Etiology of Irukandji Syndrome with particular focus on the venom ecology and life history of one medically significant carybdeid box jellyfish *Alatina moseri*

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Dedication:

"The sea, once it casts its spell, holds one in its net of wonder forever."

Jacques Yves Cousteau

To my family – and my ocean home

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Chapter 2

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Chapter 3

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Chapter 6

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ABSTRACT

Coming into contact with particular jellyfish species causes envenomation, and can trigger a range of debilitating, even fatal, set of symptoms called Irukandji Syndrome. Despite the risk to human health, potential losses in tourist revenue, and direct, substantial costs of treatment, there is confusion in the diagnosis and treatment of Irukandji Syndrome, and major gaps in our knowledge about the fundamental biology and ecology of the animals that cause it. This project examined the scope of Irukandji Syndrome and scrutinized the current ideas in relation to its distribution, definition and treatment. Focusing on one species known to cause Irukandji Syndrome, I examined the variation in morphology, reproduction, development and venom composition between two geographically distant populations (Australia and Hawaii) and explore whether Irukandji Syndrome diagnoses and treatments are likely to be affected by intraspecies variations in ecology.

My research into the occurrence of this syndrome identified that there is no encompassing diagnostic tool, making identification and accurate recording of this syndrome extremely difficult. I compiled a retrospective analysis of historical sting records, which represents the largest such database ever examined to date. From these data, I found that overwhelming, predominance of pain is a main symptom of Irukandji stings, and in many cases this can be the only symptom displayed. Notably, this illustrates that the most current definition for Irukandji Syndrome diagnosis as reported in the literature is not adequate, as an overwhelming 74% of these patients observed would not fit these guidelines (of experiencing at least three or more systemic symptoms). Based on my research, I propose a new diagnostic tool in order to provide a more encompassing definition of this syndrome and have named this Irukandji Syndrome Complex. I believe that this should be adopted on a

global scale and in doing so the full distribution of Irukandji Syndrome Complex stings will be revealed.

While examining the efficacy of different treatments administered for Irukandji Syndrome, I found that the amount of opiates required for treatment was indicative of sting severity and there is evidence to suggest that stings occurring out on the reef locations have a higher probability of resulting in a more severe sting with an increased potential for cardiac damage presenting in this region.

There is evidence that Irukandji Syndrome poses an increasing risk to human health through temporal extension of high-risk 'stinger' seasons on a local scale, with stings presenting over a significantly greater length of the year over the last 65 years. There has been a reduction in the proportion of stings that occur from beach locations in the Cairns region and this appears to be correlated with the change in Surf Lifesaving protocols for this area.

One species that is known to cause Irukandji Syndrome, *Alatina moseri,* occurs in two geographically distant populations: in the waters off Australia and Hawaii. To learn more about their general ecology, and whether there are intraspecific variations that may be relevant to the prevention, diagnosis or treatment of Irukandji Syndrome caused by this species, I examined both populations. There is evidence that these populations share similar morphology, though the Australian population was typically larger in size, and both have strong reproductive periodicity, with sexually mature, actively spawning animals appearing for circumlunar aggregations. However, analysis of the venom components present in this species showed distinct variation both geographically and temporally with a change in both number and frequency of proteins present. This highlights the potential for one of the variations in Irukandji Syndrome symptoms and severity I observed to result from intraspecific venom variation. The periodic aggregations of these animals do however make their

appearance highly predictable and therefore it may be possible to prevent the number of envenomations by redirecting marine users from higher risk waters at these times to allow for this occurrence and reduce sting potential.

Lastly the spawning populations encountered allowed for insight into the early life history of these animals that has not previously been described. My observations of *A. moseri*'s development from fertilized eggs right through to metamorphosed medusa supports recent research that these two populations are taxonomically one species, despite variations displayed in their venom composition. Additionally it gives a timeline and some environmental parameters for the development from the sessile polyp to active medusa stage, which is essential for further understanding of the overall biology of this otherwise cryptic, but medically relevant species.

In summary, this study has sought to advance our understanding of a debilitating condition that occurs globally, and may be expanding in range. By examining the medical diagnostic characteristics of Irukandji Syndrome, and clarifying these by bringing together all the relevant case studies, analysing the historic presentations to look for change over time, and analysing the ecology and venom composition of a species known to cause of Irukandji Syndrome, this research has provided key information that will help to better prevent, diagnose and treat it this debilitating disease.

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CHAPTER ONE: GENERAL INTRODUCTION

The words Irukandji Syndrome (describing a disease caused by the envenomation from a marine cubozoan) first appeared in the literature in the early 1950's (Flecker, 1952b) to describe a set of debilitating symptoms, which were being documented from north Queensland bathers since the 1920's. This was presumed to be the original documentation of this syndrome, however, one earlier description was uncovered to be previously reported from Manilla, 50 years prior (Old, 1908).

The definition of Irukandji Syndrome has been widely varied in its reporting over the years and this seems due to the diverse range of symptoms and severity experienced between patients. While this syndrome may be fairly innocuous to some, others have experienced severe symptoms and complications as a result of envenoming. In general, some pain appears to be felt at the sting site at the time of initial contact, however this seems to fade with time and when overshadowed by the onset of other systemic symptoms. Systemic symptoms appear to be delayed in onset but can include headache, backache nausea, vomiting hypertension, tachycardia and in extreme cases pulmonary or cerebral oedema and there have been two recorded deaths associated with Irukandji Syndrome (Fenner and Williamson, 1996, Little et al., 2003). The reasons as to why patients experience a difference in the severity of stings are unknown however theories of extent of exposure and species responsible abound.

Since the establishment of the name, the words "Irukandji" and "Irukandji Syndrome" have continued to cause a state of confusion among the general populous with a distinct lack of clarity over both the syndrome and the animals involved. For all the attention that those words receive "Irukandji Syndrome" is still in fact a fairly poorly-defined set of symptoms that occur after envenoming by certain species of jellyfish,

primarily cubozoans or ‘box jellyfish’. Additionally, the term “Irukandji jellyfish” is commonly used in reference to either a specific species of cubozoan that was first definitively linked to the syndrome (*Carukia barnesi*) or alternatively the collective group of species that also gives rise to this syndrome, so this confusion is understandable. Dr Jack Barnes discovered a causative agent from North Queensland waters in 1964 when he captured a small carybdeid species later named *Carukia barnesi* (Barnes, 1964). Upon application to the skin this species was found to give rise to the typical set of symptoms that had been termed Irukandji Syndrome. Initially this was the only species positively implicated however later on other species were also suspected and as such the name Irukandji has been used to describe all carybdeids that result in Irukandji Syndrome. To date there has been a total of seven species of carybdeids that have been shown to give rise to this syndrome (Little et al., 2006), however speculation abounds with up to 18 species implicated in various parts of the world, not all of which are members of the carybdeid family. As a result, confusion has surrounded exactly which species do and do not give rise to this syndrome.

Although Irukandji Syndrome represents a substantial risk to public health and tourism revenue (Carrette et al., 2012), little progress has been made to develop a clear diagnostic method. Because of their translucent appearance, often small size, the delayed response that can occur from a sting and fact that animals are rarely even seen let alone captured for identification at the time of envenomation, identification of the particular species giving rise to this syndrome has been greatly hampered. Although once thought to be a geographically confined syndrome the actual distribution of this disease has not been fully defined, and therefore the location of the sting is not diagnostic. There have been reports from not only various locations in Australia but from other tropical locations around the world and a few temperate regions as well (Cleland and Southcott, 1965), however species

responsible and timing of these events, like so many areas of this syndrome, is still very much lacking in research. Furthermore, appropriate First Aid for Irukandji stings has been debated between groups with some treatments previously used, now not supported by medical practitioners (Pereira et al., 2000) and additional to the confusion in treatment, is the potential for misdiagnosis of such a cryptic syndrome. No definitive test can be performed to confirm Irukandji syndrome and as such misdiagnosis can include a variety of conditions displaying similar symptoms (Carrette et al., 2012).

This thesis aims to summarize the current knowledge on Irukandji Syndrome and utilise a historical database to investigate commonalities of the syndrome as it presents to the North Queensland region and any highlight inconsistencies that exist in its current definition. It aims to use this database to investigate any occurring trends that may have practical application for diagnosis on a local and global scale.

Additionally, focus on a single species of carybdeid *Alatina moseri*, known to give rise to Irukandji syndrome is investigated for desperately needed ecological and venom based research for a medically significant carybdeid species with implications for human envenomings.

Thesis structure

Chapter one discusses the background to this research and justifies why this research was conducted while also summarising the aims of the thesis.

Chapter two determines the current state of knowledge on Irukandji Syndrome reviewing the spectrum of the literature encompassing the syndrome to date. It reveals the inconsistencies that exist in the description of this syndrome and attempts to expose the areas that require further research attention.

Chapter three conducts a retrospective analysis of stings from far north Queensland, utilising the existing hospital records and additional historic database to investigate the parameters of the syndrome as it is presented in this region. It extrapolates from here some of the ecological aspects of the animals associated with this syndrome and treatment protocols for the area. Analysis of the sting database is utilised to highlight areas and symptoms that may be useful as in a diagnostic capacity for professionals in the medical field.

Chapter four further investigates the syndrome via these hospital data records in detail as it presents to the North Queensland region and explores the range of symptoms presented. It attempts to highlight the predominant symptoms and draw attention to the indicative signs of concern with this syndrome. From here a new definition is described and the potential for its use as a diagnostic tool worldwide is discussed.

Chapter five focuses on two populations of a single species of carybdeid, *Alatina moseri*, that is known to cause Irukandji Syndrome and assesses their ecological and venom variation. It highlights the variation that exists within a single species in terms of venom composition and makes inferences about their occurrence both within these areas and the potential for extrapolation to a global distribution. The evidence for their spawning capacity is highlighted and suggestion for a wider spread global occurrence is implicated.

Chapter six utilises the spawning populations uncovered in the previous chapter to describe the early life history of a species (*Alatina moseri*) concerned with Irukandji syndrome. Hawaiian and Australian populations of this species are used to highlight the potential for taxonomic discrepancies to have occurred for this group of animals. Further evidence is presented demonstrating the existence of a single species (in opposition to the previously suggested theory of two distinct species) between these

geographically isolated populations and further submission of a single species is supported by these data. This chapter also brings attention to the necessity for further clarification of the classification of these animals and how further research into their life histories may also reveal integral information regarding their occurrence and distribution.

CHAPTER TWO: IRUKANDJI SYNDROME - A WIDELY MISUNDERSTOOD AND POORLY RESEARCHED MARINE ENVENOMING

Abstract

Irukandji Syndrome while historically synonymous with the North Queensland region, was originally found referenced in the literature some 40 years prior to its actually definition and from a different geographical region altogether. This is indicative of just how Irukandji Syndrome has had a vast range of signs and symptoms (ranging from headaches, nausea, vomiting, pain, cardiac complications, diaphoresis, and severe hypertension resulting in death) as well as occurrence associated with this disease and as such it remains to cause confusion both in its distribution and definition. In general it is defined as a set of systemic symptoms that occur after envenoming by a species of jellyfish, which are generally cubozoans or 'box jellyfish'. The syndrome has had a history of being misdiagnosed and consequently reported cases are most certainly an underrepresentation of the actual occurrence of this syndrome both locally and globally. Treatment techniques for this syndrome are still being debated and, with the exception of the general need for pain management across most cases, a standardized protocol does not exist. The growing awareness of this disease appears to be highlighting its prevalence in previously undocumented areas and there is increasing acknowledgement that it can no longer be considered a uniquely Australian problem, but a worldwide issue for marine users.

Introduction

The information comprised in this chapter is anything but clear-cut or consistent. This is due to a combination of factors that have historically contributed to confusion around this syndrome and its causes. I believe this confusion can be categorised into at least three areas of: a general lack of knowledge of detailed biochemistry, sparse understanding of toxicity of various envenomation pathways by most marine taxa,

and difficulties in field identification of causative stinging species, partially relating to difficulties in taxonomy of gelatinous plankton. Keeping this in mind, I have attempted here to comprehensively summarise the spectrum of knowledge that has been reported in regards to Irukandji Syndrome even though it is often contradictory in nature. The aim of this is to provide a detailed account of the literature that currently exists surrounding the etiology of this syndrome and summarise the lack of medical consensus surrounding what is an important medical phenomenon. For the purposes of this review chapter, I am referring to the term Irukandji Syndrome as it has been reported on in the literature. The evidence for this being attributable to a particular species or number of species is tenuous at times, but as these are the reported cases, they have been included in this review.

History of syndrome

It is widely published that Irukandji Syndrome is the name given by Dr Hugo Flecker in 1952 to his earlier described type A stings in 1945 (Barnes, 1964, Flecker, 1952b, Cleland and Southcott, 1965, Southcott, 1959, Southcott, 1963). This name was chosen on the absence of knowledge of the identity of the offending object causing the symptoms. Irukandji was the name of an indigenous tribe which formally inhabited the coastal regions between the Mowbray River to the north and Trinity Inlet around Cairns to the south (Flecker, 1952b) who knew that at certain times of the year, members of the tribe often left the water and showed debilitating symptoms. However, what is not well known is that these symptoms were first described much earlier in patients in Manilla and the cause had already been suggested to be a jellyfish, a schyphozoan, not a cubozoan (Old, 1908). In a series of 8 cases, what is now known as Irukandji Syndrome was documented in swimmers that had been bathing off a wharf in Manilla. This also seems to be the first report of the use of vinegar as a first aid treatment however its first aid potential appears to have been limited (Old, 1908).

Since the syndromes discovery in Australian waters in early 1922 where bathers in North Queensland noticed a marine sting and distressing symptoms that followed (Barnes, 1964) its occurrence has been regularly recorded in the literature. Primarily several cases were reported in December, 1935 (Flecker, 1952b) and then again in 1945 (Flecker, 1945) and it was at this time that Flecker reported that the causative agent was an unknown organism producing non-severe local symptoms but severe general symptoms (Flecker, 1945). However not all beaches in northern Australia recorded Irukandji Syndrome stings at this time, even though many were well patronized by bathers (Flecker, 1952b).

All jellyfish were suspects, as were marine weeds, fish larvae and other animals collected from water samples (Barnes, 1964). The causative agent was still unknown by 1964 when Barnes hypothesised that Irukandji Syndrome was caused by an agent that was

i) small
ii) colourless and transparent
iii) must at times be present in considerable numbers
iv) mobile

(Barnes, 1964)

After extensive detective work, Barnes then conducted the first published controlled envenoming by a cubozoan, namely *Carukia barnesi*, ("Car" from carybdeid and "ukia" from Irukandji, "barnesi" from Barnes (Kinsey, 1988)) which resulted in Irukandji Syndrome. He tested two jellyfish specimens, one on himself and his son, the other on a lifeguard. The initial sting was described as feeling like the envenoming of a small *Physalia* sp. and could be caused by the tentacles or the bell (Barnes, 1964). Within a short space of time all three subjects envenomed by the

small cubozoans developed Irukandji Syndrome and hence one of the causative agents of Irukandji Syndrome had been identified.

Although a great deal of time and effort was placed by Barnes and others into elucidating other causes of the syndrome, none were found and as such Carukiosis was proposed instead of Irukandji Syndrome or type A sting, to better reflect the causative agent of the disease (Southcott, 1966). However later work implicated other species and as such the term Carukiosis was abandoned (Kinsey, 1988). The term Irukandji is now used routinely to encompass any jellyfish that causes the set of systemic symptoms known as Irukandji syndrome (Fenner, 1999).

Since Barnes' undisputed discovery that *Carukia barnesi* caused Irukandji Syndrome, it was not until 2003 that further hard evidence was published implicating a second species of cubozoan in Irukandji Syndrome (Little and Seymour, 2003) with an additional five species being shown to cause Irukandji syndrome in the three years following that discovery (Little et al., 2006). Although there is suggestion that additional species may cause this syndrome (Gershwin, 2005, Gershwin, 2005b, Gershwin, 2007) presently there is no direct evidence to support those claims.

Cost to community

Although Irukandji Syndrome is a minor medical concern in Australian waters, it represents a major cost to northern Australian communities in terms of public health, leisure and tourism (Bailey et al., 2003). However, attributing a direct monetary cost to this syndrome is highly problematic. There is little doubt that Irukandji Syndrome presents a significant workload to emergency departments in presentations, retrievals and admissions (Little and Mulcahy, 1998). Approximately half of Irukandji Syndrome stings require admission (Bailey et al., 2003) and a significant amount of time and resources needed to retrieve envenomed patients, many of which are offshore and require helicopter retrieval (Fenner, 1999). For example, in northern

Australia in the 1998-99 Irukandji season there were 30 helicopter retrievals from remote locations, offshore islands and the Great Barrier Reef for Irukandji Syndrome at a cost of $2000-4000 per patient (approximate total cost of 90,000) (Fenner, 1999). In total, it is thought that costs in retrieving and treating Irukandji Syndrome patients in Australian waters alone ranges between $1-3 million per year (Fenner, 1999).

Coupled with these direct costs are the indirect effects that media presentation of the syndrome has on tourism. Like many dangerous marine animals, there is a disproportionate amount of media attention given to Irukandji Syndrome (Greenland et al., 2006) with much of it being incorrect. This often gives the impression to tourists that these waters are unsafe and may have a direct negative monetary effect on tourism, albeit unsubstantiated (Harrison et al., 2004, Leggat et al., 2005)

Local Pain

The majority of Irukandji stings are initially innocuous, with very little pain at the sting site. However wide variation in all aspects of the sting has been recorded with even initial contact displaying variation in reported reactions. Stings are described as usually, but not always, producing a local pain sensation, however this seems to vary greatly in its reported severity. Pain from first contact could be likened to that of a bee or wasp in some cases (Fenner and Carney, 1999, Flecker, 1952b) and noted as ‘quite severe’ (Holmes, 1996) and mild to insignificant (Burnett, 2001, Fenner et al., 1986, Fenner, 2000, Burnett et al., 1998) to apparently absent in others (Fenner, 1991, Flecker, 1952b). In the initial description naming the condition Irukandji Syndrome (previously recorded only as Type A stings) (Flecker, 1952b), stated in fact that some victims may be quite unaware that they have been stung at all (Barnes, 1964), while others noted a pain significant to make adults run from the water and sometimes reduce children to tears (Flecker, 1952a, Barnes, 1964). Local

pain is said to diminish with mild discomfort occasionally persisting until it is overshadowed by more dramatic symptoms (Barnes, 1964).

Sting site description

Similar to the vast majority of cnidarian envenomings, jellyfish that produce Irukandji Syndrome have nematocysts that penetrate into the papillary dermis and deposit venom theoretically in interstitial cellular spaces (Burnett and Calton, 1987). The shafts of the nematocysts penetrate the vascular epidermis and into the dermis where more vessels as well as nerve fibres are located (Burnett and Calton, 1987). However, unlike many cnidarian stings, Irukandji Syndrome stings do not give rise to dermo necrotic lesions and are not clinically present after envenomation (Burnett and Calton, 1987). This is possibly the result of a lack of phospholipase A2 in the nematocysts venom or the fact that the number of nematocysts on a sting site are probably very small (Underwood and Seymour, 2007).

The majority of Irukandji stings are initially innocuous, however, it is often possible to identify the sting site by wiping with a towel and watching where sweating re-appears (Kinsey, 1988). Interestingly, the shape and nature of the site of envenomation is usually not in accordance with the physical characteristics of the bell or tentacles of the medusae (Kinsey, 1988) as it is in the majority of jellyfish envenomings. It is often an oval area of erytherma (Tibballs, 2006) with a series of irregularly spaced papules (often referred to as “goose pimples”) which may be up to 2mm in diameter developing within 20 min of the sting, fading quickly after that (Tibballs, 2006, Fenner and Carney, 1999) however, erythema may last for days (Fenner and Carney, 1999). Many stings sites are not obvious due to the impact of scratching by the victim, application of vinegar and investigation by first aid providers, all of which often make the determination of the exact point of envenomation difficult for medical practitioners especially as it may be hours after the sting that the patient is seen. In one study,

20% of Irukandji victims had no signs of a sting when inspected by a medical practitioner, however, welts where found in 16% (Macrokanis et al., 2004). Given that not just cubozoans give rise to Irukandji Syndrome, the incidence of welts in this study may reflect envenomings by *Physalia* sp, which are common in the study's area (Williamson et al., 1996c).

It has been suggested that most stings occur by contact with the bell and stings from tentacles are less frequent (Fenner and Harrison, 2000) however there is little data to support such statements and as such can often just add confusion to this already complex issue. Known stings from just tentacles of *Carukia barnesi* have resulted in the above-described oval area of goose bumps and erythema (Figure 2.1) and as such it is all but impossible to determine the envenoming portion of the medusae for the shape of the sting site alone. In the majority of the species known to give rise to Irukandji Syndrome there is a difference in the cnidome in both density and composition between tentacles and bell and distinct differences of these cnidomes between species (Huynh et al., 2003). As such there exists a potential technique for determining both the species of cnidarian and the portion of the medusae responsible for the sting by skin scrapings. This technique has been shown to have an 80% success rate in retrieving nematocysts off the patients and subsequent nematocysts identification, when a sting site can be clearly determined (Huynh et al., 2003). The problem with this technique is that there may be few, if any, remaining nematocysts on the skin after presentation to medical aid (Fenner et al., 1986b) and this may be due to the density of nematocysts in the bell warts or the nematocysts bundles on the tentacles being very small (in the order of hundreds, not hundreds of thousands) as has been seen for *Carukia barnesi* (Underwood and Seymour, 2007). It is worth noting that nematocysts recovered from skin scrapings that do not match any occurring on a suspected jellyfish immediately exonerate it, but matching

nematocysts do not necessarily mean that species is the culprit as many species have an overlap in nematocyst types (Barnes, 1960).

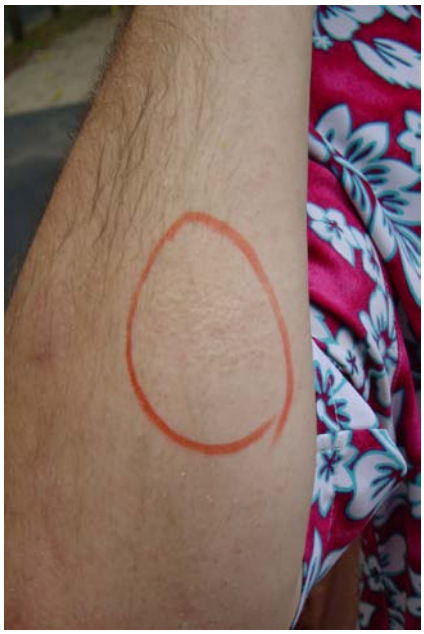

Plate 2.1 – Sting site from *Carukia barnesi* circled with a red marker on the patient's arm

In general, while sting sites that have been recorded on patients that later develop Irukandji Syndrome are small and innocuous and disappear within several hours, there are recorded cases where long lasting effects at the sting site occur. For example, consistent local itching and the presence of lesions at a sting site from *Carybdea alata* was seen for a period of seven months from the time of envenoming (Tamanaha and Izumi, 1996). In this case though the patient was initially treated with topical papain-based meat tenderiser (which causes protein disruption) and alcohol rubs (Tamanaha and Izumi, 1996) so it is possible that these effects were seen due to the protein disruption and not the cnidarian venom.

Latent period

Unlike the majority of cnidarian envenomings there is generally a delay from the time of the sting to the onset of systemic symptoms. This has been reported to range

from as little as 5 minutes (Flecker, 1952a, Fenner, 1991, Barnes, 1964) up to 40, 60 and 120 minutes (Fenner, 1991), (Flecker, 1952a), (Barnes, 1964) respectively, with an average time of 25-40minutes being the general consensus (Barnes, 1964, Flecker, 1952b, Fenner et al., 1986, Fenner, 1991, Holmes, 1996).

Range of Symptoms

Irukandji syndrome patients routinely show systemic symptoms which may include headache, backache, nausea, vomiting, abdominal cramps, overall body pain, hypertension, tachycardia, feelings of impending doom, pulmonary and/or cerebral oedema (Greenland et al., 2006), however, presently there exists no singular encompassing definition of the syndrome, with a wide and varied series of symptoms being displayed (see Table 2.1)

Table 2.1 Summary of various reported symptoms from Irukandji Syndrome patients

Symptoms	Description	Reference
Local	Perfuse sweating, generalised hyperhydrosis	(Barnes, 1964, Flecker, 1952b, Southcott, 1963)
	Stung area sweats profusely first with entire body sweating later	(Kinsey, 1986)
	Profuse sweating of sting site that reappears when wiped	(Stuart and Slagle, 1943)
	No sweating of sting site	(Southcott, 1963)
	Profuse sweating and then non-sweating a the sting site which may be from initial anhidrosis followed by compensatory hyperhydrosis	(Kinsey, 1988)
	Tremor	(Yoshimoto and Yanagihara,

		2002)
	Erythema or welts, diaphoresis or flushing	(Macrokanis et al., 2004)
	Sweating	(Fenner and Hadok, 2002, Fenner, 1991)
	Moderate to severe pain 80%	(Mulcahy and Little, 1997)
	Severe pain	(Fenner and Carney, 1999)
	Mild discomfort at sting site persisting until overshadowed by more severe symptoms, 5 minutes post sting, site marked by patch of erythema, typically oval in shape and usually 5 by 7cm wide	(Barnes, 1964)
	Acute pains in effected part, Minimal rash at sting site with slight redness and raised vesicles	(Southcott, 1963)
	Small insignificant puncture	(Flecker, 1952b)
	Absence of wheals	(Flecker, 1952b, Flecker, 1945)
Musculoskeletal	Violent cramps and muscle pain	(Flecker, 1952b)
	Jaw pain	(Fenner, 2001)
	Long lasting joint pain	(Southcott, 1963, Barnes, 1964, Fenner, 2001)
	Muscle cramps, 73% of patients in latter study	(Yoshimoto and Yanagihara, 2002, Mulcahy and Little, 1997)
	Cramping and spasms of intercostals and diaphragm	(Fenner, 1991) (Fenner, 2000)
	Chest pain and tightness from cramping	(Barnes, 1964, Fenner, 1991)
	Severe and boring pain in sacral or lower back area, 40%	(Barnes, 1964, Fenner, 1991) (Fenner, 2000)
	Muscle pains and cramps in all four limbs, limb pain 58%, abdominal pains and cramping, rigid abdominal wall, abdominal pain 92%	(Southcott, 1963, Barnes, 1964, Fenner, 2000)
	Hyperactive deep reflexes	(Southcott,

		1963)
	Lie prostrate	(Flecker, 1957)
Gastrointestinal	Painful vomiting and retching, excessive vomiting, 33% had vomiting, nausea and vomiting 50% , 57%	(Flecker, 1945, Flecker, 1952b, Yoshimoto and Yanagihara, 2002, Macrokanis et al., 2004, Fenner, 2000),(Mulcahy and Little, 1997) (Barnes, 1964, Southcott, 1963)
	Colicky pains in epigastrium	(Flecker, 1945, Southcott, 1963)
Neurological	Distressed on presentation, distress and anxiety 70%	(Mulcahy and Little, 1997, Macrokanis et al., 2004)
	Anxiety with feeling of impending doom, Restlessness	(Fenner, 1991)
	Severe frontal or global headache, mild headache	(Fenner, 2000, Barnes, 1964, Flecker, 1957, Holmes, 1996)
Cardiac	Cardiac muscle pain	(Fenner, 2000)
	Hypertension, tachycardic and systolic hypertension 50%	(Yoshimoto and Yanagihara, 2002) (Macrokanis et al., 2004, Huynh et al., 2003)
	Global cardiac dilation with left ventricular dysfunction	(Fenner and Hadok, 2002)
	Intraventricular tachycardia and transient dilated cardiomyopathy	(Hawdon and Winkel, 1997)
	22% patients elevated troponin levels, T wave inversions and ST segment depression, Mild impairment of systolic function to moderate dysfunction with segmental hypokinesis	(Huynh et al., 2003)
	Irregularly irregular heart beats	(Southcott, 1963)
	Myocardial infarction	(Salam et al., 2003)

	Cardiogenic shock	(Holmes, 1996)
Miscellaneous	Oliguria	(Fenner and Hadok, 2002)
	Priapism, allergic reactions, expiratory wheeze	(Fenner and Carney, 1999)
	Respiratory distress 7 - 10.5 hours post envenomation	(Fenner et al., 1988)
	Localised piloerection (sometimes generalised), uncontrolled tremor, hyperventilation	(Fenner, 1991)
	Difficulty in breathing, dry mouth, burning eyes, sharp prickling sensation, cold, violent shivering, Marked neutrophil leucocytosis within first hour, cough 57%	(Barnes, 1964)
	Squirting redness of sting sites	(Kinsey, 1986)
	Raised temperature may result, pyrexia	(Southcott, 1963)
	Shock, collapse	(Flecker, 1957)
	Over stimulation of the sympathetic system	(Fenner et al., 1986)
	Periorbital oedema	(Fenner and Carney, 1999)
	Pulmonary oedema	(Holmes, 1996)

Geographical variations have also been proposed for Irukandji Syndrome disparity (Fenner, 1999, Fenner and Hadok, 2002, Fenner and Carney, 1999, Macrokanis et al., 2004) both between locations such as central Queensland and Cairns (Fenner, 1999) but also within the one region, with Irukandji Syndrome in Broome reported to cluster into two distinct groups (Gershwin, 2005b). One of these clusters is allegedly distinct as it causes nausea and vomiting and symptoms of shivering cold with good response to analgesia and only mild hypertension, therefore being less life threatening variety (Gershwin, 2005b). The second cluster is suggested to lack the nausea and vomiting component of the syndrome however is more severe and results in life threatening hypertension, intense pain and paralysis (Gershwin, 2005b), although actual empirical evidence is lacking to support this hypothesis.

There has also been reported some distinct syndrome variability in Broome stings when compared with the sting data from North Queensland envenomings suggesting different potential causative agents (Macrokanis et al., 2004). Since an all-encompassing description of this disease has never been defined for either region, these observations at this stage remain purely speculative.

