The diversity and abundance of gelatinous zooplankton in north-western Australia and the association of *Ophiocnemis marmorata* (Echinodermata: Ophiuroidea) with *Aurelia aurita* (Cnidaria: Scyphozoa)

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Abstract

Gelatinous zooplankton are ubiquitous throughout the world's oceans, yet data on their abundance and diversity are scarce in the Indian Ocean, and in particular north-western Australia. Gelatinous fauna can be identified using morphological or genetic approaches, though genetic data provide more information to discriminate among species when morphology alone cannot. Moreover, a number of gelatinous species form commensal relationships with other organisms, which possibly enhances local pelagic biodiversity. The aims of this study were to: (1) assess the diversity and abundance of gelatinous zooplankton in north-western Australia; (2) use morphological and genetic approaches to identify unknown specimens from an under-sampled area; (3) use molecular approaches to assess whether an abundant gelatinous species which is highly invasive, was native or invasive in the study region; and (4) investigate a novel association between medusae and ophiuroids by assessing ophiuroid diet from stable isotope analysis and whether frequency of association was correlated with medusa size.

Underwater visual surveys (UVS) were used to measure abundance of gelatinous zooplankton 48 times between April and July in the northern region of Ningaloo Reef, Western Australia. Samples for genetic analyses were collected opportunistically offshore and during UVSs and were later sequenced for 16S and COI genes. The ophiuroid *Ophiocnemis marmorata* associated with an abundant species of medusa, *Aurelia aurita*, and was sampled for investigations into diet and size relationship with medusae. Ninety-two medusae with ophiuroids were collected over eight sites and the size of medusae and ophiuroids was measured. Thirty medusae with ophiuroids were sampled from each of two sites and potential planktonic prey were collected from each site by conducting four plankton tows for mesozooplankton and four plankton tows for seston at each site. Stable isotope analysis was used to assess whether O. *marmorata* fed upon their gelatinous hosts.

The diet of *O. marmorata* was modelled under four different trophic enrichment factors (TEFs) using the Bayesian mixing model Stable Isotope Analysis in R (SIAR).

Gelatinous zooplankton were present from April to July and morphological and genetic approaches together identified eight species of gelatinous zooplankton, one of which was *M. bella*, a dangerous cubozoan species. Phylogeopgraphic analyses indicated *Aurelia aurita*, an abundant scyphozoan species, was native to the north-west Australian region. *O. marmorata* commonly associated with large *A. aurita* medusae. All four SIAR models revealed plankton food sources form on average up to ~65% of the diet of *O. marmorata* while host medusae provided on average 0 – 10%. This suggests medusae present a platform for ophiuroid feeding and consequently may hold a significant role in the enhancement of biodiversity in pelagic communities. Future research should focus on rigorous sampling of under-sampled regions such as north-western Australia and the Indian Ocean to potentially uncover more associations between medusae and other organisms and to provide a more comprehensive assessment of the diversity and abundance of gelatinous species in this region.

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Statement of originality

The material submitted in this thesis has not been previously submitted for a degree or diploma in any university, and to the best of my knowledge contains no material previously published or written by another person except where due acknowledgement is made in the thesis itself.

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Signed: Brooke Ingram

Date: 27th October 2015

Chapter 1: General Introduction

1.1 Diversity and abundance of gelatinous zooplankton

Gelatinous zooplankton, which comprise medusae, ctenophores and salps, are ubiquitous in the world's oceans and estuaries (Lilley et al., 2011, Lucas et al., 2014). They are often conspicuous components of coastal and open-ocean ecosystems (Richardson et al., 2009) due to the propensity of many species to form blooms (Lucas et al., 2014). Gelatinous zooplankton are often short-lived, grow rapidly and are distributed patchily and so often seem to suddenly appear and disappear in the marine environment (Graham et al., 2001).

Research into gelatinous zooplankton has increased recently over concern of rising populations in some perturbed areas around the world (Pauly et al., 2009, Richardson et al., 2009). Little information exists, however, about the ecology of gelatinous zooplankton and the processes that regulate their population dynamics, despite the increasing interest in this group of marine zooplankton (Dawson and Hamner, 2009, Lilley et al., 2011, Mills, 2001). Determining the diversity and abundance of gelatinous zooplankton is an essential step towards understanding the ecology of these species and monitoring changes in the population dynamics of gelatinous zooplankton in global ocean ecosystems.

1.2 Identification of gelatinous zooplankton

Studies on the diversity of gelatinous zooplankton in Australia are quite scarce. Gelatinous fauna are relatively poorly described and most have been identified using morphological characters (Gershwin and Hannay, 2014 but see Dawson 2005a). However, morphological identification of gelatinous species can be problematic, especially if identification is made from a preserved specimen (Bentlage et al., 2010). As gelatinous zooplankton have extremely high water contents (≥ 95%) and no robust structural features or hard coverings (Thibault-Botha and Bowen, 2004), they tend to

shrink more than other zooplankton when preserved in chemical solutions (formaldehyde, chloroform and ethanol) (Condon et al., 2012, Mutlu, 1996, Thibault-Botha and Bowen, 2004). This can lead to misinterpretations of diagnostic features in preserved specimens (Bentlage et al., 2010).

Ambiguities in identifications based solely on morphology can often be resolved by genetic analyses which in turn can reveal much greater taxonomic diversity than morphology alone (Bayha and Dawson, 2010). Some gelatinous zooplankton have been misidentified using diagnostic morphological features but have later been correctly identified as different species when analysed genetically (Bentlage et al., 2010). For example, conflicting views over the validity of some medusae species in the genus *Alatina* (Cubozoa) led to the revision of two nominal *Alatina* species into one (Bentlage, 2010, Bentlage et al., 2010). Specifically, *Alatina mordens* (Gershwin, 2005a) from the Coral Sea and *A. moseri* (Mayer, 1906) from Hawaii were described as two distinct species using morphological features (Gershwin, 2005a); however Bentlage et al. (2010) subsequently found no genetic divergences corresponding to the species' locality. Thus, based on molecular genetic data (the mitochondrial 16S gene), these two cubozoans represented a single species in the genus *Alatina*, with a well-mixed population with regular gene flow (Bentlage, 2010, Bentlage et al., 2010).

The identification of cryptic gelatinous species has made possible through genetic analyses (Holland et al., 2004, Lee et al., 2013), where cryptic refers to species that are challenging or impossible to differentiate using morphological features alone (Holland et al., 2004). For example, nuclear and mitochondrial DNA sequences have provided evidence of seven sibling species of the scyphozoan medusa, *Aurelia aurita*, and two additional species, *Aurelia limbata* and *Aurelia labiata* (Dawson and Jacobs, 2001). Furthermore, genetic data have recognised six scyphozoan species in the genus *Cassiopea,* with five of those species being genetically distinct but morphologically

cryptic (Holland et al., 2004). Genetic analyses can also be used to identify introductions of invasive cryptic species and assess the potential geographic sources of large populations of gelatinous species through the application of phylogenetic and phylogeographic studies (Lee et al., 2013).

Genetic data allow for phylogenetic reconstruction independent of morphological features and provide many additional characters for analysis (Dawson, 2004, Hillis and Wiens, 2000). On the other hand, traditional morphological approaches to taxonomic characterisation of gelatinous zooplankton is necessary to facilitate reliable family- or genus-level identifications by non-specialists (Bentlage, 2012). Therefore, by combining genetic and morphological techniques, the most robust approach to identifying species can be employed (Dawson, 2005b). This approach may potentially resolve some of the taxonomic confusion that exists for gelatinous zooplankton in Australia and avoid misidentification of gelatinous species (Bentlage et al., 2010).

1.3 Ecological role of medusae in pelagic ecosystems

Despite little being known about the ecology of gelatinous zooplankton, there is evidence that large aggregations of medusae (jellyfish), may provide a number of ecosystem services to pelagic ecosystems (Doyle et al., 2014). For example, medusae provide a food source to many species of fish (Pauly et al., 2009), and are the main food source for some large predators such as the leatherback turtle, *Dermochelys coriacea* (Houghton et al., 2006). A number of organisms are also reported to associate with medusae as they provide a pelagic substrate for benthic organisms, or offer protection from predation (Ohtsuka et al., 2009). For example, many juvenile fish species shelter among the oral arms or underneath the bell of medusae and some feed on the prey and parasites of medusae (Lynam and Brierley, 2007), or the medusae themselves (D'Ambra et al., 2015). The presence of blooms of medusae in ecosystems can facilitate the survival of some species, and thus enhance local biodiversity in pelagic communities (Doyle et al., 2014).

Medusae often form symbiotic relationships with other organisms which have beneficial, harmful or no effects on the host (Leung and Poulin, 2008); these effects are usually defined as mutualistic, parasitic or commensal interactions respectively (Ohtsuka et al., 2009). Medusae share mutualistic relationships with zooxanthellae (dinoflagellates) (Ohtsuka et al., 2009, Pitt et al., 2009b). Photosynthetic products from zooxanthellae are transferred to host medusae, while medusae waste products are utilised by zooxanthellae; therefore both organisms benefit from the association (Ohtsuka et al., 2009). In contrast, parasites of medusae feed on their hosts (Phillips, 1973) or lay their eggs in the host's tissue (Crossley et al., 2009), which can be detrimental to the medusa (Ohtsuka et al., 2009). Some parasites are considered to be exclusively parasitic (Laval, 1980), though there is evidence that some organisms, generally perceived to be parasites, may actually be commensal (Condon and Norman, 1999).

Many organisms form commensal relationships with medusae, including a number of larval, juvenile and adult stages of invertebrate and fish species (Ohtsuka et al., 2009). Commensal relationships with medusae may provide organisms with protection from predation (Lynam and Brierley, 2007), a pelagic nursery (Fleming et al., 2014, Sal Moyano et al., 2012), an energy-saving means for dispersal and transport across large expanses of ocean (Sal Moyano et al., 2012), and even a source of food (D'Ambra et al., 2015, Riascos et al., 2015). For example, crabs commonly associate with medusae, utilising their host as a floating nursery during larval and juvenile stages which facilitates their dispersal to areas outside their own dispersal abilities (Sal Moyano et al., 2012). They also gain protection from predation during vulnerable molting periods and potentially feed on their host and prey captured by their host (Sal Moyano et al., 2012). Feeding on medusae hosts enhances the survival of some species, particularly fish and hyperiid amphipods (D'Ambra et al., 2015, Fleming et al., 2014, Miyajima et al., 2011). Consequently, medusae may hold a significant ecological role in global ecosystems due

to the diverse array of relationships they form with other organisms, which can lead to enhanced biodiversity in the ecosystem (Doyle et al., 2014).

1.4 General objectives

The objectives of this thesis were (1) to assess the diversity and abundance of gelatinous zooplankton in an area where they are known to bloom, but data are lacking, (2) use morphological and genetic analyses to create the most robust species identifications of unknown specimens from an under-sampled area, and (3) assess the role of medusae in the facilitation of biodiversity in pelagic communities. This will add to data needed to reliably assess the global abundance and diversity of gelatinous zooplankton and provide support for the inclusion of gelatinous zooplankton in ecosystem models.

Chapter 2: The diversity and abundance of gelatinous zooplankton found off north-western Australia

2.1 Introduction

2.1.1 Global spread of data on abundance and diversity of gelatinous zooplankton

Global estimates of gelatinous zooplankton abundance and biomass are generally limited, mostly due to unbalanced spatial coverage of data across the globe (Lucas et al., 2014). There are some areas known to be inhabited by gelatinous zooplankton, but data has yet to be published (Lilley et al., 2011), creating a void in the distribution of data available for abundance and biomass estimates. Currently, the Jellyfish Database Initiative (JeDI) is developing a scientifically coordinated global gelatinous zooplankton database to assess historical, current and future trends in gelatinous zooplankton global abundance (Condon et al., 2012, Lucas et al., 2014). Figure 1 shows the distribution of JeDI metadata sets, however most data are presence only or presence/absence rather than quantitative estimates of abundance (Condon et al., 2012). In particular, there appears to be limited data for the Southern Hemisphere, despite gelatinous zooplankton known to be abundant there (Lucas et al., 2014). Furthermore, Lucas et al. (2014) indicated that under-sampling of the Southern Hemisphere has resulted in biased estimates of global patterns of gelatinous zooplankton biomass, with whole regions having almost no data for gelatinous zooplankton.

Quantitative data on the abundance of gelatinous zooplankton are particularly limited in the Indian Ocean (Condon et al., 2012) (Figure 1) and although presence-absence data have been recorded, they do not give estimates of the quantity of gelatinous zooplankton inhabiting this region. Moreover, despite the abundance and frequency of blooms in this

region, there is a paucity of data relating to gelatinous zooplankton from the north-west coast of Australia (Condon et al., 2012) (Figure 1).

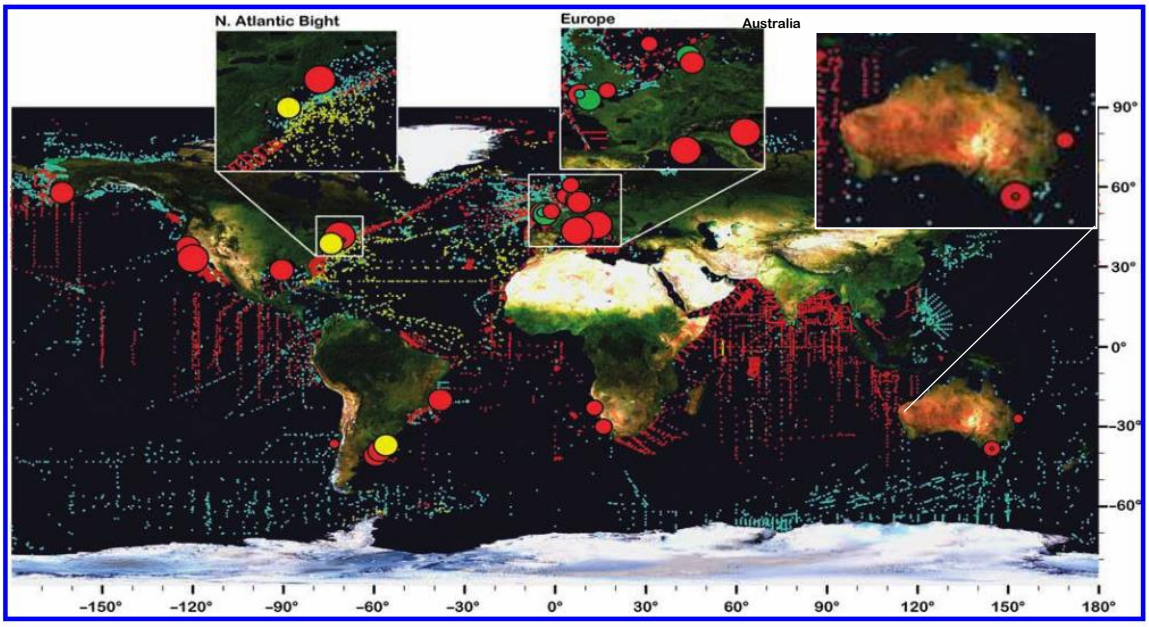


Figure 1: Derived from Condon et al. (2012). Distribution of the Jellyfish Database Initiative (JEDI) metadata sets. Metadata of gelatinous zooplankton includes quantitative (green), categorical (yellow), presence-absence (red) and presence-only (light blue). North-west Western Australia is lacking in all types of metadata compared to other regions around the world.

According to SeaLifeBase (www.sealifebase.org), there are potentially 38 species of gelatinous zooplankton that may occur in Western Australian waters. Of those, thirteen are endemic to the eastern Indian Ocean, and only four species have been described directly from locations found along the north-west Australian coast (Gershwin, 2005b, Gershwin, 2005c, Gershwin, 2014). Despite sightings of large blooms of gelatinous zooplankton at Ningaloo Reef (P. Barnes, 2014, pers. comm.), which is part of the North West Cape peninsula (Morton, 2003), only one species, a cubozoan, has been described from this region (see Gershwin, 2014). This could be due to a number of reasons, including the difficulty associated with sampling gelatinous organisms, the use of sampling techniques biased toward non-gelatinous taxa, or environmental drivers such as food availability and temperature gradients causing gelatinous zooplankton to sporadically appear and disappear (Lucas et al., 2014). Regardless, the scarcity of

information available for gelatinous zooplankton along the north-western coast of Australia needs to be addressed.

Western Australia has a variety of pristine natural areas, many of which have gained international recognition (Wood and Glasson, 2005). For example, Ningaloo Reef, which extends from Carnarvon to Exmouth in Western Australia (Wood and Glasson, 2005), is Australia's longest fringing reef (Cassata and Collins, 2008) and has gained World Heritage listing due to the high diversity of marine species supported by the relatively pristine and intact marine and coastal environments (Catlin et al., 2012). Ningaloo Reef has a low human population density due to its remote location, which has left it relatively under-developed (Cassata and Collins, 2008). However Ningaloo, like many regions in north-west Australia, is very resource-rich, and is now home to offshore oil and gas exploration projects, with port facility developments also proposed for the area (Brown et al., 2012). Given Ningaloo Reef is still in a relatively pristine condition, establishment of baseline data on marine species diversity is needed to monitor and evaluate the potential impacts from these industries (Cassata and Collins, 2008).

Ningaloo Reef supports a high diversity of marine species, and is most famous for aggregations of whale sharks, manta rays and whales (Wood and Glasson, 2005). Consequently, marine-based ecotourism has flourished in this region, providing a boost to the economy that the fishing industry and declining pastoral activities could not sustain (Wood and Glasson, 2005). However, recent sightings of dangerous medusae at Ningaloo have gained media attention and are threatening the growing ecotourism industry at Ningaloo Reef (Jones, 2014). Recently, a cubozoan medusa, *Malo bella*, presumed to cause Irukandji syndrome, was identified from the northernmost region of Ningaloo Reef (Gershwin, 2014). To address tourism operators' concerns of envenomation of tourists by dangerous gelatinous species such as *M. bella* and prevent a subsequent decline in marine-

based tourism at Ningaloo Reef, the abundance and diversity of gelatinous zooplankton, including cubozoans, in this region needs to be assessed.

2.1.2 Methods needed to comprehensively sample gelatinous zooplankton

Multiple approaches should be used to quantitatively sample gelatinous zooplankton because the wide range of body sizes and variations in the robustness of gelatinous bodies means that there is no single sampling method suitable for all gelatinous zooplankton. For example, towing a plankton net slowly and for a relatively short time either at surface or sub-surface levels can be an effective way to capture firm gelatinous zooplankton, such as medusae (Raskoff et al., 2003). Collection of gelatinous zooplankton with dip nets while snorkelling or SCUBA diving reduces the possibility of damaging the organism and allows for *in situ* observations of the animal (Pierce, 2009, Raskoff et al., 2003). Combining plankton net tows with underwater visual surveys would ensure reliable estimates of abundance, while preserving the structure of gelatinous zooplankton sufficiently well to enable identification for studies of diversity (Raskoff et al., 2003). Thus, when concerned with documenting the diversity and abundance of gelatinous zooplankton, the use of multiple approaches to sampling is essential.

2.1.3 Genes needed for the molecular identification of gelatinous zooplankton

Genetic analyses offer a degree of accuracy and reliability for the identification of gelatinous species which morphology alone cannot provide. There are, however, various factors that must be taken into account when using genetic data to identify species. For example, some genes are suitable for species identification, while others are more suitable for phylogenetic analyses (Yuri et al., 2013). Mitochondrial genes are most extensively used for species identification, as they vary only slightly within species, and have greater variation between species (Savolainen et al., 2005).

