Investigating the Potential of Jellyfishes as Marine Biomonitors and Bioindicators of Metal Pollution

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Abstract

Metal pollution has long been recognised as having a significant impact on the biodiversity and health of marine coastal systems. The use of biomonitor and bioindicator species is widely accepted for protecting ecosystem integrity and remediation of impacted systems. Species used as biomonitors in marine systems are typically benthic sessile species with a tolerance to the pollutant under investigation. In contrast, bioindicator species need to be sensitive to the pollutant. Historically, jellyfishes have not been regarded as useful bioindicators as they have been considered very tolerant of polluted environments and thus not evaluated in the suite of indicator species. Their pelagic behaviour and seasonality also mean they have not previously been considered useful biomonitor species despite their potentially high seasonal abundance in coastal systems. Notwithstanding these factors, current concerns about increased jellyfish blooms and their ability to cycle large quantities of inorganic elements suggest jellyfishes could fulfil a useful role in assessing ecosystem health.

The objective of this project was to assess the potential jellyfishes have as biomonitors or bioindicators to the effects of dissolved metals. The research focused on the response of scyphozoan and cubozoan jellyfishes to metal exposure and assessed bioaccumulative capacity and retention as well as sensitivity to metals. To assess the biomonitoring capacity, elemental concentrations in tissues of jellyfishes were measured at multiple locations on the Great Barrier Reef over a three year period. Scyphozoan jellyfishes accumulated higher concentrations of elements at coastal and inshore locations compared with off-shore locations, which likely reflected the gradient of terrestrially derived elements into the marine system. Symbiotic scyphozoan jellyfishes typically had higher body concentrations of elements than their asymbiotic relatives collected from the same location and time. Further, variations in elemental tissue concentrations among cubozoan jellyfishes were species dependent.

Laboratory assessment of the bioconcentration of aqueous copper or zinc in the symbiotic rhizostome jellyfish *Cassiopea* sp. showed that both metals readily accumulated in tissues at levels that were orders of magnitude above ambient water concentrations. However, the accumulative capacity and retention time varied between the metals. Copper was accumulated more rapidly and excreted more rapidly post-exposure (biological half-life 1.7 days), while zinc was accumulated more slowly and did not reach saturation during the study but was retained for much longer (biological half-life 9.1 days). Although the exact mechanisms of uptake and retention were not identified, it was apparent that accumulation and retention strategies in *Cassiopea* sp. were strongly metal dependent.

To assess the bioindicator potential of jellyfishes, a series of laboratory studies measured the acute effects of copper and zinc at multiple lifestages in three species of jellyfish. From the

outcomes of the acute toxicity, additional studies were conducted using the most sensitive species to determine sub-lethal responses of the jellyfish to copper or zinc exposure.

The acute toxicity of aqueous copper and zinc was assessed in three species of jellyfish representing symbiotic (*Cassiopea* sp.) and asymbiotic Scyphozoa (*Aurelia* sp.), and a cubozoan jellyfish (*Alatina mordens*). Copper was an order of magnitude more toxic to all species and lifestages than zinc. *Cassiopea* sp. was more sensitive to both copper and zinc exposure than *Alatina mordens*. *Aurelia* sp. was the least sensitive of the three species to both metals. Sensitivity to copper and zinc varied among lifestage also, with the newly metamorphosed / strobilated stages being more sensitive than the benthic polyps in the three species.

Cassiopea sp. was the most sensitive species from the acute toxicity studies. Sub-lethal toxicity to copper and zinc was assessed for both the jellyfish and its endosymbiont zooxanthellae. The response of the zooxanthellae was tested using photosynthetic yield as the end point and the host jellyfish response was assessed using change in bell diameter. There was a decrease in photosynthetic yield in the zooxanthellae exposed to increasing concentrations of copper or zinc although this was only significant at the higher concentrations (24 $\mu g.L^{-1}$ Cu, 0.88 $mg.L^{-1}$ Zn). Post-experiment counts of zooxanthellae abundances showed that the jellyfish did not expel the symbionts as a stress response to metal exposure, so that the change in photosynthetic yield resulted from decreased zooxanthellae activity rather than decreased abundance in the host tissues. Change in bell diameter of the *Cassiopea* sp. was significant at all concentrations of copper and zinc tested. This demonstrated that the host response was the more sensitive measure of exposure to copper or zinc than symbiont activity.

In conclusion, the project demonstrated that jellyfishes were sensitive to metal pollution and have potential as bioindicators. The responses were variable among species and lifestages but demonstrated high sensitivity comparable to other marine bioindicators (e.g. hermatypic corals and molluscs). When exposed to low concentrations of metals, jellyfishes were capable of concentrating metals in their tissues and retaining them for days to weeks suggesting they are of high utility as marine biomonitors. These outcomes challenge historical views that jellyfish are more tolerant to pollutants than most marine taxa and are more likely to persist under poor environmental conditions. It also demonstrates that jellyfishes have strong potential for monitoring and assessing ecosystem health.

Chapter 1 - General Introduction

1. Introduction

Marine environments are highly dynamic and productive but are subject to a range of influences, both natural and anthropogenic (Chapman 1995). These influences cause physical, chemical and biological perturbations on the marine systems, with consequential effects on the composition and abundance of biota within the environment (e.g. Sadiq 1992; Luoma & Rainbow 2008; Zhou et al. 2008). Whilst these systems have resilience to cope with natural disturbances, the added pressure from anthropogenic stresses can often lead to irreversible changes within the ecosystem (e.g. Peters et al. 1997; Luoma & Rainbow 2008). In particular, coastal urbanisation and industrialisation has resulted in increased anthropogenic discharges into coastal marine environments with consequential impacts and changes on biota (Peters et al. 1997). However, understanding the fluxes and fate of contaminants in the marine environment is rarely simple and a careful approach is required to understand the interactions between the various stressors and the biota (e.g. Goodsell et al. 2009).

Physico-chemical measurements of environmental contaminants are the most direct methods for monitoring anthropogenic discharges, but they reveal little about the ecological effects of those stressors (Goodsell et al. 2009). In addition, discrete samples of water and sediment can only provide a 'snap shot' of contaminant presence. For more time-integrated measurements, biological indicators have been considered as viable alternatives for monitoring ecological change due to anthropogenic stress (Zhou et al. 2008; Goodsell et al. 2009). Biological responses provide a measure of the ecological state of a system from an organism's perspective, in a manner that is often very difficult to measure directly (Peters et al. 1997; Goodsell et al. 2009).

Jellyfishes are an important biotic component of the marine system with the capacity to form large blooms under ideal conditions (Richardson et al. 2009). Investigations on the triggers for jellyfish blooms suggest that anthropogenic stressors such as eutrophication, may be a contributing factor (Benovic & Lucic 2001; Hay 2006; Purcell et al. 2007; Brodeur et al. 2008; Dong et al. 2010). Greater knowledge of the relationships between jellyfishes and marine pollution provides not only a basis for a better understanding of jellyfish population dynamics, but also an opportunity for exploring the wider impacts of pollution within a system.

2. Pollution in the marine environment

The terms 'pollutant' and 'contaminant' are often used interchangeably when discussing physical, biological or chemical alterations to the environment, and this has led a lack of discrimination between them. Using the definitions as described in Goodsell (2009), a contaminant is a chemical or physical measure of an element or compound that is elevated above normal background concentrations, while a pollutant is the biological or ecological response to a contaminant (Goodsell et al. 2009). Direct physico-chemical measurement can identify the presence of a contaminant. However, biological indicators provide the ability to measure environmental pollutants rather than contaminants and also allow for direct measures of environmental impact (Goodsell et al. 2009).

