completely exposed or no longer in its vertical alignment. That spurious data points (i.e. those that lie outside the ± 5 ms of a tags true interval) but no valid detections were recorded for some timestamps raises the possibility that Signal to Noise Ratio (SNR) at the receiver was an issue during this time. As such, sentinel tags would be a valuable addition to future tracking studies, with their cost (from ~$200 to ~$500 USD per tag) far outweighed by the reduced potential to misinterpret data derived from automated receivers.

Several longshore movements were evident in medusae location data. Animal 10, for instance, made four repeated movements over an ~1.6 km stretch of beachfront while Animal 11 made three such longshore movements along an ~1.8 km stretch of coastline. Given that estuary discharge is negligible at this time of year, currents along the coastline adjacent to the estuary are tidally driven, being southward on an outgoing tide and northward on an incoming tide. As such, gross medusae movement patterns appear to be with the (tidally generated) current. This is not to say, however, that medusae are passive drifters. Indeed, individual meduase showed localised behaviours on a number of occasions, remaining along a ~200 m, sometimes for several hours. Such periods potentially represent some sort of feeding behaviour. That is, *C. fleckeri* medusae employ a cruise-entangling predation strategy (Hartwick, 1991a) during which medusae swim with tentacles outstretched in an attempt to capture small fish and prawns (Barnes, 1966). Given that medusae are likely to expend large amounts of energy foraging and have been found swimming amongst prey in the shallows (Hartwick, 1991a, Rifkin, 1996), being able to remain within an area of high prey density, and hence increase encounter rates with prey would be advantageous.

That these observed localised movements occurred independently of the current was best demonstrated by four medusae tracked in November 2006. During this trial, medusae not only demonstrated localised behaviours at distinctly different sections of beachfront, but moved into and out of the gridded tracking area at different times. Medusae were not transient to the gridded area, however, with such patterns repeated over several successive days. Whether these localised movements were attributed to the favourable topography of Wooldrum Point Beach and prevailing calm onshore conditions typical for October to December, and therefore indicate that medusae can be somewhat resident in favourable areas, is worthy of further investigation. Tag retention times greater than those achieved here will be required in such studies, however.

Collectively then, the results of this chapter demonstrate that while acoustic arrays are useful in documenting medusae movement patterns over longer periods of time, a trade off in the resolution of position estimates is made. As such, acoustic arrays cannot necessarily replace manual telemetry methods, but can provide an alternative tracking method capable of documenting low resolution presence / absence data. Given the favourable conditions under which medusae tracking occurred, this study represents a base line data set to which future studies looking to quantify the response of medusae to changes in physical and environmental parameters can compare.

CHAPTER SEVEN

General Discussion

Cubozoans are found throughout the tropical waters of the world, in many cases giving rise to serious and life threatening syndromes, often within minutes of contact with their tentacles. This is particularly true for the near shore waters of tropical Australia where potentially fatal *C. fleckeri* medusae become abundant during the warmer months of the year. Yet the spatial and temporal occurrence of medusae remains sporadic and unpredictable, as does the risk of an envenomation event occurring. This study represents an initial step into the development of risk management models, having demonstrated quantitatively that while some of the long held theories relating to *C. fleckeri*'s ecology appear validated, some may be erroneous.

For instance, it is a widely accepted belief that the stinger season, or the timeframe over which medusa represent a danger to beach users, varies not only between seasons, but also between geographic locations. It is further proposed that the onset of each season is associated with seasonal fluctuations in a suite of environmental parameters such as water temperature and salinity. Within this study, however, calculations of the metamorphosis date of 461 medusae demonstrated that the onset of medusae production across six medusae seasons not only occurred much earlier than expected (2nd September ± 2 d 95% confidence limits), but was also temporally constrained (varied by only 7 d from 30 August to 5 September) between seasons. Furthermore, while water temperature, tidal amplitude, daily rainfall and moon phase were all poorly correlated with the onset of medusa production, photoperiod was strongly correlated. Manipulative trials on the polyp phase of the life cycle aimed at demonstrating a link between day length and polyp metamorphosis are required before the onset of the medusae season can be modelled reliably, however.