To help determine the species' identity of unknown samples, a method known as DNA barcoding is commonly used (Buhay, 2009, Lv et al., 2014, Savolainen et al., 2005). DNA barcoding is aimed at developing a DNA-based identification system for all taxa (Herbert et al., 2003a) and can help detect overlooked species or unknown specimens with subtle or complex morphological traits (Bucklin et al., 2011). Although, DNA barcoding relies on the basis that specimens can be identified based on sequence similarity with existing representative species sequences in a database (Ortman et al., 2010, Ross et al., 2008). When these sequences do not exist in a database, such as GenBank, only tree-based methods offer insights into species identification of cryptic or unknown specimens (Ross et al., 2008).

Multiple genes are usually needed for species identification, as individual genes have benefits and limitations (Bucklin et al., 2011, Ortman et al., 2010, Zheng et al., 2014). For example, the mitochondrial, protein-coding gene cytochrome *c* oxidase subunit 1 (COI) has been established as the core of DNA barcoding (Herbert et al., 2003a) as it is short enough to generate a large number of sequences quickly (Savolainen et al., 2005), but long enough to identify variation among species, which is crucial for reliable species identifications (Herbert et al., 2003b). However, due to gene saturation, COI genes have low phylogenetic signals at higher levels, making assessments of phylogenetic diversity difficult (Ortman et al., 2010), whereas the prevalence of indels (insertion/deletion events) in 16S, a large subunit ribosomal RNA (Savolainen et al., 2005), makes this gene useful for estimating phylogenetic relationships among animals, especially among hydrozoan medusae (Yuri et al., 2013, Zheng et al., 2014).

When concerned only with species identification, some genes present difficulties which can affect the accuracy of identifications. For example, as 16S is a non-coding gene, so it accumulates indels where protein-coding genes, such as COI, rarely do (Yuri et al., 2013).

This can complicate sequence alignments (Herbert et al., 2003a), which are performed to create sequences with 'gaps' that reflect hypothetical positions where indels would have occurred due to homology (similarity due to common ancestry) (Doyle and Gaut, 2000, Yuri et al., 2013). Consequently, species identifications based on 16S sequences may not be reliable if the alignment was complicated due to a high frequency of indels (Herbert et al., 2003b). Additionally, despite its applicability to a broad range of taxa, COI is unable to discriminate closely allied species in the phylum Cnidaria (Herbert et al., 2003a), though this conclusion related to species in the class Anthozoa, or in other words, corals and sea anemones (Bucklin et al., 2011). A study has recently reported COI can be used for species identification across the Medusazoa (Ortman et al., 2010), indicating it is still a reliable marker for medusae species identification. Thus, both COI and 16S have strengths and limitations with regards to gelatinous species identification, which is why it may be necessary to use both to obtain the most accurate identification of an unknown species.

2.1.4 Genetic analyses identify cryptic species of *Aurelia* medusae and introductions of invasive species

Aurelia sp. are perhaps the most ubiquitous medusae in the world (Dawson and Jacobs, 2001). They can be found in temperate and tropical waters (Dawson and Jacobs, 2001), occupying a range of habitats (Schroth et al., 2002). There are fourteen known species of *Aurelia* (Dawson et al., 2005), eleven of which are cryptic (Dawson et al., 2005, Dawson and Jacobs, 2001, Schroth et al., 2002). Many *Aurelia* sp. are restricted to certain regions because natural oceanographic patterns limit their dispersal range (Dawson et al., 2005), however multiple introductions of cryptic *Aurelia* sp. have been identified using genetic analyses (Dawson, 2003, Dawson et al., 2005). Typically, regionally restricted *Aurelia* sp. have higher geographic structure and genetic diversity than invasive *Aurelia* species (Dawson, 2003, Dawson et al., 2005). For example, *Aurelia* sp. 1, a globally distributed

Aurelia species, is thought to be invasive as its reduced genetic diversity does not reflect an organism with a natural distribution (Dawson, 2003). It is thought anthropogenic introductions have enabled *Aurelia* sp. 1 to exceed its natural dispersal range, allowing it to colonise warm-temperate areas outside of its original, natural distribution (Dawson, 2003, Dawson et al., 2005).

Morphological differences among populations have been used to determine whether medusae are invasive to an area where they suddenly appear (Bolton and Graham, 2004). Though, as there are many cryptic *Aurelia* species (Dawson and Jacobs, 2001) which have few distinguishing morphological features (Greenberg et al., 1996), and many regions such as the Indian Ocean and Australia are still under-sampled with regards to gelatinous zooplankton (Condon et al., 2012), and *Aurelia* sp. in particular (Dawson and Jacobs, 2001), morphological differences among populations of *Aurelia* sp. would not be an effective means for the identification of invasive or endemic populations. Thus, genetic analyses present a valuable tool for assessing whether populations of *Aurelia* species are endemic or introduced to particular regions, and are able to detect cryptic species within a population (Dawson, 2003, Dawson et al., 2005).

2.1.5 Objectives

The objectives of this chapter were: (1) to assess the abundance of gelatinous zooplankton at Ningaloo Reef using plankton tows and snorkelled transects, (2) to assess the diversity of species of gelatinous zooplankton at Ningaloo Reef by exploring the phylogenetic relationships among specimens using 16S and COI sequences for genetic analyses, and (3) to determine whether *Aurelia* sp. at Ningaloo Reef are invasive or endemic, based on phylogenetic analyses of *Aurelia* sp., including cryptic species, from various geographic localities.

2.2 Methods

2.2.1 Sampling sites

Gelatinous zooplankton were sampled in and adjacent to the northern section of Ningaloo Reef, Western Australia (Figure 2). Gelatinous zooplankton were sampled most commonly on the west coast of the Exmouth Cape, while some were sampled opportunistically within the Exmouth Gulf on the eastern side of the cape. Sampling sites included areas within the lagoon of Ningaloo Reef, at depths of 3 – 8 m, and outside the lagoon, at depths of 20 – 80 m (past the reef break). The whale shark tourism vessel Latitude 22, owned by Ocean Eco Adventures, was used to sample gelatinous zooplankton at the sampling sites. Sites within the lagoon were comprised mainly of patches of coral bomboras, while sites outside the lagoon were oceanic.

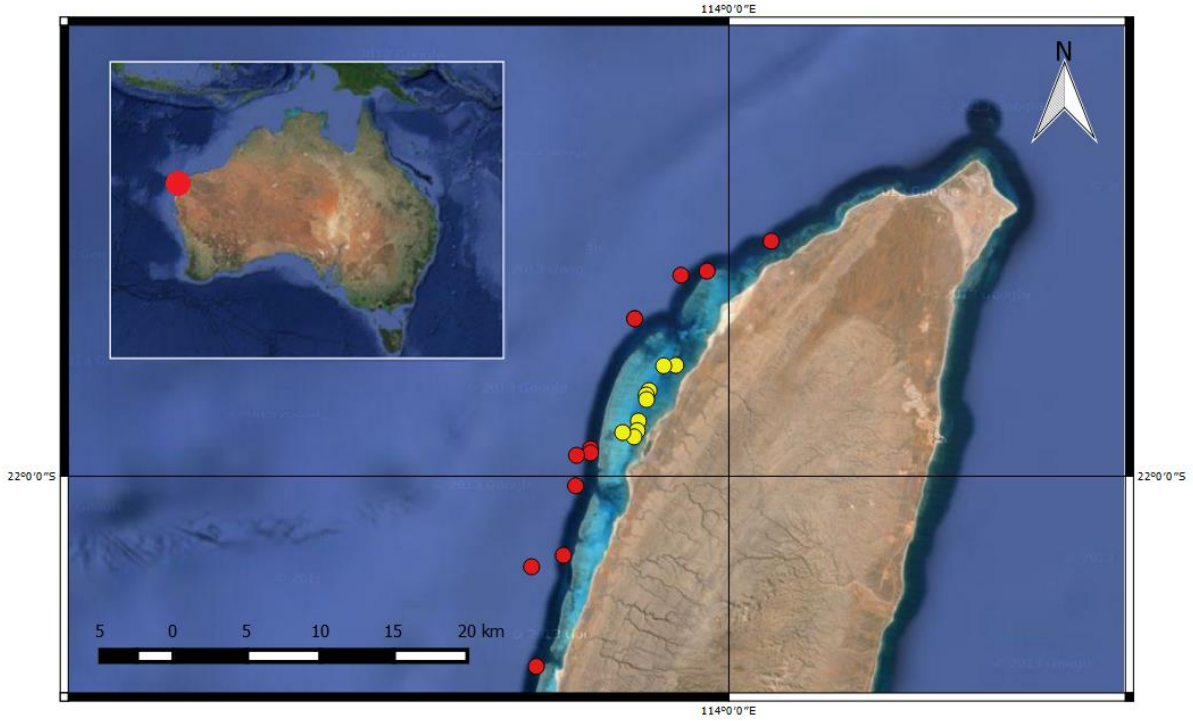


Figure 2: Locations of sampling sites. Sites within the lagoon are represented by yellow circles while sites outside the lagoon are represented by red circles.

2.2.2 Methods used to sample within the lagoon of Ningaloo Reef

Gelatinous zooplankton were sampled within the lagoon 48 times from 1st April to 22nd July 2015 at intervals of 1 – 7 days using underwater visual surveys (UVSs). Each day, one site was sampled in the morning (between 9:30 and 11:00am) and a second site was sampled in the afternoon (between 1:30pm and 3:50pm). UVSs were undertaken at a total of 18 different sites, with sites being sampled 1-12 times throughout the sampling period. The sites sampled on any given day were determined by the whale shark tourism vessel, Latitude 22, in accordance with Ocean Eco Adventure's snorkelling activities, run as part of the tour. At each site a flowmeter (General Oceanics), that was held in front of the snorkeler, was used to measure five 50 m transects, with a minimum of 10 m separating each transect. Gelatinous zooplankton 1 m either side of the snorkeler were identified and counted *in situ* or photographed for later morphological identification. Gelatinous zooplankton were also captured during UVSs using a 2 mm-mesh hand-held dip net and reserved for identification via genetic analyses.

2.2.3 Methods used to sample outside the lagoon of Ningaloo Reef

Gelatinous zooplankton were sampled outside the lagoon 13 times from 1st April to 23rd July 2015, however sampling events depended on weather conditions and whether seasick passengers necessitated the early return of the vessel. Gelatinous zooplankton were collected from horizontal tows using a plankton net (51 cm diameter net opening, 150 µm-mesh size). However, as only one medusa over the four months was caught using this method, the data were not analysed. Additional specimens were captured opportunistically outside the lagoon while snorkelling as part of the whale shark interaction tour run by Ocean Eco Adventures. All specimens were stored on ice until they were able to be processed for morphological identification and genetic analyses and were processed within 24 hours of capture.

2.2.4 Morphological identification

Preliminary order-, family-, genus- and species-level identifications were made using diagnostic morphological features of gelatinous zooplankton (Gershwin, 2014, Kramp, 1961, Wrobel and Mills, 1998). Each specimen was photographed in the laboratory, and where possible, *in situ*, to develop a photographic database of gelatinous zooplankton found at Ningaloo Reef (Appendix 1). Specimens were preserved in 10% sodium borate-buffered formaldehyde-seawater solution and lodged as voucher specimens with the Western Australian Museum (WAM).

2.2.5 Processing samples for genetic analyses

A small tissue sample from a tentacle or oral arm was extracted from gelatinous zooplankton specimens using forceps and a scalpel to prepare them for genetic analyses. When tentacles or oral arms were damaged or not visible (due to small size or morphology of some specimens) extractions were taken from the margin of the bell or muscle (e.g. subumbrella muscle bands from cnidarians) (M. Dawson, 2015, pers. comm.). The tissue sample was transferred to a 2 mL cryo tube and filled with ≥ 95% ethanol. Samples were kept at room temperature until they were sent to Dr Nerida Wilson, with the WAM, who conducted genetic analyses to sequence mitochondrial genes, 16S and cytochrome oxidase 1. DNA was extracted and purified using a DNeasy blood and tissue kit (Qiagen), following manufacturers' protocols. Whole or partial mitochondrial gene sequences from COI and 16S were amplified for species identification and phylogenetic analyses. Primers and cycling conditions are given in Table 1. Products were sent to the Australian Genome Sequencing Facility (AGSF) in Perth, Western Australia for purification and cycle sequencing on an ABI 3730 capillary sequencer. Sequences were reconciled and edited by eye in Geneious R7 before exporting for genetic analyses (N. Wilson, 2015, pers. comm.).

Table 1: Primers and cycling conditions for mitochondrial gene sequences 16S and COI.

Region	Primer	Source	Sequence (5'-3')	PCR cycling conditions
COI	LCO1490	Folmer et al. 1994	GGTCAACAAATCATAAAGATATTGG	(95 °C: 20 s; 45 °C: 30 s; 72 °C: 40 s) x 5; (95 °C: 20 s; 50 °C: 30 s; 72 °C: 40 s) x 35
COI	HCO2198	Folmer et al. 1994	CTAAACTTCAGGGTGACCAAAAAATCA	
16S	16SF2	Cunningham & Buss, 1993	TCGACTGTTTACCAAAAACATA	(95 °C: 20 s; 45 °C: 30 s; 72 °C: 40 s) x 5; (95 °C: 20 s; 50 °C: 30 s; 72 °C: 40 s) x 35
16S	R16SR2	Cunningham & Buss, 1993	ACGGAATGAACTCAAATCATGTAAG	

2.2.6 Species identification and phylogenetic analyses for Ningaloo Reef specimens

The sequences provided by WAM were identified using a GenBank non-redundant *megablast* search for highly similar sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi). E-values close to zero and sequence identities close to 100% indicate closely related sequences, and therefore potential species matches. Phylogenetic relationships among species (sequences) were explored using MEGA 6.0 (Tamura et al., 2013). COI and 16S sequences were aligned by using several gap-opening:gap-extension weighting schemes in ClustalW and alignments were corrected by eye. Phylogenetic trees were constructed by using the maximum likelihood method in MEGA 6.0 (Tamura et al., 2013) using relevant models of molecular evolution (16S, HKY+G; COI, GTR+G+I) identified by the *Find Best DNA/Protein Models (ML)* feature. The reliability of the nodes on the trees were estimated using the bootstrap method (1000 replicates). Nodes with high bootstrap values (> 70%) are considered to be closely related and specimens that share sister nodes at the tips of the tree are considered to be closely related and possibly the same species (Hall, 2013). Trees were left unrooted.

2.2.7 *Aurelia* sp. phylogeographic analysis

COI sequences from 14 known *Aurelia* species (Dawson et al., 2005) were derived from GenBank (http://www.ncbi.nlm.nih.gov/) (Benson et al., 2009, Sayers et al., 2011). Sequences were from two specimens from each species, which were sampled in different geographic locations. However, for some species (*Aurelia limbata*, *Aurelia* sp. 10 and *Aurelia* sp. 11) there was only one COI sequence from one specimen available. GenBank sequences were aligned with *Aurelia* sp. COI sequences from Ningaloo Reef to examine phylogenetic relationships among *Aurelia* sp. from different regions of the world (Table 2). Alignments were performed using several gap-opening:gap-extension weighting schemes in ClustalW and corrected by eye. A phylogenetic tree was constructed using the maximum likelihood method in MEGA 6.0 (Tamura et al., 2013) using the model of best fit, GTR+G (General Time Reversible). Reliability of tree nodes were estimated using bootstrap analyses (1000 replicates). Trees were left unrooted. A GenBank non-redundant *megablast* search for highly similar sequences was run for each Ningaloo Reef *Aurelia* sp. COI sequence to determine the locality of the species most closely related to those specimens. A *megablast* search was not conducted for Ningaloo Reef *Aurelia* sp. 16S sequences as there are too few 16S sequences available on GenBank for such a comparison. A phylogenetic tree was not constructed for the same reason.

Table 2: *Aurelia* sp. sequences derived from NCIB GenBank and the regions in which they were sampled from.

Species	16S	COI	Reference
Aurelia aurita	Baltic Sea Woods Hole, USA	Sweden; Turkey	Dawson et al. (2005), Fuchs, et al. (2014) Schroth et al. (2002)
Aurelia labiata	Bamfield, Canada; Victoria, Canada	Tomales Bay, USA; Todd Inlet, Canada	Dawson, et al. (2005), Sparmann et al. (unpublished)
Aurelia limbata	Unknown North West Pacific	Japan	Dawson et al. (2005), Gotoh et al. (unpublished), Schroth et al. (2002)
Aurelia sp. 1	China China	Perth, Australia San Diego, USA	He et al. (unpublished), Dawson et al. (2005) He et al. (unpublished), Dawson et al. (2005)
Aurelia sp. 2	No sequences available	Sao Paulo, Brazil	Dawson et al. (2005)
Aurelia sp. 3	No sequences available	Tab Kukau Cove, Palau; Tketau Lake, Palau	Dawson et al. (2005)
Aurelia sp. 4	No sequences available	Hawaii, USA; Kakaban Is., Palau	Dawson et al. (2005)
Aurelia sp. 5	No sequences available	Mljet, Croatia	Dawson et al. (2005)
Aurelia sp. 6	No sequences available	Helen Reef, Palau; New Britain, Papua New Guinea	Dawson et al. (2005)
Aurelia sp. 7	No sequences available	Tasmania, Australia	Dawson et al. (2005)
Aurelia sp. 8	No sequences available	Bay of Ston, Croatia; North Adriatic Sea	Dawson et al. (2005)
Aurelia sp. 9	No sequences available	Gulf of Mexico, Alabama	Dawson et al. (2005)
Aurelia sp. 10	No sequences available	Kachemak Bay, Alaska	Dawson et al. (2005)
Aurelia sp. 11	No sequences available	Kwajalein, Marshall Is.	Dawson et al. (2005)

2.3 Results

2.3.1 Abundance of gelatinous zooplankton inside the lagoon of Ningaloo Reef

Gelatinous zooplankton were present in all months (April, May, June and July), however appeared to be most abundant in April and June (Figure 3). Six medusae were identified to species-level, family-level and order-level based on morphology during inshore sampling. Hydrozoans in the order Narcomedusae and the scyphozoan, *Crambione mastigophora,* were most abundant in April, while the scyphozoan *Aurelia aurita* was most abundant in April and June. The cubozoan *Malo bella* was present in May, and a scyphozoan in the order Rhizostomeae and the hydrozoan medusae *Liriope tetraphylla* were present in June and July respectively (Figure 3).