Duration of syndrome

While some Irukandji Syndrome stings appear to resolve within a matter of hours (Holmes, 1996, Kinsey, 1988), it is estimated that around 10-70% of patients require hospital admission and treatment for 24-72hours (Fenner and Harrison, 2000). Duration of symptoms and effects from Irukandji Syndrome is reported as generally resolving after a few days (Flecker, 1957, Kinsey, 1988, Burnett et al., 1998, Burnett, 2001, Barnes, 1964, Holmes, 1996) however continued complications have been reported as lasting anywhere from weeks (de Pender et al., 2006, Burnett et al., 1998) to months (Little et al., 2001, Fenner and Carney, 1999, Burnett, 2001, Huynh et al., 2003) with one patient displaying mild ST depression continuing at maximal exertion for at least 6 months post sting (Fenner and Carney, 1999). While the longest admission time reported on was fourteen days in hospital with eleven of those days spent in the intensive care unit (Little, 2002)

Severe Symptoms

Primary concern for presenting Irukandji Syndrome stings aside from management of severe pain with analgesia is controlling hypertension that may potentially lead to pulmonary and cerebral oedema and serious cardiac abnormalities (Greenland et al., 2006, Little and Mulcahy, 1998, Fenner and Carney, 1999). Severe envenomings do occur, often resulting in cardiac complications (Fenner and Carney, 1999, Huynh et al., 2003, Little et al., 2001, Little et al., 2003, Fenner et al., 1988, Tibballs et al., 2001, Martin and Audley, 1990, Fenner and Hadok, 2002). Fenner (Fenner and

Carney, 1999) reported that over the period from November 1998 to May 1999 30% of Irukandji Syndrome patients experienced some degree of heart failure, however in this particular report it seems unclear as to the extent of this and where these stings originated from geographically. Perhaps more indicative of Irukandji Syndrome stings, from the Cairns region at least, are data from Huynh *et al* (2003) during which a retrospective analysis of a years' worth of presenting Irukandji Syndrome stings (totalling 128 patients) saw 22% experiencing an elevated cardiac troponin (cTnI) levels, which are indicative of cardiac damage.

Significant cardiac dysfunction has been reported in several cases (Little et al., 2003, Fenner et al., 1988, Huynh et al., 2003, Burnett et al., 1998, Burnett and Calton, 1987) with one reported case requiring ventilation (and inotropic support) for 8 days (Little et al., 2001). Pulmonary oedema has been noted in a few cases of Irukandji Syndrome (Little et al., 2001, Fenner et al., 1988, Martin and Audley, 1990, Fenner, 2000) and therefore it has been said that the Syndrome must be classed as potentially life threatening (Fenner et al., 1988) but pulmonary oedema is said to be a rare, with one report stating a 2% occurrence after 10-12 hours (Little and Mulcahy, 1998).

Deaths

Two deaths from Irukandji Syndrome have been published so far. The first reported death to have occurred was in January of 2002 at Hamilton Island (Fenner and Hadok, 2002), however, the cause is speculative as no supporting evidence of envenoming (for example the presence of nematocysts on the victim) was obtained (Bailey, 2003, Dawson, 2003), and no post mortem performed (Fenner and Hadok, 2002). In this case the patient also had a pre-existing cardiac condition with a history of an aortic valve replacement and was on Warfarin medication (Fenner and Hadok, 2002). Because of these factors it has been suggested that death may have been

due to over-coagulation not necessarily caused by the Irukandji Syndrome but as a result of this pre-existing condition (Dawson, 2003).

The second recorded death from an Irukandji Syndrome patient occurred on the outer Great Barrier Reef that same season in April 2002 (Fenner and Hadok, 2002). In this case the patient was a 44-year-old male, who suffered a sting to his chest while snorkelling 25kms north of Cairns. Within 15 minutes he displayed signs of Irukandji Syndrome and was evacuated to the Emergency ward of Cairns Base Hospital. A cranial CT was performed and an intracerebral haemorrhage was discovered in the right frontal lobe. The patient underwent a craniotomy, however, progressed to brain death 13 days post sting. Nematocysts removed from the sting site were identified as those from a large (>20mm) *Carukia barnesi* making this the first definitive death reported from this species (Pereira et al., 2010).

First Aid

In the initial treatment of potential Irukandji Syndrome patients, the main hindrance to first aid measures is the often minor initial nature of the sting (Martin and Audley, 1990).

There is a plethora of untested and ineffective treatments (Isbister, 2004a) for first aid of potential Irukandji stings patients, including but not restricted to, meat tenderiser, freshwater, aluminium sulphate, figs, mustard, manure, urine, tea, cola, aluminium sulphate, and alcohol (Holmes, 1996, Thomas et al., 2001a) and papin (Nomura et al., 2002, Thomas et al., 2001a) none of these have been shown to be effective and in cases may make the situation worse by resulting in the firing off of otherwise undischarged nematocysts (Holmes, 1996). Similarly Pressure Immobilisation Bandage (PIB) was once thought to be an appropriate first aid treatment for Irukandji Syndrome, with little if any evidence to support its introduction (Holmes, 1996). Data now exist that suggest that the application of a PIB may be

inappropriate (Tibballs et al., 2001) and has the potential to worsen patients' symptoms (Little et al., 2001) by increasing the venom load (Pereira et al., 2000, Little and Mulcahy, 1998, Little, 2002, Little, 2002).

Similarly, the use of ice packs for Irukandji Syndrome patients is now believed to have no effect on the management of the syndrome (Martin and Audley, 1990). Conversely, there is mounting evidence that the use of heat is beneficial for cubozoan envenomed victims (Carrette et al., 2002b, Taylor, 2000, Burnett, 2001, Taylor, 2007) and hence may assist the treatment of Irukandji Syndrome (Thomas et al., 2001a, Nomura et al., 2002, Yoshimoto and Yanagihara, 2002) .

Possibly the most misunderstood first aid for potential Irukandji Syndrome stings is the use of vinegar. Reports exists that dousing the envenomation site with vinegar is of little benefit (Holmes, 1996) contradicting a suggestion that vinegar alters the pH of the protein toxin, rendering it less biologically active (Nomura et al., 2002), however, there are no data to support either of these assumptions. The derivation of the use of vinegar originated from studies on the first aid for the large box jellyfish *Chironex fleckeri* (Hartwick et al., 1980). This study showed that vinegar caused permanent de-activation of all undischarged nematocysts but did not decrease pain in an envenomed victim nor did it de-activate the venom. Studies now show that vinegar is effective in nematocyst inactivation in a least five species of carybdeids (Fenner and Williamson, 1987). Given the small area of envenomation in most victims (and hence the small number of nematocyst and venom injected), and the severity of the syndrome, any prevention of nematocysts discharge is thought to be advantageous (Fenner et al., 1988).

Presently, the accepted first aid treatment for any potential Irukandji Syndrome is the application of vinegar, for a minimum of 30 seconds, and then monitoring of the patient and treating reactively (Little and Mulcahy, 1998). Victims should not re-enter

the water as delayed effects may impair breathing, muscle power and co-ordination and increase risk of drowning (Kinsey, 1986)

Medical Aid

Evidence into the most efficient medical treatment for control of Irukandji Syndrome has been described as anecdotal at best (Bailey et al., 2003) with the lack of universal approval being met from any single analgesic agent (Bailey et al., 2003). Irukandji Syndrome patients can experience potential implications from high analgesia requirements, uncontrolled hypertension, pulmonary oedema and cerebral oedema (Greenland et al., 2006), with higher risk categories including those with pre-existing cardiovascular pathologies, such as hypertension, ischemic heart disease, arrhythmias and bleeding disorders (Winkel et al., 2005).

Initial treatment of Irukandji Syndrome stings included potential use of trichlorethylene as an analgesic (Cleland and Southcott, 1965) and sodium phenobarbital given intravenously for control of spasms (Frachtman and McCollum, 1945). Both calcium chloride and calcium levulinate were trialled without apparent effect (Frachtman and McCollum, 1945), however calcium gluconate was used in two cases that saw immediate relief of pain and dyspnoea and relief of cramping in extremities (Stuart and Slagle, 1943, Southcott, 1956). Systemic antihistamines, corticosteroids and hydrocortisone were found to offer little to no benefit for symptoms (Burnett and Calton, 1987, Fenner et al., 1986b). The use of calcium channel blocking agents is reported to be speculative (Fenner et al., 1988), however, ataraxic drugs such as chloropromazine and butyrophenones which are catecholamine antagonists were suggested to be useful (Fenner et al., 1988). Excess catecholamines were treated with phentolamine intravenously until reduction in hypertension, shaking and sweating was achieved (Fenner et al., 1988, Fenner et al., 1986b, Martin and Audley, 1990). Verapamil has also been tried without

evidence of efficacy (Holmes, 1996). Diazepam was found to relieve anxiety (Fenner et al., 1986b) and some relief in patients, however, pain returned in 30 minutes time (Fenner et al., 1986b). Frusemide, dobutamine (Little et al., 2001), hydralazine (Holmes, 1996), Sodium nitroprusside or GTN (Little et al., 2001) streptokinase, asprin and propanolol have also been administered with nitrates and morphine infusions (Salam et al., 2003). There appeared to be some reduction in the symptoms and opiate requirements when *Chironex fleckeri* antivenom was administered to patients, however, the authors on this two patient trial concluded that it was not a beneficial treatment (Fenner et al., 1986b).

Control of pain is of predominant concern in the majority of Irukandji Syndrome cases with one study reporting 61% of patients requiring parenteral narcotics (Little and Mulcahy, 1998) and for this a variety of opiates have been utilized. Intravenous morphine and pethidine have been the predominant opiates used over the years (Fenner et al., 1988, Little and Mulcahy, 1998, Fenner et al., 1986b, Salam et al., 2003, Kinsey, 1986), however, concerns have been raised over the use of pethidine (Little et al., 2001, Little and Mulcahy, 1998, Greenland et al., 2006, Bailey, 2003) due to the direct myocardial and respiratory depressant effect and toxic metabolite (norpethidine) (Little and Mulcahy, 1998). Large doses of pethidine can have a myocardial depressant effect and worsen the cardiovascular function of the patient (Little and Mulcahy, 1998, Bailey, 2003), and similarly even large doses of morphine can cause respiratory depression and sedation (Greenland et al., 2006). In a Cairns study 40% of patients who were admitted and being treated for Irukandji Syndrome, required over 100mg of pethidine intravenously (Mulcahy and Little, 1997). Recent suggestions have been towards the preference of using fentanyl over either morphine or pethidine due to the myocardial depressant action in Irukandji Syndrome venom (Little et al., 2001, Mulcahy and Little, 1997, Little and Mulcahy, 1998) and the potential for detrimental effects from either of these. Interestingly, the early

application of promethazine has displayed potential for reducing the amount of narcotics required (Little and Mulcahy, 1998, Mulcahy and Little, 1997) and intravenous benzodiazepines gave improvement in Irukandji stings seen in Florida (Grady and Burnett, 2003).

Unfortunately there is still no standard for this treatment, with treatment varying with attending physician (Greenland et al., 2006). For cases requiring larger opiate loads, chest x-rays and electrocardiograms to check for pulmonary oedema are recommended (Little and Mulcahy, 1998) with oxygen therapy, vasodilators, continued adrenaline, inotropic support mechanical ventilation or application of CPAP required for severe cases (Tibballs, 2006, Little et al., 2001). Cases displaying myocardial infarction have seen administration of streptokinase, aspirin and propanolol administered with nitrates and morphine infusions (Salam et al., 2003).

Aside from pain control measures there had been little trialled in terms of control of additional systemic symptoms for Irukandji Syndrome patients until the first introduction of magnesium treatments in 2003. Magnesium sulphate was first used in February 2003 to treat hypertension suffered after an Irukandji Syndrome sting, with the patient receiving a 10mmol bolus and 5mmol per hour for 11hours, then reduced to 3mmol for 9hours (Corkeron, 2003). In this initial case the systemic features, pain and agitation of the syndrome was reportedly resolved at the end of the initial loading dose however recrudescence of hypertension, back pain and piloerection was evident with the early reduction of the magnesium treatment (Corkeron, 2003). While uncertainty remains as to how magnesium does alter Irukandji Syndrome, it is possible that it inhibits calcium influx, noradrenalin and possible acetylcholine release (Corkeron, 2003, Little, 2005). Magnesium treatment (predominantly magnesium sulphate but also magnesium chloride) theoretically both decreases the catecholamine release and sympathetic terminal receptivity to catecholamines and also reduces the catecholamine induced myocardial necrosis

(Corkeron, 2003, Winkel et al., 2005). Variability in receptiveness to magnesium treatment was observed even in this initial study with loading doses ranging between 5-20mmol (ten patients) and mean administered total dose being 66mmol. Some pain reduction was observed in these treated patients (six out of ten) and an average decrease in experienced hypertension of 18mm hg over the ten patients. There was no relationship found between severity and total magnesium load, however this trial size was fairly limited. From this initial report, suggested dose of treatment of magnesium for Irukandji stings was 10mmol loading dose bolus over 15 minutes, repeated if necessary, followed by an infusion of 5-8 mmol/hr titrated to effect and reduced slowly (Corkeron, 2003).

Treatment success of magnesium since this initial trial has had mixed success with other authors reporting that hypertension was poorly controlled by intravenous magnesium (Fenner and Lewin, 2003) however no dosages were reported in this case. Two other case reports saw magnesium infusions administered with no effect on treatment (Little, 2005).

Magnesium usage in Irukandji patients is based on its ability to decrease vascular resistance in hyperadrenergic states and the potential for it to suppress catecholamine release (Tibballs, 2006), however despite some testimonials of its proven effectiveness (Fenner and Lewin, 2003), there is great variation in its success (Fenner and Lewin, 2003, Little, 2005) and authors generally agree that the use of magnesium infusion should not be used as routine treatment until further definitive evidence arrives or until other treatment approaches have failed (Corkeron, 2003, Little, 2005, Grady and Burnett, 2003).

Similar controversy surrounds the evidence for routine usage of sublingual glyceryl trinitrate (GTN) as pre hospital treatment for Irukandji patients suffering hypertension (Isbister, 2004b, Fenner and Lewin, 2003, Little et al., 2004, Bonham, 2004, Fenner,

2004). Hydralazine, sodium nitroprusside or GTN were initially suggested in 1996 (Holmes, 1996) to control hypertension and since then the use of GTN has become far more common. Correlations were initially reported for the application of GTN with decrease in blood pressure for three patients with Irukandji Syndrome from the Whitsunday Island region (Fenner and Lewin, 2003). It has been argued however that with the limited patient study group and the addition of morphine to these patients that correlation but no causation can be drawn from this study (Little et al., 2004) so while no comprehensive patient trial has been conducted, evidence for addition of nitrates in controlling Irukandji induced hypertension is still speculative (Little et al., 2004, Fenner, 2004, Holmes, 1996). While infusions of phentolamine for reduction in hypertension have had success in patient treatment it is still suggested that a titratable nitrate for therapy would be preferable (Tibballs, 2006).

Alpha and beta-blocking drugs present potential for high risks in treatment of Irukandji Syndrome due to their associated marked hypotension side effects (Fenner and Carney, 1999, Little et al., 2001). Beta blockers are reported as occasionally leading to disastrous hypotension and potential for renal failure (Fenner and Carney, 1999) and should not be used without concomitant alpha blockade as unopposed alpha adrenergic activity may predispose to myocardial infarction and myocardial necrosis (Holmes, 1996). If alpha blocking drugs are administered, then early echocardiography and cardiovascular monitoring is recommended to occur prior to administration of these drugs (Little et al., 2001).

Irukandji treatments have had such varying success both within and between regions and remain purely supportive and non-specific (Grady and Burnett, 2003) based on treating the specific presenting symptoms (Greenland et al., 2006).

Misdiagnosis

With a historically poor understanding of the definition of Irukandji Syndrome and its possible complications, along with the fact that little or no sting mark is left at the site and the initial sting is mild with symptoms taking some time to onset and the severity arriving quickly, there seems little doubt that Irukandji Syndrome has been misdiagnosed in the past, with more commonly known ailments being blamed in the absence of knowledge regarding this disease. It has since been suggested that deaths which have been attributed to drowning may in fact have been indirectly caused by Irukandji Syndrome as a person may easily drown if in deep water at the onset of Irukandji Syndrome cramps, similarly, respiratory muscle spasm might be severe enough to cause death from asphyxia and water inhalation (Old, 1908, Stuart and Slagle, 1943). This was highlighted by Barnes who suggested that suspected sting victims should not re-enter the water as they risk drowning from the sudden onset of immense pain (Kinsey, 1986). With a historically poor understanding and diagnosis of Irukandji Syndrome and its potential complications, there is little doubt that deaths due to Irukandji Syndrome may have been missed in the past (Holmes, 1996, Fenner and Hadok, 2002). Irukandji Syndrome has previously been misdiagnosed as acute appendicitis (Kinsey, 1986, Southcott, 1959, Barnes, 1964), decompression illness (Fenner, 1999, Greenland et al., 2006, Williamson and Exton, 1985, Grady and Burnett, 2003, Fenner and Carney, 1999), gastric poisoning, peptic ulcer, ruptured spleen, ruptured ectopic pregnancy (Barnes, 1964) and myocardial infarction (Fenner and Carney, 1999, Greenland et al., 2006).

Why the delay?

The delay of the symptoms is one of the most significant indicators of the syndrome, but one of the least understood. The reasons for this delay may include but are not restricted to the possibility that the venom requires a period of time before the toxins

are metabolised in an envenomed victim (Kinsey, 1986) or that the already active venom requires time to travel from the site of initial deposition to the toxin target site.

The assumption is that venom reacts similarly in humans as it does in prey items, however, this does not appear to be correct. Although the literature on the feeding ecology is scarce, there are data that show that at least in one species, *Carukia barnesi*, the toxic effects of the venom on prey items is rapid, with envenomed fish usually succumbing within minutes (Underwood and Seymour, 2007).

Intravenous injection of venom from Irukandji Syndrome producing jellyfish *Carukia barnesi* and *Alatina* sp. into test animals causes hypertensive crisis and death in a similar time frame to that caused by *Chironex fleckeri* venom, namely within minutes (Ramasamy et al., 2005, Winter, 2008, Winkel et al., 2005, Winter et al., 2008). As such, the delay in symptoms would not appear to be caused by a latent period of time for the venom to become active.

It is well known that snake bite victims, when no first aid is administered, often have delays of symptoms. It has been suggested that this is brought about by the introduction of the toxins firstly into the slower moving, open lymphatic system before being transferred into the closed circulatory system, where it travels to target sites, after a period of about 20-30 minutes. It is known that the venom components in Irukandji venoms are large (50-100 kilodaltons) (Underwood and Seymour, 2007) thus they may travel via the lymphatics causing the characteristic delay in symptoms (Carrette, 2012, Little and Mulcahy, 1998).

Causative Agents

While initially the term Irukandji Syndrome was associated predominantly with the Cairns, North Queensland region from which the name was derived, it is now becoming evident that Irukandji Syndrome occurs from various species of jellyfish and in both hemispheres (de Pender et al., 2006, Stuart and Slagle, 1943, Otsuru et

al., 1974, Thomas et al., 2001a, Frachtman and McCollum, 1945, Burnett, 2001, Fenner et al., 1985, Gershwin, 2005b, Gershwin, 2007, Little and Seymour, 2003, O'Reilly et al., 2001, Little et al., 2001, Barnes, 1960, Fenner, 1991, Huynh et al., 2003, Gershwin and Alderslade, 2005). Symptoms experienced vary from a few systemic symptoms (Thomas et al., 2001a, Little and Seymour, 2003, Barnes, 1960, Otsuru et al., 1974) to what could be termed a 'severe' Irukandji sting (Little and Seymour, 2003, Little et al., 2001, Fenner et al., 1985, Frachtman and McCollum, 1945)

Unfortunately, the majority of cubozoans and in particular the carybdeids are generally fairly innocuous in appearance, often relatively small in size and difficult to identify precisely even when creatures are seen (Hadok, 1997), accurate identification of an envenoming event/animal are relatively rare. Evidence of species causing the symptoms range from speculative (Gershwin, 2005b) to definitive evidence where the victim managed to collect the specimen at the time of envenoming (Little et al., 2006).

Factors further complicating which species may or may not be responsible for an envenomation are the discrepancies in features that define one species from another. Ontogenetic changes have been proposed and seen in certain morphological characters used to define individual types of carybdeids i.e. gastric filaments (Uchida, 1970), velarial canals (Gershwin and Alderslade, 2005), nematocyst wart density and tentacle structure (Pereira et al., 2010, Underwood and Seymour, 2007) and so some uncertainty still surrounds accurate identification (Gershwin, 2005b). Presently 18 species of cnidarians have been implicated in causing Irukandji Syndrome (Table 2.2) however the data supporting many of these claims remains speculative.

Table 2.2: Species of cnidarians suggested to be involved in the production of Irukandji Syndrome

Species implicated	Reference
Carukia barnesi	(Southcott, 1963)
Gerongia rifkinae - a.k.a "Darwin Carybdeid"	(O'Reilly et al., 2001)
2 types of unidentified "Morbakka" carybdeids	(Fenner, 1991)
Unnamed carybdeid	(Little and Seymour, 2003)
Alatina nr *mordens*	(Little et al., 2006)
Malo maxima	(Little et al., 2006)
Carybdea alata	(Little et al., 2006, Thomas et al., 2001b)
*"*Fire jellies*" Carybdied*	(Little et al., 2006)
Carybdea xaymacana	(Little et al., 2006)
Malo maxima	(Gershwin, 2005)
Carukia shinju	(Gershwin, 2005)
Malo kingii	(Kinsey, 1988, Gershwin, 2007)
Cyanea sp. 'Lion's mane'	(Barnes, 1960, Cleland and Southcott, 1965)
Physalia physalis 'Portuguese man-o-war'	(Frachtman and McCollum, 1945, Stuart and Slagle, 1943)
Gonionemus oshoro	(Otsuru et al., 1974)
Unknown jellyfish - Thailand	(de Pender et al., 2006)
Rhizostoma sp.	(Cleland and Southcott, 1965)
Alatina rainensis	(Gershwin, 2006b)

Notably, not all carybdeids are implicated in leading to Irukandji Syndrome. *Carybdea sivickisi* has not been reported as causing Irukandji Syndrome (Hoverd, 1985, Hartwick, 1991b) neither is *Carybdea rastoni* (Cleland and Southcott, 1965, Southcott, 1958, Nagai et al., 2000, Barnes, 1964, Uchida, 1970), *Carybdea marsupialis* (Rottini et al., 1995, Peca et al., 1997), *Tripedalia binata* (Moore, 1988),

Alatina rainensis (Gershwin, 2006b) or *Gerongia rifkinae* commonly called the 'Darwin carybdeid' (Gershwin and Alderslade, 2005) however *G. rifkinae* has been reported with having some symptomatic overlap with the syndrome such as abdominal pain (O'Reilly et al., 2001).

It would appear that it is not only the carybdeids that have been implicated in this syndrome though, as there is evidence for non-carybdeid jellies to also be responsible. Contact with the scyphozoan *Cyanea* sp. has been reported to cause nausea, backache and slight abdominal pain in children when multiple but not extensive weals occur (Barnes, 1960). *Physalia* sp. is a colonial hydrozoan found in both the northern and southern hemispheres (Williamson, 1992) and has previously been stated as not producing Irukandji Syndrome (Flecker, 1945, Flecker, 1957). Conflicting reports, however, come from *Physalia* sp. stings leading to systemic symptoms from both Hawaii (Frachtman and McCollum, 1945) and Puerto Rico (Stuart and Slagle, 1943).

Another hydromedusae *Gonionemus oshoro* was applied to five experimental volunteers of which three developed systemic symptoms (Otsuru et al., 1974). This report also documented the development of systemic symptoms in people who eat raw seafood from the habitat of this species and this is believed to result from ingesting these hydrozoan specimens with the seaweed (Otsuru et al., 1974). Reports of Irukandji Syndrome symptoms from a *Rhizostoma* sp. medusae in the Swan River in 1963 are reported (Cleland and Southcott, 1965) and small medusae found in crevices in Dutch New Guinea was reported as having the potential to cause death to a diver from drowning if they were to be stung several times (Cleland and Southcott, 1965).

Taxonomic inconsistencies

Problems with determining causative agents responsible for this syndrome also lie with the dearth of circumstantial reports implicating various species while lacking empirical evidence. For example *Malo kingi* was suggested as causing the second death from Irukandji Syndrome (Gershwin, 2007) however this was based on only two of the three nematocysts types recovered from the victim and as *M. kingi* is reported to only possess two types this suggests in fact that it could not have been this species. Since then this death has been attributed to an adult *Carukia barnesi* envenoming (Pereira et al., 2010). The other stings reported to have resulted from contact with *M. kingi* are far less severe in syndrome and it would seem that this species has resulted in a purely localised reaction to mild Irukandji Syndrome where hospitalisation is not typically sought (Gershwin, 2007).

Carukia shinju and *Malo maxima* are two carybdeids from Western Australia that have also been proposed as giving rise to Irukandji Syndrome (Gershwin, 2005b) (Table 2.2). *Malo maxima* is stated to be apparently extremely dangerous, and linked to a severe case of Irukandji Syndrome that left a 41year old male in critical condition on life support for three days (Gershwin, 2005b). It is however noted by the author of this case that they lack any empirical evidence for this envenoming as skin scrapings have not been accessed, nor was any animal spotted at the scene or in the surrounding waters at the time. Other stings allegedly from this species have been reported from the pearl divers off Broome who believe this is the species they observe when stung, however, as species differentiation of carybdeids is often at a microscopic level these identifications have yet to be confirmed and severe stings in these cases have not been reported (Gershwin, 2005b). As such I believe it is speculative at best to assign sting symptoms to any species at this early stage of their identification and without additional venom or definitive patient based research.

Reasons for the assumption of the potential severity of *Carukia shinju* n. sp. are based on its morphologic and genetic similarity to *C. barnesi* (Gershwin, 2005b) however the author discloses that genetic differences are as yet unconfirmed. Morphologically the taxonomic criteria for establishing this as a new species are debatable and potentially indicative of an area for which confusion in identifiable features may occur. Both species are recorded as lacking gastric phacellae, having a small rounded-pyramidal body shape, frown-shaped rhopalia niche ostia, red pigment nematocyst warts and the cuff-like tentacular nematocyst bands (Gershwin, 2005b) a trait which *Carukia barnesi* was originally distinguished by (Barnes, 1964). Although it was initially thought that these two species could be distinguished by a larger body size and lesser density of exumbrellar warts in *C. shinju* (Gershwin, 2005b), both these features have later been described from specimens of *Carukia barnesi* also (Underwood and Seymour, 2007) with individuals greater than diameter observed (Pereira et al., 2010) (which is larger than the original description for this species (Southcott, 1966)), and a displayed ontogenetic shift in exunbrellar wart density with size (Underwood and Seymour, 2007, Pereira et al., 2010). Other features for differentiating these species such as the complexity of velarial canals, which are thought to vary ontogenetically (Gershwin, 2005b), and a small distinction in nematocyst structure and spination, should be viewed with caution as this species definition is based on one specimen and cnidarians have shown the propensity to display phenotypic plasticity (Gershwin and Alderslade, 2005). Thus, even in the hands of a specialist, in the laboratory, with access to dissecting microscopes and taxonomic keys, separation of a number of these cubozoan species is extremely challenging, and therefore impossible in the field following a sting event, especially under the circumstances.

The diversity and difficulties of identifying the species responsible and the array of envenomation symptoms experienced has no doubt led to much confusion in general

in regards to classifying and referring to this syndrome. Additionally the ontogenetic variation in morphology of *Carukia barnesi* that is still being discovered (Pereira et al., 2010) means that classification of new species on singular specimens is potentially misleading.

Distribution of Irukandji Syndrome

With a growing knowledge of the species that cause Irukandji Syndrome, we can also look to better understand its distribution and incident rates at different scales. Generally, Irukandji Syndrome is thought to be a tropical based disease, found in the waters around coral reefs between the Tropic of Cancer and the Tropic of Capricorn however no real research has been conducted into the true distribution of the syndrome. Irukandji Syndrome is recorded from many locations in Australia (Macrokanis et al., 2004, Kinsey, 1986, Williamson, 1996, Cleland, 1965, Flecker, 1957, Flecker, 1957, Cleland, 1965, Fenner, 1999, Cleland, 1965, Fenner et al., 1985, Fenner, 1988, Martin, 1990, Kinsey, 1988, Wiltshire, 2000, Fenner, 2003, Hadok, 1997, Cleland, 1965, Cheng, 1999, Williamson et al., 1996b) Hawaii (Yoshimoto and Yanagihara, 2002, Thomas et al., 2001a, Little et al., 2006, Cleland and Southcott, 1965), Fiji (Kinsey, 1988, Flecker, 1957), coastal Thailand (de Pender et al., 2006), Puerto Rico (Stuart and Slagle, 1943), Manilla Bay in the Philippines (Old, 1908), the Gulf Sea (Salam et al., 2003), Key West Florida (Grady and Burnett, 2003), the French West Indies, Bonaire, Caribbean, Timore Leste and Papua New Guinea (Barnes, 1964) Japan (Otsuru et al., 1974) and occurs throughout the Indonesian archipelago (Barnes, 1964). Surprisingly there have also been five reported stings from the temperate waters of North Wales in the United Kingdom (Cleland and Southcott, 1965). A summary of these locations is presented in Figure 2.1 below. At a finer scale, the presence of near shore reefs and islands may significantly change the presence or absence of the animals causing the syndrome. For example the higher than expected incidence of stings for particular beaches in

far northern Australia may be caused by the close location of near shore islands to beaches (Barnes, 1964).

Figure 2.1 Global distributions of documented Irukandji Syndrome stings

Australian distribution of Irukandji Syndrome

Irukandji Syndrome is recorded from Broome in Western Australia (Macrokanis et al., 2004, Kinsey, 1986) from both Roebuck Bay and Cable Bay and thought to extend over the Northern part of Australia to as far south on the Queensland coast to the East of Childers (Williamson et al., 1996a). Specifically, Onslow in Western Australia has reported stings consistent with Irukandji Syndrome in 1927 (Cleland and Southcott, 1965). Darwin (Flecker, 1957) to the Gulf of Carpentaria (Flecker, 1957) and over of the East coast of the country there have been records of Irukandji

Syndrome from Moore Park in Bundaberg (Fenner and Carney, 1999), Morton Bay (Cleland and Southcott, 1965, Fenner et al., 1985), Shute Harbour (Fenner et al., 1988) Hinchenbrook Island (Fenner et al., 1988), Michaelmas Cay (Martin and Audley, 1990), Fraser Island (Kinsey, 1988), Mackay (Wiltshire et al., 2000), the Whitsunday's (Fenner and Lewin, 2003), Brampton Island (Hadok, 1997). Further south there have also been reports of these symptoms from some marine contact in Botany Bay, Brighton Beach (Cleland and Southcott, 1965), Port Phillip Bay in Victoria (Cheng et al., 1999), Townsville (Gershwin, 2007) Swan River near Perth (Cleland and Southcott, 1965).

Similar symptoms have been recorded from Thursday Island in 1959 and 1960, however they were not recorded as such at the time (Cleland and Southcott, 1965).

Seasonal Trends

As with the disparity in the geographical variation of Irukandji Syndrome, there are also differing reports in regards to the seasonal variation of Irukandji Syndrome envenomings.