A variety of contaminants can be released into the marine environment including "heavy" metals, other trace elements, pesticides, herbicides, organic compounds, nutrients, high or low salinity waters, acidity and excess dissolved gases. With the exception of synthetic pesticides and herbicides, most of these contaminants can have both natural and anthropogenic origins with fluxes occurring in most environments. Although all contaminants can potentially be pollutants (i.e. cause a biological or ecological response), a large amount of historic work has focussed on trace element pollution due to their persistence in the environment (e.g. Calabrese et al. 1973; Bræk et al. 1976; Bloom & Ayling 1977; Furness & Rainbow 1990; Sadiq 1992; Chapman 1995).

Trace elements are those metal and non-metal elements typically present in low or 'trace' amounts. As discussed in the literature, some ambiguity surrounds the strict definition of this term and it is often used interchangeably with trace metals and sometimes heavy metals (e.g. Furness & Rainbow 1990; Luoma & Rainbow 2008). An objective definition of trace metals based on Lewis acid properties was proposed by Nieboer & Richardson (1980). Using this definition, class B and 'borderline' metals have been defined as trace metals (Nieboer & Richardson 1980; Luoma & Rainbow 2008). Class 'B' metals are considered nitrogen or sulphur seeking metals, Class 'A' metals are oxygen seeking, and 'borderline' metals are intermediate (Nieboer & Richardson 1980). The affinity of Class 'B' metals for nitrogen and sulphur allows them to exploit metabolic pathways and cause toxicity by binding to proteins and other organic compounds, inhibiting metabolism (Luoma & Rainbow 2008).

For this project, the term 'trace element' will be used. Trace element will be defined as Class B metals using the trace metal definition of Nieboer & Richardson (1980). However, it will also include aluminium (Al) which has Class A Lewis acid properties, hence the use of the term element rather than metal.

There are three main entry routes for trace elements into the marine water column; A) atmospheric (deposition from air), B) water borne (riverine / estuarine deposition); and, C) recycling within the marine environment from biota or sediment. In addition, trace elements may be natural or anthropogenic, point or non-point source in origin, and may be released as pulse or press events. These different scenarios create a dynamic system for introduction, cycling, recycling and removal of trace elements within the marine system.

The terms 'pulse' and 'press' events were initially used to define changes in species density with a 'pulse' event described as a very short term alteration to species density, while a 'press' perturbation was described as a sustained alteration to a species composition (Bender et al. 1984). However, there has been some inconsistency in their use in experimental ecological studies (Glasby & Underwood 1996). In a refinement of definition for assessing anthropogenic perturbations, Glasby & Underwood (1996) classified perturbations on the basis of both cause and effect (e.g. a pulse perturbation could result in a press organismal response). In the context of this project, the terms pulse and press will be used to describe cause rather than effect i.e. a 'pulse' event would be a perturbation of short term duration and a 'press' event will be defined as an input of sustained duration.

Trace elements in the marine environment are critical for biochemical cycles and biota. The various trace elements can be separated into two groups comprising essential and non-essential elements (Neff 2002; Luoma & Rainbow 2008). Essential elements are required in very small amounts for optimum metabolic functioning and deficiency can cause health issues, but at levels exceeding metabolic requirements they can rapidly become toxic. Essential elements are involved in key cellular functions, including, acting as catalysts for biochemical reactions, stabilising protein structures, and aiding in the maintenance of osmotic balances (Bruins et al. 2000). Examples of essential trace elements include (but are not limited to): arsenic (As), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), vanadium (V) and zinc (Zn).

In contrast, non-essential elements have no identified metabolic role and while they may be tolerated in low concentrations they are also toxic to biota if they exceed a threshold (Luoma & Rainbow 2008). Examples of non-essential trace elements include aluminium (Al), lead (Pb), cadmium (Cd), mercury (Hg) and silver (Ag) among others. Regardless of essentiality though, the presence of increased loads of these elements, whether from natural or anthropogenic sources can be harmful to the biotic structure of these systems (Luoma & Rainbow 2008).

At the level of biota, the response to trace element exposure depends on the concentration of the element, as well as its speciation and the uptake route into the organism; the latter being

defined as the bioavailability of the element (Rainbow 1990). The key uptake routes are diffusion or active transport from water, dietary uptake, and absorption from sediment or pore water (Boisson et al. 2003; Luoma & Rainbow 2008). The speciation of elements also determines their toxicity as some species (e.g. free ions) are more readily absorbed and consequently potentially more toxic than other species (Nelson & Donkin 1985; Burton & Statham 1990).

The focus on dietary uptake of metals in aquatic invertebrates has been more recent than uptake from water (bioconcentration). In part, this has been due to difficulties in assessing the assimilation of metals from diet. Research has led to a greater understanding of the role diet has in accumulation of metals and also determination of assimilation efficiencies (absorption minus excretion) in organisms (Wang & Fisher 1999a; Luoma & Rainbow 2008). The development of these parameters to assess the bioavailable fractions of metals has allowed quantitative assessments of the ability of organisms to accumulate metals (Wang & Fisher 1999a).

While the effects of trace elements on aquatic ecosystems and their biota have been widely studied, the extent to which individual elements and combinations of elements have been investigated varies greatly (Langdon et al. 2009). Copper and zinc are essential elements that have been among the most intensively studied elements due to both their presence and persistence in aquatic systems (Langdon et al. 2009). As essential elements they have the paradox of being needed in trace quantities but are toxic at concentrations that are just above the essential requirements (e.g. Neff 2002; Lee et al. 2010). Due to the combination of the availability of comparative data, their essentiality and the narrow threshold between essential concentrations and toxic levels, copper and zinc were targeted as the two elements for quantitative assessment of the biomonitoring and bioindicating potential in jellyfishes in this project.

2.1 Copper and zinc pollution

Both copper and zinc are essential trace elements required for metabolic health in both aquatic and terrestrial organisms. Copper has a number of functions in organisms, including acting as a co-factor in a number of proteins (e.g. superoxide dismutase), and having a key role in cellular respiration (Bury et al. 2003). Zinc is also considered an essential trace element and has been identified as a key co-factor in over one hundred enzymes including carbonic anhydrase (e.g. Morel et al. 1994; Einicker-Lamas et al. 2002). This enzyme is found in a wide range of symbiotic marine invertebrates and is required to convert non-bioavailable carbonates (HCO_3^-) to a form that is biologically available (CO_2) for photosynthesis (Estes et al. 2003). Thus it is critical for those organisms with photosynthetic symbionts. Zinc tends to be present in higher concentrations in biota compared with many other trace elements, which may be due to its

involvement in many co-enzymes (Outten & O'Halloran 2001; Neff 2002). Both elements though, have been shown to rapidly become toxic to biota at levels above the essential threshold (Neff 2002).

Typical copper concentrations in uncontaminated surface oceanic waters vary from 0.03 to 0.39 $\mu g.L^{-1}$ (Neff 2002; Luoma & Rainbow 2008). In seawater, copper occurs in several forms, both inorganic and organic, and free and bound, with the ionic species and some hydroxide complexes having greater bioavailability (Sadiq 1992; Lee et al. 2010). As such, direct measures of total copper concentrations rarely correlate with measures of environmental impact (Sadiq 1992; Neff 2002). Organically bound copper can range from 3 to 99 % of the total copper, with only a small amount of copper in the free, ionic species in seawater (Sadiq 1992).