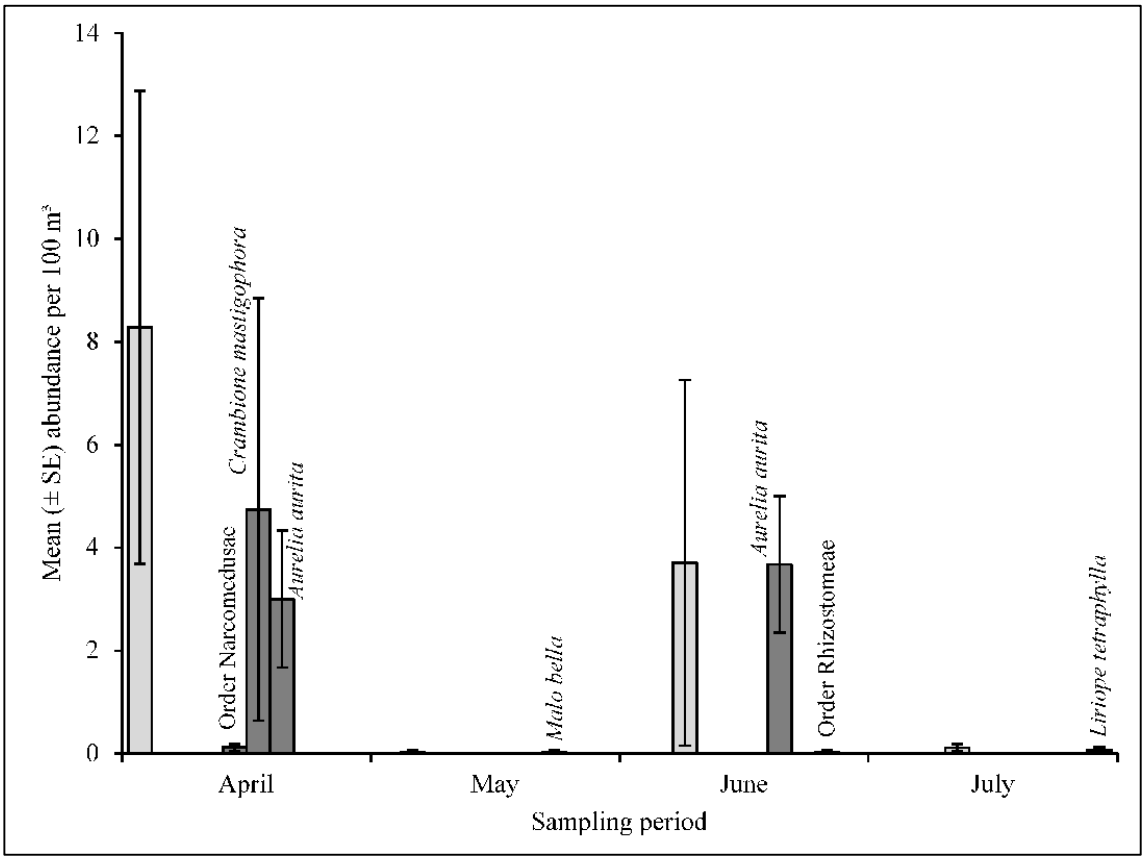


Figure 3: Mean (± SE) overall and species abundance per 100 m^3 of gelatinous zooplankton relative to time of year. Specimens were sampled within the lagoon of Ningaloo Reef. Light grey bars represent overall mean (± SE) abundance. Species mean (± SE) abundance are indicated by data labels above corresponding bars.

2.3.2 Presence-absence data for gelatinous zooplankton found outside the lagoon of Ningaloo Reef

Outside the lagoon of Ningaloo Reef, four more cnidarians were present from May to July, including: a *Cyanea* sp. scyphozoan present in July, *Aequorea australis* (Hydrozoa) present in May and June, and a hydrozoan in the family Aequoreidae and a hydrozoan in the order Leptomedusae both present in July (Table 3). *C. mastigophora* was present in both April and May and hydrozoans in the order Narcomedusae appeared in all months but July (Table 3). Though only present in May inside the lagoon, outside the lagoon *M. bella* was present in May, June and July (Table 3).

Table 3: Presence of gelatinous species outside the lagoon of Ningaloo Reef from April to July. Ticks represent sightings of a particular species on a given sampling week. Month of sampling (from April to July) is indicated by light to dark grey scale shading and month column.

Month	**Sampling week**	***Aurelia aurita***	**Order Narcomedusae**	***Crambione mastigophora***	***Malo bella***	***Aequorea australis***	**Family Aequoreidae**	***Liriope tetraphylla***	***Cyanea* sp.**	**Order Leptomedusae**
April	1 Apr - 7 Apr	✓	✓							
	8 Apr - 14 Apr			✓						
	15 Apr - 21 Apr									
	22 Apr - 28 Apr			✓						
	29 Apr - 5 May									
May	6 May - 12 May			✓	✓					
	13 May - 19 May					✓				
	20 May - 26 May									
	27 May - 2 Jun	✓	✓	✓		✓				
June	3 Jun - 9 Jun	✓								
	10 Jun - 16 Jun	✓	✓		✓			✓	✓	
	17 Jun - 23 Jun									
	24 Jun - 30 Jun				✓					
July	1 Jul - 7 Jul				✓					
	8 Jul - 14 Jul									
	15 Jul - 21 Jul				✓		✓			✓
	Total no. times species were sighted:	**4**	**3**	**4**	**5**	**2**	**1**	**1**	**1**	**1**

2.3.3 Analysis of phylogenetic relationships among medusae specimens

The GenBank *megablast* search for 16S and COI sequences with high similarity to the Ningaloo Reef specimens indicated there was variation at the species level that was not detected by morphological approaches (Table 4 and 5). The preliminary identification of some specimens as *Aequorea australis* and *L. tetraphylla* was supported by both 16S and COI *megablast* searches, indicating differences between these species and other closely-related species are large enough to differentiate them on a morphological basis (Table 4 and 5). Specimens identified to the orders Narcomedusae and Leptomedusae were identified as *Aequorea* sp. (16S: E-value 0.06E+178 – 0.0, 90-93%; COI: E-value 0.0, 87-89% sequence identity) and *Laodicea undulata* (16S: E-value 0.0, 90% sequence identity; COI: E-value 0.0, 97% sequence identity) respectively. *Cyanea* sp. identified using morphological approaches were identified with high confidence (E-value 0.0, 91% sequence identity) to be *Cyanea purpurea* (COI sequence *megablast* search) (Table 5). Similarly, *Aurelia aurita* specimens had high sequence similarity with *A. aurita* 16S sequences from GenBank (16S: E-value 0.0, 87% sequence identity) but also had high COI sequence similarity with *Aurelia* sp. 4 and *Aurelia* sp. 7 (COI: E-value 0.0, 85% sequence identity).

For *M. bella* and *C. mastigophora* specimens, 16S and COI *megablast* searches indicated there are genetic divergences among specimens undetectable by comparison of morphological differences (Table 4 and 5). However, there are no published or unpublished 16S and COI sequences available on GenBank for *M. bella* (Cubozoa) and *C. mastigophora* (Scyphozoa). Consequently, direct comparison of Ningaloo specimens identified as *M. bella* and *C. mastigophora* to existing *M. bella* and *C. mastigophora* GenBank sequences was not possible, therefore the identification of those specimens could not be verified by DNA barcoding (*megablast* search).

Table 4: Results of GenBank *megablast* 16S sequence similarity search for species identification of Ningaloo Reef specimens. Rows highlighted in light grey indicate confirmed species identifications (E-value 0.0, ≥ 90% sequence identity). Rows highlighted in dark grey indicate species for which GenBank 16S sequences do not exist, so direct comparison to species sequences was not possible. Species in column "*megablast* species identification" represent most closely-related species.

Specimen	Morphological identification	Sequence identity	E value	*Megablast* species identification
WAMZ90001	*Malo bella*	91%	0.0	*Morbakka virulenta*
WAMZ90002	*Malo bella*	91%	0.0	*Morbakka virulenta*
WAM900004	*Malo bella*	91%	0.0	*Morbakka virulenta*
WAM900007	*Aequorea australis*	98-99%	0.0	*Aequorea australis*
WAMZ90008	Aequoreidae	90%	0.0	*Phialella quadrata*
WAMZ90009	*Aequorea australis*	98-99%	0.0	*Aequorea australis*
WAMZ90010	Leptomedusae	91-93%	0.0	*Aequorea sp.*
WAMZ90011	Leptomedusae	91-93%	0.0	*Aequorea sp.*
WAMZ90012	Leptomedusae	90-92%	0.0	*Aequorea sp.*
WAMZ90013	Leptomedusae	91-93%	0.0	*Aequorea sp.*
WAMZ90014	Leptomedusae	91-92%	0.0	*Aequorea sp.*
WAMZ90015	*Liriope tetraphylla*	92-99%	0.0	*Liriope tetraphylla*
WAMZ90016	*Liriope tetraphylla*	92-99%	0.0	*Liriope tetraphylla*
WAMZ90017	Leptomeduse	92-96%	0.0	*Laodicea undulata*
WAMZ90018	Leptomedusae	90%	0.0	*Laodicea undulata*
WAMZ90020	*Crambione mastigophora*	85%	3.00E+166	*Cassiopea andromeda*
WAMZ90021	*Crambione mastigophora*	86%	6.00E+168	*Cassiopea andromeda*
WAMZ90022	*Cyanea* sp.	83%	1.00E+145	*Cyanea sp.*
WAMZ90024	*Aurelia aurita*	87%	0.00E+00	*Aurelia aurita*
WAMZ90025	*Aurelia aurita*	87%	0.00E+00	*Aurelia aurita*
WAMZ90026	*Aurelia aurita*	87%	0.00E+00	*Aurelia aurita*
WAMZ90027	*Aurelia aurita*	87%	0.0	*Aurelia aurita*
WAMZ90028	*Aurelia aurita*	87%	0.00E+00	*Aurelia aurita*

Table 5: Results of GenBank *megablast* COI sequence similarity search for species identification of Ningaloo Reef specimens. Rows highlighted in light grey indicate confirmed species identifications (E-value 0.0, ≥ 90% sequence identity). Rows highlighted in dark grey indicate species for which GenBank COI sequences do not exist, so direct comparison to species sequences was not possible. Species in column "*megablast* species identification" represent most closely-related species.

Specimen	Morphological identification	Sequence identity	E-value	*Megablast* species identification
WAMZ90000	*Malo bella*	78%	9.00E+102	*Chiropsalmus quadrumanus*
WAMZ90001	*Malo bella*	78%	9.00E+102	*Chiropsalmus quadrumanus*
WAMZ90002	*Malo bella*	78%	9.00E+102	*Chiropsalmus quadrumanus*
WAMZ90003	*Malo bella*	78%	9.00E+102	*Chiropsalmus quadrumanus*
WAMZ90004	*Malo bella*	78%	9.00E+102	*Chiropsalmus quadrumanus*
WAMZ90006	*Malo bella*	78%	9.00E+102	*Chiropsalmus quadrumanus*
WAMZ90007	*Aequorea australis*	97%	0.0	*Aequorea australis*
WAMZ90008	Aequoreidae	86%	0.0	*Blackfordia polytentaculata*
WAMZ90009	*Aequorea australis*	97%	0.0	*Aequorea australis*
WAMZ90010	Leptomedusae	89%	0.0	*Aequorea sp.*
WAMZ90012	Leptomedusae	89%	0.0	*Aequorea sp. & Eirene sp.*
WAMZ90013	Leptomedusae	87-89%	0.0	*Aequorea sp. & Eirene sp.*
WAMZ90014	Leptomedusae	87-89%	0.0	*Aequorea sp. & Eirene sp.*
WAMZ90015	*Liriope tetraphylla*	99%	0.0	*Liriope tetraphylla*
WAMZ90016	*Liriope tetraphylla*	99%	0.0	*Liriope tetraphylla*
WAMZ90017	Leptomedusae	97%	0.0	*Laodicea undulata*
WAMZ90021	*Crambione mastigophora*	82%	7.00E+143	*Crambionella stuhlmanni*
WAMZ90022	*Cyanea* sp.	91%	0.0	*Cyanea purpurea*
WAMZ90025	*Aurelia aurita*	85%	0.0	*Aurelia sp. 4*
WAMZ90026	*Aurelia aurita*	85%	0.0	*Aurelia sp. 4 & Aurelia sp. 7*
WAMZ90027	*Aurelia aurita*	85%	0.0	*Aurelia sp. 4*

All species formed distinct clusters in the 16S and COI trees constructed using the maximum likelihood (ML) tree method, with the arrangement of clusters reflecting class level groupings (Hydrozoa, Scyphozoa and Cubozoa) of specimens in the tree (Figure 4 and 5). Three species (*L. tetraphylla, A. australis* and *C. mastigophora*) formed strongly supported clades (bootstrap >90%) in both the COI and 16S trees, with *L. tetraphylla* and two specimens of *A. australis* (WAMZ90007 and WAMZ90009) clearly separated from other species (bootstraps >70%) (Figure 4 and 5). Furthermore, Leptomedusae specimens

in the 16S tree shared the same node, which was very strongly supported (bootstrap = 100%), indicating they were closely related, and potentially the same species (Figure 4).

In the 16S tree, the separation of the cubozoan species *M. bella* into distinct lineages was supported by high bootstrap values (>80 %) (Figure 4). This indicates that although the specimens are closely related, there is genetic variation, perhaps at the species or genus level, among the specimens. However, they showed very reduced genetic variation in the COI tree (Figure 5). Internal nodes in the COI tree showed strong support (bootstrap >70%) for a higher level connection between an Aequoreidae hydrozoan (WAMZ90008) and *Aequorea australis* specimens (Figure 5), indicating there is perhaps a family- or order-level relation among those specimens. Division of Leptomeduse hydrozoans into separate lineages in both trees (strongly supported in COI tree) revealed potentially family- and genus-level variation among Leptomedusae specimens (Figure 5), otherwise undetectable by morphological differences.

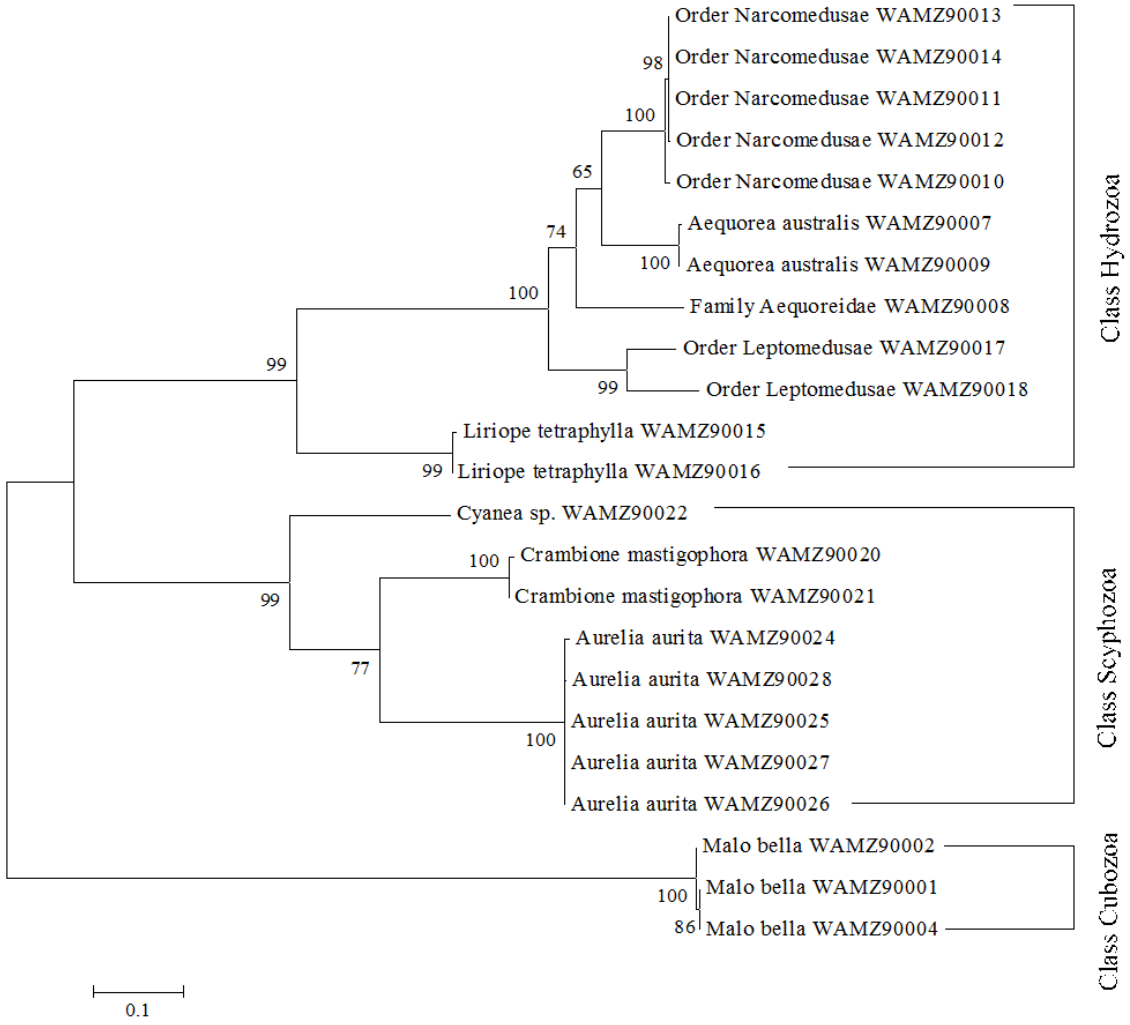


Figure 4: Maximum-Likelihood phylogenetic tree based on the mitochondrial gene 16S, a large subunit ribosomal RNA, for specimens found at Ningaloo Reef. Bootstrap values (1000 replicates) reflect the percentage of trees in which the associated taxa clustered together and are shown at each node. Class lineages are indicated. Scale bar represents evolutionary time.

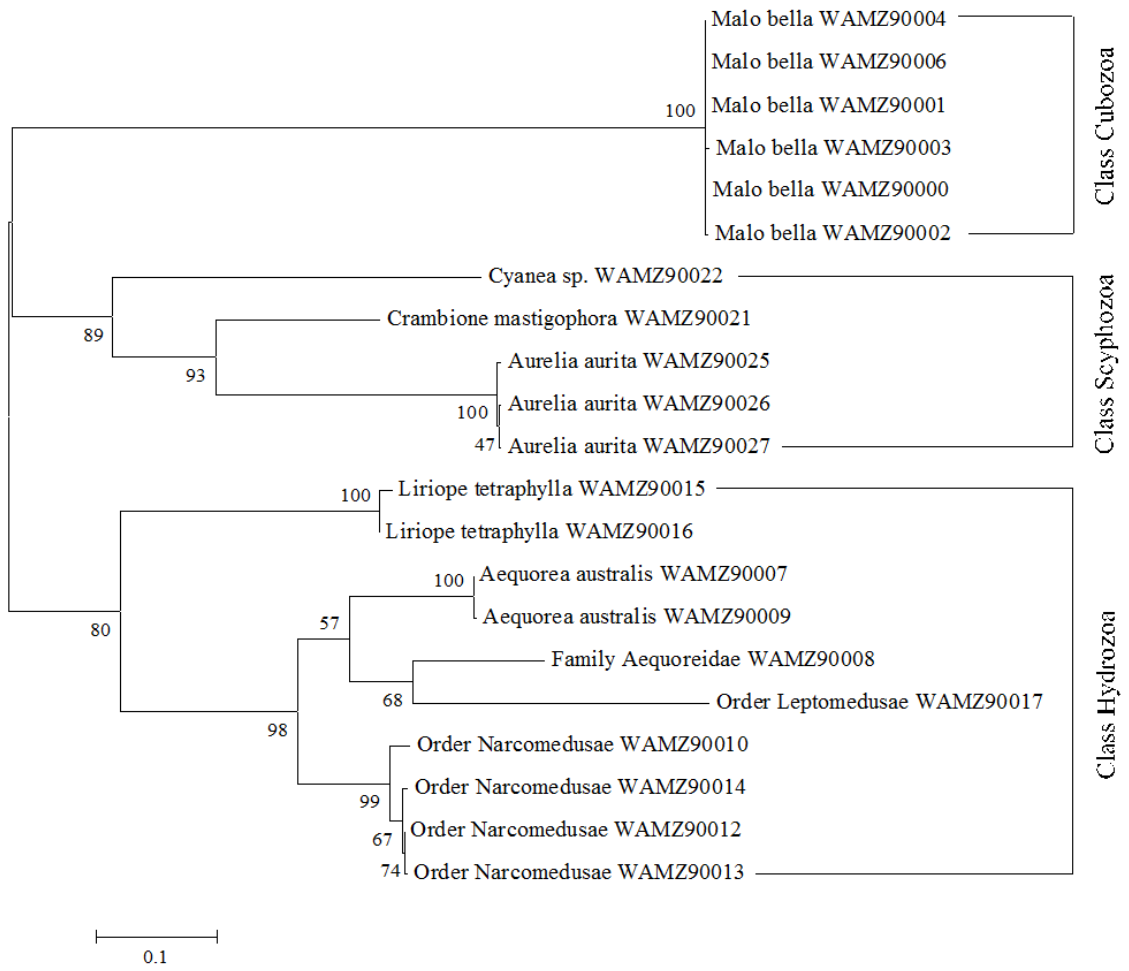


Figure 5: Maximum-Likelihood phylogenetic tree based on the mitochondrial gene cytochrome *c* oxidase subunit 1 (COI) for specimens found at Ningaloo Reef. Bootstrap values (1000 replicates) reflect the percentage of trees in which the associated taxa clustered together and are shown at each node. Class lineages are indicated. Scale bar represents evolutionary time.