That medusae begin their existence within estuary systems and undertake a downstream migration to the coastline where they accumulate and increase in size as the season progresses is a second long held theory that was not supported by the results of this research. Rather, a greater proportion of juvenile medusae was observed along the coastline, with estuarine populations typified by larger medusae. That the oldest medusa observed across seven seasons was collected from an estuarine site further suggests a downstream migration is not typical for Weipa sites. With an estimated age of 78 d, which is far less than the length of a typical medusae season (~200 d), it also appears that medusae do not accumulate as the season progresses, either. Collectively, these results suggest that the theory of a medusae downstream migration should not be arbitrarily applied throughout the tropics.

Once within the medusa phase of the life cycle, medusae are thought to grow at a rapid rate. This theory was confirmed within this study, with medusae not only shown to grow at up to 3 ± 0.2 mm d^{-1}, but to also add tentacles in pairs to each pedalium more rapidly as size increased. On an applied level, at these development rates, medusae can potentially become lethal to humans in ~50 - 65 d, with an ontogenetic shift in both their cnidome and venom reported to occur at ~100 mm IPD (Carrette et al, 2002). Being able to define both the period over which *C. fleckeri* is within the medusa phase of the lifecycle and when they pose a likely threat to humans would be a fundamental parameter to incorporate into predictive models. As such, furture research determining whether the onset of medusae production is temporally constrained, growth rates are equally as rapid and population structure is consistent between geographic locations throughout tropical Australia is required if predictive models are to be of regional, rather than local, relevance.

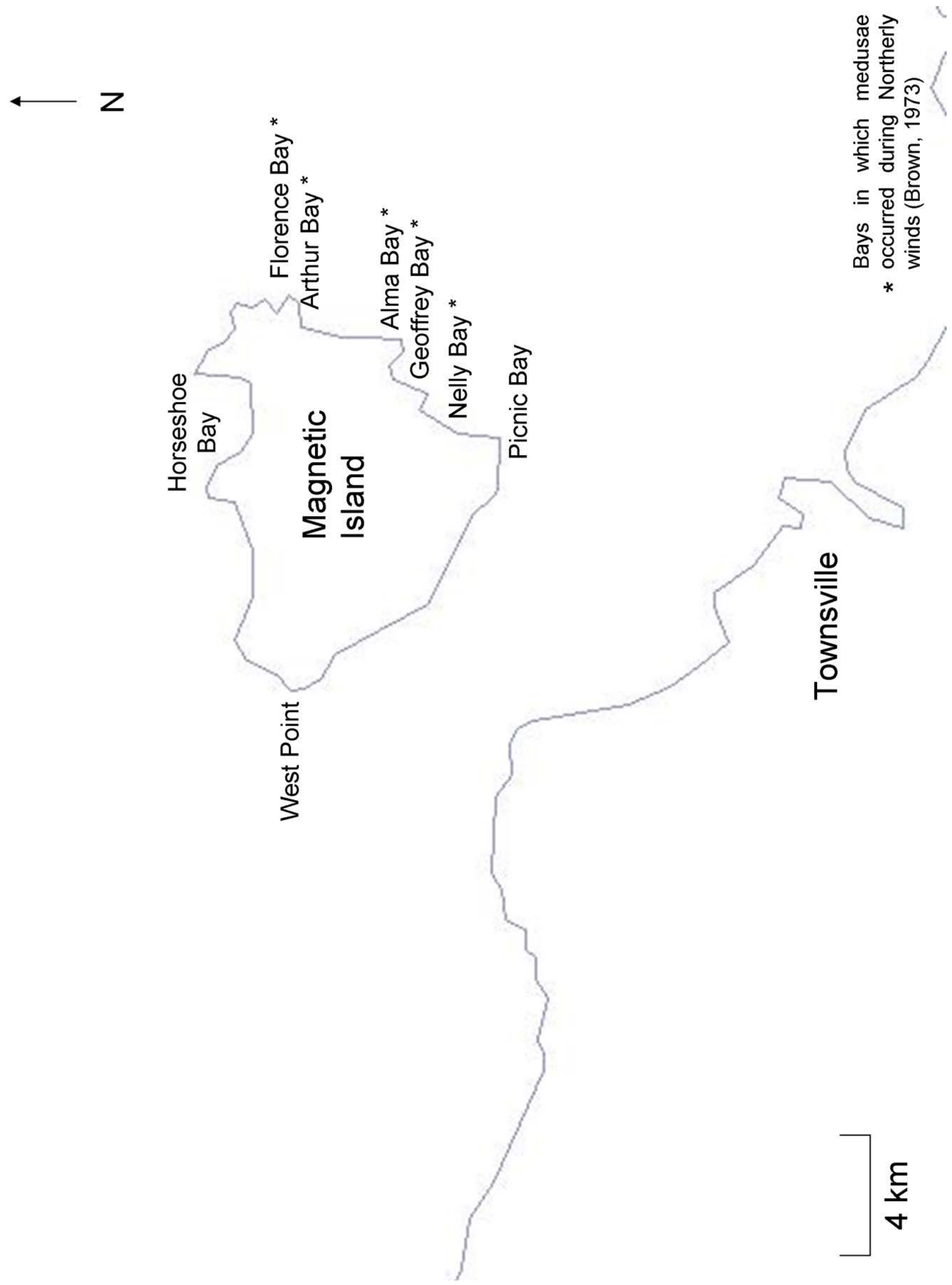
Estimating the relative risk of an envenomation occurring would also require medusae distribution within a season to be modelled. It has been long accepted that *C. fleckeri* medusae possess swimming abilities superior to other species of jellyfish, allowing them to move into and out of areas in response to changed conditions. Anecdotal evidence further suggests that elevated levels of activity can be sustained over several kilometres. These theories were supported within this study, with the successful application of acoustic telemetry, in both manual (12 medusae) and automated array (18 medusae) formats, to *C. fleckeri* medusae. Not only were medusae capable of moving several kilometres within a single tidal cycle within the estuarine habitat (average of 828 ± 306 m h^{-1}), but also possessed the ability to move independently of tidally generated currents, at least up to a certain level of flow (which could not be defined here), after which, they instead moved with the current.