In coastal and estuarine systems, copper concentrations can be much higher due to the influence of riverine flows and anthropogenic inputs (Langston 1990; Matthiessen et al. 1999; Xie et al. 2005). The lower salinities and pH found in upper estuaries often result in a greater proportion of total copper being present in the more bioavailable forms (Lee et al. 2010). Typical copper concentrations in contaminated estuaries can be up to 20 $\mu g.L^{-1}$ Cu, although concentrations high as 176 $\mu g.L^{-1}$ Cu have been measured in a UK estuary receiving acid mine drainage (Matthiessen et al. 1999; Luoma & Rainbow 2008). Major anthropogenic inputs of copper include runoff from urban areas, sewage disposal and discharges from mining operations (Matthiessen et al. 1999).

Zinc concentrations in uncontaminated surface waters are also very low, ranging from 0.003 – 0.61 $\mu g.L^{-1}$ with concentrations decreasing with increasing distance from shore and correlating with silicate concentrations (Luoma & Rainbow 2008). Atmospheric deposition is considered to be an important source of zinc to oceanic waters (Neff 2002). Zinc readily binds to organic and inorganic ligands as well as colloids in seawater. Zinc ions, which are the most bioavailable fraction, can form 17 to 46 % of total dissolved zinc in seawater but readily bind to colloidal particles and complex with dissolved organic matter (Neff 2002; Luoma & Rainbow 2008).

In contaminated estuaries, zinc concentrations as high as 20.5 $mg.L^{-1}$ Zn have been reported, although concentrations in modified systems are typically lower at approximately 20 $\mu g.L^{-1}$ Zn (Luoma & Rainbow 2008). Mining and smelting operations are significant contributors of anthropogenic zinc into estuarine and coastal systems (Luoma & Rainbow 2008). The use of sacrificial anodes on marine structures, and the increasing use of zinc antifoulants (replacing the banned tributyltin) also contribute to the zinc load in urban marine areas (Neff 2002; Xie et al. 2005; Bao et al. 2008).

3. Biomonitors and bioindicators

The use of biomonitors and bioindicators as measures of pollution in the marine environment are well known and accepted ecological tools (e.g. Rainbow & Phillips 1993; Luoma & Rainbow 2008; Zhou et al. 2008). They provide a method for measuring contaminant stress that is more ecologically relevant than direct physico-chemical measures (Goodsell et al. 2009).

Biomonitors can be defined as organisms capable of accumulating trace elements within their tissues, which in turn can provide a relative measure of the bioavailable fraction of trace elements taken up from all routes in a preceding time frame by the individual (Luoma & Rainbow 2008). They are important as they not only reflect concentrations in the environment at the time of sampling but can also provide a record of historical press and pulse events that may be missed in typical grab sample water quality monitoring programs (Anan et al. 2005; Zauke et al. 1996). Thus, biomonitors perform a very important role in helping understand the biogeochemical cycling of contaminants in the marine system. Biomonitors are also useful in that they only take up that fraction of the metal load that is "bioavailable" to organisms rather than the total metal load and, therefore, reflect the accumulated fraction that may be transferred up the food chain. The extent to which any particular organism serves as a useful biomonitor depends on a wide range of factors related to the relevant environment and specific outcomes to be investigated (Rainbow & Phillips 1993; Zhou et al. 2008).

An organism must satisfy a number of criteria to meet the requirements of an ideal biomonitor. These include being: 1) sedentary in nature; 2) abundant at the site to be investigated; 3) easily identified; 4) tolerant of physico-chemical fluctuations; 5) large enough to analyse; 6) resistant to handling stress; and importantly, 7) non-regulators of the element/s of concern (Rainbow & Phillips 1993; Zhou et al. 2008). Typically, a suite of biomonitors may be needed to assess ecosystem health to reflect the different / multiple ecological niches that may have greater or lesser exposure to any given pollutant.

In contrast, bioindicators have been defined as organisms that exhibit a change in structure or function linked to the biological effect of a contaminant at an organism, population, community or ecosystem level (McCarty & Munkittrick 1996; McCarty et al. 2002). The use of bioindicators species as a measure of ecosystem health is a useful method for discriminating between exposure and effect in an ecosystem (van Gestel & van Brummelen 1996). In part, the use of bioindicators has arisen as an outcome of the need for more sensitive markers of pollution in the environment than population mortality (Chapman 1995).

Like biomonitors, an organism needs to meet some key criteria to be considered a useful bioindicator species. These include: 1) having a key role in the ecosystem processes; 2) being present in the system under consideration (or typical of that system type); 3) being present in reasonably large numbers; and, 4) being sensitive to the contaminants of concern (Edwards et al. 1996; Goodsell et al. 2009). Again, like biomonitors it is recommended that a suite of bioindicator species be utilised when monitoring ecosystem health as no single species can occupy all ecological niches or express a response to all stresses (Wilson 1994).

Bioindicator species can also be utilised in laboratory assessments to establish criteria for ecological sensitivity, with ecotoxicological bioassays being a typical approach (e.g. LC_{50}). In this way, it is possible to assess the relative sensitivities of multiple species to pollutants (physical, biological and chemical) and pollutant combinations as part of an overall process to identify the primary response mechanisms invoked by pollutant exposure (Chapman 1995; Edwards et al. 1996; van Gestel & van Brummelen 1996).

4. Jellyfishes

Jellyfishes fill a number of important ecological niches in the marine environment. They are voracious predators on smaller zooplankton including larval fish and potentially compete for the same food as larvae of economically important fisheries species like herring (Lynam et al. 2005; Hay 2006). Jellyfishes also provide key pelagic habitat / shelter to small fish and crustaceans (Kingsford 1993; Browne & Kingsford 2005; Lynam & Brierley 2007) and are predated on by a wide range of marine organisms (Brandon & Cutress 1985; Ates 1991; Arai 2005; Richardson et al. 2009).

Jellyfishes have a very high water content (95-98 %) compared with other animals (summarised in Arai 1997) with protein (72 %) the most abundant organic fraction (Pitt et al. 2009). The proteins perform a number of functions in jellyfish, including enzymatic reactions (e.g. carbonic anhydrase), formation of collagen fibres in the mesoglea, and are part of the pigment structures and toxins (Pitt et al. 2009). Historically, jellyfishes have been considered poor dietary components due to their high water content and consequential low calorific value (Sommer et al. 2002). More recent work though, suggests that the high relative protein content, rapid digestion and assimilation may provide a useful energy source to predators (Arai 2005; Doyle et al. 2007). A rapid dietary assimilation of jellyfish by predators combined with the affinity of class ‘B’ metals for nitrogen and sulphur compounds (i.e. proteins), also suggests that the potential for trophic transfer of elements may be higher from jellyfish diets. This pathway has been implicated in the high accumulation of cadmium in leatherback turtles (Caurant et al. 1999).