2.3.4 Phylogeographic analysis of Aurelia sp. based on molecular data

Aurelia sp. from Ningaloo Reef formed distinct clusters in trees constructed using the maximum likelihood method, which were created from COI and 16S *Aurelia* sp. sequences derived from this study and GenBank. Ningaloo *Aurelia* sp. COI sequences were most closely related to *Aurelia* sp. 7, which were sampled from Tasmania, Australia (Figure 6). They also have diverging lineages connecting them to *Aurelia* sp. 4, sp. 6 and sp. 3. which were sampled from regions in the Indian and southern Pacific Oceans (Table 2), though there is weak support for the internal branch nodes (bootstraps <70 %). The

more distantly related *Aurelia* sp. were mostly sampled from regions in the Northern Hemisphere. The *megablast* sequence similarity searches revealed the Ningaloo specimens were closely related to *Aurelia* sp. from Palau, Indonesia, Hawaii (USA), Sweden, Tasmania (Australia), Israel and New Zealand.

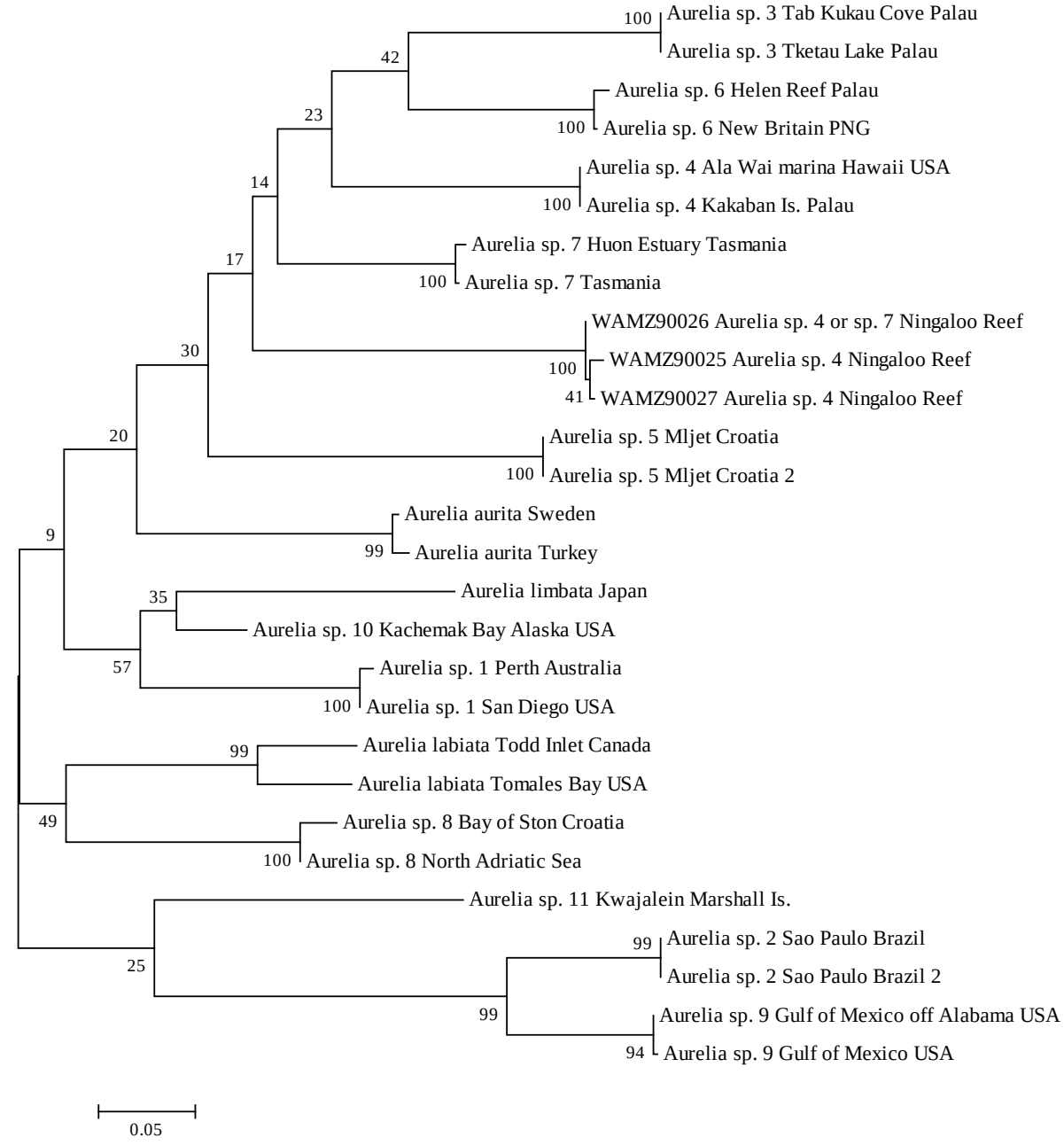


Figure 6: Maximum-Likelihood phylogenetic tree for *Aurelia* sp. from Ningaloo Reef and other regions (see Table 2), based on the mitochondrial gene cytochrome *c* oxidase 1 (COI). Bootstrap values (1000 replicates) reflect the percentage of trees in which the associated taxa clustered together and are shown at each node. Scale bar represents evolutionary time.

2.4 Discussion

2.4.1 Gelatinous zooplankton abundance in north-west Western Australia

There is a paucity of data relating to the abundance of gelatinous zooplankton found off the north-western coast of Australia (Condon et al., 2012). This study has created a baseline to assess the abundance of gelatinous zooplankton in the Ningaloo Reef region of north-western Australia and has shown species composition within the lagoon varied through time. *A. aurita* was the only recurring gelatinous species, and although *C. mastigophora* occurred in only one month, it was very abundant. All other species appeared only once in very low abundance. Presence-absence data indicated gelatinous species diversity outside the lagoon is slightly higher.

The need for data relating to the abundance and diversity of gelatinous zooplankton at Ningaloo Reef arose partly due to dangerous carybdeid cubozoan medusae being regularly sighted and occasionally stinging tourists (Gershwin, 2014, Jones, 2014, P. Barnes, 2014, pers. comm.). Although four species of carybdeid cubozoans are known from the Western Australian region (Bailey et al., 2005, Gershwin, 2005c, Gershwin, 2014), during the current study only a single species of carybdeid, *M. bella*, was found at Ningaloo Reef. A woman was also stung by one of the nine *M. bella* specimens captured, and suffered a severe case of Irukandji syndrome as a result of the envenomation. This confirmed that this carybdeid species causes Irukandji syndrome, and emphasises the need for rigorous sampling of cubozoans, particularly carybdeids, in the region. The most efficient means of capturing cubozoans is by using lights to attract the medusae at night (Garm et al., 2012, Kingsford et al., 2012). Considering cubozoans pose a threat of serious envenomation to tourists and to tourism industry professionals participating in marine-based tourism at Ningaloo Reef, intense sampling using night lights is needed to

sufficiently assess the overall abundance and diversity, and ultimately, ecology, of such a dangerous species in the north-western region of Australia.

2.4.2 Species identification and relatedness as indicated by mitochondrial DNA sequence similarity and phylogenetic analyses

COI and 16S sequences have provided additional information regarding the diversity of gelatinous zooplankton from Ningaloo Reef, in Western Australia. Overall, the COI and 16S genes, using the maximum likelihood method, produced trees that reflected the same patterns of species identification shown by the GenBank *megablast* sequence similarity searches. The maximum likelihood method is one of three methods commonly used in the construction of phylogenetic trees and was chosen as it has a firm statistical basis (Yang, 1993), does not assume a rate of constant evolution (Tamura et al., 2013) and does not ignore parsimoniously non-informative characters in sequences (Fischer and Thatte, 2010). This enabled a robust assessment of Ningaloo Reef gelatinous species diversity.

Phylogenetic trees and *megblast* sequence similarity searches assisted with discriminating among morphologically similar species. A number of Ningaloo Reef specimens shared similar morphological traits, which is why some were identified to the order- or genus-level, and not the species-level. Genetic analyses enabled species-level classifications where morphological identification could not. This is one of the benefits of using DNA barcoding, which is used for species recognition and discrimination (Bucklin et al., 2011). However, databases like GenBank need to be sufficiently populated with sequences from a broad range of medusae taxa for DNA barcoding to complement morphological identification (Ekrem et al., 2007, Ortman et al., 2010). Future studies should focus on increasing the amount of sequences available for medusae, in databases such as GenBank (Ortman et al., 2010), but sequences cannot be generated without specimens being accurately identified by taxonomists with expertise in the identification of particular

groups of medusae (Ortman et al., 2010). Thus, neither morphological nor genetic approaches represent a single method for effective identification of gelatinous species; they must be integrated to ensure accurate and reliable identification of gelatinous species (Dawson, 2005b).

Phylogenetic trees constructed from 16S and COI sequences using the maximum likelihood method revealed variation among specimens, which was undetected using morphology and GenBank *megablast* searches. For example, the 16S tree indicated there was variation among *M. bella* specimens at the species-level, though this variation was much more reduced in the COI tree. *M. bella* has been identified using morphology (Gershwin, 2014), but no DNA samples have been taken and sequenced from any specimens. Cubozoans have been sequenced using the 16S gene from the Northern Territory and south-western Australia (Bentlage et al., 2010), but no sequences for cubozoans from north-western Australia exist, despite a number of species being described from the area (Gershwin, 2005c, Gershwin, 2014). As a result, there are no 16S or COI sequences of *M. bella* or any north-west Australian carybdeid in GenBank to compare the specimens from Ningaloo Reef to (Benson et al., 2009, Sayers et al., 2011). As some cubozoans are extremely toxic (Brinkman and Burnell, 2009), it is paramount future studies aim to acquire sequences for cubozoans from north-western Australia to assist with species identification, which in turn will give an indication of the toxicity of cubozoans endemic to the north-west region (Gershwin, 2008).

2.4.3 *Aurelia aurita* specimens from Ningaloo Reef are endemic

Phylogenetic analyses offer an effective way to identify invasive or endemic populations of *Aurelia* medusae (Dawson et al., 2005). Based on *Aurelia* sp. COI sequences from different regions of the world, phylogeographic analysis revealed populations of *Aurelia* sp. from Ningaloo Reef are most likely endemic, perhaps with a small proportion of the

population comprising invasive species. Ningaloo Reef *Aurelia* sp. appear most closely related to *Aurelia* sp. 7, which were sampled from Tasmania, Australia, and may represent an invasive proportion of the population from a temperate region. Ningaloo *Aurelia* sp. also share connections with *Aurelia* sp.3, sp. 4 and sp. 6, which were sampled from warm, tropical areas to the north of Australia.

Aurelia sp. are usually regionally restricted by dispersal mechanisms (Dawson et al., 2005), so it is likely they have dispersed from warmer waters to the north of Australia, considering they are most closely related to other *Aurelia* sp. from that region. The *megablast* search reflected a similar pattern as *Aurelia* sp. with high sequence similarity matches were mostly from regions to the north of Australia. However, bootstrap values on the internal nodes of the phylogenetic tree indicated higher-level clustering of species was weakly supported, so it cannot be claimed with any certainty that populations of *Aurelia* sp. at Ningaloo Reef are endemic to the north-western Australian region.

Based on locality, it was expected Ningaloo Reef *Aurelia* sp. would be most closely related to *Aurelia* sp. 1, the specimen which was sampled from Perth, Australia. However, genetic analyses indicated Ningaloo *Aurelia* sp. are not closely related to *Aurelia* sp. 1 from Perth. In fact, the specimen from Perth shared a sister node with the *Aurelia* sp. 1 specimen from San Diego, USA, a clustering which was very strongly supported. This suggests the Perth *Aurelia* sp. 1 specimen was invasive to the area and highlights the utility of a database such as GenBank to assess whether seemingly anomalous populations of medusae are invasive or endemic.

COI is the DNA barcoding gene of choice for most studies (Dawson et al., 2005, Dawson and Jacobs, 2001, Holland et al., 2004). This has created databases with large amounts of COI sequences, which in turn has provided numerous species replicates, densely sampled across a diverse range of taxa, for DNA barcoding (Bucklin et al., 2011). The large

amount of COI sequences available for *Aurelia* sp. from a range of different localities consequently enabled a phlylogeographic analysis of the Ningaloo specimens. In contrast, *Aurelia* sp. 16S sequences were not used for this study as too few exist in GenBank (Benson et al., 2009, Sayers et al., 2011) to create a comprehensive phylogenetic tree to explore the sources of the Ningaloo Reef *Aurelia* sp. population. Future studies are needed to sequence genes other than COI for DNA barcoding, to build up a database of genetic data to enable multi-gene phylogenetic analyses of medusae populations around the world (Bucklin et al., 2011).

2.5 Conclusion

A baseline of data has been established for the diversity and abundance of gelatinous zooplankton at Ningaloo Reef, adding to data needed for the north-western Australian region (Condon et al., 2012). Although, extensive sampling is needed through space and time to sufficiently develop a database of gelatinous zooplankton in the north-western Australian region. The use of short sequences of COI and 16S mitochondrial genes assisted with discrimination of variation among and between medusae species, which was undetectable by morphological identification, and indicated the diversity of gelatinous zooplankton at Ningaloo Reef is higher than revealed by morphological identification. Further studies are needed to assess the diversity and abundance of dangerous medusae, such as cubozoans, and the level of endemism exhibited by populations of some species, in north-western Australia, and more broadly, the Indian Ocean. For reliable and accurate identification of gelatinous zooplankton species, it is recommended future studies use both morphological and genetic approaches.

Chapter 3: Commensal associations between *Aurelia aurita* (Cnidaria: Scyphozoa) and *Ophiocnemis marmorata*

3.1 Introduction

3.1.1 Associations between echinoderms and medusae

Medusae share commensal relationships with a diverse range of organisms (Ohtsuka et al., 2009). For example, fish use medusae to protect themselves from predation, and are often found living in or near the medusa (Brodeur, 1998, Lynam and Brierley, 2007). Fish may also utilise medusae as a food source, either feeding directly on their host (D'Ambra et al., 2015) or feeding on prey and parasites of the medusae (Lynam and Brierley, 2007). Other organisms, such as crabs and hyperiid amphipods, often lay eggs within the tissue of a medusa host and brood young on or inside the medusa (Fleming et al., 2014), or use it as a pelagic nursery (Sal Moyano et al., 2012). Medusae also facilitate the dispersal of benthic organisms that may "hitch a ride" on their bells (Pagès et al., 2007). Medusae, therefore, often enhance the survival of commensal organisms that associate with them, due to the protection and food sources they provide (D'Ambra et al., 2015), indicating medusae may hold a significant role in supporting pelagic biodiversity (Doyle et al., 2014).

Of all organisms that associate with medusae, associations between echinoderms and medusae are the rarest. The only echinoderm to associate with medusae is the ophiuroid, *Ophiocnemis marmorata*, which is usually a benthic organism that lives on soft substrates in the Indian and Pacific Oceans (Marsh, 1998). In Australia, *O. marmorata* has been found associating with medusae in Shark Bay and the Kimberley in Western Australia (Table 6). To date, *O. marmorata* has only been found to associate with rhizostome medusae (Table 6). This association has only been described five times (Berggren, 1994, Fujita and Namikawa, 2006, Kanagaraj et al., 2008, Marsh, 1998, Panikkar and Raghu

Prasad, 1952) and is thought to be a form of phoresy, whereby the ophiuroids use the jellyfish as a means of dispersing over large areas by 'hitching a ride' on the bell (Fujita and Namikawa, 2006, Kanagaraj et al., 2008, Marsh, 1998, Ohtsuka et al., 2010, Panikkar and Raghu Prasad, 1952). The reason for this association, however, is yet to be confirmed.

Table 6: Host rhizostome medusae associated with *Ophiocenmis marmorata*.

Family	Species	Locality	Reference
Rhizostomatidae	*Rhopilema hispidium*	Palk Bay, Sri Lanka	Pannikkar & Prasad (1952)
	Rhopilema hispidium	Vellar estuary, India	Kanagaraj et al. (2008)
	Rhopilema esculentum	Kagoshima, Japan; Palawan Island, the Phillipines	Fujita & Namikawa (2006)
	Rhopilema nomadica	Inhaca Island, Mozambique	Berrgren (1994)
Cepheidae	*Cephea cephea*	Shark Bay, Western Australia	Marsh (1998)
	Netrostoma sp.	Kimberley, Western Australia	Marsh (1998)

Despite five studies reporting an association between *O. marmorata* and medusae (Table 6), the frequency of association between *O. marmorata* and medusae has never been investigated. All studies have used few laboratory and *in situ* observations of *O. marmorata* riding on medusae to describe the association (Fujita and Namikawa, 2006, Kanagaraj et al., 2008, Marsh, 1998, Panikkar and Raghu Prasad, 1952). Most studies have not collected data relating to the number of medusae hosting ophiuroids (Fujita and Namikawa, 2006), the number of ophiuroids found on medusae (Fujita and Namikawa, 2006, Panikkar and Raghu Prasad, 1952), and have collected few measurements of medusa and ophiuroid size (Kanagaraj et al., 2008, Marsh, 1998, Panikkar and Raghu Prasad, 1952). Consequently, there are no data available to assess the frequency of

association between *O. marmorata* and medusae or whether size influences the number of ophiuroids that associate with medusae.

Ophiuroids feed in a variety of ways and although the exact feeding method and diet of *O. marmorata* is unknown (Stöhr et al., 2012, Warner, 1982), it is thought they may feed on detritus (Marsh, 1998). As these ophiuroids have usually been found attached to the oral arms of medusae, it is possible they sweep the surface of the oral arms in search of food (Fujita and Namikawa, 2006, Marsh, 1998, Panikkar and Raghu Prasad, 1952). In contrast, it has been suggested *O. marmorata* that associate with medusae are suspension feeders, utilising the pulsating movement of their host to acquire food more efficiently (Fujita and Namikawa, 2006, Kanagaraj et al., 2008). However, no studies have actually investigated the diet of ophiuroids associating with jellyfish; in fact all studies have been based on chance encounters and the association described using scarce observations.

3.1.2 Evidence needed to assess whether organisms are feeding on their medusa host

A variety of approaches can be used to assess whether organisms are feeding on their medusa host. For example, gut content analyses have been used to determine if associates are feeding on their hosts (Riascos et al., 2012, Sal Moyano et al., 2012). However, as jellyfish are not structurally robust and have no hard structures, it is difficult to identify gelatinous tissue in stomachs once digestion has begun (D'Ambra et al., 2015) and even if gut contents are preserved, gelatinous tissue often disintegrates (D'Ambra et al., 2015, Mianzan et al., 1996, Mutlu, 1996). Sal Moyano et al. (2012) have used the presence of nematocysts, which are not easily digested, in the gut contents of *Libinia spinosa* crabs to infer the crabs' diet. The species-specific structures needed to identify the type of nematocyst present, however, were not visible and the species of medusa the nematocyst came from could not be identified. Using the presence of nematocysts to infer diet is also time consuming and although it provides evidence of predation on medusae, it does not

enable the proportion of gelatinous prey that is assimilated by the predator to be determined (Sal Moyano et al., 2012). Gut contents also cannot distinguish between prey that is assimilated (and so important to the nutrition of the predator) and prey that is ingested incidentally (Fry, 2006).