Irukandji causing jellyfish were originally hypothesised by Jack Barnes to be present in the waters around tropical Australia throughout the year with the only variation being in the numbers of animals present in the water (Kinsey, 1988) which in turn can be reflected in the sting frequency trend. Cleland and Southcott (Cleland and Southcott, 1965) proposed that the season for these animals ran from December to February in North Queensland, Fiji, Darwin and New Guinea areas, however, since then evidence extending this restricted season at least in the Australian waters (Kinsey, 1988, Fenner and Harrison, 2000, Huynh et al., 2003, Little and Mulcahy, 1998) has occurred by the reporting of Irukandji syndrome producing stings over a much greater seasonal time period. This insinuates that Barnes' original hypothesis may have in fact been more accurate.

Cairns and North Queensland region

The ‘season’ for Irukandji stings in north Queensland is reported as starting as early as October (Little and Mulcahy, 1998), November (Barnes, 1960, Barnes, 1964, Kinsey, 1988, Holmes, 1996) or December (Flecker, 1957) and running until anywhere from late January (Barnes, 1964, Flecker, 1957), to mid-March (Kinsey, 1988), April (Martin and Audley, 1990) or May (Barnes, 1960, Holmes, 1996, Little and Mulcahy, 1998, Corkeron et al., 2004). Peak time for Irukandji Syndrome stings in the Cairns region appears to be around December and January (Little and Mulcahy, 1998, Fenner and Harrison, 2000), however other authors have suggested Irukandji Syndrome to be present in Queensland in all months of the year except for July and August (Fenner et al., 1986b).

Various meteorological conditions have been correlated with the incidence of Irukandji stings with some suggestion of stings being more likely to occur on hotter days, with lower than average wind speed (Little and Mulcahy, 1998) or on still, clear days, during ebbing tides (Fenner and Harrison, 2000) or during the afternoon (Burnett and Calton, 1987). As a result of these suggestions some authorities have closed the beaches when there have been three days of northerly winds followed by and afternoon high tide (Harrison et al., 2004). Irukandji stings certainly do appear to occur in ‘bursts’ and frequently only involve a few days in a season (Barnes, 1964) with often multiple swimmers effected on one beach in a single day (Flecker, 1957). Christmas day in 1985 saw 36 stings in total, making it one of the worst single days for sting incidences recorded (Fenner et al., 1986b). Maximum incidences have been reported as the last and first fortnights of each year, however, this could also reflect beach usage (Flecker, 1952b). Certain years or seasons have also seen great fluctuations of sting numbers with 128 patients presenting to Cairns Base Hospital over the 2001-2002 season (Huynh et al., 2003), 100 victims in the 1943-1944 season (Flecker, 1952a), 62 over the 1996 year (Little and Mulcahy, 1998) and

none at all over the 1965-1966 seasons (Kinsey, 1988). Barnes suggested that there was doubt as to whether this reflected the number of animals present in the water or merely the conditions under which people were in the water (Flecker, 1952b) although while he suggested that peak numbers of stings did coincide with maximum beach usage he also reported that this broke down with abnormal weather patterns (Barnes, 1964).

Other suggestions are that sting incidence may reflect the ecology of the animals and their propensity for different areas at different times (Mulcahy and Little, 1997). While initial reports suggested that Irukandji Syndrome only effected bathers on sandy beaches and not swimmers out on the reef (Flecker, 1957), it is now known that stings come from reefs and islands as well as coastal locations with some of the severe cases presenting from such offshore locations (Little et al., 2001, Little and Mulcahy, 1998, Huynh et al., 2003). There is suggestion that the percentages of cases presenting from different regions is again related to the time of year of the stings, with one study showing higher percentages of reef stings occurring later in the season and conversely a greater proportion of coastal stings earlier in the season (Mulcahy and Little, 1997), although how or if this ties into the animals distribution and ecology is again purely speculative.

Barnes noted a higher than expected incidence of stings for particular beaches of the Cairns region and he suggested that the close location of near shore islands to beaches and the water movement around islands could be having an effect on this (Barnes, 1964), certainly recent studies capturing multiple specimens of *Carukia barnesi* off a near shore island location (Underwood and Seymour, 2007) may be indicative of its role in the presence of these animals.

Australian seasonal occurrences

While the Irukandji season is reported to also range from around November to May in Darwin and the Northern Territory (Holmes, 1996), it does appear that there are different seasonal constraints in the these areas to that of the eastern Queensland coast with the maximum occurrence of stings presenting in May, which is much later in the year than that of the Queensland (Fenner and Harrison, 2000). While Irukandji stings are reported from the Darwin and Gove region (Currie, 1992, Fenner et al., 1986b, O'Reilly et al., 2001), they do not appear to be as prevalent or perhaps reported on as thoroughly in this region as those off the Queensland coast.

Western Australian regions, and Broome in particular, is another area of interest with data suggesting that this region could in fact be seeing the highest incidence of Irukandji Syndrome in the world (Macrokanis et al., 2004). In this region stings are mainly occurring from January to May (Corkeron et al., 2004) but are reported to occur all year round here (Macrokanis et al., 2004). In this region there are suggestions that stings are more common when there is a median air temperature above 28.3 degrees, after midday on an incoming tide and on windy days (Macrokanis et al., 2004).

Worldwide seasonal occurrence

Alatina moseri have been linked to incidences of Irukandji like syndrome in Hawaii and in this region they also display a very marked seasonality with aggregations occurring approximately eight to ten days after the full moon on a monthly basis (Thomas et al., 2001a, Thomas et al., 2001b, Yoshimoto and Yanagihara, 2002), with stings from these animals presenting in the days that follow such an influx. The first large influx of *Alatina moseri* is reported from December 1988 however, smaller numbers of these animals were recorded prior to this (Thomas et al., 2001b)

Carybdea alata were also found in swarms from Puerto Rico in the months of July and August in the early 1970's however since then there have been limited reports on their presence in this area. *C. alata* are also reported in swarms slightly before the full moon, throughout the year on the windward side of reefs in Kiribati (Banner, 1952) with a similar species, presumed to be a *Tamoya* sp., swarming regularly seven days before the full moon around the Gilbert Islands (Pope, 1951).

All three Irukandji sting cases in Florida were reported to occur at night and during summer/fall periods of warm water temperature (Grady and Burnett, 2003). While in these cases it has been suggested that perhaps unusual weather conditions may have introduced a more venomous species responsible for these stings to this area at this time (Grady and Burnett, 2003), it should also be considered that like so many other areas where Irukandji Syndrome was not previously reported, prior cases may just have been misdiagnosed (de Pender et al., 2006). Further research into the timing of specific species that give rise to Irukandji Syndrome may assist in sting incidence prediction and is an area for further investigation.

Venom Collection

Isolating the venom components responsible for causing Irukandji Syndrome is an important step in learning how the venom affects the human body, and allows us to learn more about the animal's potential prey items. However research into the venom components of cubozoans responsible for causing Irukandji Syndrome have been hampered by the relatively low numbers of animals collected and the low yields of venom acquired from these animals compared to that of the larger, multi-tentacled Chirodropids (Barnes, 1966).

Isolating nematocysts from animals can be achieved by a technique that removes the stinging cells from the tentacles of freshly collected specimens allowing these to be lyophilized for storage and future venom extraction (Bloom et al., 1998). Venom

extraction itself has seen a number of different techniques trialled however as the introduction of heat, chemicals and extraneous material can affect the protein composition of cnidarian venom greatly (Othman and Burnett, 1990, Carrette et al., 2002b, Endean et al., 1969) a repeatable extraction method was required. Some researchers have even bypassed this initial nematocyst isolation procedure instead using whole animal preparations (Winkel et al., 2005, Wiltshire et al., 2000), however the potential for toxic material to be present in tentacles devoid of nematocysts demonstrates how misleading these results can be (Ramasamy et al., 2005).

The use of a mini bead mill beater to extract venom from isolated lyophilised nematocysts is now used which circumvents the majority of these previously existing inconsistencies with cubozoans venom research (Carrette and Seymour, 2004) and this technique has been successfully adapted for use on *Carukia barnesi* venom (Underwood and Seymour, 2007, Ramasamy et al., 2005). The supplementation of additional washings to this original technique have been utilised with success by researchers and allows for cleaner preparations to be obtained (Kintner et al., 2005, Underwood and Seymour, 2007). Original extraction of venom from cubozoans was conducted on the larger multi tentacled Chirodropids where nematocysts are present predominantly on the tentacles of the animals and so these parts of the animals only were prepared for venom research (Bloom et al., 1998), however this same nematocyst isolation and extraction technique has since been displayed for both the bell and tentacles of *Carukia barnesi* (Underwood and Seymour, 2007). The presence of extraneous protein material from ruptured nematocyst organelles is still possible; however, this standardised technique has substantially improved the previous variations displayed between extraction techniques.

Venom components

Research conducted into extracted venom from Irukandji Syndrome producing jellyfish has shown a large number of different proteins within the venom. As many as 60 proteins were present in the venom extracted from mature bells of animals (SDS-PAGE gel analysis) and at least 45 different proteins in the tentacle venom and these proteins ranged in size from 25-250kDa in size (Underwood and Seymour, 2007) with the majority less than 100kDa. There is also a distinct difference in venom protein profiles between mature and immature specimens as well as venom extracted from bell nematocysts as compared to tentacles (Underwood and Seymour, 2007). The large number of proteins found in *C. barnesi* venom is far greater than previous studies into the components of venom, which saw only three major protein bands present (Wiltshire et al., 2000). No comparisons to date have examined geographical variations in *C. barnesi* venom components nor interspecies venom components from Irukandji Syndrome producing carybdeids.

Venom reactions

Laboratory studies into the components of Irukandji venom have predominantly revolved around the cardiac responses of extracted venom on both whole and isolated vertebrate models such as pigs, guinea pigs and rats (Winter et al., 2008, Winkel et al., 2005, Ramasamy et al., 2005, Wiltshire et al., 2000, Tibballs et al., 2001, Burnett, 1998, Burnett et al., 1998). Initial studies on the effects of *C. barnesi* venom have shown that serum levels of endogenous adrenaline increase as well as pulse rate and blood pressure, with widening pulse pressure and a positive inotropic effect when injected into rats (Burnett et al., 1998). Similarly, studies utilising crude blended whole specimens of *C. barnesi* injected into mechanically ventilated pigs (Winkel et al., 2005, Tibballs et al., 2001), saw a 200 and 100-fold increase in serum noradrenaline and adrenaline respectively (Tibballs et al., 2001) with a sustained

tachycardia and systemic and pulmonary hypertension reaction effect (Winkel et al., 2005). This research concluded that the venom effects of *C. barnesi* may not only be causing the release of catecholamines but include a component causing 'direct' vasoconstriction (Winkel et al., 2005).

Additional effects on isolated rat and guinea pig right atria demonstrated that the venom causes tachycardia in the presence of atropine, an effect that was almost completely abolished by the prophylactic addition of tetrodotoxin and was restricted to peripheral post ganglionic sympathetic sites and possibly the splanchnic nerve innervations and the adrenal medulla (Winkel et al., 2005). This indicates that this venom extract function as a neural sodium channel activator.

However, it is important to note that these studies use both crude venom extract and venom mixtures that also contain tentacle material (Winkel et al., 2005) As subsequent research has seen toxic components and cardiac response to tentacle extract devoid of nematocyst material (Winter et al., 2008, Ramasamy et al., 2005), these results must be viewed with some reservation.

Subsequent laboratory studies have utilised the refined venom extraction technique (Carrette and Seymour, 2004, Underwood and Seymour, 2007) for experimentation of venom effects. These investigations showed some comparable cardiac effects, including severe pressure responses, from venom of *Carukia barnesi* (Ramasamy et al., 2005) and *Alatina mordens* (although approximately 3-5 times less potent than that of *C. barnesi*) (Winter et al., 2008) with evidence again supporting the theory of a venom induced catecholamine release after intravenous venom administration (Ramasamy et al., 2005). Interestingly, changes in pressor response do not appear to be dose dependent (Ramasamy et al., 2005) suggesting that the venom may be inducing a release of catecholamine into the system and not actually be present in the venom itself (Ramasamy et al., 2005, Winter, 2008, Winter et al., 2008).

Administration of prazosin (an a1-adrenoreceptor antagonist) in envenomed test animals both reduced the venom induced pressor response and inhibited the tachycardia (Ramasamy et al., 2005) suggesting that it is an indirect effect of the venom due to peripheral vasculature changes and not a direct effect due to peripheral vasculature changes and not a direct b-adrenoceptor mediated effect (Ramasamy et al., 2004, Ramasamy et al., 2005).

Conversely, cardiovascular collapse in envenomed animal models does appear to be dose related suggesting the toxins may be acting directly on the myocardium (Ramasamy et al., 2005). Salivation and urination in envenomed animals is also seen suggesting parasympathetic stimulation resulting again from the venom induced catecholaminaemia (Ramasamy et al., 2005, Winter et al., 2008).

Discussion

From this review it is clear that Irukandji Syndrome is not only an area that is in need of further research but also in need of further definition. Clarification of the specific species that result in this syndrome is needed as well as the regions and timings of these events.

While a recent survey suggested that the majority of locals to one Northern Queensland tourist destination (88%) were aware of the term “Irukandji jellyfish”, a large proportion of International tourists (66%) were not (Leggat et al., 2005) and it seems clear from this that inconsistencies in information dissemination between groups visiting this location are occurring (Harrison et al., 2004) this is particularly pertinent considering this data was collected from the location nearby to which the syndrome was originally defined (Flecker, 1952b).

Irukandji Syndrome has historically been considered a problem for the waters of North Queensland; however a summary of the published sting reports here has revealed that its reach extends much further than originally perceived and

collaborative research on an international scale would greatly improve the knowledge and awareness of this medically significant disease. Globally, evidence suggests that Irukandji Syndrome appears to be a predominantly tropical occurrence and an increase in its reporting has led to speculation as to its potential increased distribution with changing ocean conditions.

Studies of jellyfish abundance and frequency are still under researched, however, the few studies that do exist seem to implicate an increase in jellyfish numbers over time (Attrill et al., 2007b). Various factors have been cited as to why this may be occurring, from anthropocentric practices such as over fishing and pollution, to climate change and ocean acidification (Purcell, 2005, Attrill et al., 2007b, Attrill et al., 2007a, Arai, 2001) (Purcell et al., 2007). Climate models of increased ocean temperatures, has led researchers to surmise that jellyfish numbers as a whole will increase over the next 100 years (Attrill et al., 2007a, Attrill et al., 2007b). There are also predictions of various gelatinous species displaying seasonal expansions, changes in blooming behaviour, and increased threat of introductions in to new areas through changing ocean currents and human relocation of jellyfish (Mills, 2001, Sullivan et al., 2001). What is clear when it comes to jellyfish trends worldwide is the need for more information on the ecology of these animals; not only in their adult form but polyp phase as well (Mills, 2001). This is certainly the case for Irukandji jellyfish with a distinct lack of knowledge of both medusa and even more so in polyp ecology.

Whether the increased reporting of Irukandji Syndrome globally is due to heightened awareness of this disease or reflective of changing ocean conditions, or even indicative of the increased number of people utilizing the oceans, it is clear that more research is needed in this area to prepare for potential future changes. The possibility of this syndrome becoming a more widely distributed phenomenon urges

further research to be conducted in all areas particularly including prevention, venom action and treatment protocols.

CHAPTER THREE: RETROSPECTIVE ANALYSIS OF STINGS WITH IMPLICATIONS FOR ECOLOGY AND MANAGEMENT

Abstract

This study reviews the occurrence, trends and severity of Irukandji Syndrome for the Cairns region of North Queensland, Australia using a retrospective analysis of patient files from two sources. The first were historic accounts kept by Dr Jack Barnes covering the period from 1942 to 1967, and the second from the Emergency Unit in Cairns Base Hospital and covers from 1995 to 2007. There has been a significant increase in the length of the Irukandji season (as determined by incidences of Irukandji Syndrome patients) since it was first consistently recorded (a low in 1961 of 15 days; a high in 2002 of 151 days); however, annual numbers of envenomations were highly variable. Traditionally, greater frequencies of Irukandji stings were reported at onshore as opposed to offshore locations. However, in recent years this trend has reversed, potentially because of increased safety protocols for beach regions by Surf Lifesaving Queensland. Geographic variation of stings was observed with mean troponin I levels higher in offshore reef envenomations compared to those from islands or coastal regions. When comparing opiates administered in terms of morphine equivalent doses, patients receiving either fentanyl or a fentanyl and promethazine-based treatment received significantly greater doses of morphine equivalent opiates compared to those treated with either morphine or pethidine. Opiate dosage was indicative of syndrome severity and correlated with other physiological parameters measured. Overall, it appeared that 5 major symptoms were associated with this syndrome, namely pain, nausea/ vomiting, diaphoresis, headache, shortness of breath and troponin leaks. Pain (but not necessarily just lumbar pain) was the overwhelming factor associated with the vast majority of envenomed victims closely followed by nausea/vomiting. Overall the duration of the Irukandji season appears to be increasing, potentially driven by increasing global seawater temperatures. Conversely the number of envenomings appears to be

decreasing and this may well be due to the modification of beach management practices in recent years for this region. Offshore envenomings appear to have a higher potential for more severe envenomings.

Introduction

Irukandji Syndrome initial envenomation is typically recorded as insignificant; however, after a delay of generally 20 to 60 minutes, recorded systemic symptoms include headache, backache, nausea, vomiting, abdominal cramps, hypertension, tachycardia and feelings of impending doom (Huynh et al., 2003, Little et al., 2003, Barnes, 1964). Historically, the syndrome was first described from the region around Cairns in North Queensland, Australia,(Barnes, 1964) and, although numerous case reports occur annually from this area, as discussed in Chapter Two, great disparity still exists not only in the reporting of the syndrome but also its seasonal occurrence (Carrette et al., 2012).

The Irukandji season in Australia has previously been reported to start as early as October and run as late as May, however, envenomations occur in all months bar July and August (Little and Mulcahy, 1998, Holmes, 1996, Corkeron et al., 2004, Fenner et al., 1986b). The peak times for Irukandji envenomations in the Cairns region have been declared around December/January; however, these observations were made over a single season with no long-term analyses being documented (Fenner and Harrison, 2000, Little and Mulcahy, 1998). There is some suggestion of a potential correlation between sting incidence and the ecology of the animals responsible, but this may only reflect conditions in which people opt to utilize the beaches with higher frequency (Mulcahy and Little, 1997, Flecker, 1952b). For example Christmas Day has one of the greatest recorded incidences of sting occurrence, potentially reflecting increased beach usage (Fenner et al., 1986b).

While the syndrome was originally described as only affecting bathers utilising the sandy coastal beaches and not on the reef, envenomations from the outer reef and island regions are now commonly reported, with some of the serious cases documented from these offshore locations (Flecker, 1957, Mulcahy and Little, 1997, Huynh et al., 2003). Anecdotal reports have suggested that there may be a pattern in the timing of the more severe envenomations that present to hospital with general consensus being that the more serious Irukandji stings present later in the season (Little et al., 2003). However, as with the sting-severity hypothesis, no empirical data currently exists to support this premise.

Presently, no detailed studies on the ecology of Irukandji jellyfish exist, and as such the only avenue available to uncover patterns in Irukandji Syndrome envenomations is the retrospective analysis of patient files. To this end, data from Irukandji Syndrome envenomations in the Cairns region, covering a total of 65 years, are analysed here to investigate trends in sting occurrence in the region on a large temporal scale. Additionally, trends in sting severity and treatment success were sought for potential insight into improving management of this syndrome.

Materials and Methods

Patient Records

Patients who suffer from Irukandji stings are typically coded as either “marine sting”, “Irukandji sting”, or “sting from venomous jellyfish or starfish” and these patients were extracted from the Cairns Base Hospital database for potential inclusion into this study. Patients included in the trial were those who had contact with seawater pre-60 minutes of symptoms developing, a delay in symptoms from an initial sting and at least one of the defined systemic clinical symptoms which included headache, nausea, anxiety, vomiting, sweating, restlessness, muscle cramps in all four limbs, abdomen and chest or severe lower back pain (de Pender et al., 2006). Any stings

that were deemed to have resulted from contact with a large chirodropid jellyfish (i.e., *Chironex fleckeri* or *Chiropsella bronzie*) or those from the hydrozoan *Physalia* sp., (noted by the visible and/or substantial welts with an absence of systemic symptoms) were removed from this study. A total of 347 envenomations were included in this study covering the years from 1995 to 2007 and were accessed under Cairns Base Hospital ethics committee approval number 287. In several cases, not all categories of data could be extracted from records, (for example geographic location, total amount of opiates administered due to treatment at other locations before transference to Cairns Base Hospital, admittance into a randomized control trial using magnesium) and in these cases these records were exclude from specific analysis if relevant data were missing.

Additional historic data inclusion

A historic sting database exists from Dr Jack Barnes' comprehensive records of sting cases from 1942 to 1967 (Kinsey, 1986). These cases were all from Irukandji Syndrome stings from the Cairns region and were all seen and documented by Barnes (Kinsey, 1986). The dates of Irukandji Syndrome stings from this report were added into this retrospective study for analysis into the occurrence of Irukandji Syndrome stings with time.

Data collected

Information extracted from patient files covered three main areas of patient demographics, sting occurrence and symptoms/treatment progression. Some files were incomplete, so not all areas could be comprehensively recorded for all stings. The following factors of envenomed patients were recorded.

1. Total opiate requirements in morphine-equivalent doses: Three opioids were used, namely pethidine, morphine and fentanyl and dosages were calculated in terms of assumed morphine equivalent doses to allow for direct

comparisons (1 mg morphine = 10 mg pethidine = 10 µg fentanyl)(Huynh et al., 2003).

2. The observed peak percentage blood pressure (BP) increase, measured as both the maximum mean arterial pressure (MAP) (approximated using the equation MAP = (2 x diastolic pressure + systolic pressure)/3) and peak systolic pressure were recorded from regular observations. Prior to discharge, patients' BP was recorded and this was deemed indicative of their 'normal' level. The peak recorded BP was then calculated as a percentage increase above this 'normal' reading.
3. The observed peak percentage heart rate (HR) increase: The maximum HR recorded during regular observations while in hospital care was calculated as a percentage increase to the HR recorded at discharge.
4. Troponin I level (cTnI): levels of cTnI greater than 0.7 g L^{-1} were recorded as significantly elevated (normal < 0.7 g L^{-1}).
5. Length of stay (hours): length of stay from first admission was recorded for each patient and is assumed to indicate the level of care needed for the syndrome to desist.

Additional to physiological information gathered, logistical information on sting events was recorded where possible including geographic location of stings. To distinguish between geographical regions, three categories were selected (these are thought to reflect the different habitats Irukandji jellyfish may inhabit). Any stings occurring from the coastal beaches were defined as 'onshore'; stings originating from water activities around the coastal islands were classified as 'islands'; and all the stings that occurred from the outer reef regions were classed as 'reef ' stings. All the documented symptoms were recorded with those that appeared in at least 5% of the cases marked into categories. These symptoms recorded throughout all the cases

examined were found to fall into the categories of pain (including limb pain, back pain, abdominal pain, and chest pain), headache, nausea and vomiting, diaphoresis, shortness of breath and finally troponin, so these six categories were used for analysis. There is a complex of three regulatory proteins that are required for muscle contraction of which troponin I is one and elevated presence of this protein in the blood stream is used to indicate the occurrence of cardiac damage.

Data analysis

For several of the analyses, data were non-normally distributed and residuals were heteroscedastic. In these cases data were transformed to normalize the distribution and produce homoscedastic residuals to avoid violation of assumptions for GLM analysis. A general linear model (ANOVA) was used to investigate the effect of year (1995/6 to 2006/7) and/or geographic location (Onshore, reef or island) on the length of the Irukandji season. *Post Hoc* analysis (LSD) was performed on significant effects to determine which treatment means were different. Only stings from 1995/6 onwards were used for this analysis as this information was lacking from many records in Barnes' published database. Similarly an ANOVA was used to elucidate any effects location and/or year had on the level of troponin I leakage seen in envenomed victims

Regression analysis was used to determine the relationship between the length of the Irukandji season and time (from 1956-2007). Chi squared analysis was used to compare the ratio of stings seen onshore versus offshore locations with each year from 1995 – 2007. Bivariate correlations were performed to determine the significance of correlations between morphine equivalent dosage with either length of stay, percentage MAP, systolic changes or troponin I level.

Finally to determine if mean morphine equivalent dosage varied with number of symptoms recorded, (pain, nausea/vomiting, diaphoresis, shortness of breath or

troponin I leakage) data were analyzed using ANOVA and LSD *post Hoc* analysis was used to determine within treatment differences.

Results

Distribution of envenomings

Patients in this study consisted of 55% male with 45% female. Patients ranged in age from 1 to 77 years old with a mean age of 24. The median age was 24, mode 20 with 25th percentile being 16 and 75th percentile being 32 yrs. Children (defined as less than 18yrs) comprised 29% of the sample population.

There was a significant positive correlation ($F_{(1x19)}$= 10.822, $P < 0.005$) between year and the length of the Irukandji Syndrome season, with a minimum of 15 days in 1961 to a maximum of 151 days in 2002 (Figure 3.1). For stings recorded from 1995 to 2007, significantly more stings occurred onshore than offshore in the earlier years, however, this trend reversed with more stings occurring offshore (Figure 3.2) ($\chi^2_{(8)}$ = 32.9, $P < 0.001$) in later years. There was a significant negative correlation ($F_{(9x1)}$ = 22.03, $P < 0.05$) between percentage of stings onshore and season with a high of 93% in 1996–1997 and a low of 26% in 2006–2007.

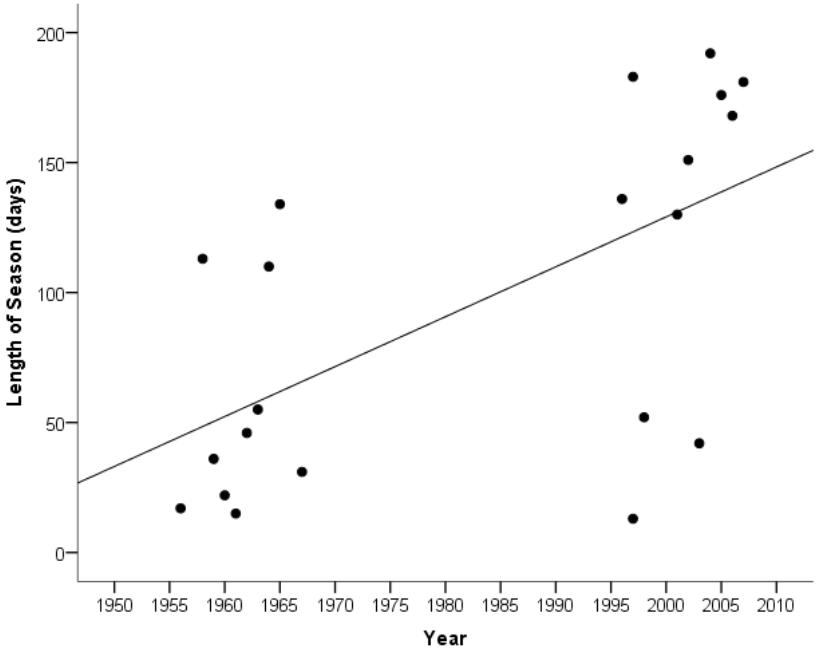

Figure 3.1 Scatter plat of total length of stinger season annually in days based on stings recorded (R^2 linear = 0.427)

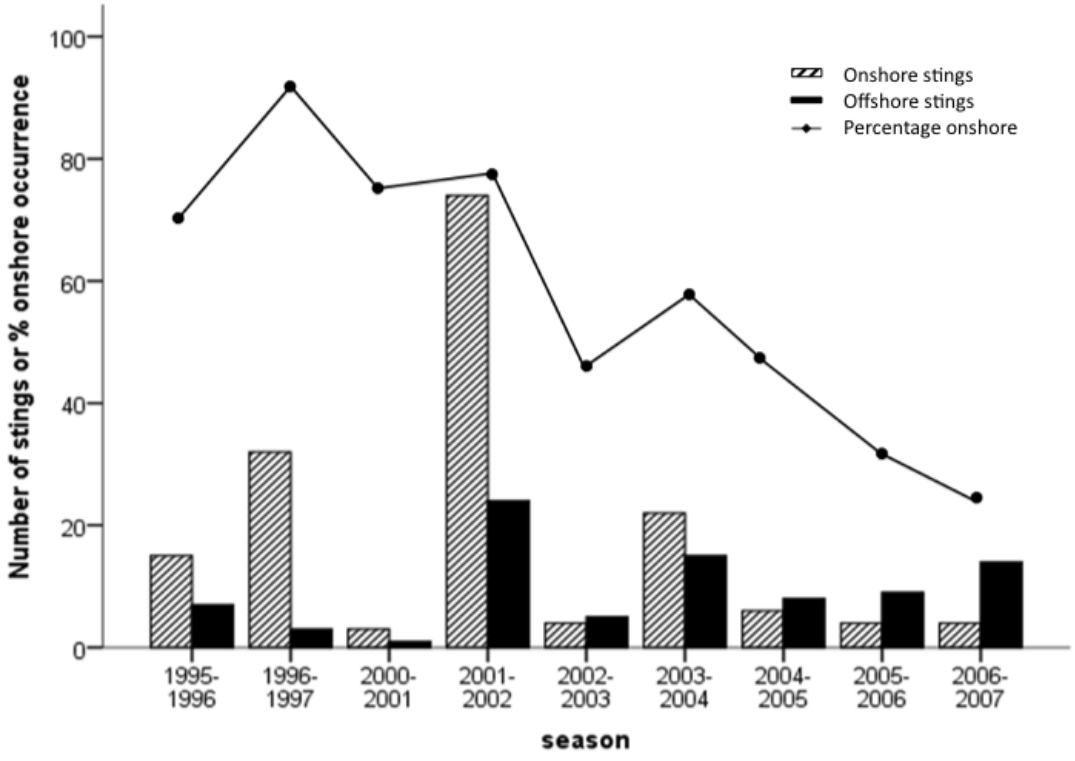

Figure 3.2 Frequency of recorded Irukandji stings from both offshore and onshore locations in the Cairns region and percentage of those stings that occur onshore.

Opioids for pain relief

The majority of patients who received opioids administered for pain relief were given fentanyl (26%) or a mix of more than two opioids (20.2%). Fewer patients were given pethidine (16.2%) or morphine (11.6%) alone or various combinations of these three opioids. Envenomed patients treated with a fentanyl/promethazine combination required significantly higher ($F_{(106x5)}$ = 6.230, P < 0.001) mean morphine-equivalent doses (mean = 59 mg) to those treated with any other opiate combination (Figure 3.3). Those treated with fentanyl alone required significantly higher morphine-equivalent dosages to those treated with morphine, pethidine or pethidine / promethazine.