Jellyfishes have been implicated in the cycling of nutrients and contaminants within the water column and between the water column and benthos (Todd et al. 2006; Pitt et al. 2009; Jantzen et al. 2010; Niggl et al. 2010). Recent work has suggested that jellyfishes may facilitate nutrient cycling in oligotrophic environments like coral reef systems (Jantzen et al. 2010). Other studies have also demonstrated that jellyfish blooms can have a significant influence on carbon, nitrogen and phosphorus cycling within the ecosystems (Pitt et al. 2009). Asymbiotic jellyfishes are net exporters of nitrogen and phosphorus and can provide a valuable additional nutrient source for phytoplankton (Pitt et al. 2009). In contrast, symbiotic jellyfishes recycle these nutrients and may instead actively compete for nutrients with phytoplankton (Pitt et al. 2009).

Combinations of processes, including changes in predation, intra- and inter- specific competition, and physical ecological processes can affect jellyfish recruitment and populations and therefore affect both jellyfish presence and abundance (Attrill et al. 2006; Brodeur et al. 2008). This can lead to regime shifts between fish and jellyfish population dominance, temporally and spatially, potentially indicating changes in ecosystem health (Lynam et al. 2006).

Recent research has indicated that jellyfish populations may be increasing in response to changes in marine systems (Mills 2001). These changes include: overfishing of commercial fish species (Daskalov 2002; Lynam et al. 2006), eutrophication (reviewed in Arai 1997), increasing atmospheric carbon dioxide (CO_2) concentrations and climate change (Purcell 2005; Hays et al. 2005). This has the potential to lead to jellyfish dominated ecosystems with their associated management issues (Purcell et al. 2007; Richardson et al. 2009).

The reported increasing occurrence of jellyfish blooms as a consequence of trophic cascades and other stressors indicate that perturbations within marine systems are driving change in the biotic components of that system (Purcell et al. 2007; Dong et al. 2010). The reported increasing dominance of jellyfish in impacted ecosystems has led to the perception they are more tolerant of poor environmental conditions than many other marine species (Arai 1997, Purcell et al. 2007; Stoner et al. 2011). Given this perceived tolerance, it would therefore be useful, to be able to utilise a response variable like jellyfish to measure and hopefully identify the potential for irreversible change before it occurs.

4.1 Jellyfish taxonomy and ecology

The living Cnidaria comprise four classes: Anthozoa (corals and anemones), Hydrozoa (hydoids and bluebottles), Scyphozoa ('true' jellyfish) and Cubozoa ('box' jellyfish and irukandji). The Scyphozoa and Cubozoa are closely related and have very similar lifecycles (Arai 1997). Unlike their anthozoan relatives they possess two adult body forms: A) the conspicuous, (generally) pelagic medusae and, B) the solitary or colonial, inconspicuous benthic polyp

(Arai 1997). Scyphozoa can be distinguished from Cubozoa by the lack of a velum extending from the margin into the subumbrellar region (Arai 1997). Both Scyphozoa and Cubozoa are considered exclusively marine although some species can tolerate reduced salinities to about 15 parts per thousand (ppt) salinity (Arai 1997).

The Scyphozoa comprises four recognised orders: Stauromedusae, Coronatae, Semaeostomeae and Rhizostomeae (Arai 1997). Representatives of the Semaeostomeae and Rhizostomeae are most commonly encountered in tropical surface waters including the Great Barrier Reef and are the focus in this study. The Semaeostomeae include the genera *Aurelia* (Péron & Lesueur 1809) and *Cyanea* (Péron & Lesueur 1809). They are characterised by large saucer shaped bells with marginal tentacles, four oral arms surrounding a single mouth, and rhopalia in niches between lappets (Aria 1997). In contrast, Rhizostomeae lack marginal tentacles and have four pairs of oral arms arising from the central manubrium. These arms fuse to form multiple mouths or ostia (Arai 1997). The bell margin has eight or more lappets with marginal sense organs. Genera include *Cassiopea* (Péron & Lesueur 1809), *Mastigias* (Agassiz 1862) and *Netrostoma* (Schultze 1898). Some rhizostome species possess symbiotic dinoflagellates of the *Symbiodinium* genus and are photosynthetic.

Scyphozoan jellyfishes display alternation of generation with both sexual and asexual phases. The most visually conspicuous lifestage is the pelagic medusae. Due to the ability of some jellyfish species to form large blooms of medusae, it is this lifestage that can cause economic and health issues, but is also the lifestage targeted in jellyfish fisheries (Kingsford et al. 2000; Kitamura & Omori 2010). Medusae tend to be gonochoristic, broadcast spawners with the resulting fertile planulae being motile for a few days to weeks before settling to a hard substrate (Fitt & Costley 1998; Bridge et al. 2004). Settled planulae develop into the smaller and more inconspicuous alternate adult phase: the polyp. For some species, the polyp lifestage has not been identified and is therefore still poorly understood (Arai 1997). Polyps are able to undergo asexual reproduction by multiple pathways to produce either additional polyps via budding, or generate the early stage ephyra via mono- or poly- strobilation (Arai 1997; Bridge et al. 2004). These ephyra subsequently develop into the much larger medusae.

Until recently, Cubozoa were included as an order within the Scyphozoa (Kramp 1961; Arai 1997). The Cubozoa have a similar life cycle to the Scyphozoa with alternation of generation between a small inconspicuous polyp and a larger (compared with polyp phase) medusae. However, cubozoan polyps tend to undergo metamorphosis to a single medusa rather than mono- or poly-strobilation as found in Scyphozoa. Typically, Cubozoa are identified by their "Box" shaped bell and the presence of a velum (Arai 1997). Tentacles may be singular or multiple, arising from the four corners of the bell and are an identifying feature of this class. The Cubozoa include *Chironex fleckerii* (Southcott 1956), *Carukia barnesii* (Southcott 1967),

Alatina mordens (Gershwin, 2005) and *Copula sivickisi* (Stiasny 1926) (reviewed in Kramp 1961; Bentlage et al. 2010).

Cubozoan jellyfish are found in tropical / sub-tropical waters around the world (Kramp 1961). The extent of species distribution is subject to debate among taxonomists with the taxonomy of species being regularly revised (e.g. Bentlage et al. 2010). Cubozoan abundance tends to be seasonal although they may be present all year round in tropical waters (Hartwick 1991). Despite the small size of many cubozoan jellyfishes, they have very potent toxins which have resulted in a number of deaths worldwide (Burnett 1991; Hartwick 1991; Fenner & Hadok 2002; Little et al. 2006). Due to the cryptic nature of their polyps and the patchiness of distribution, the ecology of many cubozoans is still poorly understood (Straehler-Pohl & Jarms 2011).

4.2 Biomonitoring and/or bioindicator potential of jellyfishes

Despite the perception of jellyfish as ecological and trophic dead ends (Verity & Smetacek 1996; Stoner et al. 2011), other research has revealed that they are ubiquitous components of marine systems, occupy many important ecological niches both as predator and prey (e.g. Brandon & Cutress 1985; Ates 1991; Arai 1997; Arai 2005; Richardson et al. 2009), and are important in recycling of nutrients (Pitt et al. 2009).

Some observations suggest that jellyfishes may be tolerant of pollution (e.g. Calton & Burnett 1981; Arai 1997). There is, however, also a body of work that suggests that jellyfish are sensitive to metal and hydrocarbons (Spangenberg 1984; Spangenberg 1986). This paradox is similar to that seen in some molluscs (e.g. oysters) where early lifestages are very sensitive to pollutants while adults are considered good bioaccumulations of pollutants and are widely utilised as biomonitors (e.g. Harrison et al. 1984; Hunt & Anderson 1993; Rainbow & Phillips 1993; Rainbow 1995). In combination with the life history traits of jellyfish, this suggests that further exploration of their potential as biomonitors and / or bioindicators is warranted.