Stable isotope analysis (SIA) can determine which foods are assimilated by a predator and detect food sources not easily visualised in gut contents (Pitt et al., 2009a). Stable isotopes reflect the history of metabolic processes of an organism (Peterson and Fry, 1987) and their use relies on potential prey sources, such as different plants or animals, having distinct ratios of the common, light isotope to the heavy, rare isotope (West et al., 2006). Carbon ($^{13}C/^{12}C$) is the foundation for the majority of energetic requirements for pelagic organisms, while nitrogen ($^{15}N/^{14}N$) is involved in protein synthesis; therefore both elements are commonly used in dietary studies (Lajtha and Michener, 2007, West et al., 2006). The preferential respiration of the lighter ^{12}C and excretion of lighter ^{14}N slightly enriches the heavier isotopes, ^{13}C and ^{15}N, therefore the carbon and nitrogen isotope ratios in an organism's tissues closely reflect the carbon and nitrogen isotope ratios of their prey (Blanchet-Aurigny et al., 2012, DeNiro and Epstein, 1978, DeNiro and Epstein, 1981).

A process known as fractionation, where isotopic ratios change slightly from one trophic level to the next (i.e. from predator to prey) (McCutchan et al., 2003, Peterson and Fry, 1987) allows for $\delta^{13}C$ and $\delta^{15}N$ to be used as isotopic tracers (Lajtha and Michener, 2007, West et al., 2006). $\delta^{13}C$ is enriched only slightly from predator to prey, which is why it is used to identify and trace potential food sources. $\delta^{15}N$ reflects the trophic level at which an organism feeds as it has a much greater stepwise enrichment from prey to predator (Fleming et al., 2011, Lajtha and Michener, 2007, West et al., 2006). In addition, trophic enrichment factors (TEFs) account for the variation in fractionation between predator and

prey and vary among species (Blanchet-Aurigny et al., 2012, Riascos et al., 2015). TEFs are necessary to generate reliable estimates of the contribution of different sources to a predator's diet, and standard TEF values can be applied across different taxa (McCutchan et al., 2003, Post, 2002, Vanderklift and Ponsard, 2003), though they are not applicable to some organisms (D'Ambra et al., 2014).

Recently, stable isotope analysis has been used to infer the dietary sources of organisms associating with medusae and has confirmed that a number of organisms, including fish, crabs and hyperiid amphipods, feed on their medusae hosts (D'Ambra et al., 2015, Riascos et al., 2015, Towanda and Thuesen, 2006). For example, D'Ambra et al. (2015) and Riascos et al. (2015) both demonstrated that medusae provide the bulk of the assimilated diet of fish and parasitic amphipods, which was confirmed by the high proportion of medusae $\delta^{13}C$ isotope ratios in their respective diets. Similarly, an earlier study by Towanda and Thuesen (2006) used stable isotope analysis to infer the diet of a brachyuran crab and a hyperiid amphipod and found that both may feed on their medusa host. Behavioural observations as well as gut content analysis and examinations of fecal pellet samples further supported their conclusion (Towanda and Thuesen, 2006). Fleming et al (2014) used stable isotope analysis to demonstrate that parasitic hyperiid amphipods use their medusae hosts as a seasonally abundant prey source. The association was observed to be limited to seasons of high amphipod abundance and the longer amphipods associated with their host, the more their diet appeared to reflect the isotopic signatures of gelatinous tissue (Fleming et al., 2014). Therefore, stable isotope analysis presents a valuable tool for the assessment of the dietary composition of organisms that associate with medusae, particularly if the associate is suspected to be actually feeding on their gelatinous host.

3.1.3 Aims and Hypotheses

The aims were: (1) to describe the prevalence of association between a medusa host, *Aurelia aurita*, and the ophiuroid *O. marmorata*, (2) to determine whether the density of ophiuroids was correlated with the size of host medusae, and (3) to use stable isotope analysis to determine if *O. marmorata* were feeding on their scyphozoan hosts. Specifically, it was hypothesised that density of ophiuroids on *A. aurita* medusae was positively correlated with the size of the medusa, and that *A. aurita* contributed a minor proportion of the ophiuroid's diet.

3.2 Methods

3.2.1 Frequency of association and relationship between medusae size and number of ophiuroids

Ninety two *A. aurita* medusae were sampled across eight sites in the northern section of Ningaloo Reef, Western Australia, between 29th May and 26th June 2015. At each site, *A. aurita* medusae were selected at random and collected from a boat using a hand-held dip net. Medusae were placed in individual buckets and any ophiuroids present on the jellyfish were removed. The number of ophiuroids present on each *A. aurita* medusa was counted and the central disc diameter of each ophiuroid was measured to the nearest 1 mm. The bell diameter of the medusa was measured to the nearest 1 mm using a ruler.

3.2.2 Collection of ophiuroids, medusae and other potential prey

Thirty medusae and their associated ophiuroids were collected at two sites separated by approximately 4km using a hand-held dip net (1 mm mesh size). All ophiuroids associating with the medusae were collected. Medusae were stored on ice during transportation to the laboratory but ophiuroids were transported alive and placed in aerated seawater where they were maintained for 24 hours to allow them to evacuate their guts.

Potential planktonic prey of ophiuroids and medusae were collected using plankton nets. Four replicate plankton tows using a small net (diameter = 30 cm; mesh size = 53µm) were conducted at each site to isolate seston (phytoplankton and particulate organic matter), while four replicate plankton tows using a large net (diameter = 51 cm; mesh size = 150 µm) were used to sample potential mesozooplankton prey. Sites were sampled two days apart.

3.2.3 Sample processing

In the laboratory, medusae were placed on a plastic tray and rinsed with filtered seawater until all associated debris was removed. Medusae were processed fresh as preservation by freezing and or in ethanol can alter the isotopic signature of medusae (Fleming et al., 2011). The bell diameter was measured to the nearest 1 mm and a sub-sample of the bell was extracted from each medusa and dried. A ca. 5 cm^2 sample of the bell was used for SIA as the isotopic composition of the bell is similar to that of the whole body (D'Ambra et al., 2014). The ophiuroid with the largest disc diameter from an individual medusa was prepared for isotope analysis by rinsing it in filtered seawater to remove any detritus. Additional ophiuroids were preserved in formaldehyde for identification purposes and lodged as voucher specimens with the Western Australian Museum in Perth, Australia.

Different size classes of mesozooplankton were separated using sieves (500 µm, 300 µm and 100 µm) and rinsed with filtered seawater. Any algal detritus present in the samples was removed during sieving using forceps. Seston (53 µm – 100 µm) samples were sieved through 100 µm mesh and filtered through pre-ashed Whatman GF/F filter papers using a single stage vacuum pump.

All samples were dried in an oven at 60°C for a minimum of 96 hours to ensure samples were at a constant weight and then homogenised to a fine powder using a mortar and pestle. Samples collected on GF/F filter papers remained firmly embedded in the glass fibre and were combusted and homogenised together (per Grey et al., 2000). Prior to analysis of $\delta^{13}C$, samples of ophiuroids and plankton were decalcified by the drop-wise addition of 1M HCl. Acid was added until the sample stopped bubbling and samples were redried and weighed (±0.01 mg) into silver capsules (5 mm, Sercon). Ophiuroid and plankton samples for $\delta^{15}N$ analyses and all medusae samples (i.e. for both $\delta^{13}C$ and $\delta^{15}N$ analyses) were left untreated and weighed into tin capsules (5 mm, Sercon).

3.2.4 Stable isotope analysis

The C and N isotopic ratios of ophiuroids, medusae, mesozooplankton and seston were measured using a continuous flow isotope ratio mass spectrometer at the University of California Stable Isotope Facility in Davis (UC SIF), California, USA, and reported as per mil values (‰) against international standards (Vienna PeeDee Belemnite for C and Air for N) using the following formula:

$$\delta^{H}\mathrm{X}\ (‰) = \left[\left(\frac{\mathrm{R_{sample}}}{\mathrm{R_{standard}}}\right) - 1\right] \times\ 10^{3}$$

where X = C or N, the superscript *H* gives the respective heavy isotope mass of the element (^{13}C or ^{15}N) and R is the ratio of either ^{13}C/^{12}C or ^{15}N/^{14}N (Fry, 2006). Precision of values as a standard are measured at 0.2‰ for ^{13}C and 0.3‰ for ^{15}N. During analyses, samples were interspersed with replicates of at least two different laboratory standards, selected to be compositionally similar to the samples being analysed.

3.2.5 Statistical analyses

The strength of the linear relationship between the density of ophiuroids and size of medusae was analysed using Pearson's Correlation Coefficient. Level of association was denoted by *r*, with *r* values >0 indicating a positive correlation between the two factors. One-way ANOVAs were used to test differences between δ^{13}C and δ^{15}N of samples among sites and between acidified and non-acidified samples. Alpha level was 0.05. Prior to the analyses, the assumption of normality was tested using residual plots (Q-Q plots) and the assumption of homogeneity of variance was tested using Levene's test. ANOVAs were run using SPSS (version 20) statistical software.

The dietary composition of ophiuroids was modelled using SIAR (version 3.2.0), a package in R (Comprehensive Archive Network site http://cran.r-project.org/). SIAR reduces error due to generalised fractionation ranges by allowing for species-specific

fractionation ranges (D'Ambra, 2012, Parnell et al., 2010). SIAR fits a Bayesian mixing model to the potential food sources of organisms, based on their derived isotopic ratios (Parnell and Jackson, 2013), to estimate the proportion of sources contributing to an organism's diet (Parnell et al., 2010). Seston, mesozooplankton and *A. aurita* were considered as potential prey of ophiuroids. For the analyses, samples from the two sites were pooled.

Species-specific TEFs for *O. marmorata* do not exist so TEFs were selected based on a previous study that estimated TEFs for two ophiuroid species (Blanchet-Aurigny et al., 2012) and TEF values that are standard across different taxa (Post, 2002). The sensitivity of the analysis to variation in TEFs was tested by comparing four different models using four different TEFs (*sensu* Riascos et al. 2015) (Table 7). The TEFs in Model A were derived from Post (2002) and are standard across different taxa (Table 7). The TEFs in Model B were from Blanchet-Aurigny et al. (2012) and represent TEFs specific to the fractionation ranges of ophiuroids (Table 7). Models C and D used TEFs modified from the TEFs used for Model B (Blanchet-Aurigny et al., 2012) (Table 7). The TEFs in Model C were increased by 0.5‰, while the TEFs in Model D were decreased by 0.5‰, to simulate more variation in ophiuroid fractionation ranges (TEFs).

Table 7: Trophic enrichment factors (TEFs) used for SIAR analyses to assess the contribution of each source to the diet of *O. marmorata*.

	Δ^{13}C TEF	Δ^{15}N TEF	Reason for use	Reference
Model A	1.4 ± 1‰	3.0 ± 1‰	Standard across taxa	Post (2002)
Model B	1.5 ± 0.2‰	4.0 ± 0.3‰	Ophiuroid-specific	Blanchet-Aurigny et al. (2012)
Model C	2.0 ± 0.2‰	4.5 ± 0.3‰	Positively modified ophiuroid-specific	Blanchet-Aurigny et al. (2012)
Model D	1.0 ± 0.2‰	3.5 ± 0.3‰	Negatively modified ophiuroid-specific	Blanchet-Aurigny et al. (2012)

3.3 Results

3.3.1 Size-frequency distribution of medusae with and without ophiuroids and relationship between medusa size and density of ophiuroids

Seventy three of the 92 *A. aurita* sampled (79%) hosted at least one *O. marmorata*. Ophiuroids were generally located underneath the bell or amongst the oral arms (Figure 7). Disc diameter of ophiuroids ranged from 1 to 6 mm, with 67% of ophiuroids having a disc diameter of 3 mm or larger (Figure 8).

Figure 7: Photo courtesy of 3 Islands (Whale Shark Tourism Operator). *O. marmorata* riding on an *A. aurita* medusa (Ningaloo Reef). The ophiuroids are attached to the underside of the bell, sitting underneath the oral arms.

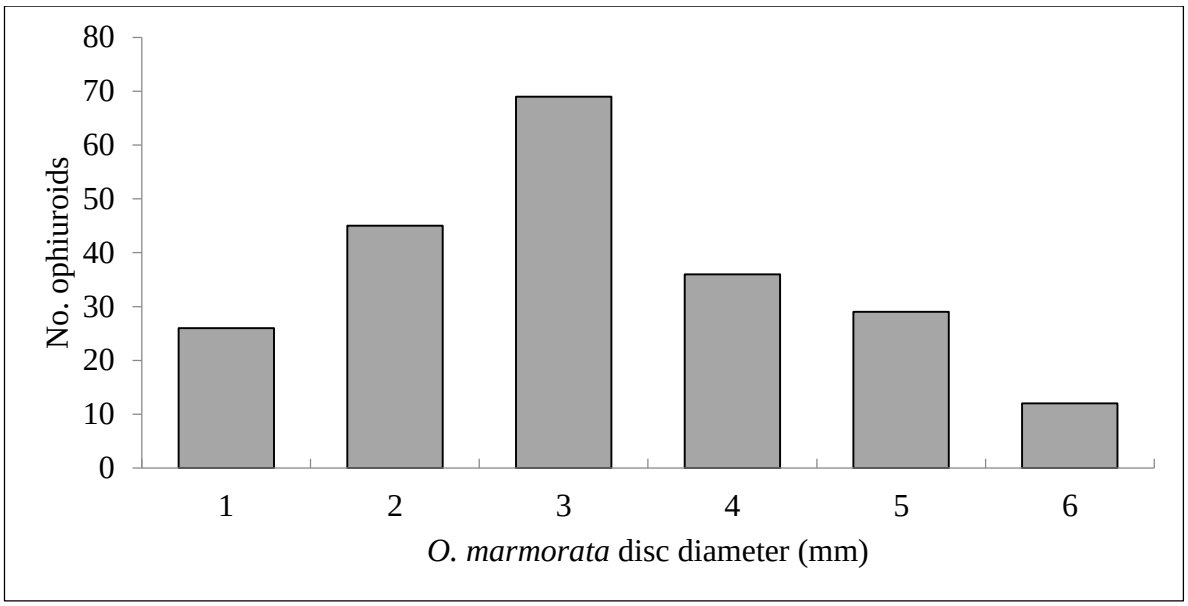


Figure 8: Size-frequency distribution of ophiuroids associated with *A. aurita* medusae captured in June 2015 on Ningaloo Reef (*N = 217*).

Size frequency distributions of *A. aurita* showed that medusae hosting ophiuroids ranged from 55 to 230 mm bell diameter, while medusae without ophiuroids ranged from 60 to 130 mm (Figure 9). Every medusae >135 mm bell diameter hosted ophiuroids (Figure 9). Size of medusae had a significant effect on whether or not they hosted an ophiuroid, thus medusae hosting ophiuroids were larger than those without (χ^2 = 10.759, df = 1, p = 0.013). There was a significant (p <0.001) but weak ($r(90)$ = 0.39) positive relationship between the size of medusae and the number of ophiuroids hosted by medusae (Figure 10). The largest number of ophiuroids recorded (N = 14) occurred on a medusa 160 mm bell diameter. No conspicuous damage to the bell or oral arms of medusae was observed in medusae hosting ophiuroids.

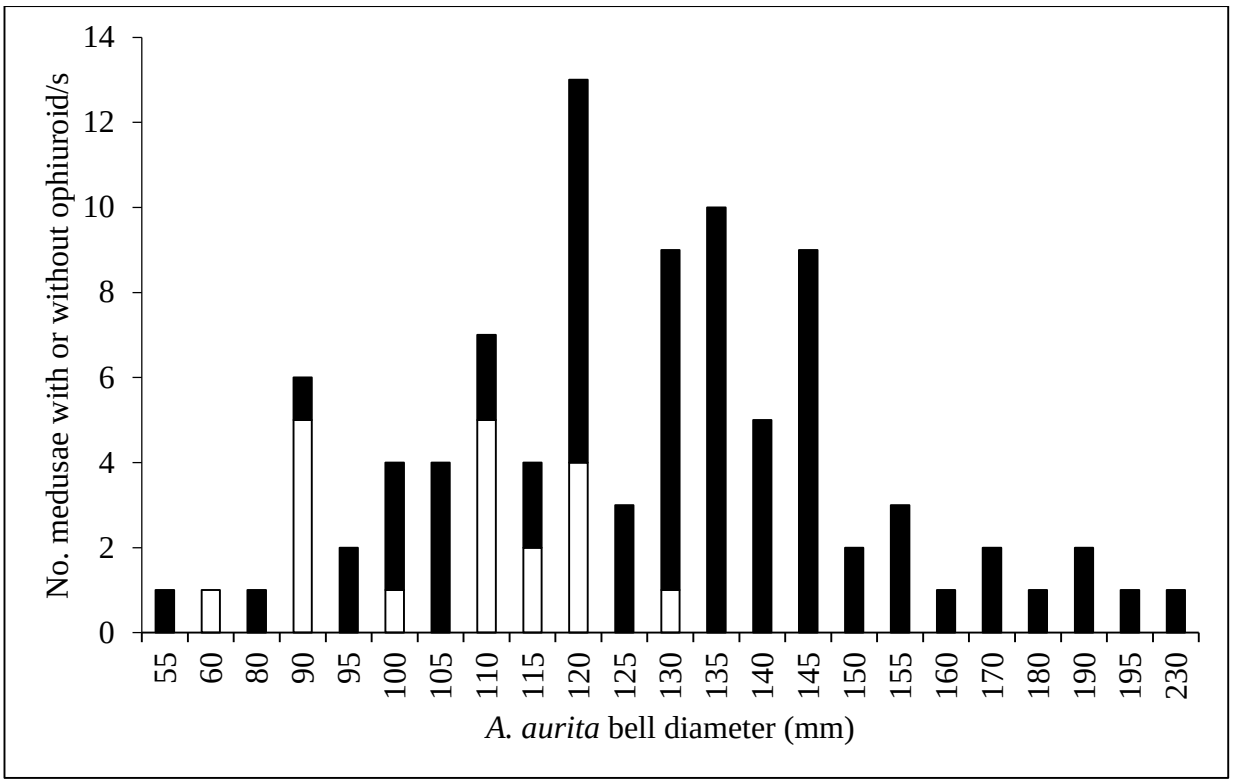


Figure 9: Size-frequency distribution of *A. aurita* medusae; medusae not associated with ophiuroids (*white bars, N = 19*) and medusae associated with ophiuroids (*black bars, N = 73*), captured in June 2015 on Ningaloo Reef.