Movement patterns were not necessarily consistent between habitats, however. Within the coastal habitat, medusa movements were not only more localised, but rates of travel were typically slower (52 ± 26 SE m h^{-1} to 187 ± 53 m SE h^{-1}) than those of estuarine medusae (110 ± 53 SE m h^{-1} to 828 ± 306 SE m h^{-1}). Longshore movements along ~2 km of coastline were evident in the movement patterns of several medusae within the coastal habitat, occurring over several successive days. Whether the periods during which coastal medusae moved independently of tidally generated currents represented a foraging behaviour is currently unclear, but is worthy of further investigation.

Importantly, these data also represent a baseline data set to which the movement patterns medusae during less favourable conditions can now be compared. That is, the coastal habitat of Weipa during November and December is typically calm and stable, with offshore breezes resulting in little more than riffles to the sea surface. Estuary discharge is also minimal at this time, with salinity typically being elevated and stable. Turbidity too is low and remains so until the onset of the wet season. As such, future tracking studies should document medusae movement patterns when winds become onshore, rainfall and estuary runoff is elevated, salinity is unstable and turbidity increases, a suite of environmental parameters typically associated with the wet season. Quantifying medusae movements patterns under such conditions would greatly increase the degree to which medusa occurrence and distribution, and hence the risk of an envenomation event occurring, could be modelled.

While the findings of this study are relevant to tropical Australia, evidence is emerging that being able to predict the occurrence of highly venomous Chirodropids would be of advantage throughout the South Western Pacific, Indonesia and Malaysia (Pearn, 1995) as well as Japan (Lewis and Bentlage, 2009). Similarly, while management of the risks inherent within a stinger season may remain the mechanism driving future research, it is only once long term data sets have been established that broader questions of whether medusae abundances, stinger season length and distributional range are increasing can be addressed adequately.

Appendix 2.1 Map of Magnetic Island (Townsville) showing the bays into which medusae move under influence of northerly breezes



Appendix 4.1 – Months in which Weipa sample sites were sampled throughout study

Season Site	1999	2000	2003	2005	2006	2007	2010
Andoomajetti Point		Ω.		∗		∗	Ω.
Rocky Point					∗		
Wooldrum Point Beach				∗	∗	∗	Ω.
Westminster South					∗		
Jessica Point				∗			
Hey Point		Ω.		∗	∗	∗	Ω.
Pine River		Ω.					
Landfall Point				∗			
Weipa (generic)	Ω.		Ω.				