Data on the bioaccumulative capacity of jellyfishes are scarce but there is some indication that they are capable of accumulating metals above ambient concentrations (Romeo et al. 1987; Hanaoka et al. 2001; Fowler et al. 2004; Templeman & Kingsford 2010). The range and quantification of elemental bioaccumulative capacities is lacking though, and needs to be addressed more fully to assess the biomonitoring and bioindicator potential jellyfishes.

5. Aims

The overall objective of this study was to assess the bioaccumulative and bioindicator capacity of jellyfishes for trace elements. These responses would indicate whether there is merit in utilising jellyfishes as marine biomonitors and / or bioindicators for trace element pollution.

The specific aims to meet this objective were to:

- Determine whether jellyfishes accumulate trace elements in marine environments and compare spatial and temporal variation among species to determine extent of accumulation on the Great Barrier Reef (GBR) (Chapter 2);
- Measure the uptake and retention capacity, and the biological half-life of aqueous copper or zinc in *Cassiopea* sp., thereby quantifying their biomonitoring capacity (Chapter 3);
- Establish the bioindicator potential of jellyfishes by deriving acute toxicity responses (LC_{50}) of three jellyfish species to copper or zinc for the different lifestages (Chapter 4); and,
- Quantitatively assess the bioindicator potential of *Cassiopea* sp. and it's endosymbiont to copper and zinc stress using a novel sub-lethal bioassay (Chapter 5).

Chapter 2 - Variation in Soft Tissue Chemistry Among Scyphozoan and Cubozoan Jellyfishes from the Great Barrier Reef, Australia

1. Introduction

Knowledge of environmental levels of dissolved metals in marine waters is important for monitoring ecosystem health. A large body of research exists on the levels, fluxes and cycling of chemicals in marine environments (e.g. Sadiq 1992; Luoma & Rainbow 2008), however, concentrations of metals do not necessarily reflect their ecological significance to biota. The use of organisms as marine biomonitors is an important tool for understanding how time-integrated changes in water quality can affect the diversity and abundance of local biota (Bresler et al. 2003; Luoma & Rainbow 2008; Creighton & Twining 2010). The term biomonitor, in this context, is defined as the ability to accumulate metals from the surrounding environment in an organism's tissues (Luoma & Rainbow 2008).

The ability to absorb, store and detoxify metals is important for organisms exposed to dissolved metals in the aquatic environment, as exposure and accumulation above a threshold can be damaging or deleterious (e.g. Chapman 1995). A wide variety of invertebrates and vertebrates have been investigated to determine their ability to regulate or accumulate dissolved metals (e.g. Benson & Summons 1981; Rainbow & Phillips 1993; Ruus et al. 2005). The ability of some organisms (e.g. barnacles, molluscs) to readily accumulate metals makes them very useful as marine biomonitors (e.g. Rainbow & Phillips 1993; Bresler et al. 2003). Unfortunately, there are few published data on accumulation of dissolved metals in scyphozoan jellyfishes (Templeman & Kingsford 2010) and no reported elemental tissue concentrations for cubozoan jellyfishes. However, from the few data that exist, jellyfishes do seem to have the capacity to absorb an elemental load that can alter the relative concentrations in the surrounding seawater (Heymans & Baird 2000; Kingsford et al. 2000; Fukuda & Naganuma 2001; Hay 2006; Pitt et al. 2009; Jantzen et al. 2010). In addition, due to their ability to consume plankton from large volumes of water, low ambient concentrations of dissolved metals may still result in high body loads in jellyfish.

Metals in tissues can be classified as essential or non-essential elements (Chapter 1; Luoma & Rainbow 2008). Trace amounts of these elements are essential to metabolic activity in organisms, but levels either below or above a narrow concentration range can result in sub-optimal health. In general, organisms are able to regulate essential elemental concentrations to

meet their metabolic requirements through storage in a detoxified form or excretion if thresholds are exceeded (Rainbow 2007). In contrast, non-essential elements have no identified role in metabolic activity but can also be accumulated in tissues above ambient concentrations.

Accumulation of dissolved metals in jellyfish has the potential to influence the health of higher-ordered predators through trophic transfer (Kingsford et al. 2000). Despite historic arguments of jellyfish as 'trophic dead ends' (Verity & Smetacek 1996; Stoner et al. 2011), other evidence (Purcell & Arai 2001; Arai 2005; Pauly et al. 2009) suggests that jellyfish form a significant proportion of the diet of many marine animals, including other gelatinous zooplankton (Purcell 1991), cephalopods (Heeger et al. 1992), *Fungia* sp. (Alamaru et al. 2009), nudibranchs (reviewed in Arai 2005), turtles (Caurant et al. 1999), seabirds (Harrison 1984), and fish (Pauly et al. 2009). Furthermore, jellyfish fisheries are important industries providing dried tissue for human consumption, particularly in Asian cuisine (Kingsford et al. 2000; Kitamura & Omori 2010).

To determine whether jellyfishes play any role in the biotransfer of trace elements within marine food webs requires information on the elemental loads in jellyfish tissues. Thus, the objectives of this study were to: 1) determine elemental concentrations in scyphozoan and cubozoan jellyfish collected from multiple locations along the Great Barrier Reef (GBR); 2) determine the extent of elemental accumulation above ambient seawater for each jellyfish species; and, 3) assess whether there were variations in elemental concentrations within tissues among species, time and location.

2. Materials and methods

2.1 Specimen and water collection and handling

Jellyfish and water samples were collected between December 2007 and March 2010 from multiple locations along the Great Barrier Reef (GBR), Australia (Figure 2.1; Table 2.1). In order to obtain a range of jellyfish species, sampling was conducted annually at four latitudes and multiple distances from the mainland (coast, inner, mid- and outer-shelf). In addition, opportunistic collections of medusae were made at coastal locations when possible. Due to the patchy nature of jellyfish abundances, species were not able to be collected at each location at each sampling event.

Medusae of five species of Scyphozoa and two species of Cubozoa were collected at or near the surface using either dip or seine nets, with targeted collections during the day or under lights (1000W) at night. *Cassiopea* sp. were collected in plastic bags by SCUBA divers at depths between 7 and 12 m. At least three medusae were collected at each sampling location except

one (Table 2.1). Surface water samples were collected at the same time as the medusae, except for a single collection of *Chironex fleckeri*, when no water samples could be taken. All equipment and containers used in jellyfish collections and processing were cleaned using 10 % nitric acid (HNO_3), triple rinsed in deionised water and allowed to air dry in a Class 100 laminar flow unit before use. Sampling containers were stored in clean, plastic bags until needed to avoid metal contamination.

After collection, jellyfish were rinsed with seawater from the collection location to remove any visible sediment or other material adhering to the animals. Animals less than 40 mm in diameter were placed in clean, acid-washed vials. Animals greater than 40 mm in diameter were sub-sampled using a corer consisting of an acid-washed 30 mL plastic vial. Five random cores were taken from the swimming bell, stomach, gonads (if present), oral arms, and tentacles of each medusa. Due to their small size, 4 to 5 *Copula sivickisi* medusae were pooled per replicate sample. Tissue samples were frozen as soon as possible and kept at –18 ^{0}C until processed.