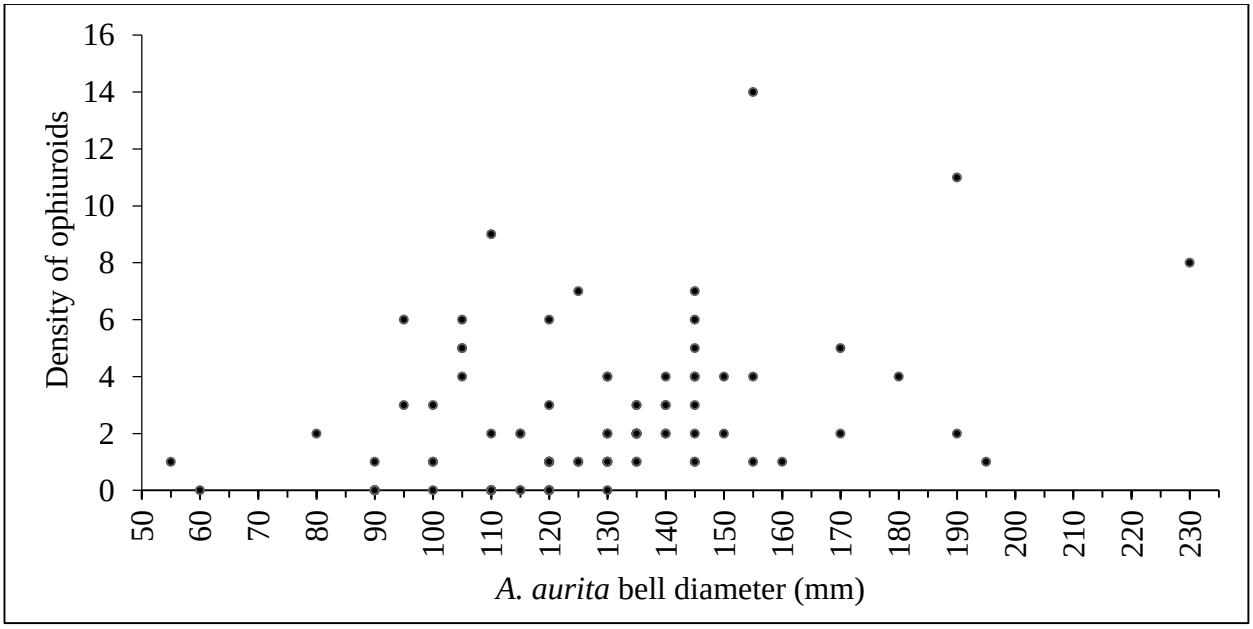


Figure 10: Relationship between size of medusae (mm) and density of associating ophiuroids.

3.3.2 Dietary composition of *Ophiocnemis marmorata* defined by stable isotope analysis

A comparison of $\delta^{13}C$ and $\delta^{15}N$ values showed isotopic differences between *O. marmorata* and their medusa hosts (*A. aurita*) (Figure 11). *O. marmorata* were enriched in ^{13}C by ca. 1.3‰ compared to their hosts, and were enriched in ^{15}N by ca. 0.2 ‰ (Figure 11, Table 8).

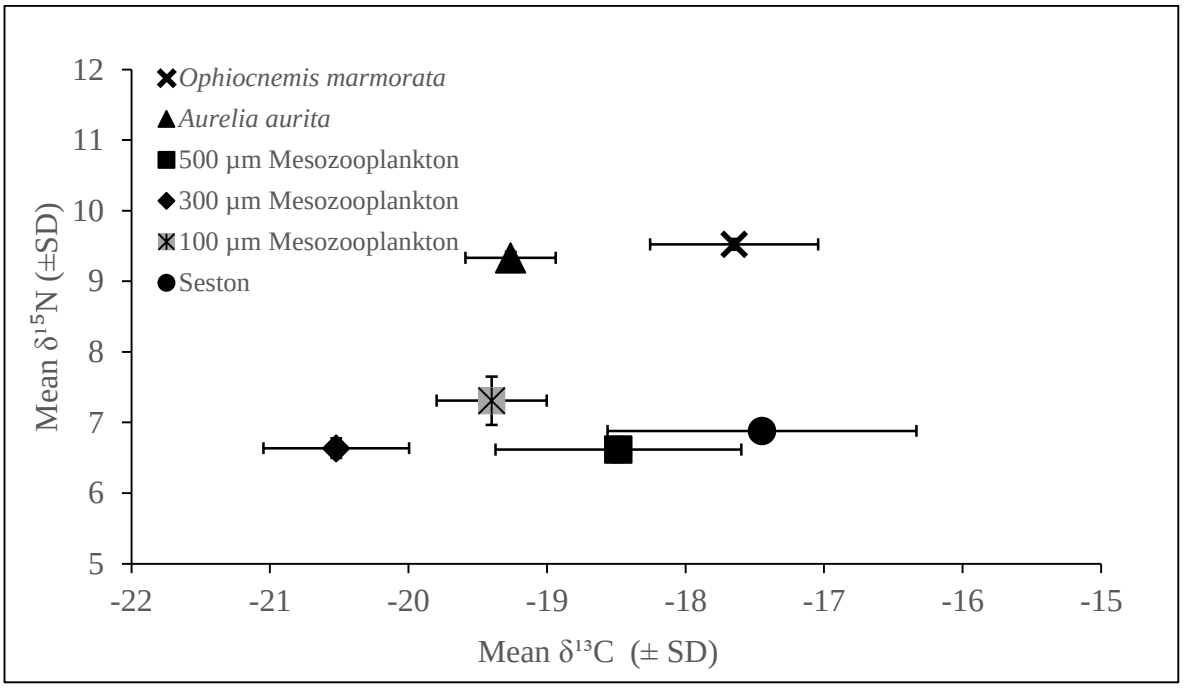


Figure 11: Isotopic biplot showing variation in mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ (‰) values calculated for *Ophiocnemis marmorata* and key food sources potentially contributing to *O. marmorata* diet: *Aurelia aurita*, mesozooplankton (500 µm, 300 µm and 100 µm size classes) and seston (phytoplankton and particulate organic matter).

Table 8: Mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ for *O. marmorata* and potential prey.

Taxon	*n*	$\delta^{13}C$	$\delta^{15}N$
Ophiocnemis marmorata	17	-17.65 (2.5)	9.52 (0.3)
Aurelia aurita	20	-19.26 (1.2)	9.33 (0.3)
500 µm mesozooplankton	8	-18.48 (2.5)	6.62 (0.5)
300 µm mesozooplankton	6	-20.52 (1.5)	6.64 (0.4)
100 µm mesozooplankton	7	-19.40 (1.1)	7.31 (1.0)
Seston (phytoplankton and POM)	8	-17.45 (3.2)	6.88 (0.4)

Four separate SIAR model runs using different TEFs (Table 7) indicated 500 µm-sized mesozooplankton contributed the greatest proportion, ~50 – 75%, of the diet of *O. marmorata* (CI = 95%) (Figure 12). The 300 µm-sized mesozooplankton contributed ~45 – 60% of the diet of *O. marmorata* while seston made up ~40 – 55% (CI = 95%) (Figure 12). However, the models showed 500 µm-sized mesozooplankton, 300 µm-sized mesozooplankton and seston also contributed low amounts (0.3 – 7%; 0 – 5%; and 0 – 0.7% respectively) to the diet of *O. marmorata* (CI = 95%) (Figure 12), indicating individuals of *O. marmorata* were feeding on a range of planktonic food sources, causing variation in proportional ranges of sources. All four models indicated 100 µm-sized mesozooplankton contributed a small amount (0 – 31% on average) to the diet of *O. marmorata*, while the host medusa, *A. aurita*, contributed only 0 – 10% on average under any scenario.

Acidification had no effect on $\delta^{15}N$ values of *O. marmorata* ($F(1,32) = 0.252$, $p = 0.619$), mesozooplankton ($F(1,48) = 0.599$, $p = 0.443$) and seston ($F(1,17) = 0.267$, $p = 0.612$) samples (Table 9). $\delta^{13}C$ for mesozooplankton was significantly different between sites and $\delta^{15}N$ for seston was also significantly different between sites (Table 10). Site had no effect on $\delta^{13}C$ and $\delta^{15}N$ for *O. marmorata* and *A. aurita* (Table 10).

Table 9: One-way ANOVA of differences between acidified and untreated $\delta^{15}N$ samples.

	Ophiocnemis marmorata			
	Source	*df*	*F*	*p*
$\delta^{13}N$	Between groups	1	.252	.619
	Within groups	32		
	Total	33		
	Mesozooplankton (all sizes pooled)			
	Source	*df*	*F*	*p*
$\delta^{13}N$	Between groups	1	.599	.443
	Within groups	48		
	Total	49		
	Seston			
	Source	*df*	*F*	*p*
$\delta^{13}N$	Between groups	1	.267	.612
	Within groups	17		
	Total	18		

Table 10: One-way ANOVA of differences in $\delta^{13}C$ and $\delta^{13}N$ of all samples among sites.

	Ophiocnemis marmorata			
	Source	*df*	*F*	*p*
$\delta^{13}C$	Between groups	1	.815	.318
	Within groups	15		
	Total	16		
$\delta^{13}N$	Between groups	1	1.272	.277
	Within groups	15		
	Total	16		
	Aurelia aurita			
	Source	*df*	*F*	*p*
$\delta^{13}C$	Between groups	1	.076	.786
	Within groups	18		
	Total	19		
$\delta^{13}N$	Between groups	1	.015	.903
	Within groups	18		
	Total	19		
	500 µm-sized mesozooplankton			
	Source	*df*	*F*	*p*
$\delta^{13}C$	Between groups	1	12.274	.013
	Within groups	6		
	Total	7		
$\delta^{13}N$	Between groups	1	.021	.890
	Within groups	6		
	Total	7		
	300 µm-sized mesozooplankton			
	Source	*df*	*F*	*p*
$\delta^{13}C$	Between groups	1	24.726	.008
	Within groups	4		
	Total	5		
$\delta^{13}N$	Between groups	1	2.407	.196
	Within groups	4		
	Total	5		
	100 µm-sized mesozooplankton			
	Source	*df*	*F*	*p*
$\delta^{13}C$	Between groups	1	43.370	.001
	Within groups	6		
	Total	7		
$\delta^{13}N$	Between groups	1	4.150	.088
	Within groups	6		
	Total	7		
	Seston			
	Source	*df*	*F*	*p*
$\delta^{13}C$	Between groups	1	2.021	.205
	Within groups	6		
	Total	7		
$\delta^{13}N$	Between groups	1	7.022	.038
	Within groups	6		
	Total	7		

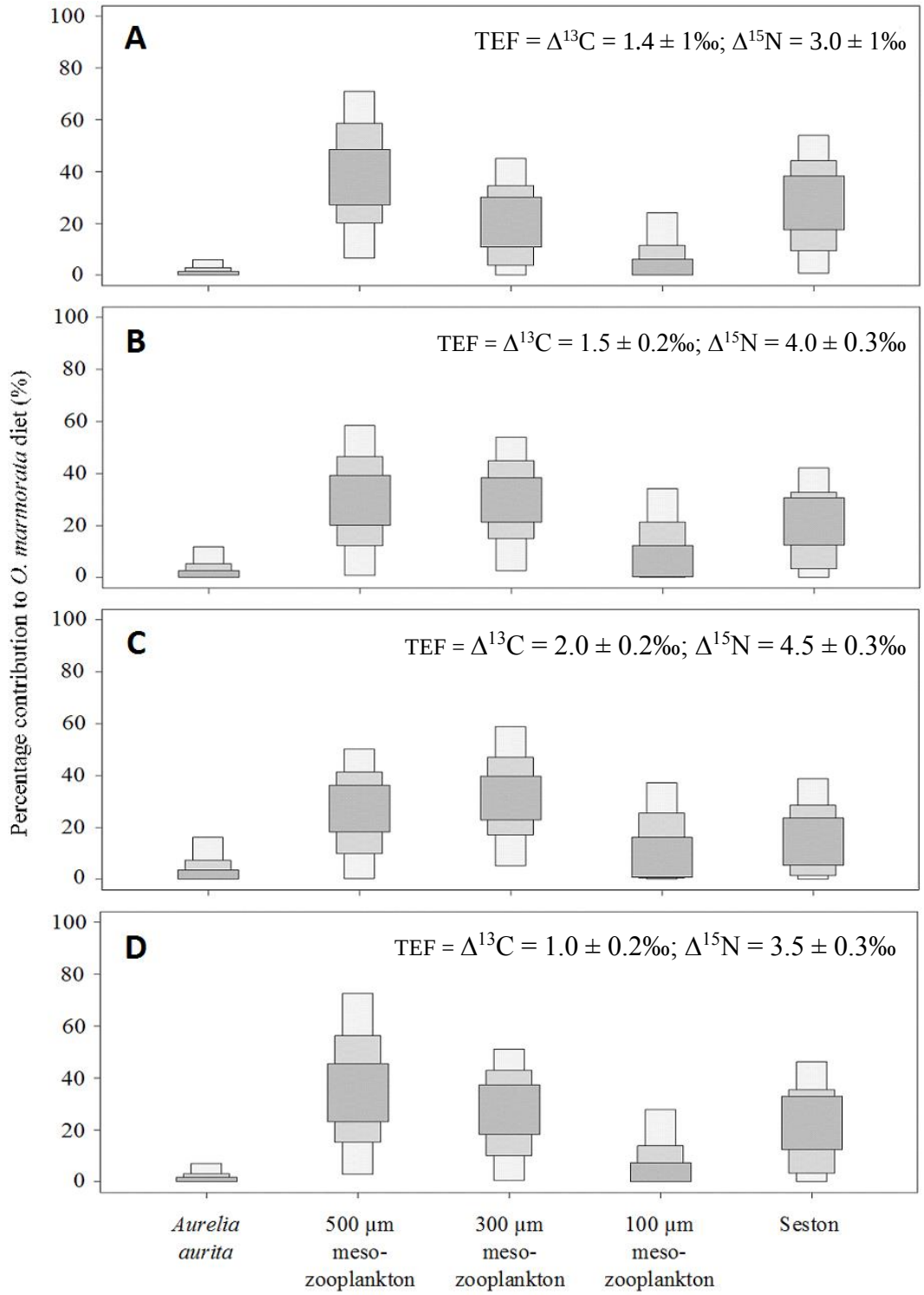


Figure 12: Percentage contribution of each source to the assimilated diet of *Ophiocnemis marmorata* with **A** standard TEF values (Post, 2002), **B** ophiuroid-specific TEF values (Blanchet-Aurigny et al., 2012), **C** positively modified ophiuroid-specific TEF values (+0.5 to values) and **D** negatively modified ophiuroid-specific TEF values (-0.5 from values). *Grey scale* (from *light to dark*) indicates 95, 75 and 50% confidence intervals, respectively.

3.4 Discussion

3.4.1 The association between *O. marmorata* and *A. aurita*

The ophiuroid *O. marmorata* has been found to associate with medusae in the Indo-Pacific region, including India (Kanagaraj et al., 2008), Sri Lanka (Panikkar and Raghu Prasad, 1952), Japan (Fujita and Namikawa, 2006), Mozambique (Berggren, 1994), the Philippines (Fujita and Namikawa, 2006) and Western Australia (Marsh, 1998). *O. marmorata* is now reported to associate with *A. aurita* medusae at Ningaloo Reef, which is close to other areas in Western Australia where it has previously been found (see Marsh, 1998). Furthermore, medusae hosting *O. marmorata* in previous studies have all been members of the order Rhizostomeae, and it is thought the stiff body, complicated form of oral arms, coastal habitat and vertical movements of many of these types of medusae facilitates their colonisation by *O. marmorata* (Fujita and Namikawa, 2006). Interestingly, the medusae reported here, *A. aurita*, are members of the order Semaeostomeae, which lack complex appendages (Yasuda, 1973). Therefore, it is now recognised that *O. marmorata* associate with two orders of medusae, and that factors like complex appendages, useful for attachment (Fujita and Namikawa, 2006, Kanagaraj et al., 2008), may not significantly influence whether ophiuroids colonise medusae.

This study presents the first data on the frequency with which *O. marmorata* associates with medusae and the relationship between the size of medusae and density of ophiuroids. O. marmorata associated with 79% of *A. aurita* at Ningaloo Reef indicating the association is a common occurrence. This is supported by a number of other studies that have reported the association (Fujita and Namikawa, 2006, Marsh, 1998, Panikkar and Raghu Prasad, 1952). The number of ophiuroids associating with medusae differed based on the size of a medusa. There was a significant, positive relationship between the density of ophiuroids and size (bell diameter) of medusae, suggesting ophiuroids tend to more

prolifically colonise medusae with larger bell diameters. Despite the correlation being significant, however, only 39% of the variability between density of ophiuroids and size of medusae was accounted for. A larger sample size may have revealed a stronger relationship, though these results are still a robust indication of how the size of a medusa may influence the amount of ophiuroids it hosts, and how frequent the association is.

There are a number of reasons why large medusae may be more likely to host ophiuroids and host a higher number of them. Multiple cohorts of medusae were present at the time of sampling, as indicated by the range of *A. aurita* size classes (Pitt and Kingsford, 2003). The older cohort may have been present when the ophiuroids were available to recruit to the medusae. Large medusae also provide a larger surface for colonisation, perhaps increasing the likelihood of ophiuroids coming into contact with them and colonising the medusae (Fujita and Namikawa, 2006). However, the method of colonisation is still unclear (Fujita and Namikawa, 2006, Kanagaraj et al., 2008, Marsh, 1998).

As *O. marmorata* are typically benthic, two methods for colonising medusae have been suggested (Fujita and Namikawa, 2006, Kanagaraj et al., 2008, Marsh, 1998). Firstly, medusae may occasionally encounter the benthos as they swim thereby creating opportunity for ophiuroids to attach to the medusae (Fujita and Namikawa, 2006, Marsh, 1998, Ohtsuka et al., 2009). *O. marmorata* have been found on the benthos in Exmouth Gulf, which is 70 km south east of the Ningaloo Reef sampling locations used in this study. Like rhizostome medusae, *A. aurita* medusae undergo vertical migration through the water column (Yasuda, 1973). However, rates at which medusae encounter the benthos may be insufficient to allow the high numbers of *O. marmorata* observed on *A. aurita* at Ningaloo Reef to colonise the medusae so prevalently (Yasuda, 1973).

A second method of colonisation has been proposed by two authors (Kanagaraj et al., 2008, Marsh, 1998), who believe ophiuroids may settle on the medusa body during their

planktonic larval stage. When compared to their counterparts on the benthos, ophiuroids found riding on medusae were observed to be smaller (Fujita and Namikawa, 2006) suggesting they may live and grow on the medusa in place of a benthic substrate (Kanagaraj et al., 2008, Marsh, 1998). Similarly, *O. marmorata* found on *A. aurita* were very small, ranging in size from 1 – 6 mm disc diameter. As some ophiuroid species reach sexual maturity at 5 mm disc diameter (Hendler, 1975), it's possible the ophiuroids found on *A. aurita* were mostly in the juvenile developmental stage (Fujita and Namikawa, 2006). A number of other studies have found larger, and perhaps more mature, *O. marmorata* specimens on medusae, ranging from around 6 – 124 mm disc diameter (Fujita and Namikawa, 2006, Kanagaraj et al., 2008, Marsh, 1998). However, the 124 mm ophiuroid disc diameter seems erroneous, as benthic ophiuroids are usually around 20 mm (Ambrose et al., 2001). Regardless, *O. marmorata* specimens found on *A. aurita* medusae may have colonised their hosts during their planktonic larval stage (Marsh, 1998), although further research is needed to determine the reproductive status of the commensal *O. marmorata* to assess whether they were juveniles.

It remains unclear as to whether *O. marmorata* live on medusae hosts for the majority of their life, or return to the benthos after to reaching a particular stage of life (or due to the death of the medusa host) (Marsh, 1998). In the laboratory, *O. marmorarata* were observed to detach from their host medusae after 8 hours, though it was unclear whether that was a natural behaviour or due to the stress of being in captivity (Kanagaraj et al., 2008). Further studies, employing both laboratory and *in situ* experiments, would be needed to determine the duration for which *O. marmorata* associates with medusae to assess whether the association is obligate, facultative or opportunistic (Fujita and Namikawa, 2006).