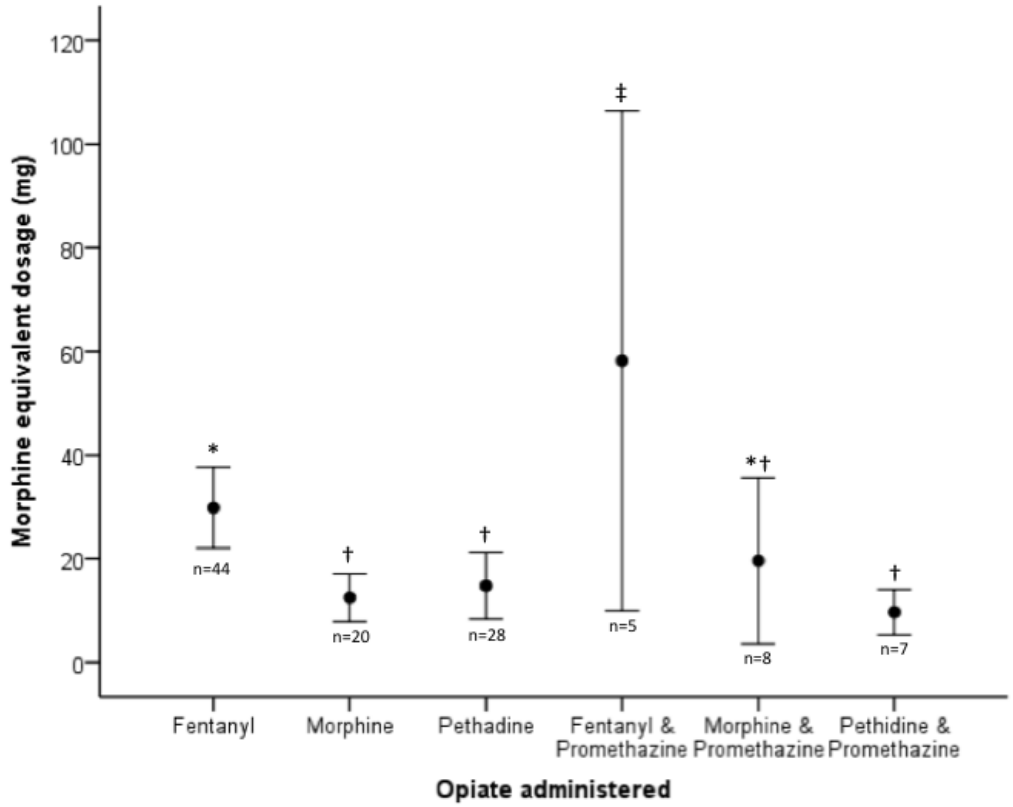

Figure 3.3: Mean total of morphine-equivalent doses for different opioids (means followed by same symbol are not statistically different at the 0.05 level by LSD post hoc analysis) (n=143)

There was a significant positive correlation (Pearson's correlation 0.499) between opiates and symptoms experienced, with patients receiving significantly higher amounts of opiates as more symptoms developed (Figure 3.4).

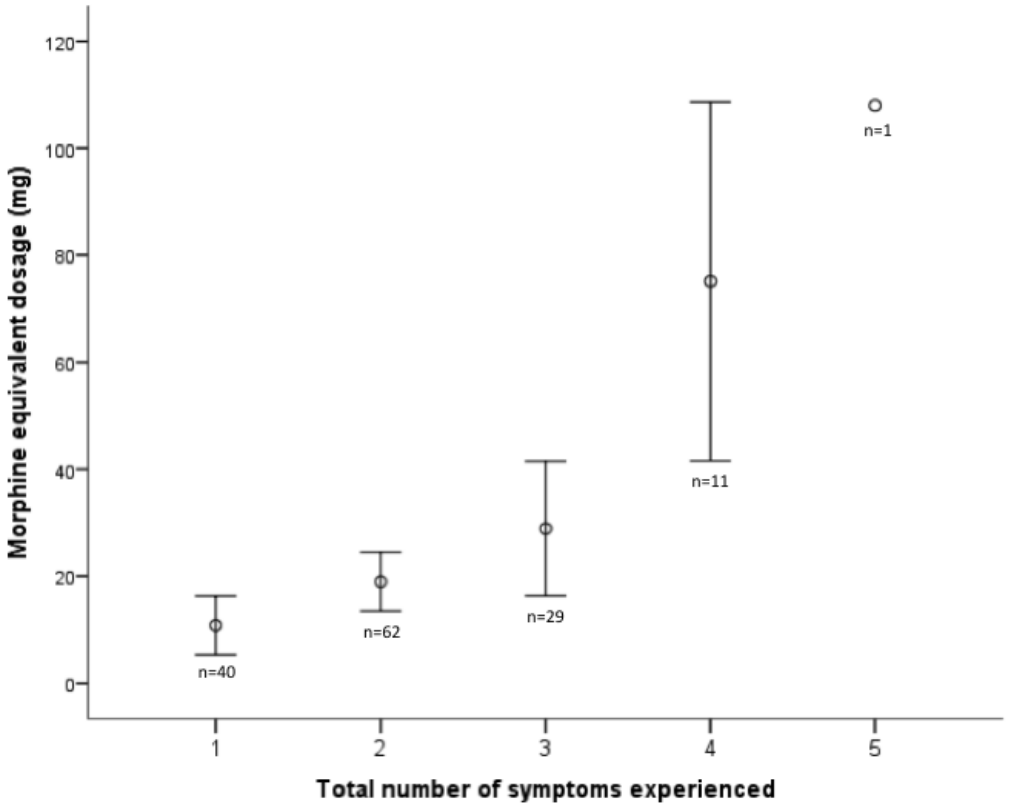

Figure 3.4 Mean morphine-equivalent dose received (mg) for number of symptoms reported

Approximately 31% of patients diagnosed as suffering from Irukandji Syndrome showed only one of these defined symptoms with 43% recording 2 symptoms (Figure 3.5). Notably only one patient showed five combined symptoms and no patients showed a combination of all six.

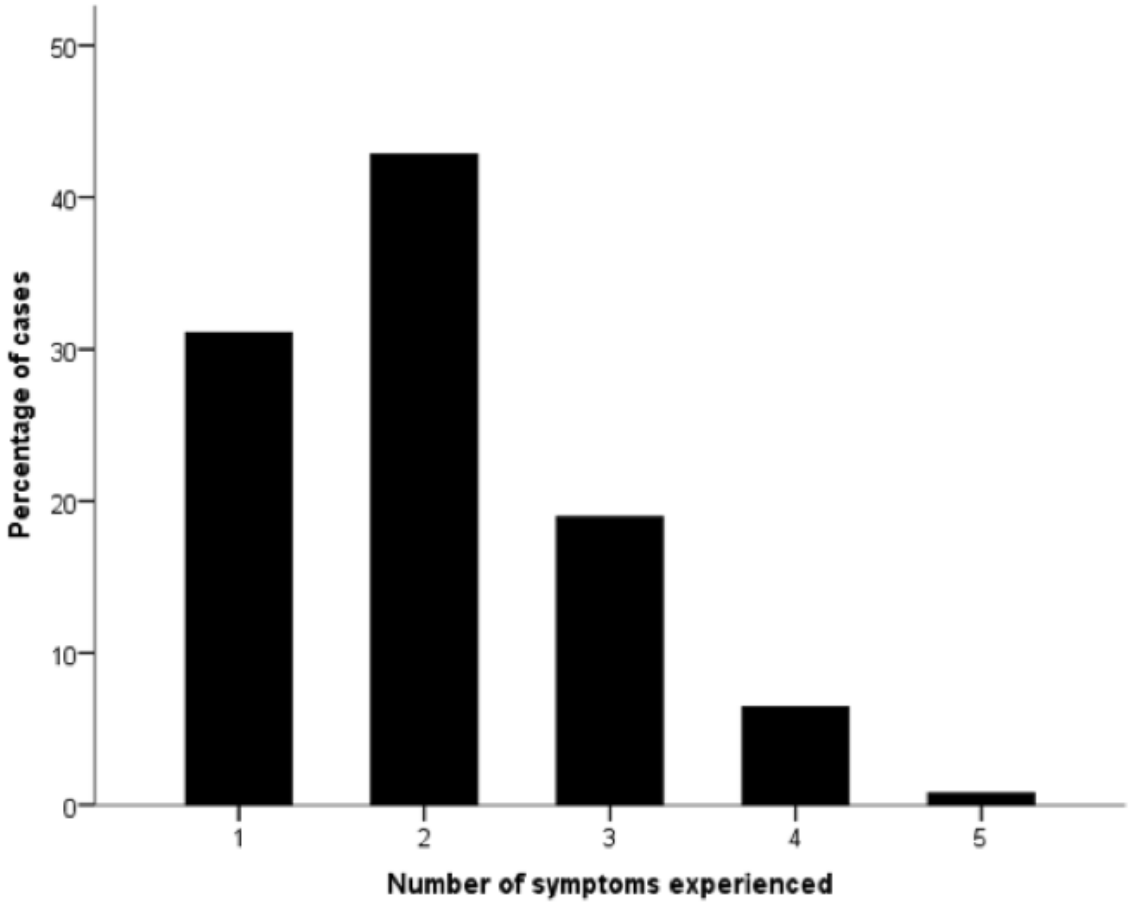

Figure 3.5: Mean percentages of cases presenting with the number of symptoms experienced (n=143)

For patients that only experienced one symptom, over 80% of this was pain of some description (chest, back, limb pain) (Figure 3.6). The second most predominant symptom was nausea/vomiting with troponin rises, being displayed only in patients who showed 3 or more clinical symptoms (Figure 3.6).

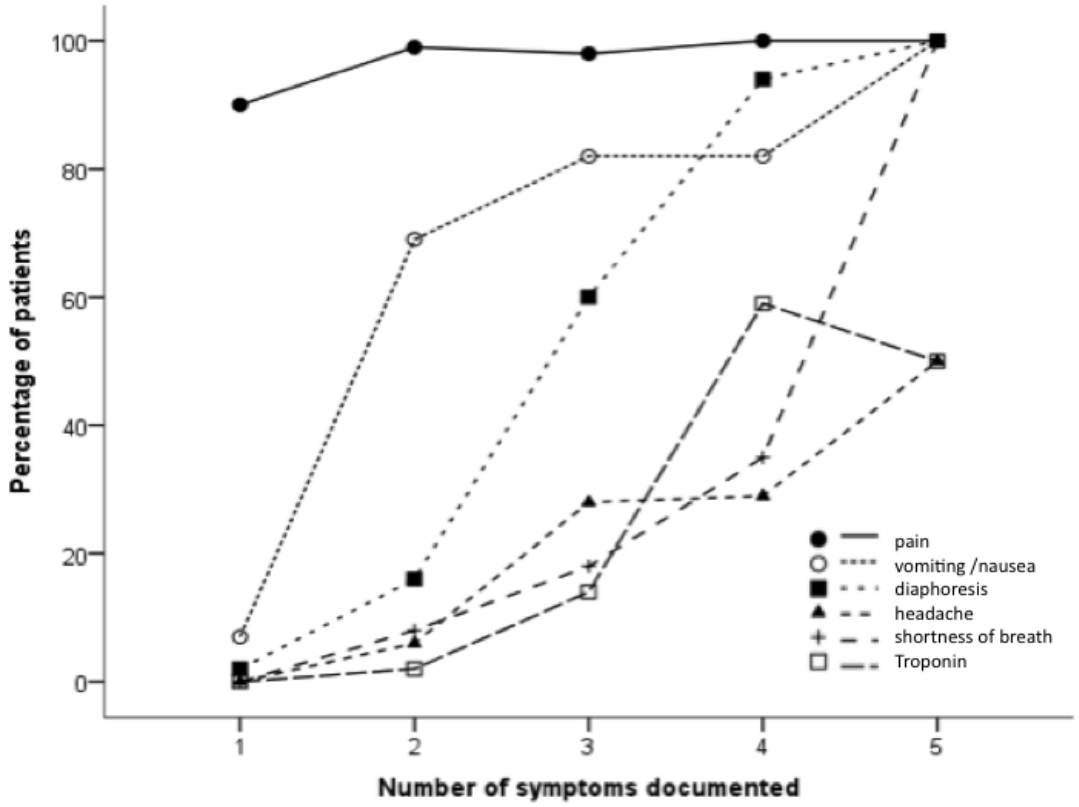

Figure 3.6 Percentage of patients presenting with the number and type of symptoms reported (n=143)

Morphine-equivalent dosage was shown to have a strong positive correlation with all variables examined but was most strongly positively correlated with length of hospital stay (Pearson corr (r) = 0.665), than with troponin levels (r = 0.509), percentage BP change (r = 0.441), % MAP (r = 0.328) or percentage HR change (r = 0.195). The mean, minimum, maximum and median data for morphine equivalent dosage, peak BP, peak MAP, peak heart rate, troponin level and length of stay are presented in Table 3.1.

Table 3.1 Range of physiological parameters recorded for envenomed patients documented

		Morphine equivalent dosage (mg)	Peak Blood Pressure	Peak M.A.P.	Peak Heart Rate (bpm)	Troponin (cTnI) score in ug/L	Length of stay in hours
Sample size		264	251	249	204	189	265
Mean		25	141	105	95	1.5	16
Median		13	140	105	93	0.0	12
Mode		0	130	100	80	0.0	3
Range		255	140	115	156	34.0	167
Minimum		0	90	68	47	.0	1
Maximum		255	230	183	203	34.0	167
Percentiles	25	1.2	125	93	80	0.0	3.9
	75	35	155	116	107	0.1	21

Troponin I

Envenomed patients from reef locations had significantly higher levels of troponin I ($F_{(2x152)}$ = 7.577, *P* = 0.001) (mean = 3.78 g L^{-1}) than those from either the island (mean = 0.99g/L) or onshore locations (mean = 1.06 g/L) (Figure 3.7).

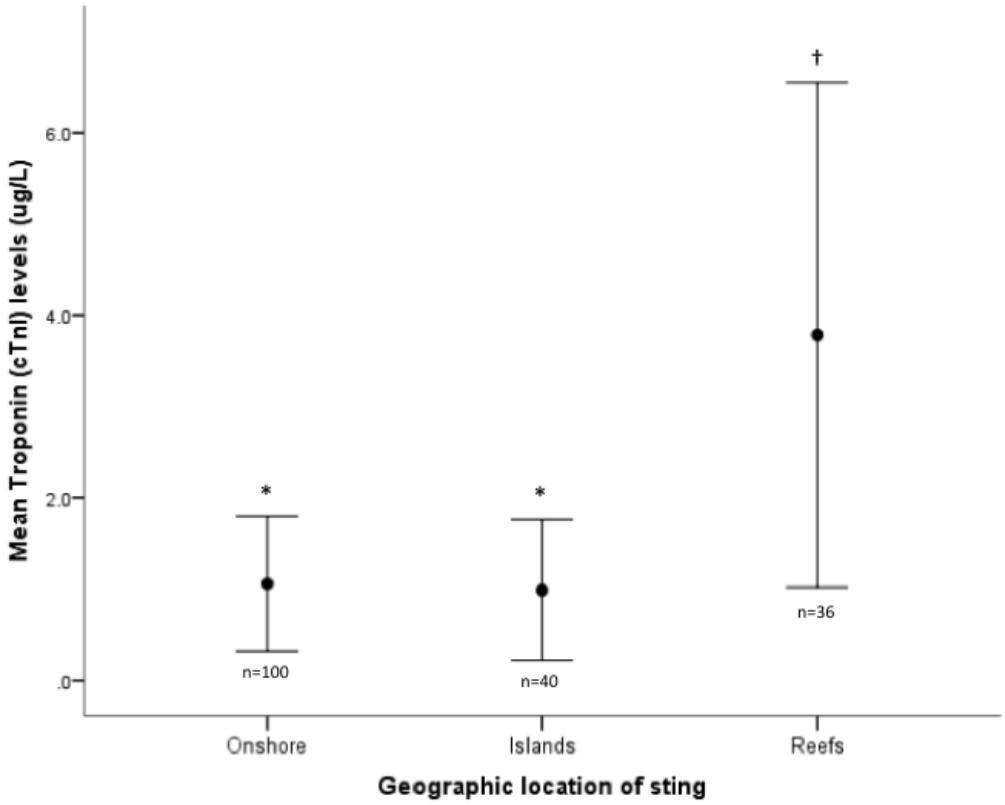

Figure 3.7: Mean detected troponin I (cTnI) levels in patients stung from different geographical locations (means followed by same symbol are not statistically different at the 0.05 level by LSD post hoc analysis) (n=176)

Discussion

To date, this is the largest data set analysis of Irukandji envenomings, spanning 65 years, with the majority of previous studies focusing on only a single season's records. These findings give insight into the seasonal occurrence of the syndrome, symptoms that are displayed and supply information into the treatment and management of this disease.

These data suggest the length of the Irukandji season in the Cairns area appears to be increasing with time. With the global rise in seawater temperatures a prolonged state of optimal temperature conditions may exist, which in turn may allow medusa to survive until later in the year then they have in the past (Solomon et al., 2007). Certainly there has been a trend in other cnidarian species to increase in numbers

due to sea temperature increases, eutrophication and possible over-fishing (Pauly et al., 2009). My data, while not conclusive, may be an example of increased season length because of increased water temperatures. This has direct consequences to physicians who may be under the impression that this syndrome only occurs over a few months of the year.

These data also show that recently, there has been a change in the ratio of onshore/offshore stings, with onshore sting numbers decreasing. Although not testable, there is speculation that the potential for this reversal in trend is due to the increase in beach safety protocols that have been instigated in recent years. In 2001, there was a change in the Irukandji sting protocols of Surf Lifesaving Queensland for onshore, beach locations, to include a 24-hr mandatory closure if a positive Irukandji sting occurred, and the addition of daily drags conducted by surf lifesavers to check for the presence of carybdeids on a routine basis (current SLSQ Stinger protocol). The sting data that coincides with this new management strategy is supportive of this increased safety approach for beachgoers in decreasing the sting incidence. No such strategy has been initiated for offshore reef and island regions and, with increasing numbers visiting the reef and island regions, there is great potential for occurrence to increase. It is apparent that a preventative strategy and risk management system is essential in these regions if occurrence of this syndrome is to be controlled.

Of additional concern for the reef envenomings is the higher levels of cTnI measured in patients from this region. While the presence of cTnI is used as an indicator of myocardial damage and its link to Irukandji Syndrome patients experiencing myocardial dysfunction has been previously reported, there is uncertainty as to the relationship of these levels and their association with cardiac dysfunction (Huynh et al., 2003). Patients with elevated cTnI levels have been flagged as potentially developing cardiac complications and, as patients originating from reef regions show higher mean levels, origin of sting would seem a potential tool for severity diagnosis

in Irukandji Syndrome presentation. Further evidence for this comes from the only recorded Irukandji Syndrome-related death in this region that occurred from an offshore reef location, with this patient also displaying vastly elevated cTnI levels (Pereira et al., 2010, Huynh et al., 2003).

Additional to geographical origin for sting severity prediction would be the number of symptoms experienced, with patients who display three or more of the defined symptoms increasing their propensity for cTnI presence. This suggests that a combination of sting origin, opiate requirements and number of symptoms displayed, could be used as a risk assessment technique for patient severity prediction, assuming that these with an elevated cTnI are at a higher risk.

Opioid requirements varied and, with its substantially shorter half-life than that of either morphine or pethidine, higher total doses of fentanyl were required for pain relief; the shorter duration of action requires more frequent doses to maintain effective blood concentrations (Torda and Pybus, 1982, Woodhouse et al., 1996). Morphine has been reported to be more likely to induce vomiting in Irukandji Syndrome patients, whilst pethidine in large doses may possibly causing seizures and cardiac depression, worsening myocardial function of patients with Irukandji Syndrome (Little and Mulcahy, 1998, Barnes, 1964). Therefore, pethidine is not recommended in Irukandji Syndrome (Little and Mulcahy, 1998). Fentanyl would appear to be the opioid of choice because of its relatively low toxicity and good tolerance profile reported in other studies (Pergolizzi et al., 2008, Little and Mulcahy, 1998). Having said this, individual patient responses to drugs do vary and each case must be assessed with patient safety considerations foremost (Pergolizzi et al., 2008).

Promethazine has previously been suggested as improving the outcome of patients with this syndrome by lowering the amount of opiates required and because of its

antiemetic, antihistamine and sedation effects (Little and Mulcahy, 1998). However, our data appears contrary to this view, with no significant decrease in morphine or pethidine dosages and a higher average dosage of fentanyl in patients receiving promethazine. While promethazine may provide some favourable effects for the patient, there is no evidence in these cases to suggest it affects opiate dosage advantageously.

Basic ecological data on the types of carybdeids giving rise to Irukandji Syndrome are still lacking and such fundamental questions as which species are involved and how these animals vary both geographically and seasonally are urgently required. Reactive control measures for onshore coastal locations have been shown to reduce the number of Irukandji envenomings on a short-term scale; however, a broader-spectrum, proactive warning system for all regions would be highly valuable, and this cannot be accomplished without further wide scale investigations. Irukandji Syndrome represents a significant health problem in the North Australian region, but also there is evidence for its increased reporting from other global locations (Thomas et al., 2001a, Yoshimoto and Yanagihara, 2002, Grady and Burnett, 2003, Mulcahy and Little, 1997). With evidence for the season increasing in length, medical practitioners are now facing exposure to patients presenting with this syndrome for approximately six months of the year so further clarification of protocols and treatment strategies is paramount to ensure optimal patient treatment.

This retrospective study indicates that the season for Irukandji stings is increasing. The percentage of these stings originating from the offshore reef areas is also increasing and these stings have shown an increased potential for cardiac complications as indicated by higher levels of cTnI in these patients. Opioid dosage appears to correlate with the severity of the syndrome. Although total dosage (in morphine-equivalents) is greater with fentanyl, it is probably the opioid of choice, as it appears to have fewer adverse reactions than, for instance, pethidine, which may

increase complications in Irukandji Syndrome. Finally I believe that a new definition for the syndrome should be made based on the findings of this database and this is discussed in further detail in the following chapter.

CHAPTER FOUR: DEFINITION OF IRUKANDJI SYNDROME FROM THE NORTH QUEENSLAND REGION

Abstract

This study uses the historical database of patients presenting with Irukandji Syndrome to the Emergency Department of the Cairns Base Hospital from 1995 to 2007 as well as the historic database from Dr. Jack Barnes, which describes stings presenting from 1942 to 1967. The spectrum of systemic symptoms is presented as well as their occurrence in patients. It was observed that patients presenting with either elevated troponin levels or diaphoresis required a significantly higher levels of morphine equivalent opiates, than those presenting with any other symptoms, however troponin level rise does not occur until at least three symptoms are experienced. Of all the presentations, pain was the overwhelming symptom with it accounting for 58.1% of the total percentage of symptoms displayed. In conjunction with the data analysis from Chapter Three, I propose that a new definition of the syndrome is required to provide a more encompassing diagnosis of this syndrome and I have termed this Irukandji Syndrome Complex. The definition is: Irukandji Syndrome Complex must be considered if a patient has (1) Recent contact with seawater (2) A delay of 5 to 60 minutes between an initially mild sting sensation and the onset of constitutional symptoms (3) With one or more of the following symptoms; pain (regardless of location), headache, nausea and vomiting, diaphoresis, shortness of breath and/or troponin leak. I believe this is applicable for not only this region but for other areas of the world and using this modified definition would bring to light previously undocumented or misdiagnosed cases of this syndrome. While this does still not assist in revealing the cause of an envenoming, this definition can be used regardless of the biological source of the sting, and the term Irukandji Syndrome Complex can be used as a term for diagnostic purposes to indicate certain course of treatment.

Introduction

Dr Hugo Flecker published the original description of Irukandji Syndrome from North Queensland, Australia in The Medical Journal of Australia in January 1945. In this original description, there was variation reported in the syndrome between sting victims, and this trend is one that has continued since that time with a wide range of symptoms associated with this infliction. It is little wonder then that confusion in the specific definition of this syndrome has abounded for so long.

As highlighted in Chapter Two, variation seems to be the only consistency when it comes to Irukandji Syndrome envenoming and even initial contact shows a wide range of reported reactions. Pain from first contact could be likened to that of a bee or wasp in some cases (Fenner and Carney, 1999, Flecker, 1952b) and has been noted as being 'quite severe' (Holmes, 1996), mild, insignificant (Burnett, 2001, Fenner et al., 1986, Fenner, 2000, Burnett et al., 1998) to apparently absent in others (Fenner, 1991, Flecker, 1952b).

Following the initial envenoming there is generally a delay before systemic symptoms set in. This has been reported to range from as little as 5 minutes (Flecker, 1952a, Fenner, 1991, Barnes, 1964) up to 40, 60 and 120 minutes (Fenner, 1991), (Flecker, 1952a), (Barnes, 1964) respectively, with an average time of 25 - 40minutes being the general consensus (Barnes, 1964, Flecker, 1952b, Fenner et al., 1986, Fenner, 1991, Holmes, 1996), with a wide range of symptoms experienced, which are presented in full in Chapter Two, Table 2.1.

Distinct differences/variation

Variation displayed between envenomed casualties has led to many theories on the reason for such deviations. Suggestions of a correlation between the duration of jellyfish contact (Barnes, 1964) or the time to first aid and sting severity have been postulated, however this observation was based on only three cases and there was

acknowledgement that there were too many other factors involved and too few test cases to support this (Kinsey, 1988). Additionally thicker skin regions or presence of body hair was suggested as maybe providing partial protection from an Irukandji sting (Barnes, 1964), with the shaved legs of females more often resulting in stings then that of males. In a similar fashion the thin and hairless skin of children was said to make them more susceptible to stings, however it was also noted that the tendency of children to utilise the shallower inshore sections of the beach where the majority of the stings occurred could also be the reason for their increased susceptibility (Barnes, 1964).

To further complicate the situation, when nematocyst retrieval has implicated *Carukia barnesi* as the offending animal the range of syndrome has still been quite vast (Huynh et al., 2003). In fact hospital studies have seen *C. barnesi* victims experiencing the whole gambit of symptoms from no/mild effects through to severe illness including cardiac dysfunction (Little et al., 2006) and even death (Pereira et al., 2010). This may be in response to an ontogenetic change in the venom of the species (Huynh et al., 2003, Pereira et al., 2010), as has been seen to occur for both this (Underwood and Seymour, 2007) and other cubozoan species (Underwood and Seymour, 2007, Carrette et al., 2002). However it is unclear if variation in the venom components from immature to mature individuals (Underwood and Seymour, 2007) translates to variation in envenoming risk or susceptibility. Though in the Cairns region, there are suggestions of smaller animals being able to move inside the stinger nets while larger animals are restricted to outside the 'stinger resistant' enclosures, perhaps increasing the likelihood of swimmers being sting by young individuals (Fenner, 1999).

Stings from the Cairns region in general are reported as relatively mild, without serious complications and despite one fatality recorded in this region from Irukandji Syndrome (Huynh et al., 2003) the majority of stings presented do not require

hospital admission (Mulcahy and Little, 1997, Huynh et al., 2003), however diagnosis of the syndrome remains dependant on the treating physician accurately identifying this specific syndrome. Multiple stings have been rarely reported so the potential for increased resistance to Irukandji stings is unknown and anecdotal, however, what few reports exist suggest that there is no additional risk from second encounters (Cleland and Southcott, 1965, Barnes, 1964).

In an effort to define specific criteria that can be reliably used to categorise Irukandji Syndrome as it occurs in the Cairns region this study uses a retrospective approach on a the currently existing database (as discussed in Chapter Three) to examine a comprehensive list of the common symptoms displayed. The range of symptoms patients may experience and the frequency in which these occur was investigated.

Materials and Methods

As per methods section Chapter Three, patient files from the Cairns Base Hospital were retrieved and examined for cases of Irukandji Syndrome and the additional database from Dr Jack Barnes was also analyzed. Defined systemic clinical symptoms included headache, nausea, anxiety, vomiting, sweating (diaphoresis), restlessness, muscle cramps in all four limbs, abdomen and chest or severe lower back pain (de Pender et al., 2006). Added to these symptoms was shortness of breath and the presence of troponin I (if measured as higher than normal, gauged as cTnI, normal < $0.7gL^{-1}$) as these have also been reported in the literature from this area (Huynh et al., 2003, Barnes, 1964). As this study aimed to investigate the scope of symptoms displayed, the grouping together of pain (Chapter 3 Materials and Methods section) was not conducted and similarly, vomiting and nausea were also left as separate symptoms. A total of 347 envenomings were included in this study covering the years from 1995 – 2007.

To determine if mean morphine-equivalent dosage altered with the type of symptom experienced a general linear model (ANOVA) was used with LSD post hoc analysis.

Results

There was a significant effect of the type of symptom experienced with the morphine equivalent dosage received ($F_{(9x432)}$=2.19, $p < 0.05$) with patients experiencing symptoms of troponin I or diaphoresis receiving significantly higher morphine equivalent doses (Figure 4.1) regardless of number of symptoms experienced.

When only one symptom is seen, the greatest percentage of cases experience either abdominal pain or back pain and these (along with nausea as symptom number increases) remain the dominant symptoms displayed as patients experience more symptoms (Figure 4.2).