Duplicate 30 mL water samples were collected at the water surface, immediately filtered through a 0.45 µm pore syringe filter, and stored in acid-washed vials. Water samples were acidified on-site with 20 % Suprapur grade HNO_3 and stored at 4 ^{0}C until analysis.

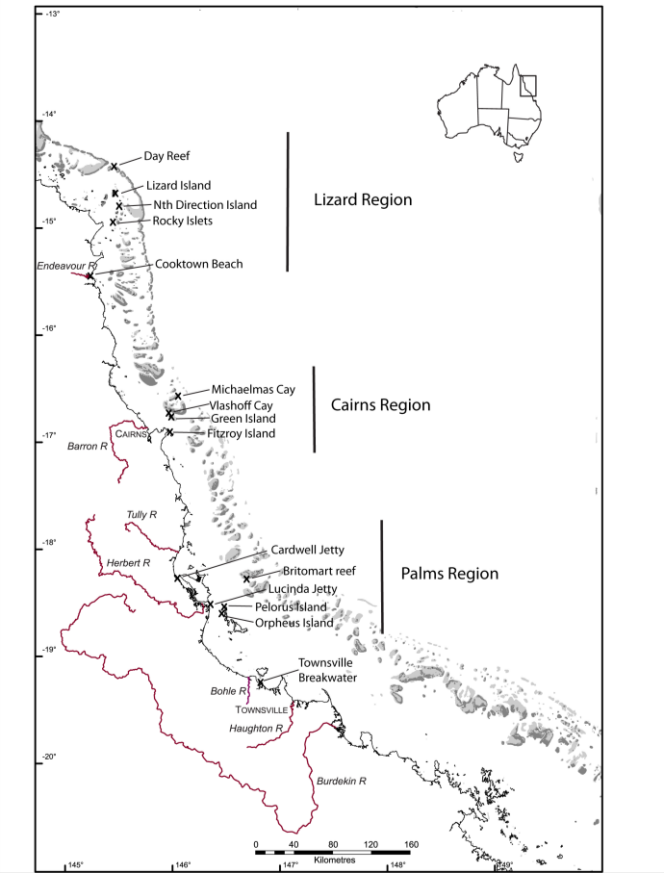


Figure 2.1: Field sampling locations for jellyfish collections between 2007 and 2010 along the Great Barrier Reef, Australia. Locations are separated by region.

Table 2.1: Sampling locations for elemental analysis of medusae collected between 2007 and 2010. Shelf locations represent position across the Great Barrier Reef. Sample coding used in the legend of Figure 2.4. *C. sivickisi* = *Copula sivickisi*; *C. fleckeri* = *Chironex fleckerii.*

Collection date	Sampling site	Region	Shelf location	Species	No. medusae	Sample coding
10 Dec 2007	North Direction Island	Lizard	Mid	*Aurelia* sp.	5	A1
11 Dec 2007	Day Reef	Lizard	Outer	*Aurelia* sp.	5	A2
07 Jan 2008	Michaelmas Reef	Cairns	Outer	*Aurelia* sp.	5	A3
09 Feb 2008	Britomart Reef	Palms	Mid	*Aurelia* sp.	5	A4
10 Feb 2010	Britomart Reef	Palms	Mid	*Aurelia* sp.	3	A5
11 Dec 2007	Mermaid Bay	Lizard	Mid	*C. sivickisi*	3	B1
13 Dec 2008	Mermaid Bay	Lizard	Mid	*C. sivickisi*	4	B2
20 Jan 2009	Green Island	Cairns	Mid	*C. sivickisi*	5	B3
11 Dec 2007	Mermaid Bay	Lizard	Mid	*Mastigias* sp.	5	C1
14 Dec 2008	Mermaid Bay	Lizard	Mid	*Mastigias* sp.	2	C2
17 Dec 2009	Rocky Islets	Lizard	Inner	*Mastigias* sp.	1	C3
18 Dec 2009	Cooktown Beach	Lizard	Coastal	*Mastigias* sp.	11	C4
29 Dec 2009	Townsville Breakwater	Townsville	Coastal	*Mastigias* sp.	3	C5
09 Jan 2008	Fitzroy Island	Cairns	Coastal	*Netrostoma* sp.	5	D1
22 Jan 2009	Michaelmas Reef	Cairns	Outer	*Netrostoma* sp.	5	D2
09 Feb 2010	Pelorus Island	Palms	Inner	*Netrostoma* sp.	3	D3
09 Feb 2010	Orpheus island	Palms	Inner	*Netrostoma* sp.	4	D4
07 Feb 2008	Pelorus Island	Palms	Inner	*Cyanea* sp.	5	E1
15 Mar 2008	Townsville Breakwater	Townsville	Coastal	*Cyanea* sp.	5	E2
09 Feb 2010	Lucinda Jetty	Palms	Coastal	*Cyanea* sp.	10	E3
14 Dec 2008	Lizard Island Lagoon 1	Lizard	Mid	*Cassiopea* sp.	5	F1
14 Dec 2008	Lizard island Lagoon 2	Lizard	Mid	*Cassiopea* sp.	5	F2
21 Jan 2009	Vlashoff Cay	Cairns	Mid	*Cassiopea* sp.	6	F3
17 Dec 2009	Lizard Island Lagoon 1	Lizard	Mid	*Cassiopea* sp.	5	F4
17 Dec 2009	Lizard island Lagoon 2	Lizard	Mid	*Cassiopea* sp.	11	F5
05 Jan 2010	Vlashoff Cay	Cairns	Mid	*Cassiopea* sp.	9	F6
18 Feb 2010	Cardwell Jetty	Palms	Coastal	*C. fleckerii*	3	G1

2.2 Tissue processing and analysis

Tissue samples for subsequent chemical analysis were digested with a heated acid solution. Samples ranging from 0.3 g to 3.0 g wet weight were digested in 5 mL concentrated (69 %) Suprapur grade HNO_3 on a hot plate for approximately 2 hours. Samples were evaporated to approximately 2 mL. To remove residual organic carbon and colour, 3 to 5 mL of AR grade hydrogen peroxide was added to the digested samples. Once all samples were clear with no residual colour, they were evaporated again to a final volume of approximately 2 mL. Samples were cooled to room temperature and then brought to a final volume of 25 mL with Milli-Q water. This digestion method is similar to that used previously for jellyfish tissue digestions (Templeman & Kingsford 2010).

Both digested tissue and water samples were analysed using a Varian 820-MS Inductively Coupled Plasma Mass Spectrometer (ICP-MS) and Varian Liberty Series II Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). ICP-MS was used to determine aluminium (Al), arsenic (As), barium (Ba), copper (Cu), cadmium (Cd), chromium (Cr), lithium (Li), manganese (Mn), lead (Pb), strontium (Sr), and zinc (Zn), while ICP-AES was used to measure calcium (Ca), magnesium (Mg) and iron (Fe). The detection limit was 0.1 $\mu g.L^{-1}$ for Ba, Cr, Cu, Li, Mg, Mn, and Sr, 0.05 $\mu g.L^{-1}$ for Pb and Cd, 0.5 $\mu g.L^{-1}$ for Al, 1.0 $\mu g.L^{-1}$ for As and Fe, 2.0 $\mu g.L^{-1}$ for Zn, and 10.0 $\mu g.L^{-1}$ for Ca. Elements chosen for analysis were based on either their importance as essential metabolic elements or their consideration as anthropogenic or priority pollutants. Due to issues with signal suppression, it was necessary to dilute seawater samples 1:10 (seawater:diluent) prior to analysis.