3.4.2 Food sources of *Ophiocnemis marmorata* associating with *Aurelia aurita* are largely plankton-based

Stable isotope analysis of ophiuroids and their potential sources of prey indicated that mesozooplankton (500 µm- and 300 µm-sized) and seston represent a large proportion of the diet of *O. marmorata*, while medusae hosts provided a negligible contribution. Also, as *A. aurita* may have contributed up to 10% on average of the diet of *O. marmorata*, it is possible the ophiuroids were scavenging prey from the oral arms of their medusa host, and incidentally ingesting some of the medusa as they consume prey captured by their host. Therefore, *O. marmorata* may display kleptoparasitism, defined as "a form of competition that involves the stealing of already-procured items" (Iyengar, 2008). Alternatively, as the majority of the ophiuroid's diet is comprised of larger mesozooplankton (500 µm- and 300 µm-sized) and seston, *O. marmorata* may also filter-feed while sitting on the bell of a medusa (Fujita and Namikawa, 2006). Thus, *A. aurita* present a platform for *O. marmorata* feeding, with ophiuroids possibly switching between filter-feeding and scavenging, depending on food availability.

Different SIAR mixing models run using a range of TEFs indicated 500 µm-sized mesozooplankton consistently contributed the largest proportion of the diet of *O. marmorata* and that 300 µm-sized mesozooplankton and seston were also assimilated. The models also consistently showed that medusae made a minor or no contribution to the diet. Thus, ophiuroids feed on a range of different-sized plankton and any medusa tissue in their diet is most likely due to incidental ingestion. To discern which source truly contributes the largest proportion to their diet, species-specific TEFs for *O. marmorata* are needed. It is recommended future studies follow the procedures outlined by Blanchet-Aurigny et al. (2012), where the TEFs of two other ophiuroid species were estimated using laboratory experiments. *O. marmorata* specimens would be fed a specific diet (e.g. fish, mussel) daily (Blanchet-Aurigny et al., 2012). Individuals would be sampled

randomly over time, and kept in filtered seawater overnight to allow them to evacuate their guts before being dried and ground to a fine powder for isotope analysis (Blanchet-Aurigny et al., 2012).

Identification of food sources of *O. marmorata* may have been affected by variation in $\delta^{13}C$ values of prey. Large variation in $\delta^{13}C$ values existed for 500 µm-, 300 µm- and 100 µm-sized mesozooplankton and in $\delta^{15}N$ values for seston. The $\delta^{13}C$ variation could be due to the differences in the species composition of mesozooplankton among sites, or due to the division of samples into size-classes, rather than division by species or genera groups. Mesozooplankton species present in different-sized samples may have been more depleted in $\delta^{13}C$, as indicated by previous studies (Jerling and Wooldridge, 1995, Tamelander et al., 2006). The size of phytoplankton within seston samples may also have created variation, as larger phytoplankton typically have more enriched $\delta^{15}N$ values (Parker et al., 2011). Moreover, upwelling events can influence N signatures of phytoplankton (Brzezinski and Washburn, 2011), but given the sampling sites were in close proximity (approximately 4 km apart) and were sampled two days apart, an upwelling is not a likely explanation for variation in $\delta^{15}N$ seston values between sites. Overall, despite the variation among sites, all four TEF models still indicated plankton formed the majority of the diet of *O. marmorata*, while under any scenario, *A. aurita* contributed a minor proportion to the diet, if any.

3.5 Conclusion

The use of quantitative data in this study has led to a better understanding of the unusual association between *O. marmorata* and medusae. The size of a medusa has an effect on the prevalence of association, with larger medusae more frequently hosting higher densities of *O. marmorata*. This could be due to a number of reasons, including the recruitment and maturity of large medusae coinciding with ophiuroid colonisation, or

large bell sizes of medusae offering a larger surface for colonisation (Fujita and Namikawa, 2006).

Stable isotope analysis provided evidence that *O. marmorata* is not feeding on its host. Instead, stable isotopes allowed host medusae to be identified as a platform for ophiuroid feeding. The association may also be a form of phoresy, allowing the ophiuroids to disperse over large areas by riding on medusae (Fujita and Namikawa, 2006, Kanagaraj et al., 2008, Ohtsuka et al., 2009). Both phoresy and the platform for feeding may potentially enhance the survival of *O. marmorata* (D'Ambra et al., 2015). Through the provision of ecosystem services, such as presenting a platform for pelagic feeding and phoresy to organisms that typically adopt a benthic lifestyle, blooming medusae populations may actually facilitate the biodiversity of pelagic communities (Doyle et al., 2014).

Chapter 4: General Discussion

The perceived widespread increase in the global abundance of gelatinous zooplankton still lacks rigorous foundation, as there is a scarcity of data relating to the abundance and diversity of gelatinous zooplankton in most ecosystems (Brotz et al., 2012, Condon et al., 2012). Detecting trends in gelatinous zooplankton populations is hindered by the lack of a defined baseline for many ecosystems, caused by a dearth of long-term data on gelatinous zooplankton (Condon et al., 2012). Blooms of gelatinous zooplankton have been overlooked in older scientific literature (Galigher, 1925), causing many recent blooms to be considered outbreaks (Condon et al., 2012). Furthermore, the study of the diversity of gelatinous species can be impeded by the absence of data for species prior to their discovery (Condon et al., 2012). Species may be misidentified as invasive species due to a lack of prior observation of the native species (Bentlage et al., 2010, Gorokhova et al., 2009).

Some regions of the world are more under-sampled than others for data on gelatinous zooplankton (Condon et al., 2012, Lucas et al., 2014). While a large amount of data exists for gelatinous zooplankton in the Northern Hemisphere, there is limited data for the Southern Hemisphere (Condon et al., 2012, Lilley et al., 2011, Lucas et al., 2014). Sampling efforts may be reduced in the Southern Hemisphere due to it being far more sparsely populated (Small and Cohen, 2004) and having a greater area of ocean to sample (see Figure 5 in Condon et al., 2012). Put simply, there are far less people available to sample a wider expanse of ocean compared to the Northern Hemisphere, making it inherently more difficult to obtain data on gelatinous zooplankton in the Southern Hemisphere (Lucas et al., 2014).

This study has provided baseline data on the abundance and diversity of gelatinous zooplankton for a region of the Southern Hemisphere where almost no gelatinous

zooplankton data exist: north-western Australia (Condon et al., 2012). It has also provided baseline data for dangerous species, such as cubozoans, and highlighted the need for more genetic data relating to cubozoans from north-western Australia, which, when integrated with morphological diagnoses, will ensure correct identification of these dangerous animals (Dawson, 2005b). Future studies would need to employ long-term temporal sampling to detect trends in populations of gelatinous zooplankton in north-western Australia, however there is now a reference point from which to base descriptions of gelatinous zooplankton abundance and diversity. This will ultimately fill some of the knowledge gaps relating to gelatinous zooplankton in the Southern Hemisphere and perhaps uncover changes in abundance and diversity over time (Condon et al., 2012, Lucas et al., 2014).

Through their association with a number of organisms, gelatinous zooplankton may actually be enhancing the biodiversity of many other organisms in pelagic communities (Doyle et al., 2014, Graham et al., 2014). In this study, a novel association between ophiuroids and *A. aurita* medusae was observed. Medusae made a minor contribution, if any, to the diet of the ophiuroid *O. marmorata* and instead provided their associated ophiuroids with a platform for feeding and, potentially, a means of dispersal. In contrast, a number of other studies have demonstrated that medusae usually contribute a significant food source to the diet of their associates (D'Ambra et al., 2015, Riascos et al., 2015, Sal Moyano et al., 2012). Intense temporal sampling of severely under-sampled regions in the Southern Hemisphere may reveal similar outcomes, where an association with gelatinous zooplankton is actually facilitating the survival of another organism. Thus, gelatinous zooplankton may hold a significant role in the facilitation of biodiversity in pelagic communities, which is why the paucity of gelatinous zooplankton data for the Southern Hemisphere needs to be addressed.

References

Ambrose, W., Clough, L., Tilney, P. & Beer, L. 2001. Role of echinoderms in benthic remineralization in the Chukchi Sea. *Marine Biology,* 139**,** 937-949.

Bailey, P. M., Bakker, A. J., Seymour, J. E. & Wilce, J. A. 2005. A functional comparison of the venom of three Australian jellyfish—Chironex fleckeri, Chiropsalmus sp., and Carybdea xaymacana—on cytosolic Ca 2+, haemolysis and Artemia sp. lethality. *Toxicon,* 45**,** 233-242.

Bayha, K. M. & Dawson, M. N. 2010. New family of allomorphic jellyfishes, Drymonematidae (Scyphozoa, Discomedusae), emphasizes evolution in the functional morphology and trophic ecology of gelatinous zooplankton. *The Biological Bulletin,* 219**,** 249-267.

Benson, D., Karsch-Mizrachi, I., Lipman, D., Ostell, J. & Sayers, E. 2009. GenBank. *Nucleic Acids Research,* 37**,** D26-D31.

Bentlage, B. 2010. Carybdea alata auct.(Cubozoa): rediscovery of the Alatina grandis type. *Zootaxa,* 2713**,** 52-54.

Bentlage, B. 2012. PHYLOGENETIC SYSTEMATICS, TAXONOMY, AND BIOGEOGRAPHY OF JELLYFISH (CNIDARIA: MEDUSOZOA).

Bentlage, B., Cartwright, P., Yanagihara, A. A., Lewis, C., Richards, G. S. & Collins, A. G. 2010. Evolution of box jellyfish (Cnidaria: Cubozoa), a group of highly toxic invertebrates. *Proceedings of the Royal Society B: Biological Sciences***,** rspb20091707.

Berggren, M. 1994. Periclimenes nomadophila and Tulеariocaris surec, two new species of pontoniine shrimps (Decapoda: Pontominae), from Inhaca Island, Mocambique. *Journal of Crustacean Biology,* 14**,** 782-782.

Blanchet-Aurigny, A., Guillou, M., Pernet, F., Gaffet, J.-D. & Dubois, S. F. 2012. Tissue-diet discrimination factors of isotopic ratios (Δ δ 13 C andΔ δ 15 N) in two brittle star species: Effect of reproductive state, diet and tissue composition. *Journal of Experimental Marine Biology and Ecology,* 426**,** 68-77.

Bolton, T. F. & Graham, W. M. 2004. Morphological variation among populations of an invasive jellyfish. *Marine ecology. Progress series,* 278**,** 125-139.

Brinkman, D. L. & Burnell, J. N. 2009. Biochemical and molecular characterisation of cubozoan protein toxins. *Toxicon,* 54**,** 1162-1173.

Brodeur, R. D. 1998. In situ observations of the association between juvenile fishes and scyphomedusae in the Bering Sea. *Marine Ecology Progress Series,* 163**,** 11-20.

Brotz, L., Cheung, W. L., Kleisner, K., Pakhomov, E. & Pauly, D. 2012. Increasing jellyfish populations: trends in Large Marine Ecosystems. *Hydrobiologia,* 690**,** 3-20.

Brown, A., Bejder, L., Cagnazzi, D., Parra, G. J. & Allen, S. J. 2012. The North West Cape, Western Australia: A Potential Hotspot for Indo-Pacific Humpback Dolphins Sousa chinensis? *Pacific Conservation Biology,* 18**,** 240-246.

Brzezinski, M. A. & Washburn, L. 2011. Phytoplankton primary productivity in the Santa Barbara Channel: Effects of wind-driven upwelling and mesoscale eddies. *Journal of Geophysical Research: Oceans,* 116.

Bucklin, A., Steinke, D. & Blanco-Bercial, L. 2011. DNA barcoding of marine metazoa. *Annual Review of Marine Science,* 3**,** 471-508.

Buhay, J. E. 2009. "COI-like" Sequences are Becoming Problematic in Molecular Systematic and DNA Barcoding Studies. *Journal of Crustacean Biology,* 29**,** 96-110.

Cassata, L. & Collins, L. B. 2008. Coral Reef Communities, Habitats, and Substrates in and near Sanctuary Zones of Ningaloo Marine Park. *Journal of Coastal Research,* 24**,** 139-151.

Catlin, J., Jones, T. & Jones, R. 2012. Balancing commercial and environmental needs: Licensing as a means of managing whale shark tourism on Ningaloo reef. *Journal of Sustainable Tourism,* 20**,** 163-178.

Condon, R. & Norman, M. 1999. Commensal associations between the hyperiid amphipod, Themisto australis, and the scyphozoan jellyfish, Cyanea capillata.

Condon, R. H., Graham, W. M., Duarte, C. M., Pitt, K. A., Lucas, C. H., Haddock, S. H. D., Sutherland, K. R., Robinson, K. L., Dawson, M. N., Decker, M. B., Mills, C. E., Purcell, J. E., Malej, A., Mianzan, H., Uye, S.-I., Gelcich, S. & Madin, L. P. 2012. Questioning the Rise of Gelatinous Zooplankton in the World's Oceans. *Bioscience,* 62**,** 160-169.

Crossley, S. M., George, A. L. & Keller, C. J. 2009. A method for eradicating amphipod parasites (Hyperiidae) from host jellyfish, Chrysaora fuscescens (Brandt, 1835), in a closed recirculating system. *Journal of Zoo and Wildlife Medicine,* 40**,** 174-180.

D'ambra, I. 2012. *Application of stable isotopes in the analysis of trophic interactions between jellyfish and fish.* University of South Alabama.

D'ambra, I., Carmichael, R. H. & Graham, W. M. 2014. Determination of δ13C and δ15N and trophic fractionation in jellyfish: implications for food web ecology. *Marine Biology,* 161**,** 473-480.

D'ambra, I., Graham, W. M., Carmichael, R. H. & Hernandez Jr, F. J. 2015. Fish rely on scyphozoan hosts as a primary food source: evidence from stable isotope analysis. *Marine Biology***,** 1-6.

Dawson, M. 2003. Macro-morphological variation among cryptic species of the moon jellyfish, Aurelia (Cnidaria: Scyphozoa). *Marine Biology,* 143**,** 369-379.

Dawson, M. 2004. Some implications of molecular phylogenetics for understanding biodiversity in jellyfishes, with emphasis on Scyphozoa. *Hydrobiologia,* 530**,** 249-260.

Dawson, M. N. 2005a. Morphologic and molecular redescription of Catostylus mosaicus conservativus (Scyphozoa: Rhizostomeae: Catostylidae) from south-east Australia. *Journal of the Marine Biological Association of the United Kingdom,* 85**,** 723-731.

Dawson, M. N. 2005b. Morphological variation and systematics in the Scyphozoa: Mastigias (Rhizostomeae, Mastigiidae)–a golden unstandard? *Hydrobiologia,* 537**,** 185-206.

Dawson, M. N., Gupta, A. S. & England, M. H. 2005. Coupled biophysical global ocean model and molecular genetic analyses identify multiple introductions of cryptogenic species. *Proceedings of the National Academy of Sciences of the United States of America,* 102**,** 11968-11973.

Dawson, M. N. & Hamner, W. M. 2009. A character-based analysis of the evolution of jellyfish blooms: adaptation and exaptation. *Hydrobiologia,* 616**,** 193-215.

Dawson, M. N. & Jacobs, D. K. 2001. Molecular Evidence for Cryptic Species of Aurelia aurita (Cnidaria, Scyphozoa).

Deniro, M. J. & Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et cosmochimica acta,* 42**,** 495-506.

Deniro, M. J. & Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et cosmochimica acta,* 45**,** 341-351.

Doyle, J. J. & Gaut, B. S. 2000. Evolution of genes and taxa: a primer. *Plant Molecular Biology,* 42**,** 1-23.

Doyle, T. K., Hays, G. C., Harrod, C. & Houghton, J. D. 2014. Ecological and societal benefits of jellyfish. *In:* PITT, K. A. & LUCAS, C. H. (eds.) *Jellyfish Blooms.* Springer.

Ekrem, T., Willassen, E. & Stur, E. 2007. A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular phylogenetics and evolution,* 43**,** 530-542.

Fischer, M. & Thatte, B. 2010. Revisiting an equivalence between maximum parsimony and maximum likelihood methods in phylogenetics. *Bulletin of mathematical biology,* 72**,** 208-220.

Fleming, N. E., Harrod, C., Griffin, D. C., Newton, J. & Houghton, J. D. 2014. Scyphozoan jellyfish provide short-term reproductive habitat for hyperiid amphipods in a temperate near-shore environment. *Mar Ecol Prog Ser,* 510**,** 229-240.

Fleming, N. E., Houghton, J. D., Magill, C. L. & Harrod, C. 2011. Preservation methods alter stable isotope values in gelatinous zooplankton: implications for interpreting trophic ecology. *Marine biology,* 158**,** 2141-2146.

Fuchs, B., Wang, W., Graspeuntner, S., Li, Y., Insua, S., Herbst, E. M., Dirksen, P., Bohm, A. M., Hemmrich, G., Sommer, F., Domazet-Loso, T., Klostermeier, U.C., Anton-Erxleben, F., Rosenstiel, P., Bosch, T. C. & Khalturin, K. 2014. Regulation of polyp-to-jellyfish transition in *Aurelia aurita*. *Current Biology,* 24 (3), 263-273.

Fry, B. 2006. *Stable isotope ecology,* New York, Springer.

Fujita, T. & Namikawa, H. 2006. New observations of Ophiocnemis marmorata (Echinodermata: Ophiuroidea) associated with Rhopilema esculentum (Cnidaria: Scyphozoa: Rhizostomeae) in the Philippines and Japan. *Memoirs of the National Science Museum, Tokyo,* 44**,** 3-28.

Galigher, A. 1925. On the occurrence of the larval stages of scyphozoa in the Elkhorn Slough, Monterey Bay, California. *American Naturalist***,** 94-96.

Garm, A., Bielecki, J., Petie, R. & Nilsson, D. E. 2012. Opposite patterns of diurnal activity in the box jellyfish Tipedalia cystophora and copula sivickisi. *The Biological Bulletin,* 222**,** 35.

Gershwin, L.-A. 2005a. Carybdea alata auct. and Manokia stiasnyi, reclassification to a new family with description of a new genus and two new species. *Memoirs of the Queensland Museum,* 51**,** 501-523.

Gershwin, L.-A. 2005b. *Taxonomy and phylogeny of Australian Cubozoa*. PhD Thesis, James Cook University, Townsville, pp. 1-202.

Gershwin, L.-A. 2005c. Two new species of jellyfishes (Cnidaria: Cubozoa: Carybdeida) from tropical Western Australia, presumed to cause Irukandji Syndrome. *Zootaxa,* 1084**,** 1-30.

Gershwin, L.-A. 2014. Two new species of box jellies (Cnidaria: Cubozoa: Carybdeida) from the central coast of Western Australia, both presumed to cause Irukandji syndrome. *Records of the Western Australian Museum,* 29**,** 10-19.

Gershwin, L. 2008. Morbakka fenneri, a new genus and species of Irukandji jellyfish (Cnidaria: Cubozoa). *Memoirs of the Queensland Museum–Nature,* 54**,** 23-33.

Gershwin, L. & Hannay, K. 2014. An anomalous cluster of Irukandji jelly stings (Cnidaria: Cubozoa: Carybdeida) at Ningaloo Reef. *Records of the Western Australian Museum,* 29**,** 78-81.

Gorokhova, E., Lehtiniemi, M., Viitasalo-Frösen, S. & Haddock, S. H. 2009. Molecular evidence for the occurrence of ctenophore Mertensia ovum in the northern Baltic Sea and implications for the status of the Mnemiopsis leidyi invasion. *Limnology and Oceanography,* 54**,** 2025-2033.

Gotoh, R. O., Ito, C., Mochizuki, S. & Hanzawa, N. Unpublished. Development of the rapid identification method for the giant jellyfish, *Nemopilema nomurai*, in possession of Yamagata University library, Japan, 2012.