∗	denotes samples collected within PhD project
Ω.	denotes samples collected by others prior to PhD project

Appendix 5.1 Permutations of tags detected

1

1 1

1 2 1

1 3 3 1

1 4 6 4 1

Number of Permutations	**1**	**5**	**10**	**10**	**5**	**1**
Number of tags heard per cycle	0	1	2	3	4	5

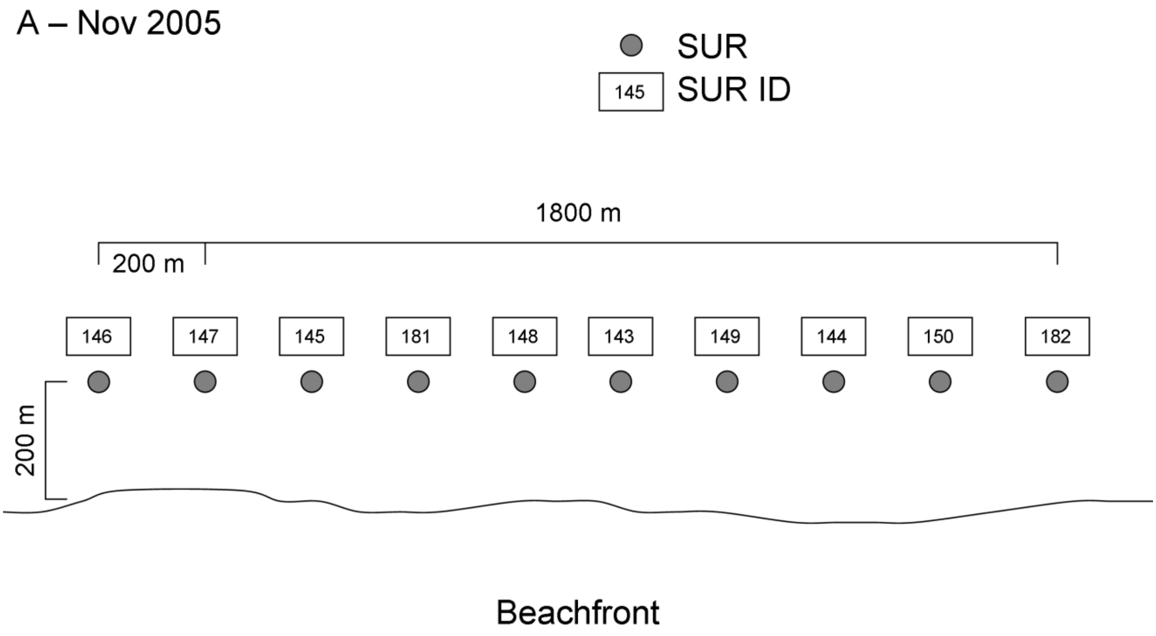
Appendix 5.2 Matrix of mean cycle time for all permutations of tags detected