Indium, gallium and yttrium were used as internal standards to correct for potential instrument drift and matrix effects. Subsets of samples were spiked with known concentrations of all elements for quality control and to determine recoveries. With the exception of a low Pb and Zn recovery for one sample batch (72%), recoveries were good and ranged from (80 - 116 %). Analytical data were checked to ensure that signal strength of results exceeded three standard deviations for all analyses. Digestion blanks were included to ensure integrity of the digestion process. Digestion blanks had low levels of contamination; therefore, tissue data were corrected for blank results before statistical analyses.

2.3 Data analysis

Bioconcentrations were calculated by dividing the metal concentration in tissue by the metal concentration in seawater for each species (Sadiq 1992; Parametrix 1995). Univariate data for distance from the mainland were analysed with one-way ANOVA (Statistica Version 9.0); data were transformed where necessary to try and meet assumptions of normality and homogeneity of variance (Bartlett's Test). If data could not meet the assumptions after transformation they

were only considered significant if $p < 0.01$ (Underwood 1997). Principal components analysis (PCA) was performed using SYSTAT Version 10 (Crane Software) after log n+1 transformation to describe spatial and temporal variation in multi-element signatures, following the recommendations of Legendre & Legendre (2003).

3. Results

3.1 Elemental concentrations in jellyfishes

Concentrations of elements in jellyfish tissues varied among species, with a range of values found among both years and locations (Table 2.2). The cubozoan *Copula sivickisi* had the highest mean concentration of most elements except for the osmoconforming elements Ca, Mg and Sr (Table 2.2). The other cubozoan species, *Chironex fleckeri*, had much lower concentrations of all elements except for Mg, Ca, Sr and Fe than did *C. sivickisi*.

Among the scyphozoans, the rhizostome jellyfishes with symbiotic zooxanthellae generally had higher mean tissue element concentrations than asymbiotic species. Among the symbiotic species, *Cassiopea* sp. and *Mastigias* sp. had higher concentrations of most elements than did *Netrostoma* sp. (Table 2.2). *Cassiopea* sp. also had higher concentrations of Al, Ba, Cd, Cu, Fe, Mn, and Sr than all other scyphozoan species. Among the Semaestomeae, *Cyanea* sp. typically had higher concentrations of most elements than *Aurelia* sp. The major osmoconforming elements of Ca, Li, Mg, and Sr were similar across all scyphozoan species (Table 2.2).

The concentration range for individual elements differed among species in a similar way to the mean concentrations. *Cassiopea* sp. and *C. sivickisi* had high variation in elemental concentrations for many elements. For *Cassiopea* sp. there were large variations in Al (4-2840 $\mu g.kg^{-1}$), Cr (1.6-276 $\mu g.kg^{-1}$) and Cu (48-261 $\mu g.kg^{-1}$). In *C. sivickisi* the elemental variation in was greatest for Al (65-5883 $\mu g.kg^{-1}$), Cr (12-349 $\mu g.kg^{-1}$) and Fe (751- 5216 $\mu g.kg^{-1}$) (Table 2.2). *Cyanea* sp. also showed high variation among locations and years for As (53-2100 $\mu g.kg^{-1}$) and Fe (74-3440 $\mu g.kg^{-1}$), while the concentration ranges tended to be lower for *Aurelia* sp. and *Netrostoma* sp. for most elements (Table 2.2).

Table 2.2: Mean and range of elemental tissue concentrations by species and in the water. Results are combined data from all years and locations. All concentrations in jellyfish tissues are given in $\mu g.kg^{-1}$ wet weight except calcium and magnesium, which were measured as $mg.kg^{-1}$ wet weight. Numbers in parentheses alongside species represents the number of animals collected. For water samples, all concentrations are given in $\mu g.L^{-1}$ except Ca, Mg, and Sr, which were measured in $mg.L^{-1}$. Aluminium (Al), arsenic (As), barium (Ba), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), manganese (Mn), lead (Pb), strontium (Sr), and zinc (Zn), * represents the number of pooled samples. < DL = less than reported detection limit.

Species		Al	As	Ba	Ca	Cd	Cr	Cu	Fe	Li	Mg	Mn	Pb	Sr	Zn
Aurelia sp. (23)	Mean	11.4	63.0	32.9	348	5.72	6.58	15.0	82.3	154	1118	12.8	1.51	6380	93.7
	Range	1.78-58.9	5.45-135	9.19-125	293-400	2.50-12.6	1.86-9.80	8.10-43.0	12.4-455	131-208	970-1230	6.80-22.2	0.55-6.56	4800-8750	47.0-313
Water		<DL-1.41	<DL	<DL-4.88	359-395	0.45-7.16	<DL-7.66	<DL-3.03	<DL	127-145	1130-1235	<DL-2.48	<DL	6.40-8.70	<DL-8.21
Cassiopea sp. (41)	Mean	519	186	173	438	33.2	44.9	86.0	2756	120	1116	193	9.49	28267	572
	Range	4.24-2840	65.4-313	18.7-1670	295-811	10.4-69.6	1.57-276	48.2-261	1147-5510	11.4-226	809-1620	68.3-522	0.61-60.0	11800-73900	298-1980
Water		<DL	<DL	5.22-5.94	363-444	<DL-3.77	<DL	<DL-5.23	<DL	144-182	1037-1280	<DL-3.71	<DL-0.14	7.72-9.11	<DL-2.35
Chironex fleckerii (3)	Mean	157	236	17.9	260	0.52	3.21	65.3	681	81.2	765	57.5	3.05	5023	553
	Range	92.5-206	139-365	15.2-22.9	255-268	0.50-0.57	2.39-4.49	42.4-84	374-901	71.3-90.6	737-816	44.2-84.0	2.06-4.57	4680-5380	314-714
*Copula sivickisi** (12)	Mean	1266	4137	50.9	609	410	90.0	475	3391	173	1357	224	115	10913	6028
	Range	64.6-5883	2380-5806	13.4-144	372-1040	220-691	11.8-349	234-751	751-5216	123-282	900-1980	102-382	12.1-207	6564-19400	4280-9292
Water		<DL-10.9	<DL	<DL-6.14	364-378	0.70-2.96	<DL	<DL	<DL	145-180	1030-1175	<DL-2.52	<DL-1.44	6.60-8.77	<DL
Cyanea sp. (20)	Mean	75.3	473	37.3	263	38.7	11.1	63.3	908	114	865	22.2	2.41	5437	537
	Range	7.35-636	52.8-2100	6.63-180	210-329	4.23-184	1.19-22.7	24.2-116	73.9-3440	89.3-142	6652-1110	5.11-85.1	0.74-7.55	4880-6290	109-1600
Water		<DL-1.17	<DL	<DL-8.11	317-380	<DL-1.48	<DL-8.6	<DL-1.97	<DL	107-182	974-1120	2.09-7.52	<DL-0.74	5.80-7.23	<DL-11.62
Mastigias sp. (22)	Mean	160	177	59.5	353	12.0	17.3	50.8	981	144	1124	91.8	18.6	8002	586
	Range	3.99-468	61.5-542	7.88-142	326-385	5.68-23.1	0.80-35.2	19.6-74.3	130-1940	123-170	1030-1210	12.8-161	2.27-35	6690-9460	203-1100
Water		<DL-10.9	<DL	<DL-13.0	364-378	0.70-3.23	<DL	<DL-1.78	<DL	146-188	1030-1175	<DL-16.95	<DL-1.44	6.60-8.77	<DL-5.83
Netrostoma sp. (17)	Mean	37.1	104	27.3	335	12.0	7.94	31.8	280	140	1056	20.7	3.83	6974	295
	Range	12.4-115	9.84-200	5.57-79.3	282-419	3.01-33.6	1.20-21.0	13.3-80.3	26.2-1490	103-171	872-1320	5.76-41.1	0.56-29.8	5750-9270	65.7-954
Water		<DL-2.58	<DL	<DL-5.50	317-374	0.51-1.43	<DL-4.58	<DL-4.41	<DL	123-151	1045-1132	<DL-2.53	<DL	6.06-8.59	<DL-8.36