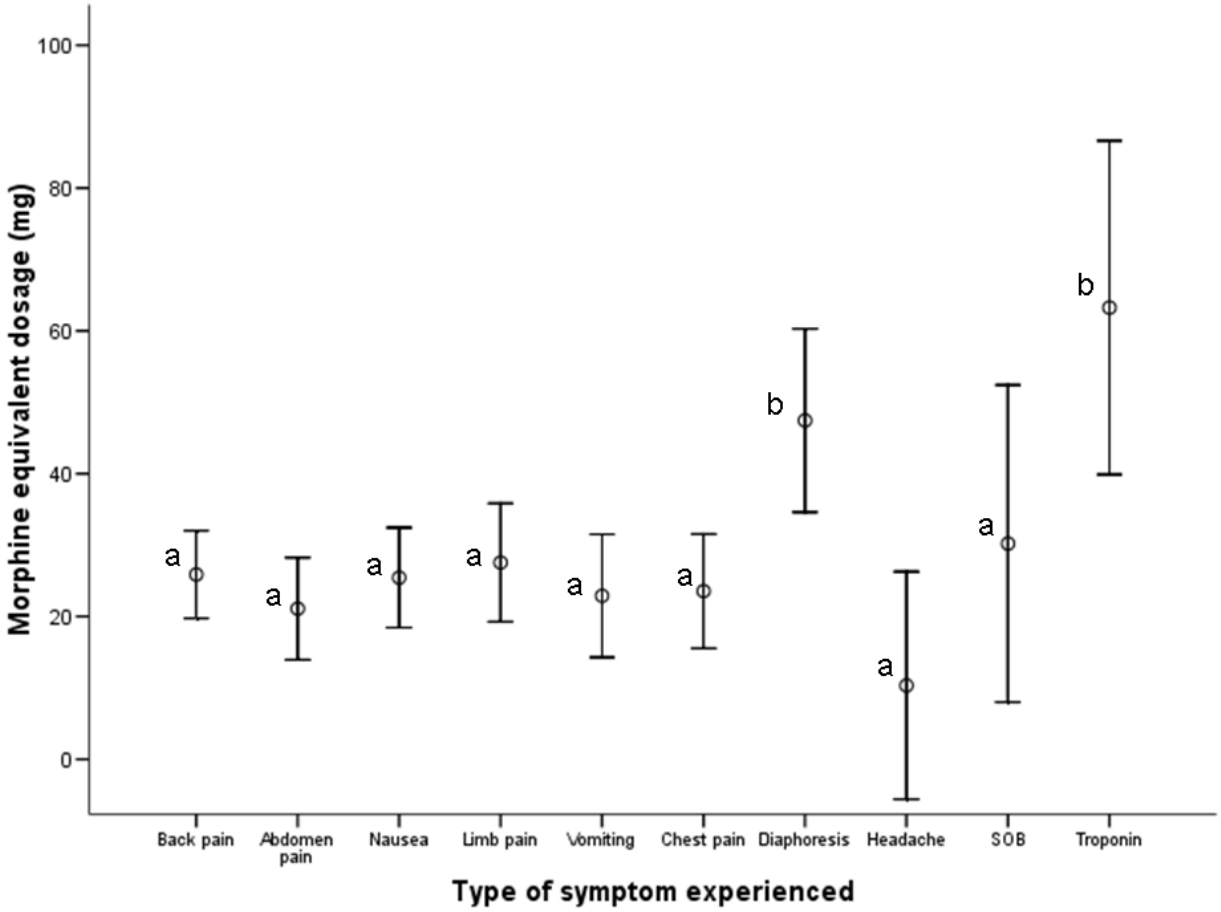

Figure 4.1: Mean morphine equivalent dosage administered for type of symptom experienced (Error bars are 95% Confidence limits; letters denote significantly different means)

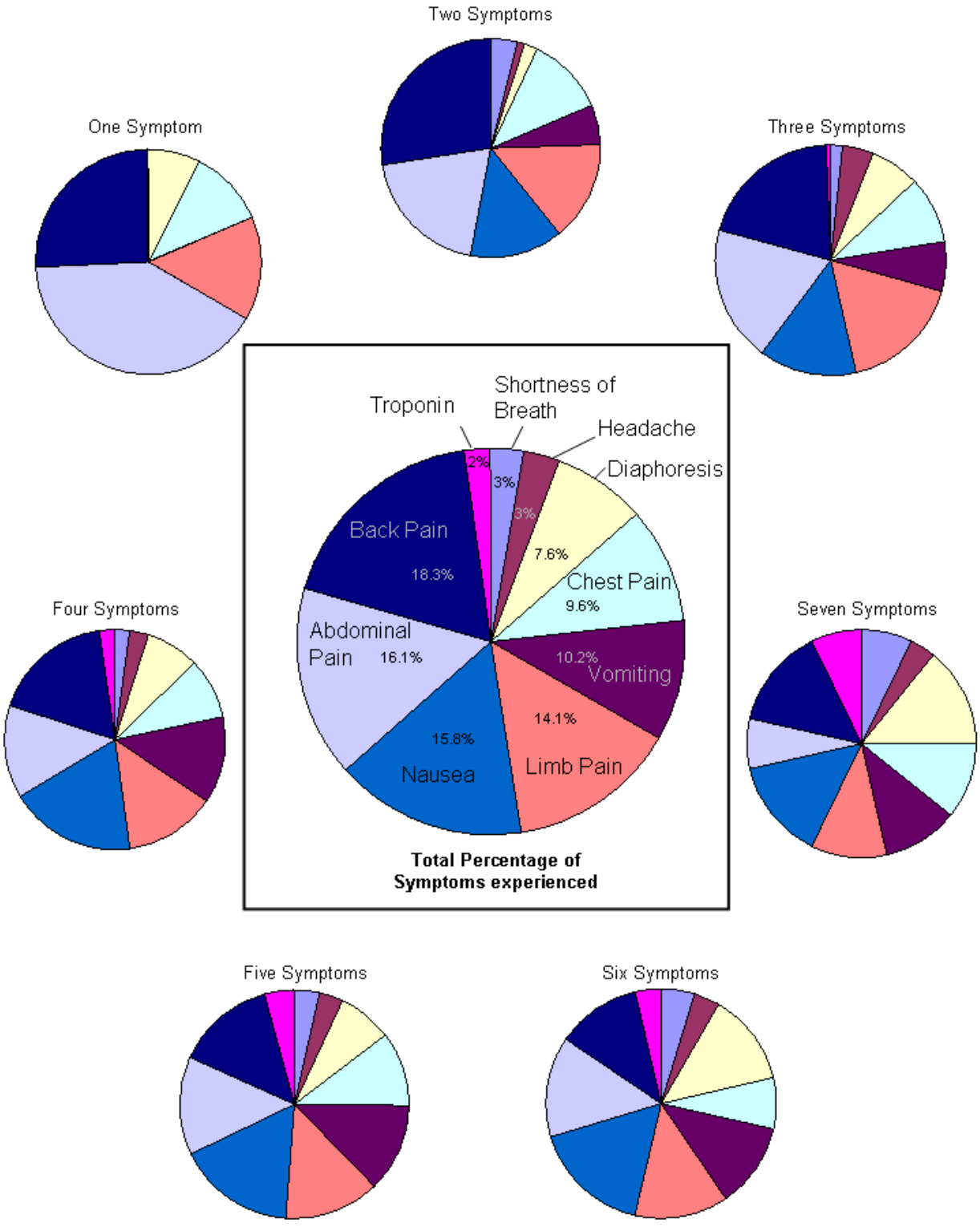

Figure 4.2: Total percentage of symptoms experienced and the relative percentages for the number of symptoms that developed

Discussion

Cardiac dysfunction has been linked with this species, however, with troponin rise, ECG changes and a reduction in cardiac output has previously been seen in only one patient where *Carukia barnesi* nematocysts were retrieved from the victim's skin (Huynh et al. 2003), In the Cairns region which the majority of case studies have been conducted, only a small percentage of cases had been seen to have abnormal ECG readings with this syndrome (Huynh et al., 2003) and cardiac problems have been reported to resolve on follow up inspections (Martin and Audley, 1990, Fenner, 1999) even if this can take many months (Fenner, 1999). This study illuminates the presence of troponin rise in a number of patient cases, and shows it does appear to be a significant symptom of Irukandji Syndrome in this area. While a relatively small proportion of patients are recording elevated troponin levels, there is a significant increase in the amount of opiates these patients require. Interestingly, there is also a significant increase in opiates required for patients who are presenting with diaphoresis and, while the reason for this is unclear this is potentially useful from a diagnostic perspective for clinicians.

While not all patients receive the whole spectrum of symptoms (in fact some only experience one or two), with an increased number of symptoms experienced there is an increase in the amount of morphine equivalent dosage administered (Carrette and Seymour, 2013), which would infer a more severe sting is experienced. Diagnostically this is an interesting observation with patients suffering from a wide range of symptoms likely to need more opioids than those suffering from only a few of these symptoms. From the percentages of symptoms experienced, it appears that overwhelmingly the major symptom experienced is some element of pain (be it back, limb, chest or abdominal) with this comprising of 58.1% of the total percentage for all the symptoms experienced. Diagnostically, for patients presenting with only

one symptom, it is some element of pain or diaphoresis that appears to be experienced for all cases examined.

Irukandji Syndrome, while historically difficult to determine, has most recently been defined as requiring at least three systemic clinical symptoms including nausea; vomiting; headache; sweating; anxiety; restlessness; muscle cramps in all four limbs, the abdomen and chest or severe low back pain (de Pender et al., 2006). Investigation of our records for stings in this part of the world show that using this definition of at least three symptoms would in fact exclude 74% of all Irukandji sting cases (under the currently definition by de Pender 2006), with 31% of patients examined displaying only one symptom (Chapter Three Figure 3.5).

Given that with increased awareness regarding this syndrome, there is increased reporting of it's occurrence across larger regions both within Australia and globally (Carrette et al., 2012, de Pender et al., 2006, Grady and Burnett, 2003, Macrokanis et al., 2004) I propose a more accurate description would see more of these cases being accurately identified.

As such I suggest that a new description for the syndrome be developed and I propose that this be termed Irukandji Syndrome Complex, to reflect the more encompassing nature of this definition.

Irukandji Syndrome Complex must be considered if a patient has

(1) Recent contact with seawater

(2) A delay of 5 to 60 minutes between a relatively mild sting and the onset of constitutional symptoms

(3) With one or more of the following six symptoms; pain (regardless of location), headache, nausea and vomiting, diaphoresis, shortness of breath and/or troponin leak.

I believe this would provide a more encompassing diagnostic tool for physicians to identify this syndrome worldwide.

CHAPTER FIVE: ECOLOGICAL VARIATIONS IN *ALATINA MOSERI* AGGREGATIONS FROM WAIKIKI BEACH (HAWAII) AND OSPREY REEF (AUSTRALIA)

Abstract

Populations of the carybdeid *Alatina moseri* were found to be displaying lunar–based aggregations at two locations from Hawaii and Australia consistently forming at multiple locations 8-12 days after the full moon. These aggregations have been previously reported from both Hawaii and Australia with evidence suggesting that these are spawning events. Populations of animals were collected on multiple occasions from both locations to examine their structure and venom capacity both spatially and temporally. *Alatina moseri* specimens from the Australian population at Osprey Reef were significantly larger with a mean bell height of 72mm (n=118) compared to those from Hawaii who had a mean bell height of 62mm (n=2331). The limited data that was obtainable for near successive events (both locations had collection success two months apart) no increase in mean bell height was shown with Osprey Reef animals actually showing a significant decrease in bell height in this time. This, coupled with the presence of dying animals observed in the days following collections, supports the theory that these are one off spawning events. For Hawaii based populations, there is evidence to suggest that the males are arriving at these events in greater numbers initially, followed by the females and then the spent animals make up the aggregation after that.

Additionally, venom from the tentacles of these animals was collected at both locations during aggregations and analyses through HPLC and SDS page gels were performed. Venom composition showed intraspecies variation both between geographical populations and also seasonally at one location meaning these animals have the propensity to change their venom composition in some way. The implications for this variation may be pertinent for human envenomings from this and related species.

Introduction

When humans are envenomated by toxic species, small differences in venom profile may produce different symptoms in the patient (Chippaux et al., 1991) highlighting the importance of assessing how venomous species vary in ecology, morphology and venom structure across multiple populations (Francischetti et al., 2000). In the previous Chapters I highlighted the lack of clarity that exists in identifying and understanding the mechanisms of Irukandji Syndrome. *Alatina moseri* has been demonstrated to cause this syndrome in both the Hawaii and Osprey Reef (Australia) regions (Chung et al., 2001, Yoshimoto and Yanagihara, 2002, Thomas et al., 2001b, Thomas et al., 2001a, Carrette et al., 2012, Little et al., 2006) however a comparison of the venom from these populations does not currently exist. There is a growing body of evidence, ecological, morphological and molecular, that the two cubozoan species, *A. mordens* (Hawaii) and *A. moseri* (Australia) are in fact metapopulations of a single species, *A. moseri* (Bentlage and Lewis, 2012, Bentlage et al., 2009). Our knowledge of the two groups suggests that they share morphology; share a reproductive seasonality and strategy unusual for cubozoans, making them ideal model populations with which to examine intraspecies variation in ecology and venom composition.

Morphology

Lengthy geographical, spatial or ecological isolation of populations within a species can lead to divergence in morphology, physiological state and/or behaviour as a result of different selection pressures associated with their particular ecological niche, seasonal variations, or predation pressures (Endler, 1977, West-Eberhard, 1989, Price et al., 2014). Morphological variations in jellyfish species have previously been reported for populations of *Phyllorhiza punctata* from geographically distinct regions (Botton and Graham, 2004) and likewise adaptive polymorphism has been recognised in populations of the moon jellyfish *Aurelia* sp. (Dawson and Martin,

2001) potentially driven by various localized environmental factors. While intraspecific variation in jellyfish venom profiles has yet to be evaluated, differences have been noted between geographically and seasonally distinct populations of venomous cone snails (Duda et al., 2009, Jakubowski et al., 2005) with potential variation being related to prey utilization for distinct populations. Research is discovering an increasing number of animals that show marked variation in venom profiles on an intraspecies level (Daltry et al., 1996, Menezes et al., 2006, Francischetti et al., 2000) and factors associated with this variation have been attributed to an individual's sex, diet, age, geography, season and venom regeneration time (McClounan and Seymour, 2012, Chippaux et al., 1991, Binford, 2001) although how this variation is controlled still remains unknown (Jakubowski et al., 2005).

Reproduction

Previously, lunar based spawning events have not been reported in other cnidarians, however, recent evidence has demonstrated lunar-linked spawning activity in *A. moseri* (Chiaverano et al., 2013). The lunar linked periodicity of *A. moseri* in Hawaii has been documented since the late 1980's with their predictable arrival on Waikiki beach being reported as the 8^{th} to 12^{th} days after every full moon for a 2-4 day period (Chiaverano et al., 2013, Thomas et al., 2001a) with more than 800 animals being recorded at several of these events. Animals are reported to be fully mature at this time with active gonads expelled during this period (Chiaverano et al., 2013) suggesting that this is a spawning aggregation. Additionally, populations of *A. moseri* from Australia have documented this same seasonal pattern (Courtney and Seymour, 2013, Carrette et al., 2014) with sexually mature, spawning individuals forming these aggregations (Carrette et al., 2014). Very little documentation on the sexual reproduction of cubozoans in the wild exists, with the majority of successful cultures occurring from a few collected 'ripe' specimens that have consequently

spawned in aquaria or buckets (Toshino et al., 2012, Studebaker, 1972, Hartwick, 1991a) which may potentially be stress induced. These aggregations present the opportunity to observe spawning behaviour in a naturally occurring situation with wild populations of animals.

Venom

Cubozoan venom studies of such type are in their infancy, however, intraspecies variation has been shown for both *Chironex fleckeri* (Winter et al., 2010) and *Carukia barnesi* (Underwood and Seymour, 2007). With these species showing an ontogenetic venom change and a geographical variation for *Carukia barnesi* and *Chironex fleckeri* respectively (Winter et al., 2010, Underwood and Seymour, 2007). Carybdeid research in particular has been historically hampered by the small quantities of venom available for collection, due to the unpredictability of their presence and the relatively small individual size and tentacle number (Barnes, 1966). Aggregations of *Alatina moseri* present a rare opportunity to collect substantial volumes of venom from a carybdeid species for analysis. Venom from these events will then allow for comparison of both seasonal and geographical variation in this species.

Members of the *Alatina* genus (reported as *Carybdea alata)* have been reported as occurring at or close to the continental shelf (Arneson, 1976), which is unlike many other cubozoans, and therefore observations of animal position and movement in the water column during this time will be detailed. Additional understanding of the movement patterns and ecology of these medically significant jellyfish is an essential step towards further risk management protocols. The aim of this study was to investigate the population dynamics, reproductive strategies, and venom profiles of aggregations of *Alatina moseri* from Australia and Hawaii and compare both between and within locations.

Materials and Methods

Species collection sites

Collections of *Alatina moseri* were made from both Hawaii and Australia opportunistically over a three-year period. In Australia the primary site was Osprey Reef (13°52′55.17″S, 146°32′56.74″E) (Figure 5.1) and animals have been recorded there at a semi-regular basis from the years between 1999-2005 with aggregations appearing 8-10 days after the full moon (Undersea Explorer – dive log books).

This is a highly remote site and access is very weather dependent. Opportunistic trips were taken on board research dive vessel “The Undersea Explorer” which operated to this site on a regular basis but collection success was dependent on availability and access to the specific site. Additional trips were supplemented on board the charter vessel “Deepstar”, however again, collection success was extremely weather dependent. Trips where aggregations were discovered, physiological data could be collected and venom samples were extracted was made on the 26/09/2005, 25/11/2005, 13/11/2006.

Waikiki beach, Oahu was the sole collection site in Hawaii (21°16′12.90″N, 157°49′24.22″W) (Figure 5.2) as, like in Australia, large aggregations of animals have been reliably recorded on a monthly basis (Chiaverano et al., 2013, Yoshimoto and Yanagihara, 2002, Thomas et al., 2001b, Thomas et al., 2001a). These chosen sites allowed for reliable collection of large aggregations of individuals at one time period. As this type of collection trip also involves considerable expense, efforts were concentrated to a few main occasions when animal abundance seemed optimal.

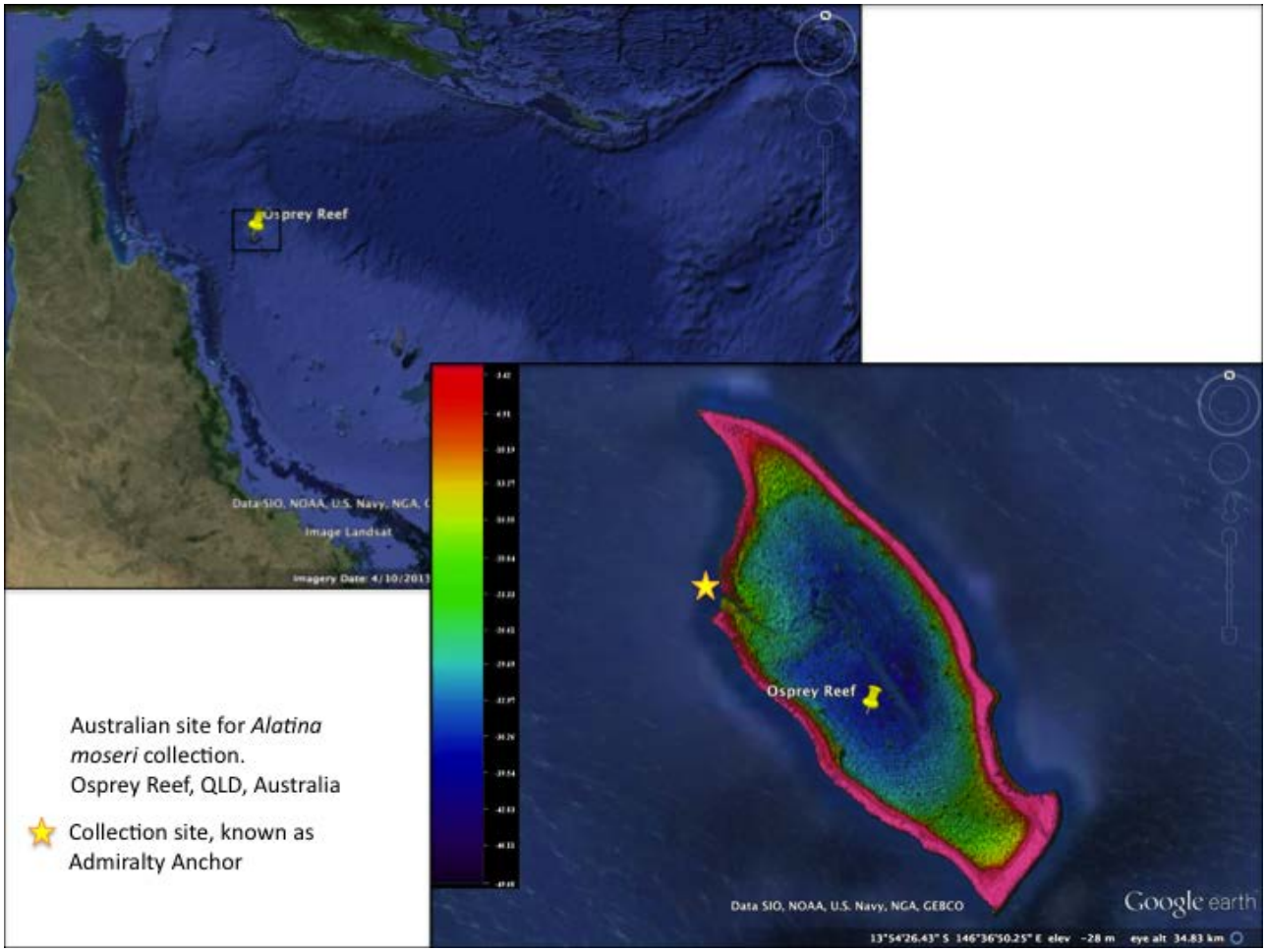

Figure 5.1 Field site map for collection of *Alatina moseri* from Osprey Reef, Australia. Images sourced from Google Earth and adapted from www.deepreef.org

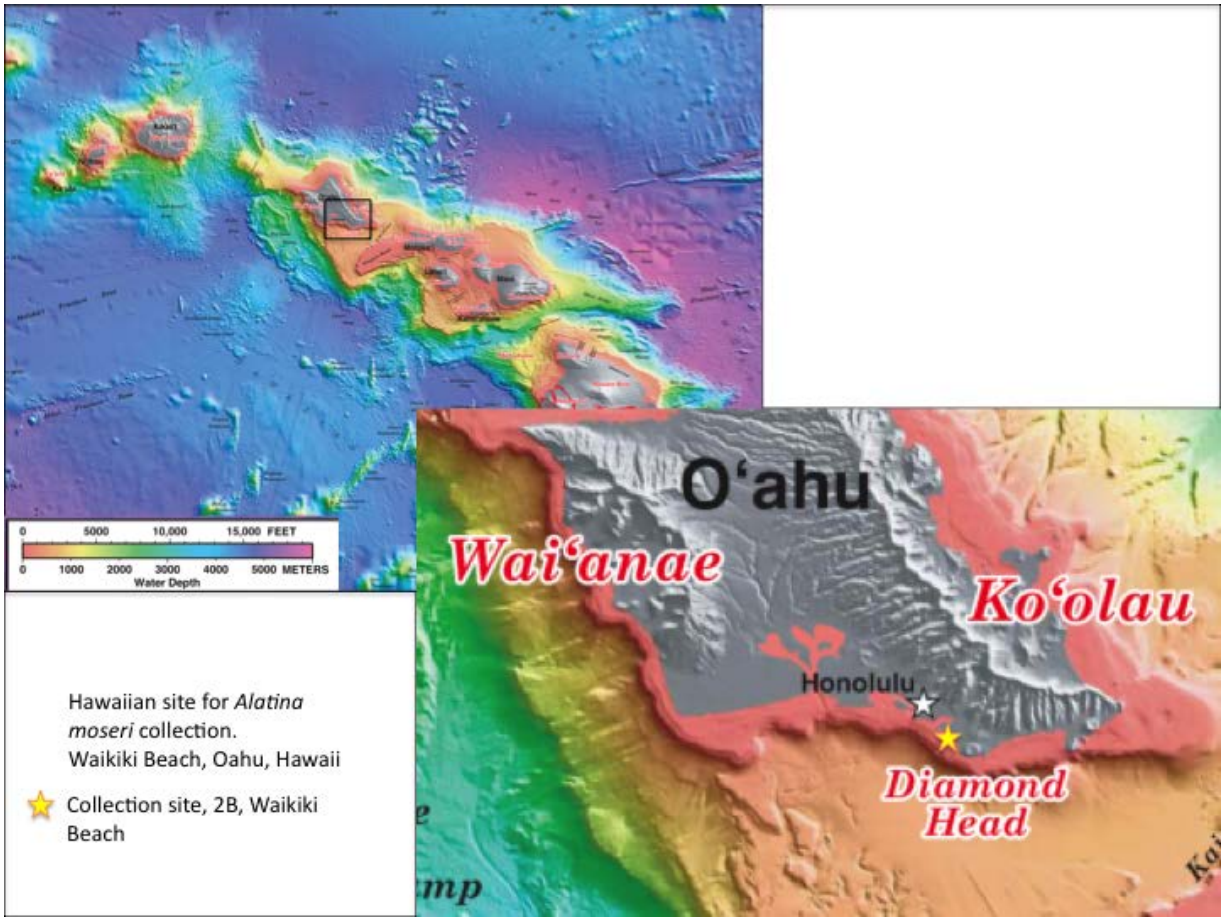

Figure 5.2 Field site map for collection of *Alatina moseri* from Waikiki Beach, Oahu, Hawaii. Images adapted from Eakins *et al.* (2012)

Successful aggregations were encountered on the 22/10/2000, 19th-21st/11/2000, 22/04/2006, 19th-21st/06/06 and the 05-06/09/2007. Physiological parameters were taken for all these events, with venom collection occurring from the 06/09/2007 and 22/04/2006. A general linear model (ANOVA) was used to investigate the variation on size of animals (as determined by bell height) between geographic locations and also between collections from the one location. Post hoc analysis (LSD) was performed on significant effects to determine which treatment means were significantly different.

Specimen collection technique

Animals collected from Osprey Reef, Australia were collected between the hours of 1900hrs and 0400hrs from a research dive vessel anchored at the reef's North Western shelf. Carybdeids are reported as being readily attracted to light sources at night time (Lewis and Long, 2005, Arneson, 1976) and large numbers have been reported as aggregating around dive boat spotlights in this area (Undersea Explorer dive staff pers comms). As such aggregations of medusa were attracted towards the boat for collection using 1000watt underwater halogen lights, set at a depth of 1meter off the diving platform. As animals surfaced near the lights, they were extracted from the water with dip nets and transferred into collection bins filled with fresh seawater. Time interval of collection (noted in 15 minute increments) was also recorded. At the end of the collection, individual animals were processed, which included bell measurements, sex determination and collection of tentacles. Tentacles from each collection were excised and placed in fresh seawater for venom extraction. Additional to sampling the animals present at the surface, secondary observations of the population were made via diving within the aggregations. This was done to ensure the subset sampled was representative of the entire aggregation.

Collections of animals from Waikiki were done between the hours of 1900hrs and 0600hrs from a location of beach stretching no more than 400mts in length in 15minute time intervals.

Individual animals that freshly washed up onto the sand were collected by hand and placed individually into plastic click-seal bags with fresh seawater. At the end of a collection event, individual animals were then measured, sexed, had their tentacles removed and placed in fresh seawater. As with the Osprey animals, potential for sampling a subset of the population that were washing onto shore existed, so

supplementary diving offshore within the aggregation allowed for a visual inspection of the larger aggregation.

Population Dynamics

While there are a number of morphometric measurements that are commonly used for cubozoan medusae (bell height, diagonal bell width, total body length, interradial bell width) (Collins et al., 2011, Gershwin, 2007, Gordon et al., 2004), the fragility of these animals renders some of these more speculative and prone to sampler variation especially on these less robust *Alatina moseri* specimens. For this reason, measurements for this study were taken as bell height, (measured from the top of the rhopalial niche, to the apex of the bell when animals were positioned flat on a surface) and also bell width (taken as interpedalia distance through rhopalial niche).

Sex determination was made by direct observation of mature gametes in the bell tissue with eggs having a more solid grainy consistency to the milky sperm of the males. Any specimens that were in doubt had a small sample of gonad tissue removed and this was confirmed using light microscopy. Animals where neither male nor female gametes could be easily determined had canal tissue removed to inspect for underdeveloped or absent gonad material. Animals were consequently identified as male, female or spent (if gonad tissue had been released already and no immature gonads were noted).

Venom comparison

Specimens of jellyfish were collected as described above and tentacles were excised and immediately placed in fresh sea water and stored at 4ºC for nematocyst extraction as per Bloom *et al* 1998. This technique involves refrigerating the tentacle material with daily agitation of the suspension to release nematocysts from the decaying animal tissue. After four days, the whole suspension is then drained through a fine mesh filter and rinsed through with fresh filtered seawater. This

process isolates the nematocysts in the seawater suspension so that the remaining decomposing tissue can then be discarded. The filtered sediment containing the concentrated nematocysts settles and is then lyophilized and stored at -20ºC until use. As all specimens collected in these events were deemed to be mature (see population dynamics section), therefore venom was pooled for analysis as any potential ontogenetic variation, if present, should not apply. Lyophilized samples had venom extracted as per Carrette and Seymour (Carrette and Seymour, 2004). This involves introducing lyophilized nematocysts and distilled water into 3ml screw top vials with 0.5mm glass beads (approximately 8000 per 3ml vial). This mixture is placed into a mini bead mill beater and shaken at 5000rpm for 10 second intervals a total of four times with 1 minute period of ice bath in between each interval. The disrupted mixture is then centrifuged at a speed of 3000rpm for a period of 1 minute allowing for the rehydrated venom to be extracted from the top of the solution by pipette.

Protein determination was performed using a Pierce BCA protein assay kit following manufacturer's instructions. Briefly, bovine serum albumin (BSA) was used as a reference standard. Twenty-five (25)μL venom was added to an individual well of a 96-well micro-titre plate. MilliQ water was used as a blank and all experiments were performed in triplicate. Color development was assessed using Fusion a microtitre plate reader set at a wavelength of 562nm (Perkin Elmer; Massachusetts, USA).

Sodium dodecyl sulphate -polyacrylamide gel electrophoresis (SDS-PAGE)

Polyacrylamide mini gels (10 well, 1.5 mm thick) were cast using BioRad gel casting system (BioRad Laboratories; Hercules, CA, USA). Gels comprised of a 12 % polyacrylamide separating gel: 40%(v/v) 30% acylamide/bisacylamide (BioRad Laboratories; Hercules, CA, USA), 375mM Tris Base (Sigma; St Louise, Missouri), 0.1% (w/v) sodium dodecyl sulphate (SDS), 0.05% (w/v) ammonium persulfate (biorad), 0.05% (v/v) TEMED (BioRad Laboratories; Hercules, CA, USA)/ milliQ H_2O

up to 20mL) and a 4% polyacrylamide stacking gel: 13.2 %(v/v) 30% acylamide/bisacylamide (BioRad Laboratories; Hercules, CA, USA), 126mM Tris Base (Sigma; St Louise, Missouri), 0.1% (w/v) SDS (Ameresco; Solon, Ohio), 0.05% (w/v) ammonium persulfate (BioRad Laboratories; Hercules, CA, USA) 0.05% (v/v) TEMED (BioRad Laboratories; Hercules, CA, USA)/ milliQ H2O up to 6.25mL). Thirty (30)μg protein was diluted in Laemmli's sample buffer (50% glycerol; 0.5% Bromophenol Blue; 3.1% Tris-HCl pH 6.8 0.05% b-mercaptoethanol) at equal volume (1:1 (v/v) before heating for 5 min at 95°C. Proteins were loaded in triplicate on to gels and electrophoresed for 10 min at room temperature at 60V and then for 1.5 hrs at 130V. The molecular weight standard (Dual colour Precision Plus Protein standards, Bio-Rad, C.A. USA) was also run in parallel. Gels were placed in fixative solution (40% (v/v) methanol (Merck; Darmstadt, Germany),10% (v/v) acetic acid (Merck; Darmstadt, Germany) for one hour then transferred to BioSafe Commassie G-250 solution (BioRad Laboratories; Hercules, CA, USA) and left incubating on orbital shaker overnight. Gel was destained using 1% acetic acid (Merck; Darmstadt, Germany). Destain solution was changed regularly until background was reduced and protein bands could be visualised clearly. Gel image was captured utilising Typhoon Trio Scanner (GE Healthcare; Uppsala, Sweden).