Graham, W. M., Gelcich, S., Robinson, K. L., Duarte, C. M., Brotz, L., Purcell, J. E., Madin, L. P., Mianzan, H., Sutherland, K. R. & Uye, S.-I. 2014. Linking human well-being and jellyfish: ecosystem services, impacts, and societal responses. *Frontiers in Ecology and the Environment,* 12**,** 515-523.

Graham, W. M., Pagès, F. & Hamner, W. M. 2001. A physical context for gelatinous zooplankton aggregations: a review. *Hydrobiologia,* 451**,** 199-212.

Greenberg, N., Garthwaite, R. & Potts, D. 1996. Allozyme and morphological evidence for a newly introduced species of Aurelia in San Francisco Bay, California. *Marine Biology,* 125**,** 401-410.

Hall, B. G. 2013. Building Phylogenetic Trees from Molecular Data with MEGA. *Molecular Biology and Evolution,* 30**,** 1229-1235.

He, J. R., Zheng, L. M, Lin, Y. S., Zhang, W. J. & Cao, W.O. Unpublished. DNA barcoding Medusazoa of China Seas, Xiamen University library, China, 2014.

Hendler, G. 1975. Adaptational significance of the patterns of ophiuroid development. *American Zoologist,* 15**,** 691-715.

Herbert, P. D. N., Cywinska, A., Ball, S. L. & Dewaard, J. R. 2003a. Biological identifications through DNA barcodes. *Proceedings of the Royal Society: Biology Letters,* 270.

Herbert, P. D. N., Ratnaslngham, S. & Dewaard, J. R. 2003b. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society: Biology Letters,* 270**,** S96-S99.

Hillis, D. & Wiens, J. 2000. Molecules versus morphology in systematics: conflicts, artifacts, and misconceptions. *Phylogenetic analysis of morphological data***,** 1-19.

Holland, B. S., Dawson, M. N., Crow, G. L. & Hofmann, D. K. 2004. Global phylogeography of Cassiopea (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. *Marine Biology,* 145**,** 1119-1128.

Houghton, J. D., Doyle, T. K., Wilson, M. W., Davenport, J. & Hays, G. C. 2006. Jellyfish aggregations and leatherback turtle foraging patterns in a temperate coastal environment. *Ecology,* 87**,** 1967-1972.

Iyengar, E. V. 2008. Kleptoparasitic interactions throughout the animal kingdom and a re-evaluation, based on participant mobility, of the conditions promoting the evolution of kleptoparasitism. *Biological Journal of the Linnean Society,* 93**,** 745-762.

Jerling, H. & Wooldridge, T. 1995. Relatively negative δ13C ratios of mesozooplankton in the Sundays River estuary, comments on potential carbon sources. *Southern African Journal of Aquatic Science,* 21**,** 71-77.

Jones, N. 2014. Two newly discovered irukandji jellyfish species found off WA coast. *ABC News* [Online]. Available: http://www.abc.net.au/news/2014-08-06/irukandji-jellyfish-species-malo-bella-keesingia-found-off-wa/5650650.

Kanagaraj, G., Kumar, P. S. & Morandini, A. C. 2008. The occurrence of Ophiocnemis marmorata (Echinodermata: Ophiuroidea) associated with the rhizostome medusa Rhopilema hispidum (Cnidaria: Scyphozoa). *Journal of Ocean University of China,* 7**,** 421-424.

Kingsford, M., Seymour, J. & O'callaghan, M. 2012. Abundance patterns of cubozoans on and near the Great Barrier Reef. *Jellyfish Blooms IV.* Springer.

Kramp, P. L. 1961. Synopsis of the medusae of the world. *Journal of the marine biological Association of the United Kingdom,* 40**,** 7-382.

Lajtha, K. & Michener, R. H. 2007. *Stable isotopes in ecology and environmental science,* Malden, MA; Oxford, Blackwell.

Laval, P. 1980. Hyperiid amphipods as crustacean parasitoids associated with gelatinous zooplankton. *Oceanogr. Mar. Biol. Ann. Rev,* 18**,** 11-56.

Lee, P. L., Dawson, M. N., Neill, S. P., Robins, P. E., Houghton, J. D., Doyle, T. K. & Hays, G. C. 2013. Identification of genetically and oceanographically distinct blooms of jellyfish. *Journal of The Royal Society Interface,* 10**,** 20120920.

Leung, T. & Poulin, R. 2008. Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. *Vie et Milieu,* 58**,** 107.

Lilley, M. K. S., Beggs, S. E., Doyle, T. K., Hobson, V. J., Stromberg, K. H. P. & Hays, G. C. 2011. Global patterns of epipelagic gelatinous zooplankton biomass. *Marine Biology,* 158**,** 2429-2436.

Lucas, C. H., Jones, D. O., Hollyhead, C. J., Condon, R. H., Duarte, C. M., Graham, W. M., Robinson, K. L., Pitt, K. A., Schildhauer, M. & Regetz, J. 2014. Gelatinous zooplankton biomass in the global oceans: geographic variation and environmental drivers. *Global ecology and biogeography,* 23**,** 701-714.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C. & Wang, Q. 2014. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasit Vectors,* 7**,** 93.

Lynam, C. P. & Brierley, A. S. 2007. Enhanced survival of 0-group gadoid fish under jellyfish umbrellas. *Marine Biology,* 150**,** 1397-1401.

Marsh, L. 1998. Hitch-hiking ophiuroids. *Echinoderms: San Francisco***,** 393-396.

Mayer, A. 1906. Medusae collected in the Hawaiian Islands by the steamer Albatross in 1902. *Bull. US Fish Comm. 1903,* 23**,** 1131-1143.

Mccutchan, J. H., Lewis, W. M., Kendall, C. & Mcgrath, C. C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos,* 102**,** 378-390.

Mianzan, H. W., Mianzan, H. W., Mari, N., Prenski, B. & Sanchez, F. 1996. Fish predation on neritic ctenophores from the Argentine continental shelf: A neglected food resource? *Fisheries research,* 27**,** 69-79.

Mills, C. E. 2001. Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? *Jellyfish Blooms: Ecological and Societal Importance***,** 55-68.

Miyajima, Y., Masuda, R., Kurihara, A., Kamata, R., Yamashita, Y. & Takeuchi, T. 2011. Juveniles of threadsail filefish, Stephanolepis cirrhifer, can survive and grow by feeding on moon jellyfish Aurelia aurita. *Fisheries Science,* 77**,** 41-48.

Morton, B. 2003. Ningaloo. *Marine Pollution Bulletin,* 46**,** 1213-1214.

Mutlu, E. 1996. Effect of formaldehyde on the gelatinous zooplankton (Pleurobrachia pileus, Aurelia aurita) during preservation. *Turkish Journal of Zoology,* 20**,** 423-426.

Ohtsuka, S., Koike, K., Lindsay, D., Nishikawa, J., Miyake, H., Kawahara, M., Mujiono, N., Hiromi, J. & Komatsu, H. 2009. Symbionts of marine medusae and ctenophores. *Plankton and Benthos Research,* 4**,** 1-13.

Ohtsuka, S., Kondo, Y. & Sakai, Y. 2010. In-situ Observations of Symbionts on Medusae Occurring in Japan, Thailand, Indonesia and Malaysia< Article>. *広島大学総合博物館研究報告***,** 9-18.

Ortman, B. D., Bucklin, A., Pagès, F. & Youngbluth, M. 2010. DNA Barcoding the Medusozoa using mtCOI. *Deep-Sea Research Part II,* 57**,** 2148-2156.

Pagès, F., Corbera, J. & Lindsay, D. 2007. Piggybacking pycnogonids and parasitic narcomedusae on Pandea rubra (Anthomedusae, Pandeidae). *Plankton Benthos Res,* 2**,** 83-90.

Panikkar, N. K. & Raghu Prasad, R. 1952. Interesting association of ophiuroids, fish and crab with the jellyfish Rhopilema hispidum. *Journal of The Bombay Natural History Society,* 51**,** 295-296.

Parker, A. E., Wilkerson, F. P., Dugdale, R. C., Marchi, A. M., Hogue, V. E., Landry, M. R. & Taylor, A. G. 2011. Spatial patterns of nitrogen uptake and phytoplankton in the equatorial upwelling zone (110°W–140°W) during 2004 and 2005. *Deep-Sea Research Part II,* 58**,** 417-433.

Parnell, A. C., Inger, R., Bearhop, S. & Jackson, A. L. 2010. Source partitioning using stable isotopes: coping with too much variation. *PloS one,* 5**,** e9672.

Parnell, A. C. & Jackson, A. 2013. Package 'siar'. *Stable Isotope Analysis in R.* 4.2 ed: CRAN.

Pauly, D., Graham, W., Libralato, S., Morissette, L. & Palomares, M. D. 2009. Jellyfish in ecosystems, online databases, and ecosystem models. *Hydrobiologia,* 616**,** 67-85.

Peterson, B. J. & Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual review of ecology and systematics***,** 293-320.

Phillips, P. J. 1973. The Occurrence of the Remarkable Scyphozoan, Deepstaria enigmatica, in the Gulf of Mexico and Some Observations on Cnidarian Symbionts. *Gulf and Caribbean Research,* 4**,** 166-168.

Pierce, J. 2009. Prediction, location, collection and transport of jellyfish (Cnidaria) and their polyps. *Zoo biology,* 28**,** 163-176.

Pitt, K. A. & Kingsford, M. J. 2003. Temporal and spatial variation in recruitment and growth of medusae of the jellyfish, Catostylus mosaicus (Scyphozoa: Rhizostomeae). *Marine and Freshwater Research,* 54**,** 117-125.

Pitt, K. A., Connolly, R. M. & Meziane, T. 2009a. Stable isotope and fatty acid tracers in energy and nutrient studies of jellyfish: a review. *Jellyfish Blooms: Causes, Consequences, and Recent Advances.* Springer.

Pitt, K. A., Welsh, D. T. & Condon, R. H. 2009b. Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. *Hydrobiologia,* 616**,** 133-149.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology,* 83**,** 703-718.

Raskoff, K. A., Sommer, F. A., Hamner, W. M. & Cross, K. M. 2003. Collection and Culture Techniques for Gelatinous Zooplankton. *Biological Bulletin,* 204**,** 68-80.

Riascos, J., Vergara, M., Fajardo, J., Villegas, V. & Pacheco, A. 2012. The role of hyperiid parasites as a trophic link between jellyfish and fishes. *Journal of fish biology,* 81**,** 1686-1695.

Riascos, J. M., Docmac, F., Reddin, C. & Harrod, C. 2015. Trophic relationships between the large scyphomedusa Chrysaora plocamia and the parasitic amphipod Hyperia curticephala. *Marine Biology,* 162**,** 1841-1848.

Richardson, A. J., Bakun, A., Hays, G. C. & Gibbons, M. J. 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in ecology & evolution,* 24**,** 312-322.

Ross, H. A., Murugan, S. & Li, W. L. S. 2008. Testing the reliability of genetic methods of species identification via simulation. *Systematic biology,* 57**,** 216-230.

Sal Moyano, M., Schiariti, A., Giberto, D., Briz, L. D., Gavio, M. & Mianzan, H. 2012. The symbiotic relationship between Lychnorhiza lucerna (Scyphozoa, Rhizostomeae) and Libinia spinosa (Decapoda, Epialtidae) in the Río de la Plata (Argentina–Uruguay). *Marine biology,* 159**,** 1933-1941.

Savolainen, V., Cowan, R. S., Vogler, A. P., Roderick, G. K. & Lane, R. 2005. Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 360**,** 1805-1811.

Sayers, E. W., Barrett, T., Benson, D. A., Bolton, E., Bryant, S. H., Canese, K., Chetvernin, V., Church, D. M., Dicuccio, M. & Federhen, S. 2011. Database resources of the national center for biotechnology information. *Nucleic acids research,* 39**,** D38-D51.

Schroth, W., Jarms, G., Streit, B. & Schierwater, B. 2002. Speciation and phylogeography in the cosmopolitan marine moon jelly, Aurelia sp. *BMC Evolutionary Biology,* 2**,** 1.

Small, C. & Cohen, J. 2004. Continental Physiography, Climate, and the Global Distribution of Human Population. *Current Anthropology,* 45**,** 269-277.

Sparmann, S. F., Ortaman, B. D. & Leander, B.S. Unpublished. Utility of 16S rDNA for phylogenetic inference and phylogeography, University of British Colombia, Vancouver, 2012.

Stöhr, S., O'hara, T. D. & Thuy, B. 2012. Global diversity of brittle stars (Echinodermata: Ophiuroidea). *PLoS One,* 7**,** e31940.

Tamelander, T., Renaud, P. E., Hop, H., Carroll, M. L., Ambrose, W. G. & Hobson, K. A. 2006. Trophic relationships and pelagic-benthic coupling during summer in the Barents Sea Marginal Ice Zone, revealed by stable carbon and nitrogen isotope measurements. *Marine ecology. Progress series,* 310**,** 33-46.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution,* 30**,** 2725-2729.

Thibault-Botha, D. & Bowen, T. 2004. Impact of formalin preservation on Pleurobrachia bachei (Ctenophora). *Journal of experimental marine biology and ecology,* 303**,** 11-17.

Towanda, T. & Thuesen, E. V. 2006. Ectosymbiotic behavior of Cancer gracilis and its trophic relationships with its host Phacellophora camtschatica and the parasitoid Hyperia medusarum. *Marine Ecology Progress Series,* 315**,** 221-236.

Vanderklift, M. A. & Ponsard, S. 2003. Sources of variation in consumer-diet δ15N enrichment: a meta-analysis. *Oecologia,* 136**,** 169-182.

Warner, G. 1982. Food and feeding mechanisms: Ophiuroidea. *Echinoderm nutrition. Balkema, Rotterdam***,** 161-181.

West, J. B., Bowen, G. J., Cerling, T. E. & Ehleringer, J. R. 2006. Stable isotopes as one of nature's ecological recorders. *Trends in Ecology & Evolution,* 21**,** 408-414.

Wood, D. & Glasson, J. 2005. Giving the environment a voice: the transformational potential of valuing tourism in sensitive natural environments: the case of the Ningaloo coastal region, Western Australia. *Planning, Practice & Research,* 20**,** 391-407.

Wrobel, D. & Mills, C. 1998. *Pacific coast pelagic invertebrates: a guide to the common gelatinous animals*, Sea Challengers.

Yang, Z. 1993. Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution,* 10**,** 1396-1401.

Yasuda, T. 1973. Ecological studies on the jelly-fish, Aurelia aurita (Linné), in Urazoko Bay, Fukui Prefecture-VIII. Diel vertical migration of the medusa in early fall, 1969.

Yuri, T., Kimball, R. T., Harshman, J., Bowie, R. C., Braun, M. J., Chojnowski, J. L., Han, K.-L., Hackett, S. J., Huddleston, C. J. & Moore, W. S. 2013. Parsimony and model-based analyses of indels in avian nuclear genes reveal congruent and incongruent phylogenetic signals. *Biology,* 2**,** 419-444.

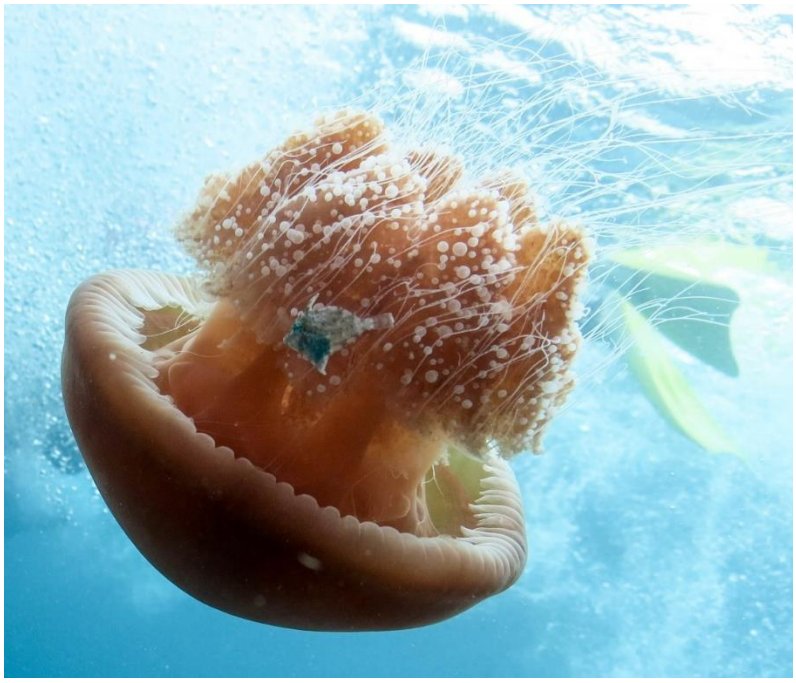
Zheng, L., He, J., Lin, Y., Cao, W. & Zhang, W. 2014. 16S rRNA is a better choice than COI for DNA barcoding hydrozoans in the coastal waters of China. *Acta Oceanologica Sinica,* 33**,** 55-76.

Appendices

Appendix 1: Photographic database of gelatinous zooplankton found at Ningaloo Reef

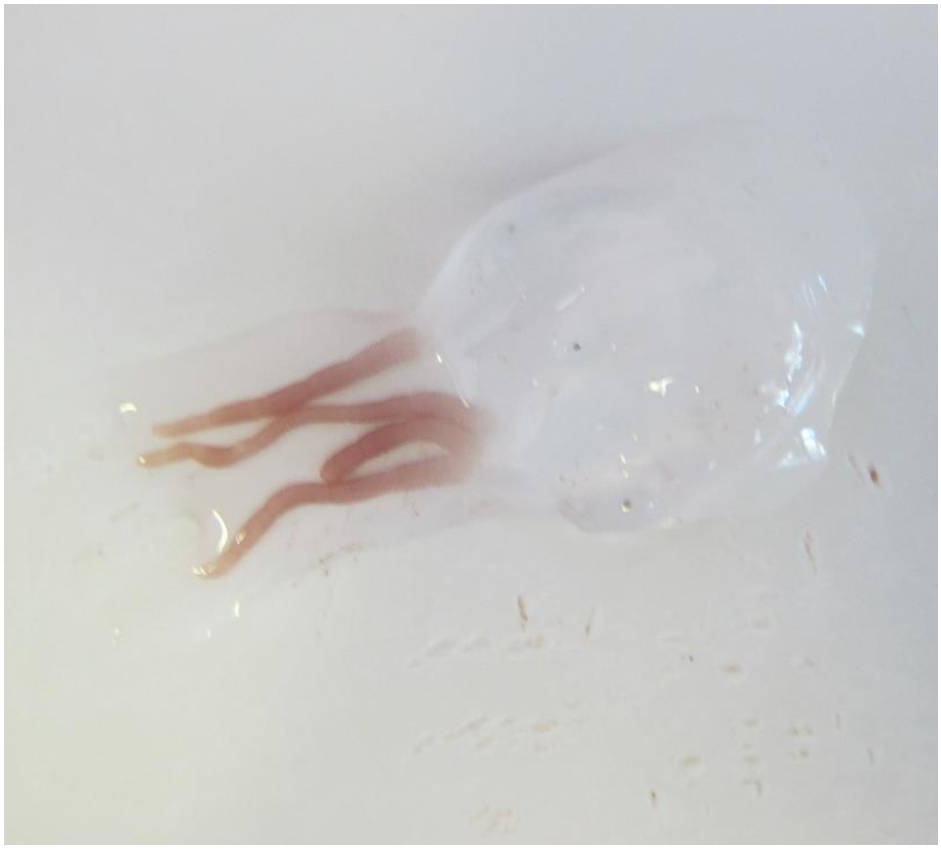
Aurelia aurita

Crambione mastigophora with juvenile leatherjacket (Photo courtesy of Cindy White)



Cyanea sp.

Malo bella



Leptomedusae specimen

Aequorea australis

Rhizostome specimen