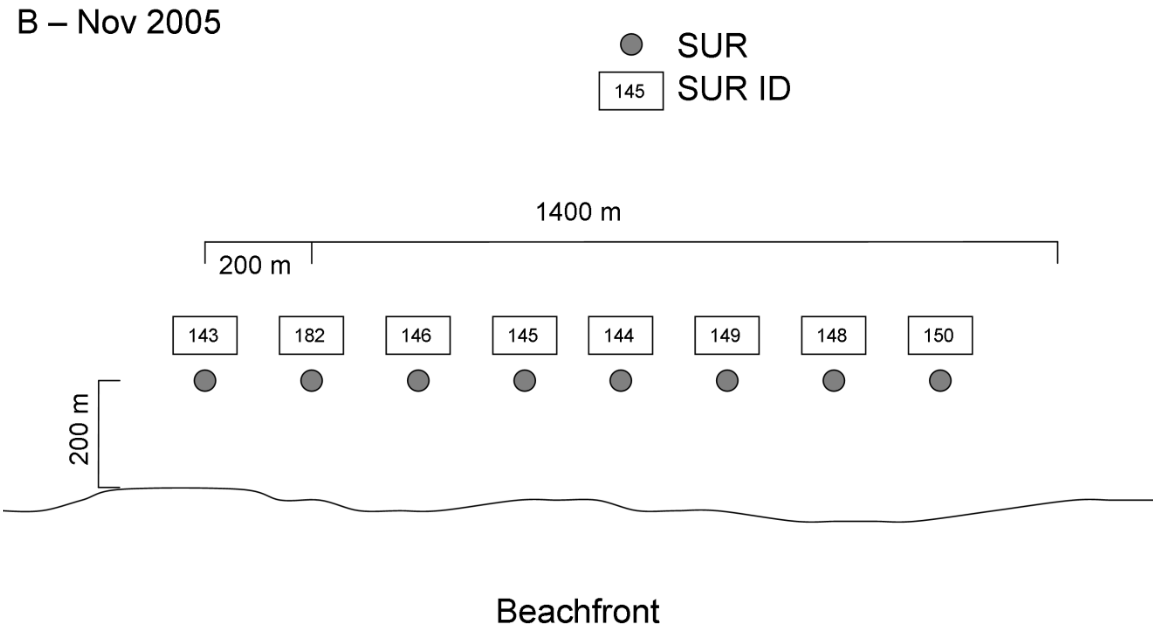
Number of Tags Detected	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Handling Time Tag 1	Handling Time Tag 2	Handling Time Tag 3	Handling Time Tag 4	Handling Time Tag 5	# Pulse Sequence Intervals	Tag Detection Time	Total Cycle Time
5	1	2	3	4	5	3890.74	3495.19	2966.67	3012.73	3090.00	0	16455.32	18.96
4	1	2	3	4		3890.74	3495.19	2966.67	3012.73	0	1	13365.32	18.37
4	1	2	3		5	3890.74	3495.19	2966.67	0	3090.00	1	13442.59	18.44
4	1	2		4	5	3890.74	3495.19	0	3012.73	3090.00	1	13488.65	18.49
4	1		3	4	5	3890.74	0	2966.67	3012.73	3090.00	1	12960.13	17.96
4		2	3	4	5	0	3495.19	2966.67	3012.73	3090.00	1	12564.58	17.56
3	1	2	3			3890.74	3495.19	2966.67	0	0	2	10352.59	17.85
3	1	2		4		3890.74	3495.19	0	3012.73	0	2	10398.65	17.90
3	1	2			5	3890.74	3495.19	0	0	3090.00	2	10475.93	17.98
3	1		3	4		3890.74	0	2966.67	3012.73	0	2	9870.13	17.37
3	1		3		5	3890.74	0	2966.67	0	3090.00	2	9947.41	17.45
3	1			4	5	3890.74	0	0.00	3012.73	3090.00	2	9993.47	17.49
3		2	3	4		0	3495.19	2966.67	3012.73	0	2	9474.58	16.97
3		2	3		5	0	3495.19	2966.67	0	3090.00	2	9551.85	17.05
3			3	4	5	0	0	2966.67	3012.73	3090.00	2	9069.39	16.57
3		2		4	5	0	3495.19	0	3012.73	3090.00	2	9597.91	17.10
2	1	2				3890.74	3495.19	0	0	0	3	7385.93	17.39
2	1		3			3890.74	0	2966.67	0	0	3	6857.41	16.86
2	1			4		3890.74	0	0.00	3012.73	0	3	6903.47	16.90
2	1				5	3890.74	0	0	0	3090.00	3	6980.74	16.98
2		2	3			0	3495.19	2966.67	0	0	3	6461.85	16.46
2		2		4		0	3495.19	0	3012.73	0	3	6507.91	16.51
2		2			5	0	3495.19	0	0	3090.00	3	6585.19	16.59
2			3	4		0	0	2966.67	3012.73	0	3	5979.39	15.98
2			3		5	0	0	2966.67	0.00	3090.00	3	6056.67	16.06
2				4	5	0	0	0	3012.73	3090.00	3	6102.73	16.10
1	1					3890.74	0	0	0	0	4	3890.74	16.39
1		2				0	3495.19	0	0	0	4	3495.19	16.00
1			3			0	0	2966.67	0	0	4	2966.67	15.47
1				4		0	0	0	3012.73	0	4	3012.73	15.51
1					5	0	0	0	0	3090.00	4	3090.00	15.59
0						0	0	0	0	0	5	0.00	15.00
											Overall Mean		16.98