Size exclusion HPLC analysis

Additional analysis on samples was performed using size exclusion HPLC analysis. This was to investigate the proportion of proteins present in venom samples collected. Chromatography was performed using a Shimadzu (Kyoto, Japan) high-performance liquid chromatography system (LC-10ATvp pump and SPD-10AVP detector).

Venom was prepared as per preparation methods described above, and then filtered through a 0.2 μm membrane, low protein binding, syringe filter. The venom was applied to a Superdex G200 (10/300 GL, GE Healthcare) column that had been

equilibrated with a 0.05M phosphate buffer (Na_2HPO_4 0.0375M, NaH_2PO_4 0.125M) pH 7.4 containing 0.15M Sodium chloride. The sample was eluted at a flow rate of 0.5ml/min. The eluent were monitored at 214nm. Output data was then entered into computer software Prism version 6.0 for graph generation.

Results

Population dynamics

Bell width from Hawaii varied from 19mm to 45mm with mean of 33mm and a standard error of 0.32 (n=234) with bell width from Osprey ranged between 18mm and 38mm with a mean of 26mm and a standard error of 0.51 (n=70). This measurement of bell width was difficult to reliably record as the animals lost structural integrity and pedalia did not maintain their shape and positioning well. Bell height was deemed a more robust and repeatable measurement of size, so was used for the majority of the animals collected. Correlation between bell height and bell width was examined (Figure 5.3) for 234 specimens from Hawaii and 70 individuals from Osprey Reef.

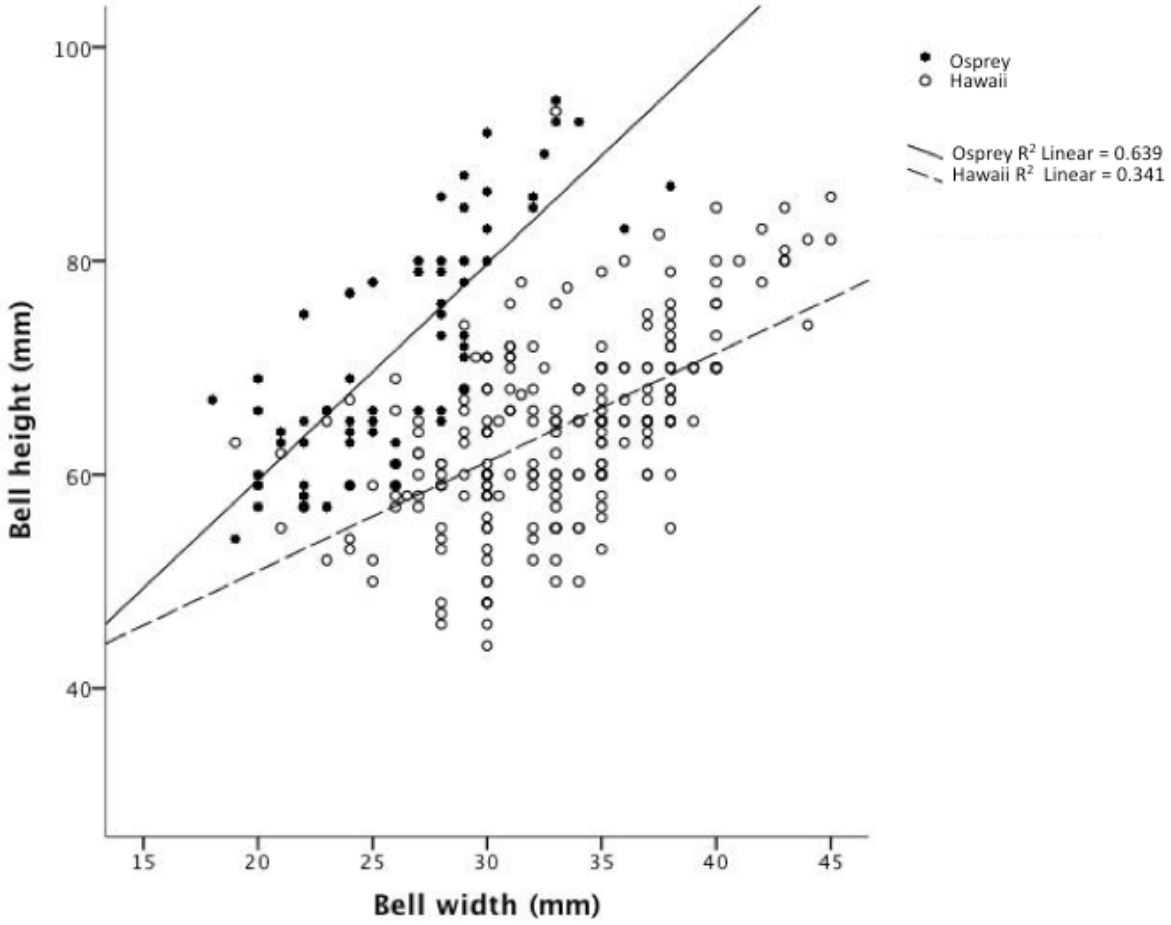

Figure 5.3: Correlation of size in bell height (mm) and bell width (mm) for *Alatina moseri* collected from Hawaii (n=234) and Osprey (n=70)

Alatina moseri specimens collected from Hawaii had a mean bell size (as measured by bell height) of 62mm (n=2331) (Figure 5.4) with the minimum height being 38mm and the maximum 94mm. Osprey Reef individuals were significantly larger (Figure 5.5) with a mean bell size of 72mm with a minimum size of 54mm and a maximum height of 95mm (n=118).

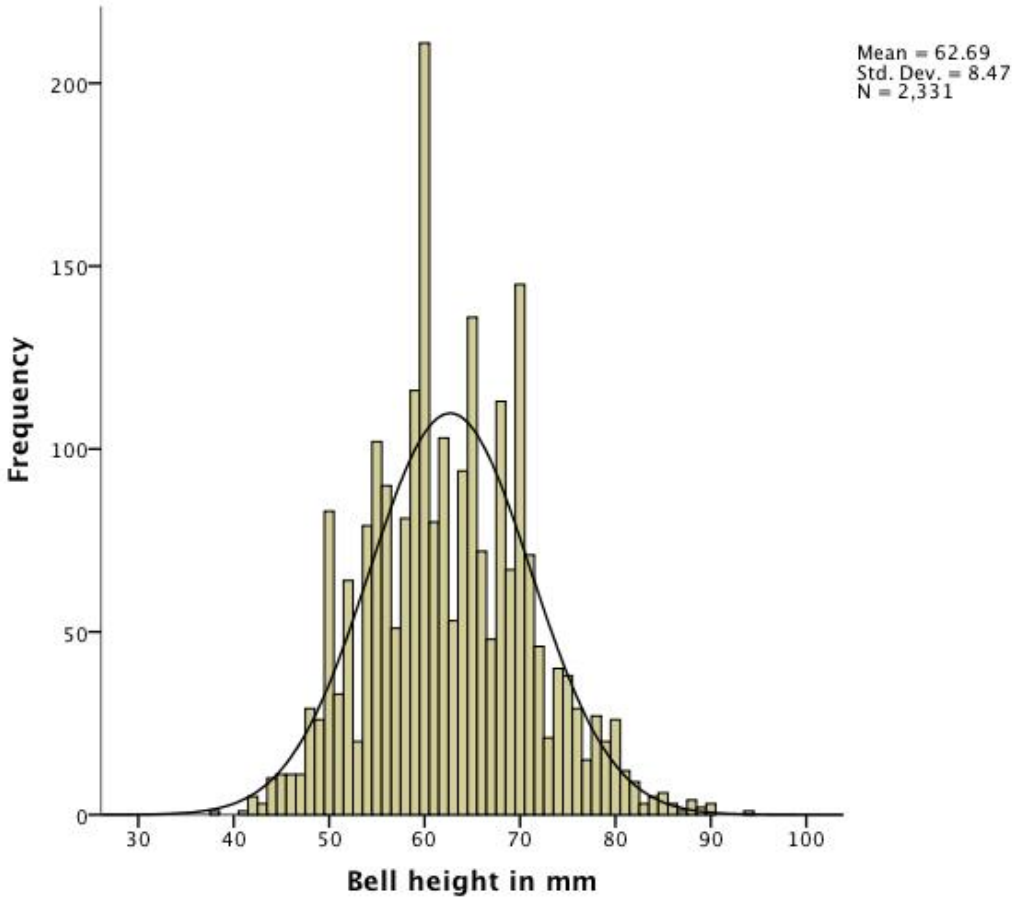

Figure 5.4: Distribution of size in bell height (mm) for *Alatina moseri* collected from Hawaii (n=2,331)

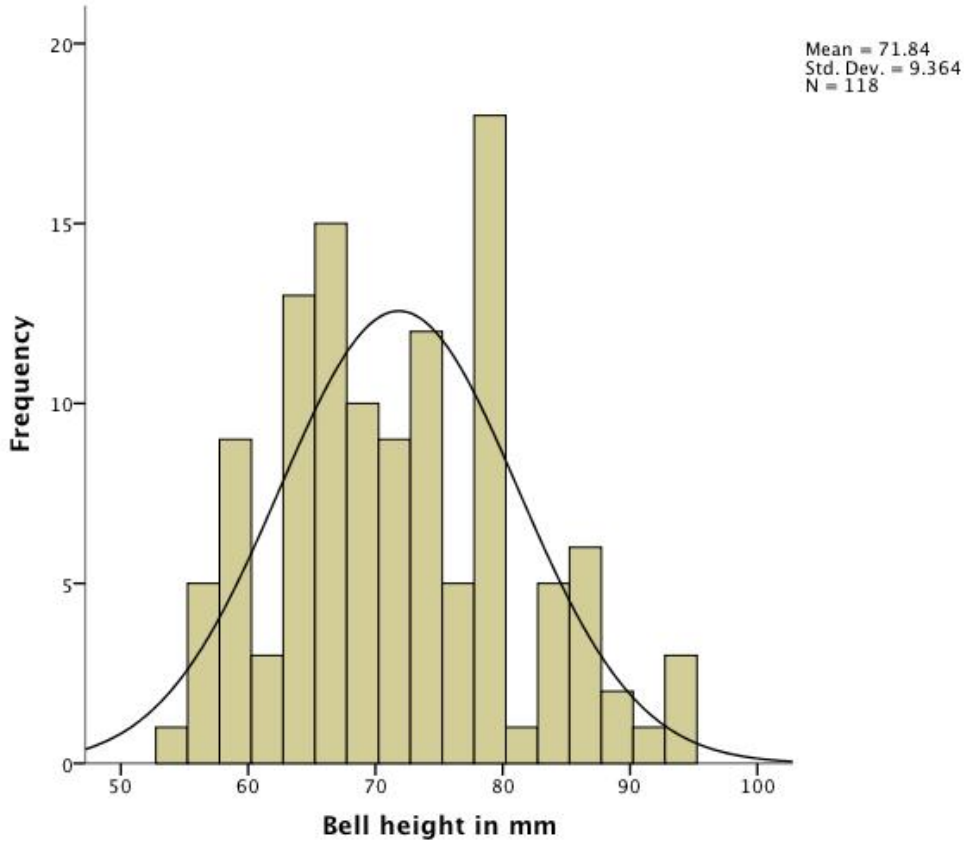

Figure 5.5 Distribution of size in bell height (mm) for *Alatina moseri* collected from Osprey Reef (n=118)

While there was variation in the bell height of animals within a population they were found to all be normally distributed within their size range. There was a significant effect of location on bell height ($F_{(1x2447)}$ =129.8, P<0.005) with the Osprey animals attaining a larger bell size than those collected from Hawaii (Figure 5.6).

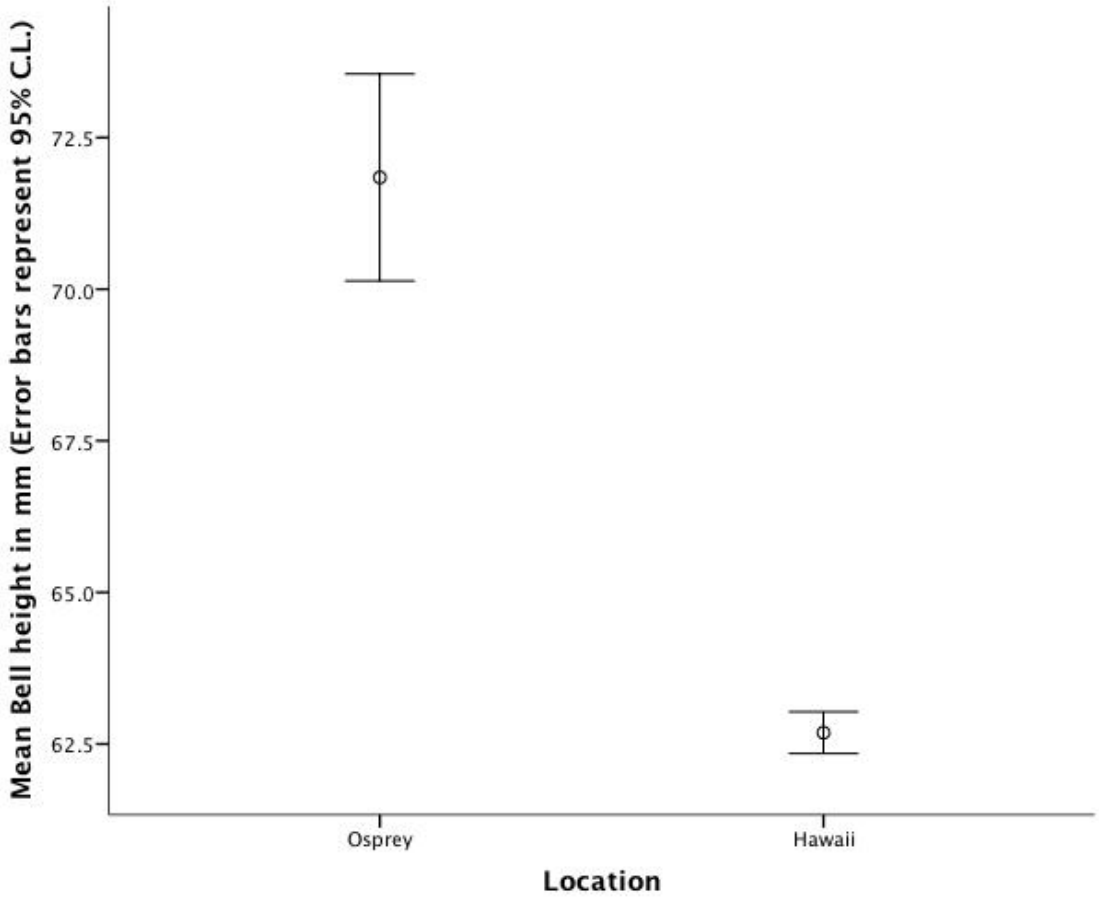

Fig 5.6 Mean bell height (in mm) recorded of *Alatina moseri* populations from Hawaii (n = 2,331) and Osprey Reef (n = 118) aggregations

There was a significant difference in the bell height of animals collected from Osprey Reef ($F_{(1,115)}$ =18.29, P<0.005) with animals from September 2005 having a larger mean bell height then those collected in November 2005 or November 2006 (Figure 5.7).

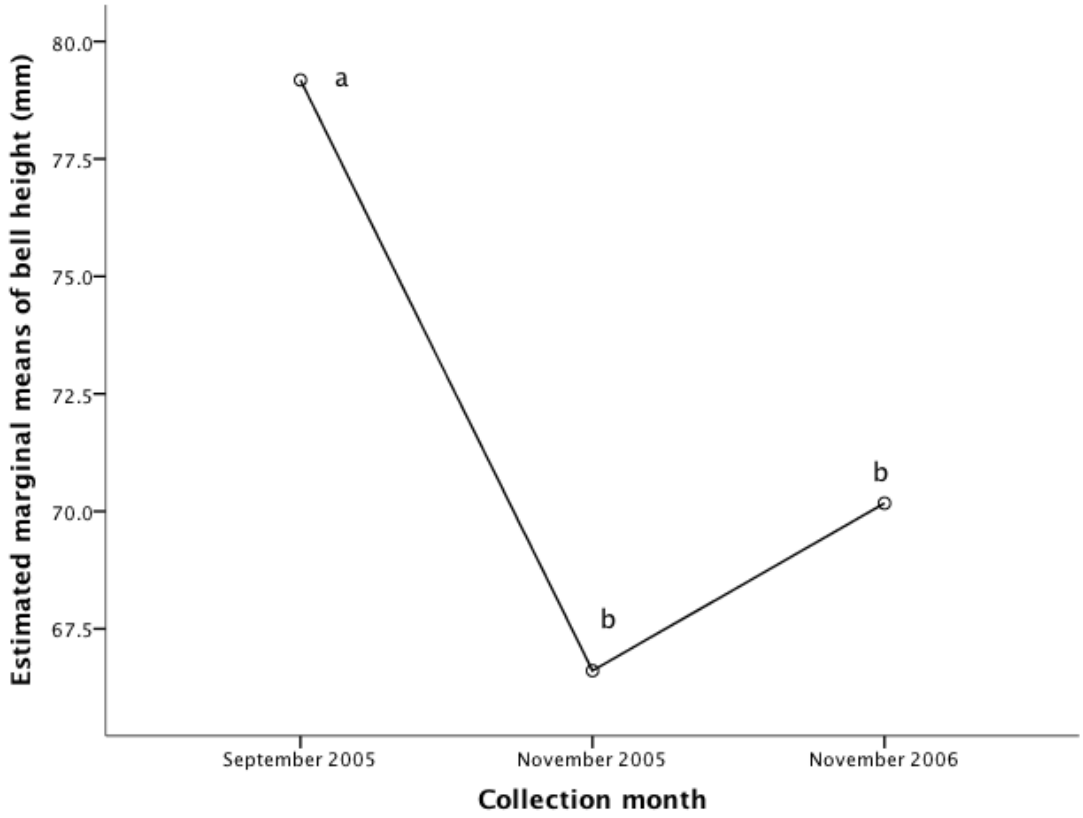

Figure 5.7 Mean bell height (in mm) recorded for Alatina moseri populations from Osprey Reef for September 2005 (n=31), November 2005 (n=23) and November 2006 (n=64) (means followed by same letter are not statistically different at the 0.05 level by LSD post hoc analysis)

There was no significant difference in the bell height of animals collected from Hawaii at different times (Figure 5.8).

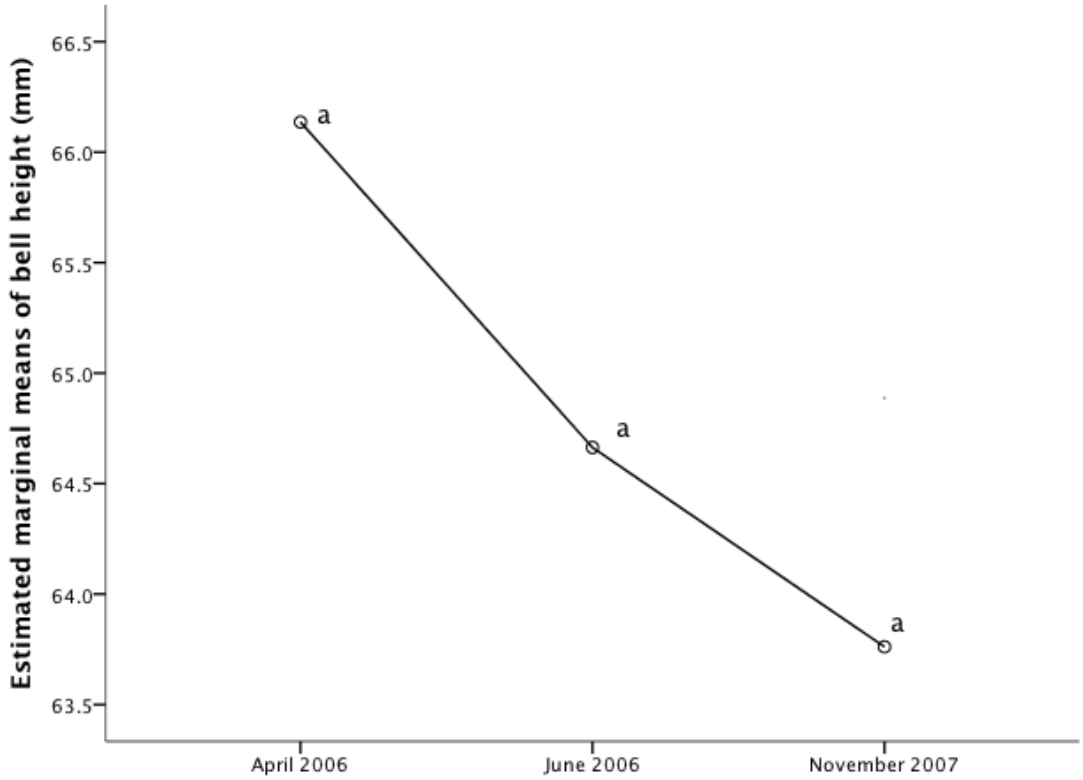

Figure 5.8 Mean bell height (in mm) recorded for *Alatina moseri* populations from Hawaii for April 2006 (n=22), June 2006 (n=52) and November 2007 (n=80) (means followed by same letter are not statistically different at the 0.05 level by LSD post hoc analysis)

All animals were sexually mature, with visible gonads held in their bell cavity, or spent (devoid of any gamete material) with no immature individuals observed within either of these populations. Animals that were holding gametes within their body cavity were seen to release these up to 4 hours post capture into the containers they were being held in.

Data that included sex determination with time increment for the Hawaiian population of animals showed that a larger proportion of males were present for the first 2 hours of collection with females then spent animals following after that time (Figure 5.9).

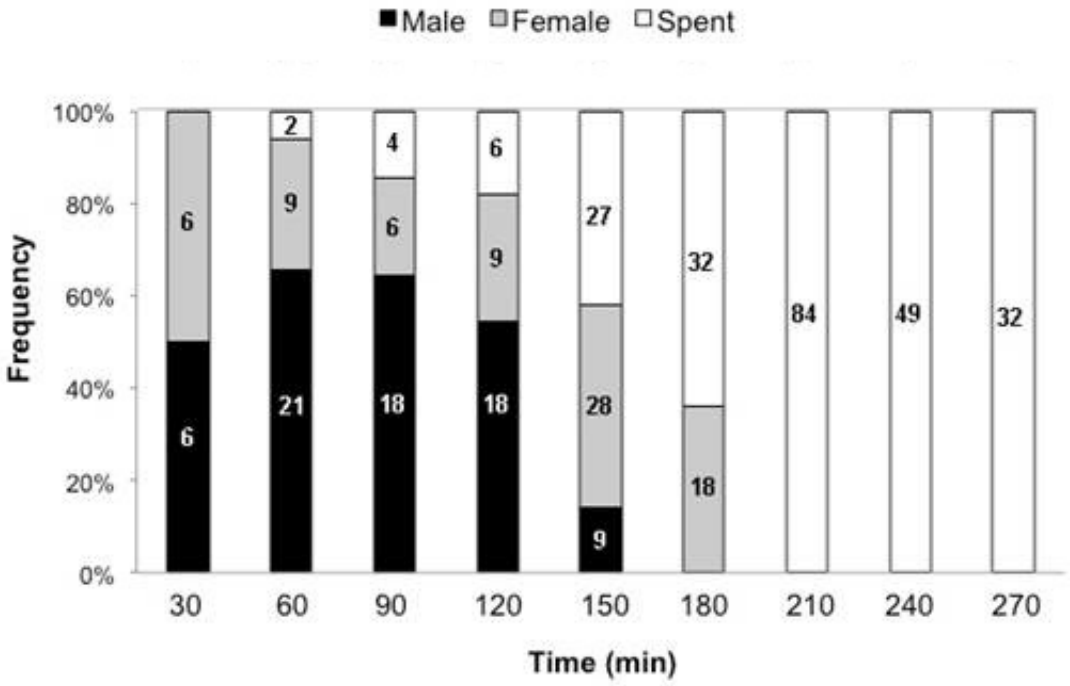

Figure 5.9 Ratio of male, female and spent animals present in aggregation of Hawaiian *Alatina moseri* with time in minutes since first appearance (numbers on bars = n values)

Diving within the aggregations at both sites allowed for visual assessment of the wider population. Osprey Reef individuals appeared to be moving in from the deeper water (from the west, heading east towards the reef wall) and were accumulating (albeit artificially around the dive lights) in the top ten meters of the water column at the anchoring point known as “Admiralty Anchor” (Figure 5.1). No animals were observed any deeper than 15meters in this area.

Diving for visual inspection of aggregations in Hawaii involved swimming directly offshore from collection site 2B (Figure 5.2) to approximately 150mts from the beachfront and supplementary diving from small dive vessel approximately 5 nautical miles from shore. Individuals were hard to observe from the beachfront due to the substantial wave action at this distance. Animals observed from the dive vessel showed distinct unidirectional swimming behaviour directly towards the shore in an

easterly direction. Observations of in water specimens showed that sampled population was representative of the larger aggregate, and that animal's presence at this time appeared to be restricted to the top 15meters of the water column.

Venom comparisons

HPLC analysis of venom from three separate aggregations from Osprey Reef was performed. While there were similarities in the time of venom peaks, there were variations between collections in the height of these peaks. November 2005 venom collection showed greater peaks across the whole time span, with November 2006 peaks appearing lower but displaying small peaks at around 20-30 mins that are not apparent in the other samples (Figure 5.10).

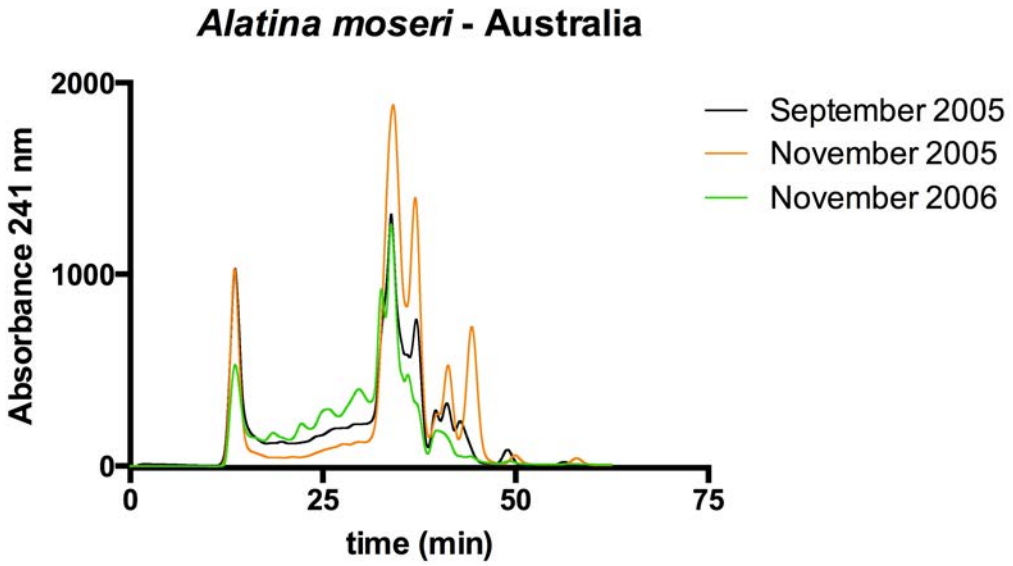

Figure 5.10 HPLC analysis of venom from *Alatina moseri* specimens collected from Osprey Reef at an absorbance of 241nm over time

Venom collection from both April 2006 and September 2007 aggregations were also analysed and again these showed variation in the height and time of peaks (Figure 5.11) showing increased proportion of proteins in these complexes.

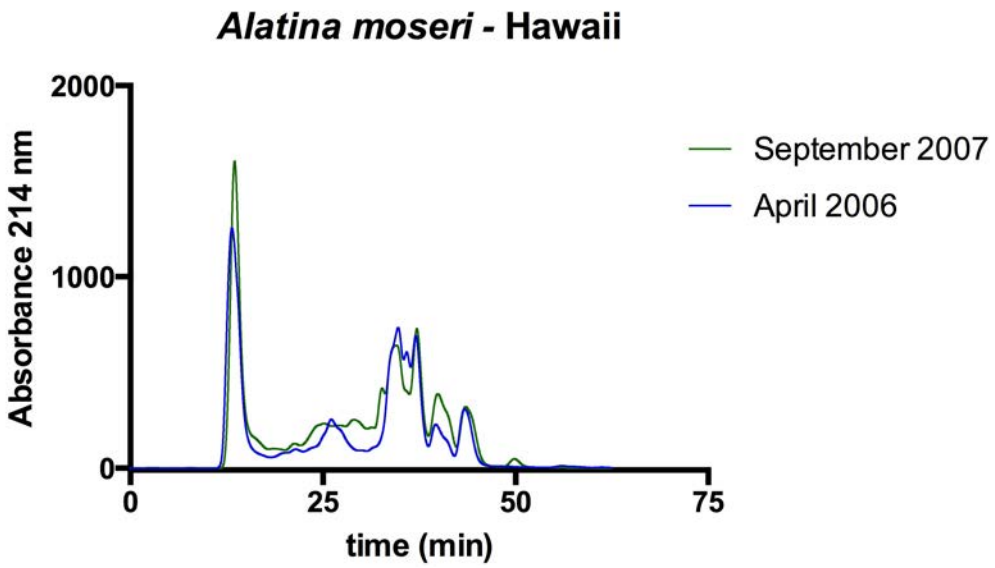

Figure 5.11 HPLC analysis of venom from *Alatina moseri* specimens collected from Hawaii

SDS page gel analysis of venoms from all of these occasions was examined to investigate the presence of specific size proteins between venoms collected and any variation in these. Several major protein components were highlighted in these samples with some notably large proteins around the 100kDa range and a spread of others showing up right down to below 15kDa (Figure 5.12) however there were distinct differences in the abundance and occurrence of proteins in the venom profiles of all these samples. Proteins of highest intensity across the samples were seen at around the 100kDa mark and again at around 37kDa. While the spread and intensity of these bands showed marked variation between samples, there was great consistency for the replicate samples of each category.