Appendix 5.2 Matrix of mean cycle time for all permutations of tags detected

Number of Tags Detected	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Handling Time Tag 1	Handling Time Tag 2	Handling Time Tag 3	Handling Time Tag 4	Handling Time Tag 5	# Pulse Sequence Intervals	Tag Detection Time	Total Cycle Time
5	1	2	3	4	5	3553.55	3466.67	3122.86	3423.33	3347.39	0	16913.80	19.41
4	1	2	3	4		3553.55	3466.67	3122.86	3423.33	0	1	13566.41	18.57
4	1	2	3		5	3553.55	3466.67	3122.86	0	3347.39	1	13490.46	18.49
4	1	2		4	5	3553.55	3466.67	0	3423.33	3347.39	1	13790.94	18.79
4	1		3	4	5	3553.55	0	3122.86	3423.33	3347.39	1	13447.13	18.45
4		2	3	4	5	0	3466.67	3122.86	3423.33	3347.39	1	13360.25	18.36
3	1	2	3			3553.55	3466.67	3122.86	0	0	2	10143.07	17.64
3	1	2		4		3553.55	3466.67	0	3423.33	0	2	10443.55	17.94
3	1	2			5	3553.55	3466.67	0	0	3347.39	2	10367.61	17.87
3	1		3	4		3553.55	0	3122.86	3423.33	0.00	2	10099.74	17.60
3	1		3		5	3553.55	0	3122.86	0	3347.39	2	10023.80	17.52
3	1			4	5	3553.55	0	0	3423.33	3347.39	2	10324.27	17.82
3		2	3	4		0	3466.67	3122.86	3423.33	0	2	10012.86	17.51
3		2	3		5	0	3466.67	3122.86	0	3347.39	2	9936.92	17.44
3			3	4	5	0	0	3122.86	3423.33	3347.39	2	9893.58	17.39
3		2		4	5	0	3466.67	0	3423.33	3347.39	2	10237.39	17.74
2	1	2				3553.55	3466.67	0	0	0	3	7020.22	17.02
2	1		3			3553.55	0	3122.86	0	0	3	6676.41	16.68
2	1			4		3553.55	0	0	3423.33	0	3	6976.88	16.98
2	1				5	3553.55	0	0	0	3347.39	3	6900.94	16.90
2		2	3			0	3466.67	3122.86	0	0	3	6589.52	16.59
2		2		4		0	3466.67	0	3423.33	0	3	6890.00	16.89
2		2			5	0	3466.67	0	0	3347.39	3	6814.06	16.81
2			3	4		0	0	3122.86	3423.33	0	3	6546.19	16.55
2			3		5	0	0	3122.86	0	3347.39	3	6470.25	16.47
2				4	5	0	0	0	3423.33	3347.39	3	6770.72	16.77
1	1					3553.55	0	0	0	0	4	3553.55	16.05
1		2				0	3466.67	0	0	0	4	3466.67	15.97
1			3			0	0	3122.86	0	0	4	3122.86	15.62
1				4		0	0	0	3423.33	0	4	3423.33	15.92
1					5	0	0	0	0	3347.39	4	3347.39	15.85
0						0	0	0	0	0	5	0.00	15.00
											Overall Mean		17.21

Appendix 6.1 Grid arrays for individual medusae tracking trials





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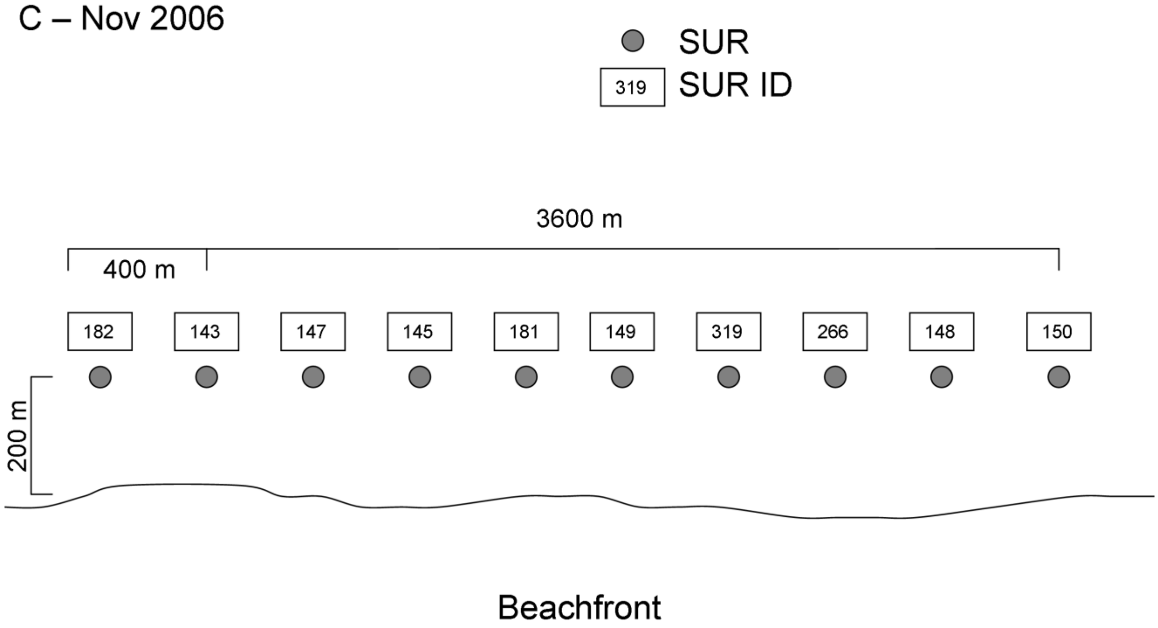
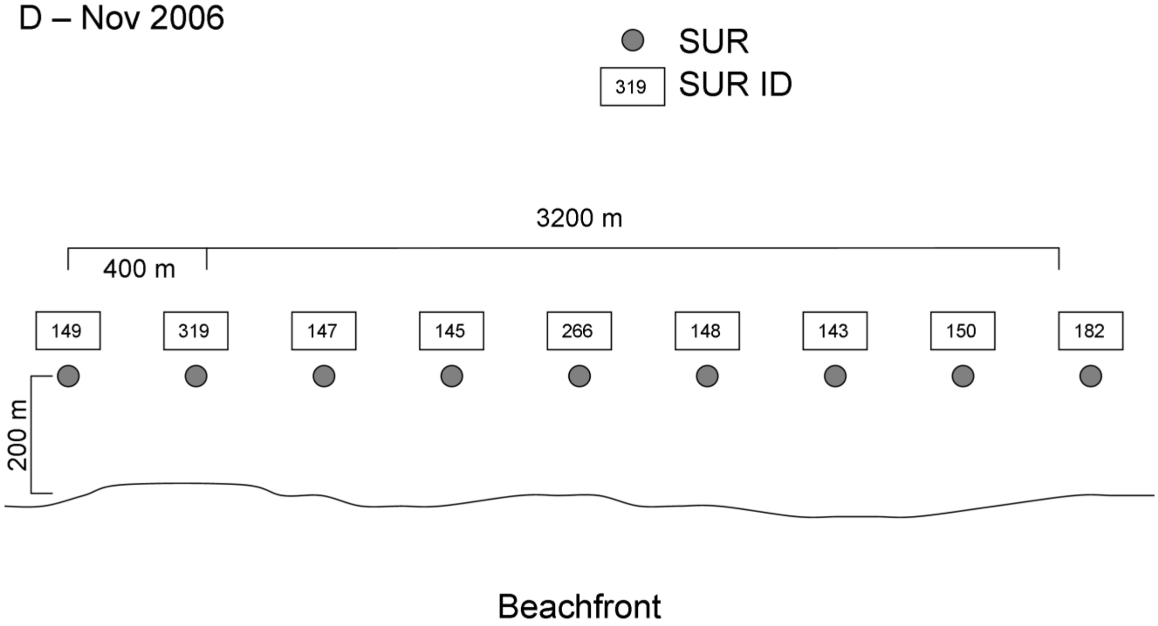
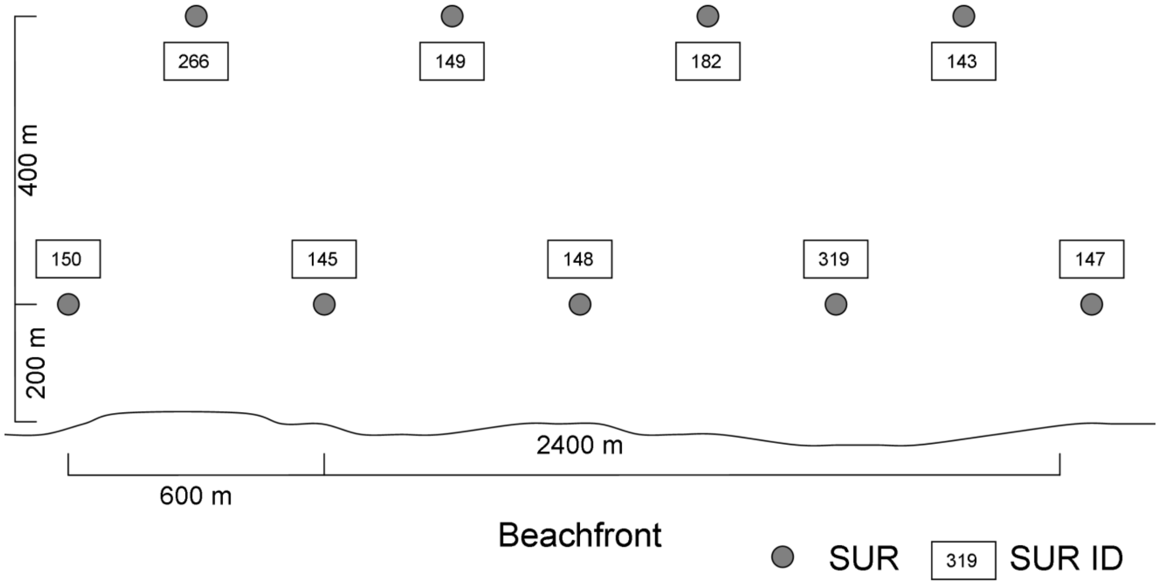