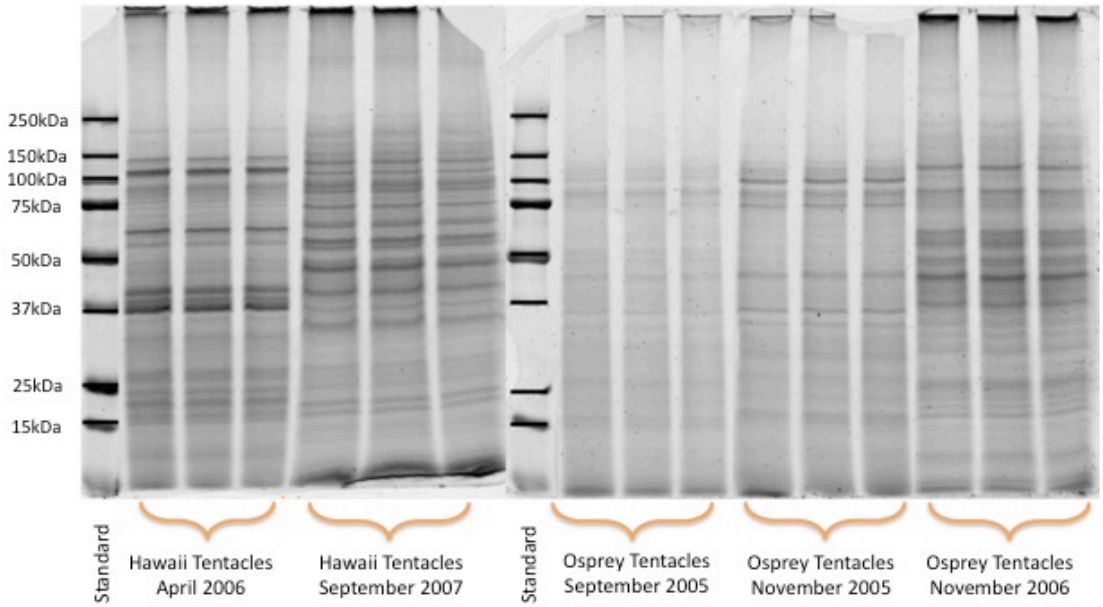

Figure 5.12 SDS PAGE gels from venom of *Alatina moseri* specimens collected from Osprey Reef and Hawaii at different time periods

Discussion

Populations dynamics

Intraspecies variation is displayed between geographically distinct populations of *Alatina moseri* with animals from Osprey reef attaining a larger overall body size than those from Hawaii. There are many potential reasons for this displayed plasticity with temperature, resource availability and predator interactions all having been suggested for morphological variations in other species (West-Eberhard, 1989) however, without further research this currently remains speculative.

The previous reports of *Alatina* sp (reported as *Carybdea alata*) being collected from

great water depths (>100mts) off the coast of Brazil (Morandini, 2003) and observations suggesting they are living on or near the edge of the continental shelf from Puerto Rico (Arneson and Cutress, 1976) (as *Carybdea alata*) would certainly also fit into the topographical region that the *Alatina moseri* appear to be inhabiting in both Hawaii and Osprey Reef areas with previous suggestions of this species living an oceanic lifestyle (Morandini, 2003). Where this species is occurring outside these spawning aggregations is still speculative, however, most of the available data would suggest that *A. moseri* spends most of its adult life as a medusa in the mesopelagic (twilight) zone.

Alatina moseri from both collection sites in this study appear to form night time aggregations that are moving in to the shallow waters *en mass*. These animals seem to congregate after dark and turn up on the beachfront or reef front in the late evening. The limited variation in size range of the animals collected from this area and the consistency of the reproductive condition of animals collected seem to insinuate that these are all reproductively active specimens with no immature animals involved. Sexual reproduction via mass spawning events is a well-documented global tropical phenomenon (Omori, 2011, Edwards, 2010), however there are many other motile aquatic animals both vertebrates and invertebrates that also utilize this strategy (Sadovy de Mitcheson et al., 2012). One of the distinct advantages hypothesized to be to increase mate encounter rates, especially when occurring around a spatially rare feature or resource (Sadovy de Mitcheson et al., 2012). Large numbers of individuals in very close proximity may also ensure high fertilization rates (whether internal or external fertilization is occurring) especially if gametes are broadcast (Levitan, 2005). As these populations of *Alatina moseri* occur in topographically remote regions, shallow water reef fronts would assist in aggregation density, leading to increased fertilization success.

There are associated costs involved for mass spawning events with animals

becoming more conspicuous in large numbers for predation (of both adults and larva) (Sadovy de Mitcheson et al., 2012) and potential for increased energy expense if substantial migration to sites is required (Sadovy de Mitcheson et al., 2012). Preliminary observations suggest that reproduction in *A. moseri* may occur only once per adult, with animals dying after spawning has occurred. This is suggested due to the tight size range of animals in aggregations and the fact that dying and deceased animals have been observed following spawning events in both locations. Additionally, the fact that there is not an observed size increase in subsequent collections (in fact there was a size decrease in Osprey animals collected only 2 months apart (Figure 5.5)) would also add weight to the fact that animals do not return for sequential events. It is possible that the energetic expense involved to migrate to these locations is such that recovery from this is not possible and all effort is therefore put into this event. The energetic costs of migration for animals travelling to spawning grounds has certainly been documented for several fish species (Crawford et al., 1986, Slotte and Fisken, 2000) and would also apply if these animals are in fact living an oceanic lifestyle. Certainly the fact that so many individuals are washing up and dying on the beaches of the Hawaiian site would also insinuate that they are paying the ultimate cost for this mass reproductive event.

From data directly collected from Hawaii and Osprey during this study and the additional records from Bonaire, Puerto Rico and the various reefs around North Queensland, it is suggested that particular species of carybdeids, at various locations around the world, may be exhibiting similar cyclic lunar spawning behaviour. This is the first recorded event of a cubozoan species displaying such cyclic spawning activity and in fact one of only few “natural” spawning aggregations events witnessed for this class. As no clear visualization of broadcast spawning was witnessed, it still remains uncertain as to whether this is an external event (as in the majority of corals) or if females are taking up sperm and fertilization is occurring internally as has been

seen in *Tripedalia cystophora* and *Carybdea sivikisis* (Hartwick, 1991b, Werner, 1973b) but no evidence of internal fertilization was seen in any of the collected individuals.

The predictability of these aggregations does also highlight the potential for management of stings in these areas at these times with dive boats off the coast of Queensland already using this information to alter diving practices (Undersea Explorer dive staff pers coms) in this region. Potential future application should also be investigated to extend to other regions where these events may also be occurring such as Fiji, Puerto Rico and Bonaire. It is my belief that with increased awareness of animals operating in these regions coupled with the more encompassing definition of Irukandji Syndrome (Chapter Four) it will highlight previously under reported envenomings.

Venom comparisons

I found that intraspecies variation in venom composition is displayed in these animals, both geographically and temporally with some variation in protein presence and size displayed between all samples collected. This adds to the two other studies demonstrating intraspecies variation in jellyfish venoms (*Carukia barnesi:* (Underwood and Seymour, 2007) and *Chironex fleckeri:* (Winter et al., 2010)), but is the first carybdeid that has examined this both geographically and seasonally.

There appears to be greater variation between geographic locations then between seasonal variations but there is also a large number of proteins present which insinuate a large protein complexity to the venom from this species. Differences in venom composition between these populations could relate to changes in diet/prey composition, ocean chemistry, thermal regime and any number of other regional and seasonal oceanic variations. As minimal research currently exists on the specific components of *Alatina moseri* venom and their individual function, the implication for

the variation seen in these proteins is limited. Previous research has isolated a hemolytic protein from the Hawaiian population of *A. moseri* (as *Carybdea alata*) at approximately 42kDa which was termed CAH1 (Chung et al., 2001) and two additional toxins at 43kDa, termed CaTX-A and 45kDa, termed CaTX-B (Nagai et al., 2000b) but notably only CaTX-A was present in the nematocysts of specimens. While the hemolytic properties of CAH1 were investigated in some detail, the study did also estimate that at a rough estimate, this fraction probably only constituted about 5-10% of the total protein in the crude venom extracted and that this is also reflected in the spectrum of proteins highlighted in this study. Additionally (Chung et al., 2001) noted that the pain and suffering endured by envenomation from this species is indicative that the potential hemolysis activity of this protein can only represent one of several venomous actions of this venom and this is supported by the presence of many other additional prominent proteins in our samples. While much further research into the spectrum of proteins is required before specific actions of individual proteins can be elucidated, this study has highlighted the potential for variations to occur between venom samples of this species. Venom variation may be occurring due to changes in prey acquisition with season, as has been displayed for the ontogenetic shift of *Chironex fleckeri* (McClounan and Seymour, 2012) but this remains purely speculative at this stage. What is apparent from this research is that there is definitive intraspecies variation in the venom composition from *Alatina moseri* with the potential for this to cause a variation in envenoming potential and this should be a consideration for future research in this area.

CHAPTER SIX: EARLY LIFE HISTORY OF *ALATINA MOSERI* POPULATIONS FROM WAIKIKI BEACH (HAWAII) AND OSPREY REEF (AUSTRALIA)

Abstract

Understanding the early life history and development strategies of venomous jellyfish species is an important under-researched area of cubozoan studies, with implications for occurrence both temporally and seasonally as well as environmental constraints and adaptiveness of a species (Courtney and Seymour, 2013, Hartwick, 1991a, Gordon and Seymour, 2012). The early life stages of the cubomedusa *Alatina moseri* from Osprey Reef (North Queensland, Australia) and Waikiki (Oahu, Hawaii) were studied using laboratory-based culturing conditions. Spawning populations from both regions were observed with reliable periodicity (Chapter Five) allowing polyp cultures from these locations to be collected and established under laboratory conditions. The polyps of this species were successfully reared from spawning adults. Polyps of *Alatina moseri* were cultured at temperatures of 23–28°C, developed up to 19 tentacles and reached up to 1.70 mm in height. The balloon-shaped hypostomes possessed 4 well-defined lips.

The polyps increased their numbers by means of formation of either sedentary polyp buds or creeping-polyp buds, which attached after 2-3 days. Metamorphosis occurred at temperatures of 25-28°C. Development of polyps and medusae were achieved for the first time within the genus *Alatina* and allowed comparisons of early life history between these and other families within the order Carybdeida. The metamorphosis and young medusa of this genus showed characters that differed distinctly from those noted for other Carybdeida species, but are very similar to the one described from Puerto Rico by Arneson and Cutress in 1976 (Arneson and Cutress, 1976) for *Alatina* sp. (as *Carybdea alata*). Based on this evidence, the discrepancies in original specimen descriptions and the previous genetic

comparisons, we support the suggestion that the two previously described species of *Alatina* from Australia and Hawaii (*Alatina mordens* and *Alatina moseri*) appear to represent artificial taxonomic units and may in fact be the same as the original *Carybdea alata* species first described from Puerto Rico. Further taxonomic studies are desperately needed in order to clarify the various species and description discrepancies that exist within this newly proposed genus.

Introduction

The early life history has not yet been described for Carybdeida species belonging to the newly formed genus *Alatina* (Gershwin, 2005) In fact, life cycle knowledge is missing for the majority of the cubozoan species (Carrette et al., 2012) only a small number having descriptions of the early stages of the life cycle (Hartwick, 1991a, Studebaker, 1972, Okada, 1927, Cutress and Studebaker, 1973, Arneson, 1976, Arneson and Cutress, 1976, Yamaguchi and Hartwick, 1980, Hartwick, 1991b, Stangl et al., 2002, Lewis and Long, 2005, Straehler-Pohl and Jarms, 2005, Straehler-Pohl and Jarms, 2011, Lewis et al., 2008, Toshino et al., 2012). Only one complete life cycle, from spawning medusa through sessile stages to spawning medusa, has been published to date for the Caribbean cubozoan *Tripedalia cystophora* Conant, 1897 (Werner, 1976, Werner, 1973a, Werner et al., 1971, Werner, 1973b, Werner, 1975, Werner, 1983, Laska-Mehnert, 1985).

Mass spawning of corals and their link to the lunar cycle is a well-known phenomenon across various parts of the globe (Edwards, 2010, Omori, 2011) but very few such lunar based spawning events have been reported in other cnidarians (Carrette et al., 2012). The lunar linked periodicity of *Alatina moseri* (Mayer, 1906) (previously *Charybdea moseri* Mayer, 1906 or *Carybdea alata* Reynaud, 1830) in Hawaii has been documented since the late 1980's with their predictable arrival on Waikiki beach being reported as the 8^{th} to 10^{th} days after every full moon (Thomas et al., 2001b, Thomas et al., 2001a, Yoshimoto and Yanagihara, 2002, Carrette et al.,

2012). A similar observation of sexually mature medusae with regular lunar periodicity (also 8 to 10 days after a full moon) was noted from *Alatina* populations from 1999 onwards at Osprey Reef, which allowed for reliable collections to be made from both these sites (as per Chapter Five).

The order Carybdeida is under revision and the subject of some debate, however, there are currently five accepted families within this group, which are Alatinidae (Gershwin, 2005), Carukiidae (Bentlage et al., 2009), Carybdeidae (Lesson, 1843), Tamoyidae (Haeckel, 1880) and Tripedaliidae (Conant, 1897). Within the Alatinidae family there are several species that are under revision and debate continues as to the validity of the different *Alatina* species (Bentlage and Lewis, 2012) in particular and how these relate to the original description of *Carybdea alata* that was described by Reynaud (Reynaud, 1830) for a species from the Atlantic Ocean.

Species Identification

The population of *Alatina moseri* used in this study, collected from Osprey Reef, is similar in morphology to the described alatinid *Alatina mordens* also reported from the Great Barrier Reef region (Gershwin, 2005), however, the specimens reported here have 6 eyes per rhopalium (not 2 or 4) and their statoliths are a distinctly light amber colour and jellybean in shape (as opposed to deep garnet reddish and tear drop shaped). This suggests that either these specimens are a different species to those described as *Alatina mordens* by Gershwin (Gershwin, 2005) or that the original description of *Alatina mordens* is inaccurate regarding this character. As specimens of *Alatina mordens* used for genetic comparative analysis by Bentlage et al. (Bentlage and Lewis, 2012, Bentlage et al., 2009) were provided from my collection, this discrepancy in identification may simply be from the original description (Gershwin, 2005). Additionally, the original description of *Alatina moseri* (Gershwin, 2005) was made purely from preserved specimens and discrepancies in

described features also exist when compared to specimens collected for this study. The predominant difference and a feature used to distinguish *Alatina moseri* is the presence of only 4 eyes in total (Gershwin, 2005), whereas the specimens collected for these studies have 6 eyes per rhopalial niche. As type specimens of *Alatina moseri* do not match those used for this study, it again suggests that either these are a different species or that the original description is inaccurate as was solely based on preserved specimens and preservation can alter the appearance of certain features. Adult medusae from both collection sites most closely resemble the former species *Carybdea alata* and show identical morphological key taxonomic characteristics to each other. As *A. moseri* is the earliest named species of the two, the specimens are referred to as *Alatina moseri.*

Early developmental stages have been recently identified as important taxonomic indicators for both scyphozoans and cubozoans (Straehler-Pohl and Jarms, 2005, Straehler-Pohl, 2009, Straehler-Pohl et al., 2011, Fuentes et al., 2011, Straehler-Pohl and Jarms, 2011, Toshino et al., 2012) and could help shed light on the current species, family, order and even class debate. This study details the early life history of Australian and Hawaiian populations of *Alatina moseri* comprehensively for the first time and compares to the description of *Carybdea alata* development from Puerto Rico (Arneson and Cutress, 1976) to see if there is evidence of developmental basis for species segregation. Additionally, these developmental observations will be compared to the current databases of cubozoan polyp life histories described and serve as a resource for ongoing research into cubozoan polyp ecology with future application into venom ontogeny and environmental constraints of this species.

Materials and Methods

Specimens from Waikiki Beach, Oahu, Hawaii

Specimens of *Alatina moseri* (Plate 6.1, Fig. A) were collected from the shore of Waikiki, (Chapter Five - Materials and Methods) Hawaii on the 22nd of October 2000, 10 days after the full moon. Individual animals that washed up onto the sand were collected by hand and placed individually into plastic click-seal bags with seawater. Over 80 individuals with milky white gonads and deemed to be in spawning condition (making the normally transparent bells cloudy) were retrieved on this occasion.

Specimens from Osprey Reef, Northern Queensland, Australia

Large aggregations of *Alatina moseri* (Plate 6.1, Fig. B) were observed at Osprey Reef, North Queensland (Chapter Five) and this synchronicity allowed for predictable collection of sexually mature specimens. On November 15th, 2006, 10 days after full moon, specimens of *Alatina moseri* were collected between 0000 h and 0230 h at Osprey Reef (as per Chapter Five - Materials and Methods).

Fertilization

Alatina moseri *from Hawaii*

The sexes of individual medusae were determined by eye and confirmed by light microscopy observation. For fertilization, approximately 0.5 mL of egg solution was extracted from each spawning female with a glass pipette and transferred into individual Petri dishes filled with clean, seawater. A collection of the water in which spawning males were kept was made, and from this “sperm mixture” a single drop was added to each of the egg solution dishes. Regular observations of cultures were made using a dissecting microscope with additional photographic documentation.

Alatina moseri *from Australia*

Due to the mass storage of specimens in large buckets at the time of collection, individual fertilization of eggs was not required as the individuals spawned directly into the bucket. Multiple 650 mL plastic containers were filled with clean seawater and in each container approximately 1 mL of gamete mixture was added. The plastic containers were closed with lids and stored in a darkened room for transportation back to land. When back on land, observed planulae in these plastic containers were extracted and placed in new 650 ml containers with filtered seawater. Detailed observations concerning polyp formation were also documented via observations with a dissecting microscope and recorded with still images.

Polyp cultures

The polyps of both *A. moseri* populations were primarily cultured in the plastic containers and Petri dishes in which the planulae had settled. Additionally to the plastic containers, cultures of *Alatina moseri* from Osprey Reef initially and later on cultures from Waikiki Beach were also cultured inside 30-L-BiOrb Fish Tanks® with filter systems. These were filled with fresh seawater and the whole content of plastic containers was suspended in the water column.

Cultures of both populations were held in a constant temperature aquarium room (24-26°C). Observations were made regularly over the following 18 months.

The polyps of both populations were fed with *Artemia* nauplii every second to third day from day 11 post fertilization onwards. Complete water changes of all the plastic containers occurred two hours post feed, with the large BiOrb® containers receiving half water changes every 3 to 6 months due to the filter systems. Salinity levels were also kept at a constant range of 33-36 psu.

Abbreviations: MDD: mouth disc diameter; TBL: total body length; UD: umbrella diameter; UH: umbrella height

Results

Thorough observations of these two populations of polyps showed no difference between timelines of developmental stages or morphological features. As such all the data on development and anatomy have been combined from here on with any discrepancies noted.

For both populations the planulae were observed from 24 h to 48 h post gamete mixing in Petri dishes and at this stage showed the presence of “eye spots”. The planulae were then extracted and placed in clean plastic containers with seawater.

72 h post fertilization the planulae were observed on the bottom of the Petri dishes and had attained irregular shapes. Although predominantly round, 2 to 4 tentacle buds protruded from their circumference containing 2-4 small eurytele nematocysts in their tips. The tentacle buds continued to grow over the next 24 h.

On day 5, primary polyps were visible at the bottom of the container and moving in a creeping way. They had cone shaped bodies and 2-4 tentacles that were longer than the body length. They creep with one of the tentacles stretched out on the substrate like an antenna.

On day 6, approximately 50% of the primary polyps settled down on the bottom of the plastic containers and hypostomes were evident (Plate 6.1, Fig. D). At this stage, four 30-L-BiOrb® aquaria were filled with clean, filtered seawater and the plastic containers, with the settled polyps attached, were suspended in each of these BiOrbs®.

Table 6.1 Time spans for embryology and primary polyp development in Cubozoa

Event	**Species**								
	Alatina moseri **(both populations from Hawaii and Australia**	***Alatina*** **sp. (formerly described as *Carybdea alata* from Puerto Rico)**	***Carybdea*** **sp. (formerly described as *C. xaymacana*[1] or *C. marsupialis*[3] from Puerto Rico)**	***Carybdea rastoni*** **(from Japan)**	***Carybdea sivickisi*** **(from Japan)**	***Carybdea sivickisi*** **(from Queensland)**	***Tripedalia cystophora*** **(from Puerto Rico)**	***Morbakka virulenta*** **(from Japan)**	***Chironex fleckeri*** **(from Queensland)**
Mating behaviour	spawning aggregation	spawning aggregation	spawning aggregation	spawning aggregation	courtship	courtship	courtship	spawning aggregation	courtship
Type of fertilization	artificially external	internal	internal	internal	internal	internal	internal	external	artificially external / external
Cite of planula development	external	external	internal / external	internal	external in embryo strand	external in embryo strand	internal	external	external
Embryological development (Age in hours (h) / days (d) / weeks (w), post fertilization (p.f.) or post planula release (p.p.r.)									
Blastulae / embryo strand	8 h p.f. / no	1 d p.f. / no	no data / no	no data / no	mean: 55 h p.f. (range: 44-66 h p.f.) / yes	no data / yes	no data	4 h p.f.	no data / no
Blastocyst	-	-	-	-	-	-	-	3 d p.f.	
Planulae	24 - 48 h p.f.	2 d p.f.	10 h p.f.	no data	6 d p.f.	5 d p.f.	2 d p.f.	24 d p.f.	12 h p.f.
Planulae settled	3 d p.f.	6 d p.f.	2 d p.f.	2-3 d p.p.r.	9.5 d p.f.	8-10 d p.f.	2-3 d p.f.	-	2 d p.f.
Primary polyp development									
1-4 Tentacle buds with 2-4 euryteles in tips	4 d p.f	8 d p.f.	4 d p.f. (no nematocysts in tentacle tips)	4-5 d p.p.r. (no nematocysts in tentacle tips)	14.5 d p.f.	9-11 d p.f.	4-6 d p.f.	26 d p.f.	3-5 d p.f. (no nematocysts in tentacle tips)

2-4 Tentacles & hypostome	5-6 d p.f.	no data	7-8 d p.f.	4-5 d p.p.r.	17.5 d p.f.	14-16 d p.f.	4-6 d p.f.	39 d p.f.	19 d p.f.
Stenotele replaces euryteles	10 – 13 d p.f.	31 d p.f.	no data	no data	no data	14-16 d p.f.	-	-	19 d p.f.(no euryteles)
6-8 Tentacles	21 d p.f.	31 d p.f.	no data	no data	no data	no data	8-10 w p.f.	9 w p.f.	no data
Metamorphosis (start)	31 d p.f.	68 d p.f.	no data	no data	no data	no data	10-12 w (70-84 d) p.f.	no data	58 d p.f.
Reference	present study	(Arneson, 1976)	(Cutress and Studebaker, 1973, Studebaker, 1972, Berger, 1900b, Conant, 1897)	(Okada, 1927)	(Lewis and Long, 2005, Lewis et al., 2008)	(Hartwick, 1991b)	(Werner, 1973b, Werner et al., 1971, Conant, 1898)	(Toshino et al., 2012)	(Hartwick, 1991a, Kinsey, 1986, Yamaguchi and Hartwick, 1980)

- : event does not occur in this species

d: day(s) p.f.: post fertilization w: week(s) h: hour(s)

From day 10-13 a distinct shift in the morphology of the primary polyps was noted: they appeared to flatten against the substrate while their four tentacles were stretched out to the sides. The tentacles elongated and the euryteles in the tentacle tips were replaced by single stenoteles. At this stage polyps were fed for the first time on either finely sliced crayfish meat, ground up egg yolk, or the more successful diet of 1-day old *Artemia* nauplii (Plate 6.1, Fig. F). A feeding rate of approximately 30% of the individuals was noted on day 11. The polyp density was approximately 1 per 5 cm^2. Polyps were subsequently fed with *Artemia* nauplii every second day.

From day 18 onwards, polyps increased in size and by day 21 approximately 40% of the polyps had developed six (Plate 6.1, Fig. E) or eight tentacles. These polyps with increased tentacle number started asexual reproduction (approximately 20% of polyps with buds at their calyx base, Plate 6.1, Fig. J). Asexual reproduction continued with polyps having as many as five buds at any time. These buds appeared to be in one of two forms and would either remain next to the base of the original 'parent' polyp after detachment, or undergo a creeping phase and relocate to another position. The creeping polyps from budding (Plate 6.1, Fig. K) did neither resemble the parent polyp nor the primary polyps during creeping phase (see creeping polyp description below).

Feeding regimes continued and asexual reproduction was apparent throughout the culture with exponential numbers of creeping polyps apparent over the sides of the Bio-Orb® tanks.

On day 29-31 the first signs of metamorphosis were apparent in all cultures with an observed change in polyp shape, a darkening of the polyp pigment and migration of the tentacles into four distinct clusters. At this time the hypostome of the polyp lengthened, as did the stalk. Continual and daily metamorphosis of polyps was observed and is noted in detail below.

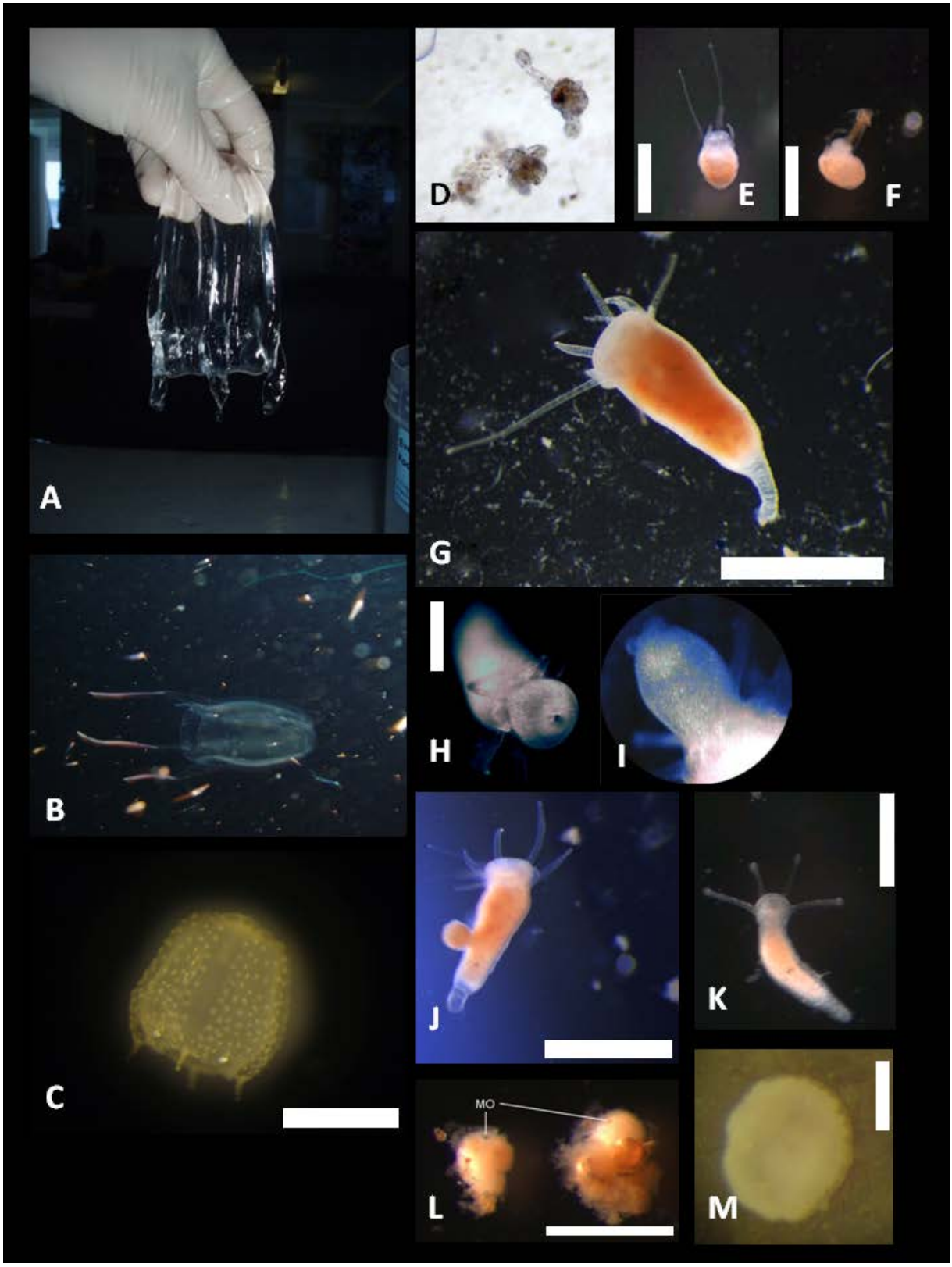

Plate 6.1 Medusae, polyps and asexual reproduction in two *Alatina moseri* populations (Hawaii, Australia)

A: adult medusa from Hawaii, bell height ca. 100 mm – tentacles removed; B: adult medusa in the open water column (Australia); C: newly detached medusa (Hawaiian population); D: primary polyps (only few µm in height, no scale, picture was taken by camera through 400x

microscope objective); E: young polyp (21 days post fertilization); F: young polyp feeding on *Artemia* nauplium; G: adult polyp (>21 days post fertilization), lateral view (Australian population); H: adult polyp, oral view – note lips (Australian population); I: hypostome, note lips (hypostome ca. 0.5 mm in length) (Hawaiian population); J: budding adult polyp (Hawaiian population); K: creeping polyp (bud); L: cyst, stage right after encystment, note the still visible mouth opening (MO); M: cyst, stage after a week, note the unstructured tissue inside the mucus shell.

Scale bars: A=100mm; C, G, J, L=1mm; E, F, H, K=0.5mm; M=0.25mm

Polyp anatomy

The polyps of the two observed *Alatina moseri* populations showed no distinct differences. Polyps both populations were solitary (Plate 6.1, Fig. G). The bodies were divided into three parts, the hypostome, the calyx and a stalk region including a basal disc and a tiny periderm beaker enveloping the pedal region.

The polyps of both populations had a total body length (=TBL) of up to 1.70 mm (TBL: 1.46-1.70, mean: 1.58mm). The polyps of the Hawaiian population were only slightly larger with a total body length (=TBL) of up to 1.70 mm (TBL: range: 1.46-1.70 mm) with a mean value of 1.58 mm (n = 25) than the observed polyps of the Australian population (TBL: 1.43-1.63 mm, mean mm: 1.52 (n = 25)). The hypostome region included a single circlet of up to 19 tentacles (11-19, mean: 16, n = 25) in the Australian population. The tentacle numbers in the Hawaiian population ranged between 12-18 with a similar average of 16. The tentacles in both populations were solid, translucent, and bore a single stenotele in the knob-like tip. The calyces of both populations were shaped like a bottle, creamy-white with a tinge of orange when recently fed and about 60% of the TBL. A belt of nematocysts, mainly stenoteles and ovoid heterotrichous microbasic euryteles, was clearly visible around the lower calyx. The mouth disc diameter in both populations (MDD: Australian: 0.41-0.46, mean: 0.43; Hawaiian: 0.43-0.50; 0.48) was the widest body diameter and about 0.3fold of the calyx length.

The hypostome of polyps of both populations was four-lipped (Plate 6.1, Figs. H, I), balloon-shaped and approximately 14-15% of the TBL. It was not completely contractible into the body. The stalk, which was only slightly set off from the bottle shaped calyx, was short and colourless translucent.

Observations on feeding behaviour noted that the polyps of both populations showed a muscular ring at the level of the tentacular circlet which seemed to function as a water gate by constricting the calyx underneath the peristome until the opening to the gastro vascular cavity underneath the hypostome lumen was completely shut. In this way, the captured prey was placed into the hypostome and remained held there until the outer mouth opening was completely closed. After the opening had completely shut, this muscle ring relaxed and the opening to the stomach widened so that the prey could be transferred into the main gastric cavity.

Asexual reproduction

Creeping polyps

There were no observed differences in the creeping polyps of either populations. Asexual proliferation in both populations occurred by lateral budding of polyps (origin: junction point of calyx and stalk, nematocyst developing zone), typically one to five buds per polyp at a time, depending on the feeding conditions. The creeping polyps showed 4 to 8 very short, still filiform tentacles around a cone-shaped hypostome and a high number of large stenotele nematocysts at the rear end of the body. Single stenoteles were located at the bases of the tentacles that migrated to the tentacle tips as soon as the polyps settled. The morphology of the creeping polyps differed a lot from the primary polyps by having a distinctly larger body size (TBL up to 2 mm) and by the “head region” which resembled a “daisy flower” resulting from the very short, nematocyst-lacking tentacles surrounding the mouth cone. These creeping polyps glided oral pole first, and, in contrary to the primary polyps, on their body

midst with raised “head region” for 2 to 3 days over the substrate before they settled. After settlement they grew to a 9 to 12-tentacle stage before start budding themselves.

Cysts

During extremely adverse conditions (drastic changes in temperature or salinity or absence of food for prolonged periods) the polyps of both populations encysted. To induce this cyst form, temperatures were lowered from 25°C to 19°C or salinity was increased to 42 psu or feeding was ceased for a period of four to five weeks. In this state polyps retracted their tentacles completely into their calyx and contracted the whole body into a ball-shape (Plate 6.1, Fig. L). The different parts of the body like tentacle tips, mouth opening and calyx fused into an indistinguishable white tissue ball, enclosed by a transparent and flexible mucus coat (Plate 6.1, Fig. M). The cysts of both populations stayed attached to the substrate. These survived for up to 3 months in these cyst forms during starvation events and both polyp populations subsequently regenerated within 3 days after feeding restart.

There were no obvious differences in the morphology of the endurance stages of populations from Australia or Hawaii, however, the longer conditions stayed unfavourable the more polyps died inside the cysts. The longest observed cyst state was by the Australian culture and this was for a twelve-month period before recovery was commenced.

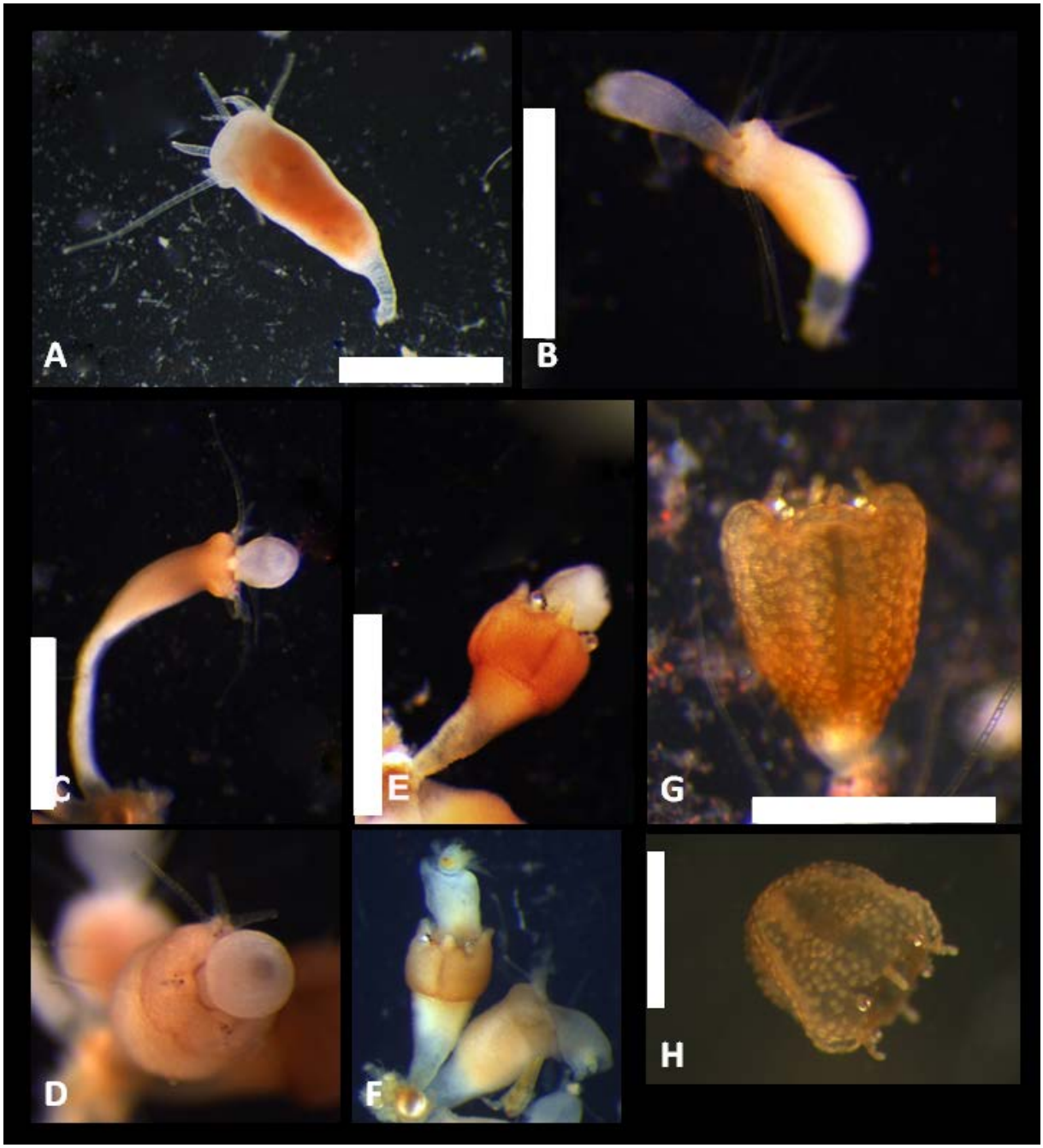

Plate 6.2 Metamorphosis in *Alatina moseri* (Australian population).

A: adult polyp; B: Stage 1: elongation of hypostome, calyx and stalk, tentacles cluster at four spots; C (lateral view), D (oral view close up of same animal as C): Stage 2: clustered tentacles fuse at base, note the red-violet pigmentation around hypostome and eye spots at the fused tentacles bases (D); E: Stage 3: medusa tentacles appear in space between rhopalia, F (same animal as E): note that this stage is still feeding on *Artemia* nauplii; G: Stage 4: nematocyst clusters appear on the developing exumbrella; H: Stage 5: after reabsorption of the remaining stalk tissue, the newly detached medusa free swimming.

Scale bars: A, C, E, G, H=1mm; B=2mm

Metamorphosis (Plate 6.2)

The metamorphosis in cultures has occurred spontaneously since their original collection, however, only once in mass numbers as was initially observed six weeks after initial fertilization took place. Populations showed a complete metamorphosis of approximately 14 days from start of metamorphosis to medusa detachment. There were no morphological or time differences noted when comparing both populations. The stages of metamorphosis were documented photographically and described in detail (both populations combined) below:

Phase 1 (Plate 6.2, Fig. B): Elongation of calyx, stalk and especially hypostome, the hypostome in some cases being as long as the calyx and in all cases baseball bat-shaped. The tentacles formed clusters at 4 points around the peristome. Reddish violet pigmentation appeared at 4 spots between the tentacle at the base of the hypostome.

Phase 2 (Plate 6.2, Figs. C,D): The clustered tentacles fused at the bases, distal tentacle ends were reabsorbed and rhopalia developed at the fused tentacles bases. Statolith formation occurred initially while the tentacles were still reabsorbed. A horizontal ring groove appeared at the oral end of the calyx, dividing the transforming calyx into two parts: the upper part with already transformed medusoid tissue (plate-like cells) marked by reddish dark brown colour and the lower part with the not-yet transformed, unpigmented polypoid tissue (cylindrical cells) marked by white/pinkish colour (Plate 6.2, Fig. C). The reddish violet pigmentation sprat edgeways until a red-violet coloured pigment ring (Plate 6.2, Fig. D) was formed around the hypostome base.

Phase 3 (Plate 6.2, Figs. E, F): Between the rhopalia, 4 medusa tentacle buds appeared. A deep cleft around the hypostome marked the transformation of the polypoid hypostome into a medusoid manubrium. Single gastric filaments emerged in

the interradii at the inner base of the manubrium. The red-violet pigmented region transformed into a velarium. The horizontal ring groove moved down towards the calyx base due to transformation of the remaining calyx tissue into medusoid tissue. Until this stage, the metamorphosing polyps were still catching and consuming prey (Plate 6.2, Fig. F).

Phase 4 (Plate 6.2, Fig. G): The medusoid tentacle buds grew out to very short, hollow and moniliform tentacles with four thick, yellowish brown coloured rolls of nematocyst batteries. The transformed calyx grew further in size, forming a slightly transparent umbrella and changing again colours from reddish dark brown to yellowish light brown. Small nematocyst clusters appeared, densely covering the developing exumbrella. The remaining stalk was reabsorbed.

Phase 5 (Plate 6.2, Fig. H): Pulsing of the medusae was initiated at least 48 h prior to detachment. At this stage no feeding behaviour in any of the newly metamorphosed polyps was observed. The medusa detached from the substrate while still a little remnant of the basal disc was visible at the apex of the umbrella. The basal disc was reabsorbed a few hours after detachment.

Young medusa (Plate 6.2, Fig. H)

There were absolutely no differences found between the young medusae of these two populations. Newly detached medusae of both populations had tetraradial symmetry. The umbrella was spheroidally-pyramidal (Hawaiian and Australian population: umbrella height (=UH): 1.2-1.6 mm, mean: 1.35 mm, umbrella diameter (=UD): 1.1-1.5 mm, mean: 1.24 mm).

The bell warts of the medusae were small (0.06 mm in diameter), innumerable, round and densely covering the whole exumbrella and contained microbasic euryteles and spherical holotrichous isorhizas. The manubria of the young medusae were short

(0.24-0.3 mm, mean: 0.27; ≈ 20% of UH) and four-lipped. One gastric filament per quadrant was visible through the apex of the umbrella.

The medusae had 4 medusa tentacles that were much shorter than the length of the umbrella (approx. 16% of umbrella height). No additional medusa tentacles developed after detachment. Extended tentacles resembled short pearl strings because of the up to 4 thick, round, yellowish brown nematocyst batteries containing only ovoid heterotrichous microbasic euryteles. The young medusae were yellowish brown to yellowish olive in colour. Feeding of young medusae proved challenging with *Artemia* naupilli seeming insufficient to sustain them. The longest time span medusae (n=6) were kept alive was for 29 days and were fed small pieces of raw *Acetes australis* three times a day. At their time of death, medusae ranged in size from 15 mm to 25 mm.

Discussion

Several observations on the early life history of Carybdeida species have now been reported, however this is the first comprehensive description of the early life stages of two populations of the newly established genus *Alatina* (Gershwin, 2005).

Fertilization & Polyp formation (Table 6.1)

Spawning aggregations have been previously reported in several species of Cubozoa (Conant, 1897, Berger, 1900b, Studebaker, 1972, Cutress and Studebaker, 1973, Arneson, 1976, Werner, 1976, Yamaguchi and Hartwick, 1980, Hartwick, 1991a, Lewis and Long, 2005, Straehler-Pohl, 2011, Toshino et al., 2012), however, the investigations on *Alatina* sp. (= *Carybdea alata* from Puerto Rico Arneson & Cutress (Arneson, 1976, Arneson and Cutress, 1976)), *Carybdea xaymacana* Conant, 1897 (Berger, 1900a, Conant, 1898, Conant, 1897) , *Carybdea* sp. (= *Carybdea marsupialis* from Puerto Rico, (Studebaker, 1972, Cutress and Studebaker, 1973), *Carybdea rastoni* Haacke, 1887 (Haacke, 1887, Okada, 1927),

Copula sivickisi (Stiasny, 1926) (Stiasny, 1926) (Lewis et al., 2008, Lewis and Long, 2005, Hartwick, 1991b) and *Tripedalia cystophora* Conant, 1897 (Werner, 1976, Werner, 1973b, Conant, 1898, Conant, 1897) have all suggested internal fertilization occurs except in *Morbakka virulenta* (Kishinouye, 1910) where the gametes were released into the open water (Toshino et al., 2012).

While it is unclear if internal fertilization was occurring in the observed populations of *Alatina moseri* as was reported for Caribbean population of Alatina (Arneson, 1976) there were obvious mass spawning events occurring for both these populations (Chapter Five).

The time spans of embryonic development stages are similar in most cubozoan species compared in Table 6.1. Fertilised eggs developed into blastulae within 24 h post fertilization (p.f.) that transformed into “eye spot” bearing planulae within 1–2 days p.f. and then settled approximately 2–3 days p.f. The only exceptions to this are the development of *Copula sivickisi* and *Morbakka virulenta*. In *C. sivickisi* the developmental time in the different stages were extended by up to 150% compared to the other described cubozoan species. This might be due to the unique reproductive strategy in this species where they produce an embryo strand and the embryos are not released from it until they have reached the planula stage (Hartwick, 1991b, Lewis and Long, 2005, Lewis et al., 2008). In *Morbakka virulenta* the embryonic development was postponed for 21 days after reaching the blastula stage by forming a cyst as resting stage (Toshino et al., 2012). After the resting period the blastula transformed into an “eye spot”-lacking planula which stayed inside the egg shell until a 1-tentacled primary polyp hatched directly from the egg, using the shell as substrate (Toshino et al., 2012).

In the original life cycle description of *Alatina sp.* (*Carybdea alata* from Puerto Rico*)* the settlement of the planulae was completed after 5–6 days p.f. (Arneson, 1976)

which is about twice as long as in these cultures of *Alatina moseri*. Likewise, for the subsequent developmental rates, the stages were approximately one third slower in the *Alatina* sp. description compared to the developmental shown in these *Alatina moseri* cultures. Potentially these differences might be caused by the different culturing conditions, such as a lower rearing temperature, and not necessarily that these are different species as temperature and food supply can lead to differences in developmental rates (Spangenberg, 1968, Hartwick, 1991b). The planulae of *Alatina moseri* settled on the bottom of PET-containers while the planulae of *Alatina* sp. settled on clean glass (Arneson, 1976). PET-substrate was used as this has previously been suggested as being the artificial substrate of choice for settlement of scyphozoan planulae (Holst and Jarms, 2006, Brewer, 1976, Brewer, 1978).

Polyps (Plate 6.1)

When comparing the polyps of both populations neither the measured sizes nor the morphology showed any significant difference. The general polyp morphology of the observed species conforms with that of other known cubopolyps in having (1) a bottle-shaped body with a clear structure and an aseptate gastric cavity, (2) solid, capitate tentacles with a stenotele nematocyst in their tips, and (3) a distinct stalk with a basal region covered by a thin periderm cuticle. The number of tentacles on polyps has been shown to differ between species (Werner et al., 1971, Studebaker, 1972, Cutress and Studebaker, 1973, Stangl, 1997). Polyps of *Alatina* sp.(Arneson, 1976, Arneson and Cutress, 1976) from Puerto Rico seem to be similar in structure and number with the polyps of *Alatina moseri* when comparing the photos and descriptions and it is of note that the mean number of tentacles was identical. The polyps of *Carybdea* sp. (formerly described as *Carybdea marsupialis* by (Studebaker, 1972)), *Chironex fleckeri* (Hartwick, 1991a)and *Carybdea morandinii* (Straehler-Pohl and Jarms, 2011) do also show this general morphology in polyp tentacle structure (Studebaker, 1972, Cutress and Studebaker, 1973, Yamaguchi and Hartwick, 1980,

Golz and Thurm, 1993, Stangl, 1997). The main exceptions to this polyp organisation are the polyps of *Tripedalia cystophora*, which have a bag-shaped body, fewer tentacles, and 20–40 heterotrichous euryteles in their tips instead of stenoteles (Werner et al., 1971, Werner et al., 1976, Werner, 1973a, Werner, 1975, Werner, 1983, Chapman, 1978, Mehnert, 1984, Laska-Mehnert, 1985, Straehler-Pohl, 2001, Straehler-Pohl, 2009) and the polyps of *Morbakka virulenta* which have a tulip-shaped body which resembles more a scyphopolyp, and up to 17 tentacles with up to 30 nematocysts of two types (tri-rhopaloids and small spherical *p*-rhopaloids) located in their tips (Toshino et al., 2012). Additionally the polyps of *Carybdea morandinii* host zooxanthellae in their bodies (Straehler-Pohl and Jarms, 2011), which were not found in either of the *Alatina moseri* populations examined here.

Polyps of *Alatina moseri* show a muscular ring at the level of the tentacular circlet that seems to function in a similar way to the diaphragm found in *Carybdea morandinii* (Straehler-Pohl and Jarms, 2011, Straehler-Pohl, 2009, Straehler-Pohl, 2001) by constricting the calyx underneath the peristome until the opening to the stomach underneath the hypostome lumen is completely shut. Like in *Carybdea morandinii* the muscle ring divides the gastric cavity horizontally into an antechamber and a main chamber but the diaphragm structure, which was found in *C. morandinii*, was not detected in the *Alatina moseri*. These features can be observed with light microscopy while polyps are feeding.

A distinguishing characteristic of the observed *Alatina moseri* populations is the four lips detected in the balloon shaped hypostome which resembles the club-shaped, four-lipped hypostomes of the scyphistoma of the Rhizostomida species (Calder, 1973, Calder, 1982, Pitt, 2000, Kawahara et al., 2006, Holst et al., 2007, Schiariti et al., 2008, Straehler-Pohl, 2009, Fuentes et al., 2011). For Cubozoa this structure was previously unknown and was not noted in the *Alatina* sp. description by (Arneson, 1976).

Asexual reproduction

Creeping polyps

The budding process and the outer appearance of the creeping polyps resemble the description for other Carybdeida species (Studebaker, 1972, Cutress and Studebaker, 1973, Arneson and Cutress, 1976, Arneson, 1976, Stangl, 1997, Fischer and Hofmann, 2004, Straehler-Pohl, 2001, Straehler-Pohl, 2009, Straehler-Pohl and Jarms, 2011) except the creeping polyps of *T. cystophora* (Werner et al., 1971, Werner, 1976, Werner, 1973a, Werner, 1975, Werner, 1983, Werner, 1993) which are very different from other species and resemble more the primary polyp stage than the creeping polyps and are also similar to those described for the chirodropid species *Chironex fleckeri* (Hartwick, 1991a, Yamaguchi, 1982). An exception is again found in *Morbakka virulenta* which produces two-tentacled buds which do not develop head first but in a lateral position and which do not creep after detachment but swim with oral pole first near the bottom or the water surface (Toshino et al., 2012). This kind of swimming behaviour was not observed in the *Alatina moseri*.

Cysts

Encystment of cubopolyps were not originally described for *Alatina* sp (Arneson, 1976) but were noted in *Tripedalia cystophora* (Werner, 1975), *Chironex fleckeri* (Hartwick, 1991a) and *Carybdea morandinii* (Straehler-Pohl and Jarms, 2011) during drastic salinity or temperature changes in culture waters. Encysting polyps were briefly described by Hartwick (Hartwick, 1991a) as “a state that is probably equivalent to encystment where the polyps are highly contracted and non-responsive (*sensu* Werner (Werner, 1975))”. This state was further affirmed with the cyst formation being described again in *Tripedalia* and also noted in *Carybdea* sp. and *Carybdea morandinii* (Straehler-Pohl, 2009, Straehler-Pohl, 2001). The cysts of the two *Alatina moseri* populations stuck to the substrate, similar to *Tripedalia,* and did not float

around as seen in *Carybdea* sp. but as with those two species, the *Alatina moseri* lacked structures like the anchor strings found in *Carybdea morandinii* (Straehler-Pohl, 2009, Straehler-Pohl, 2001, Straehler-Pohl and Jarms, 2011).

For *Chironex fleckeri* it was noted that encysted polyps could last for at least two weeks in this form and would re-emerge to again feed if the salinity levels were returned to the original level (Hartwick, 1991a). Additionally, *Tripedalia* and *Carybdea* sp. cysts can survive for short periods (up to 14 days) of unfavourable conditions while the anchored cysts of *Carybdea morandinii* can survive for periods of up to 3 months (Straehler-Pohl, 2001, Straehler-Pohl and Jarms, 2011). If the conditions did not return to normal after this time, the cysts died and macerated within 2–3 days. In comparison, the observed polyp cysts of the *Alatina moseri* cultures seemed to be highly resilient, as they endured high salinities and starvation conditions for more than 12 months. The regeneration of *C. morandinii* polyps took 7 days after 14 days of encystment and up to 3 weeks after 1.5 months (Straehler-Pohl and Jarms, 2011, Straehler-Pohl, 2001) while the culture of Australian *Alatina moseri* polyps regenerated back to their healthy feeding polyp state within 7 to 9 days after an encystment period of 12 months.

Metamorphosis

Metamorphosis in both *Alatina moseri* populations is complete and without any noted residuum (Metamorphosis Type 2 (Straehler-Pohl and Jarms, 2005, Straehler-Pohl and Jarms, 2011, Straehler-Pohl, 2009, Straehler-Pohl, 2001)), as in other cubozoan life cycles (Werner et al., 1971, Werner et al., 1976, Werner, 1973a, Werner, 1975, Werner, 1983, Studebaker, 1972, Cutress and Studebaker, 1973, Arneson, 1976, Arneson and Cutress, 1976, Yamaguchi and Hartwick, 1980, Mehnert, 1984, Laska-Mehnert, 1985, Stangl, 1997) .

The whole process is very similar to the other cubozoan species, except for the highly extended hypostome, the capability of still feeding until a late stage (Phase 3) of the metamorphosis and the conspicuous, red-violet pigmentation of the future velarium region. This pigmentation was not mentioned for the metamorphosis of *Alatina* sp from Puerto Rico (Arneson, 1976, Arneson and Cutress, 1976).

The metamorphosis of the *Alatina moseri* populations results in a single 4-tentacled juvenile medusa as in *Alatina* sp, *Copula sivickisi*, *Carybdea* sp. (from Puerto Rico), *Tripedalia cystophora*, *Carybdea morandinii* and *Chironex fleckeri* (Uchida, 1970, Werner et al., 1971, Studebaker, 1972, Cutress and Studebaker, 1973, Werner, 1973a, Werner, 1975, Werner, 1976, Werner, 1983, Arneson, 1976, Arneson and Cutress, 1976, Yamaguchi and Hartwick, 1980, Hartwick, 1991a, Mehnert, 1984, Laska-Mehnert, 1985, Straehler-Pohl, 2001).

A subsequent study on the same Australian population of *Alatina* (cited as *A latina* nr. *mordens*) has shown that while thermal and osmotic variations did not seem to vary the rate of metamorphosis in this species, the reduction of available food did (Courtney and Seymour, 2013).

Newly detached medusae

As in *Alatina* sp. (Arneson and Cutress, 1976, Arneson, 1976) in the exumbrella of the young medusae of the two *Alatina moseri* populations, clusters of microbasic euryteles and spherical holotrichous isorhizas are found. This distribution of nematocyst types observed differs from *T. cystophora* and *C. marsupialis*, two species in which nematocyst clusters on the exumbrella mainly comprise atrichous and holotrichous isorhizas (Stangl, 1997, Werner, 1975) while in nettle warts on the exumbrella of *C. morandinii*, ovoid heterotrichous microbasic euryteles are predominant and isorhizas are rare. In the annular nematocyst batteries of the tentacles of *A moseri* only ovoid heterotrichous microbasic euryteles are found like in

Alatina sp from Puerto Rico, *Tripedalia cystophora* and *Carybdea* sp. (Werner, 1975, Stangl, 1997) while in the tentacles of *C. morandinii* several categories of nematocysts (holotrichous isorhizas, atrichous isorhizas, and single ovoid heterotrichous microbasic euryteles) are apparent (Straehler-Pohl and Jarms, 2011). The morphology of the newly detached medusae of *A*. *moseri* are very similar to the medusae of *Alatina* sp described by (Arneson and Cutress, 1976) from Puerto Rico and the medusa drawn and described by Mayer (Mayer, 1900) as *Charybdea aurifera* from the Tortugas.

Notes on taxonomy

Early life history stages have recently been discussed as ancestral-relatedness-reflecting characters in Scyphozoa (Straehler-Pohl, 2009, Straehler-Pohl et al., 2011, Fuentes et al., 2011) and in Cubozoa (Straehler-Pohl and Jarms, 2005, Straehler-Pohl, 2009, Straehler-Pohl and Jarms, 2011) and also this study highlights that early life stages may be important indicators for taxonomical evaluations.

Not only does this study show that the early life stages from polyp to medusa of *Alatina moseri* from both the Osprey Reef, Australia and Waikiki beach, Hawaii populations follow the identical developmental lines and show no significant differences in body sizes or morphology in both polyps and young and adult medusae. The morphological features of these populations are also remarkably similar to the stages described for the *Alatina* sp. (former *Carybdea alata*) from Puerto Rico, with noted variations potentially attributable to incomplete observations at that time. Additionally, it supports previous suggestions that within the *Alatina* genus there may be multiple species named which represent artificial taxonomic units (Bentlage and Lewis, 2012, Bentlage et al., 2009). Previous DNA analysis has suggested that prior naming of *Alatina mordens* and *Alatina moseri* in these areas are in fact the same species as there was no significant genetic divergences which

corresponded to the geographical localities of these populations (Bentlage et al., 2009, Bentlage, 2012). Through observations of the sessile stage, development times, both adult and young medusa morphology as well as the structure of the cysts, this study supports the conclusion that the two populations of adult *Alatina* used in this study have displayed no evidence for species differentiation. However, further investigation is required into the other species comprising the newly formed genus *Alatina* in order to clarify the taxonomic issues that remain for this group. Recent analysis of the cubozoan group has also highlighted the desperate need for improved taxonomy of these species and its role in leading to improved phylogenetic forecasting and abolishing existing inconsistencies due to species misidentification within this group (Bentlage et al., 2009). The implications of accurate species identification on risk management is apparent and the suggestion of closely related species having closely related venom toxicity also is relevant for such taxonomic clarity (Bentlage et al., 2009).

CHAPTER SEVEN: GENERAL DISCUSSION

The aims of this research were:

- to investigate the spectrum of Irukandji Syndrome as it has been historically reported and highlight any inconsistencies that exist in its current definition.

- to analyse existing sting records to illuminate trends that can have practical application from a diagnostic perspective as well as for potentially global application.

- to focus on a species known to cause Irukandji Syndrome, examine its general ecology and venom structure including tests of whether there is any intraspecific variation that may affect diagnosis or treatment of the syndrome.

The outcomes of this study include redefinition of the symptoms that identify Irukandji Syndrome in presenting patients and this I believe will result in a more encompassing approach to identifying victims of this marine envenomation both locally and globally. I have termed this new definition Irukandji Syndrome Complex and it use should be instigated by medical practitioners worldwide. Dissemination of this new diagnostic tool will allow for increased awareness of this affliction and its potential in various regions of the world. This syndrome can no longer be thought of as being restricted to Australian waters and the potential for its increase with correlating water temperature rise makes this even more pertinent.

The predictable and synchronised arrival of large jellyfish aggregations presents a unique opportunity to research aspects of these syndrome-producing animals that are typically elusive and cryptic. Populations of animals from these aggregations were reliably identified, and existing records suggest that a similar spawning synchronicity may be occurring in various other global locations that have yet to be scientifically investigated. It is my belief that descriptions of multiple *Alatina* (reported as *Carybdea alata*) from Puerto Rico (Arneson, 1976) and Bonaire in the past have

been observations of these same spawning aggregations and with further investigations in these areas, additional occurrences would be uncovered. Not only does this open up another avenue for comparative study, but also may highlight potential for risk management strategies to better protect marine users in these areas. I believe that these large carybdeids are a single species that have a much wider distribution than previously thought. Additionally, its expansion of range in recent years as recently reported in Hawaii highlights their potential for further increased distribution.

The venom variation of this species both geographically and temporally highlights another avenue for variation displayed in this syndrome. Previously, two predominant proteins have been isolated from the Hawaiian *Alatina moseri* (as *Carybdea alata*) (Chung et al., 2001, Nagai et al., 2000b) but from my research these represent only a fragment of the full spectrum of proteins that are occurring in these populations. Future research should now look at isolating additional proteins from this species and attempting to describe the various action potential of these in relation to both the animal ecology and envenomation potential. The variation of composition is undoubtedly playing a role in the syndrome variation displayed and by isolating these, we can move closer to both treatment and prevention of severe stings.

Finally the early life history data and investigation into the comparative morphology of the two populations was described in detail with reference to other cubozoan species. This supports the recent reclassification of *Alatina mordens* (Gershwin, 2005) as an Australian population of *Alatina moseri,* which also occurs off the coast of Hawaii (Bentlage et al., 2009). This highlights the need for further research into the taxonomic inconsistencies that are evident in this group as well as the distribution patterns of these populations with the implications for envenomings in previously under documented areas. I believe further taxonomic investigations will reveal

additional inconsistencies of wider cubozoan “species” classification due to the small numbers often encountered difficulty in preservation techniques prior to identification.

Cultivating and maintaining polyps of animals provides unique insight into a stage of cubozoan development that is rarely seen but represents an important development period with the occurrence of asexual reproduction for proliferation of the population occurring at this stage (Straehler-Pohl and Jarms, 2011, Straehler-Pohl et al., 2011, Arneson and Cutress, 1976). This is the first detailed description of this species life stage and highlights the likely relatedness of these populations to those described from Puerto Rico (Arneson and Cutress, 1976). From here, additional studies have already been performed on these cultures for insight into the theoretical environmental limits of their distribution (Courtney and Seymour, 2013), which will provides additional clues regarding these animals natural occurrence, and how their distribution may change in the future. The resilience displayed by the polyp phase of this animal shows it’s potential for continued population even when placed under increasing environmental pressures.

In conclusion, this thesis has highlighted three main outcomes of research. Firstly the need for my boarder definition of the syndrome “Irukandji Syndrome Complex” to be applied and used worldwide for further identification of this syndrome on a global scale. Secondly, that presenting symptoms, region of sting occurrence and treatment requirements can be indicative of syndrome severity and indicate potentially ‘severe’ sting cases. Thirdly, it exposes the potential for certain species of carybdieds to be occurring in synchronised aggregations at previously undocumented regions and the application of this for a risk management strategy of marine use. Finally, it has also demonstrated the need for multidisciplinary approach to be taken on this global syndrome, as there are many factors affecting the distribution, occurrence and severity of stings experienced. In future research, this approach should be considered for additional regions experiencing Irukandji Syndrome envenomings,

with a focus on a more global view essential to what appears to be an increasingly global phenomenon.

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