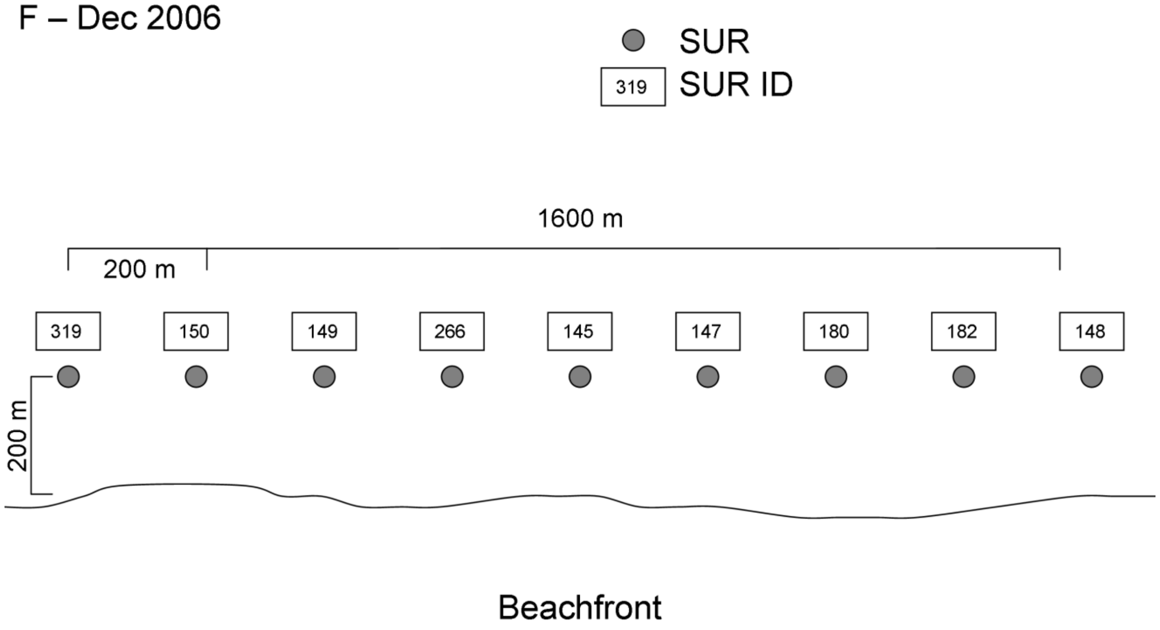
D – Nov 2006



E – Dec 2006



F – Dec 2006



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