3.2 Bioaccumulation of elements by jellyfishes

Bioaccumulation differed among elements and among species (Figure 2.2). Except for a few individual samples, tissue concentrations of all elements were present above detectable levels at all locations in all years (Table 2.2). Water concentrations of As and Fe were below the detection limit for all water samples and were excluded from the analysis to avoid skewing the results (Table 2.2). In addition, other elements were below detection in water samples at some locations (Table 2.2). Samples where water concentrations were below detection, but measurable in the tissues, also were excluded from the analysis because bioaccumulation could not be calculated. Tissue concentrations of Cr, Cu and Zn were above detection in *C. sivickisi* but these elements were below detection in the seawater samples where this species was collected (Table 2.2). Similarly, although concentrations of Pb were above detection in *Aurelia* sp. and *Netrostoma* sp. tissues and Al in *Cassiopea* sp. tissue, water concentrations were not. Water concentrations of Cr were below the detection limit at all sites where *Cassiopea* sp., *C. sivickisi* and *Mastigias* sp. were collected (Table 2.2).

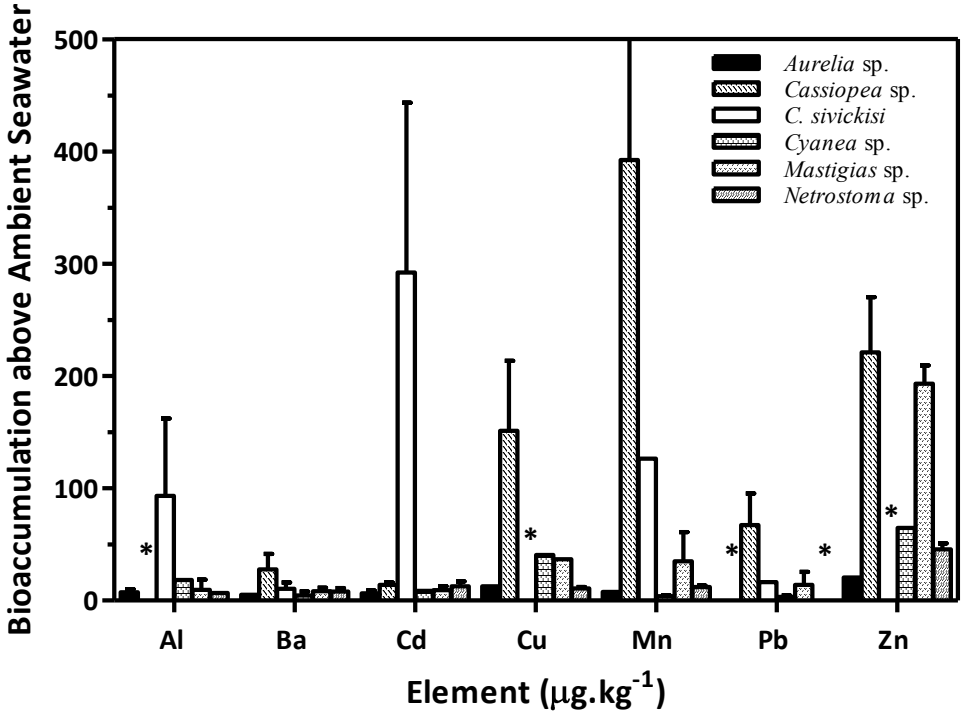


Figure 2.2: Bioaccumulation of elements above ambient seawater concentration in jellyfish tissues. Data pooled from all locations and all years for each species. * indicates seawater concentration below detection level for individual species (data removed from plot). Bioaccumulation = metal concentration in tissue (μg/kg) / metal concentration in seawater. Error bars represent standard errors.

C. sivickisi had the greatest accumulation among species for Al and Cd (Figure 2.2). Accumulation of Ba, Cu, Mn, Pb, and Zn was highest in *Cassiopea* sp. (28, 151, 392, 67 and 221 times seawater concentrations, respectively). *Mastigias* sp. also had high accumulation of Zn (193 times seawater). Copper accumulation was similar for *Cyanea* sp. (41 times) and *Mastigias* sp. (37 times), but much lower for *Aurelia* sp. (13 times) and *Netrostoma* sp. (11 times). *Netrostoma* sp. and *Aurelia* sp. generally had the lowest levels of accumulation among species, while the extent of accumulation in *Cyanea* sp. was element dependent (Figure 2.2). Lithium seemed to be actively regulated by *Cassiopea* sp., *Cyanea* sp. and *Mastigias* sp., with tissue concentrations of 0.71, 0.88 and 0.89 times the concentration of the ambient seawater, respectively. Calcium and magnesium were measurable in the tissues and present at concentrations similar to ambient water concentrations. Strontium was 3.3 times seawater concentration in *Cassiopea* sp. tissues as compared to 0.75-1.42 times seawater concentration for other species. Concentrations of Cr were twice the concentration in water for *Aurelia* sp., *Cyanea* sp. and *Netrostoma* sp.

3.3 Variation in elemental concentrations relative to distance offshore

Only *Mastigias* sp. and *Netrostoma* sp. were found at more than two distances (shelf locations) from the mainland over the three year sampling period. *Mastigias* sp. had an inverse relationship between tissue concentration and distance from the mainland for Al, As, Cu, Zn, and Fe (Figure 2.3a). The effect of distance from the mainland was significant for Al ($F_{2,18} = 101.8$, $p < 0.001$), As ($F_{2,18} = 10.712$, $p < 0.001$), Cu ($F_{2,18} = 107.1$, $p < 0.001$), Fe ($F_{2,18} = 185.4$, $p < 0.001$), and Zn ($F_{2,18} = 17.48$, $p < 0.001$). Other elements (Cd, Cr and Pb) showed no relationship with distance from the mainland.

In contrast to *Mastigias* sp., *Netrostoma* sp. tissue concentrations differed with distance from the mainland in that coastal and inner-shelf locations had similar concentrations while outer-shelf locations had lower concentrations (Figure 2.3b). Significant differences among distances were found for As ($F_{2,14} = 84.736$, $p < 0.001$), Cu ($F_{2,14} = 46.458$, $p < 0.001$), Fe ($F_{2,14} = 34.503$, $p < 0.001$), and Zn ($F_{2,14} = 18.563$, $p < 0.001$); however, Al was not significantly different ($p > 0.05$). Results were only considered significant if $p < 0.01$ because the data were heterogeneous (Underwood 1997).

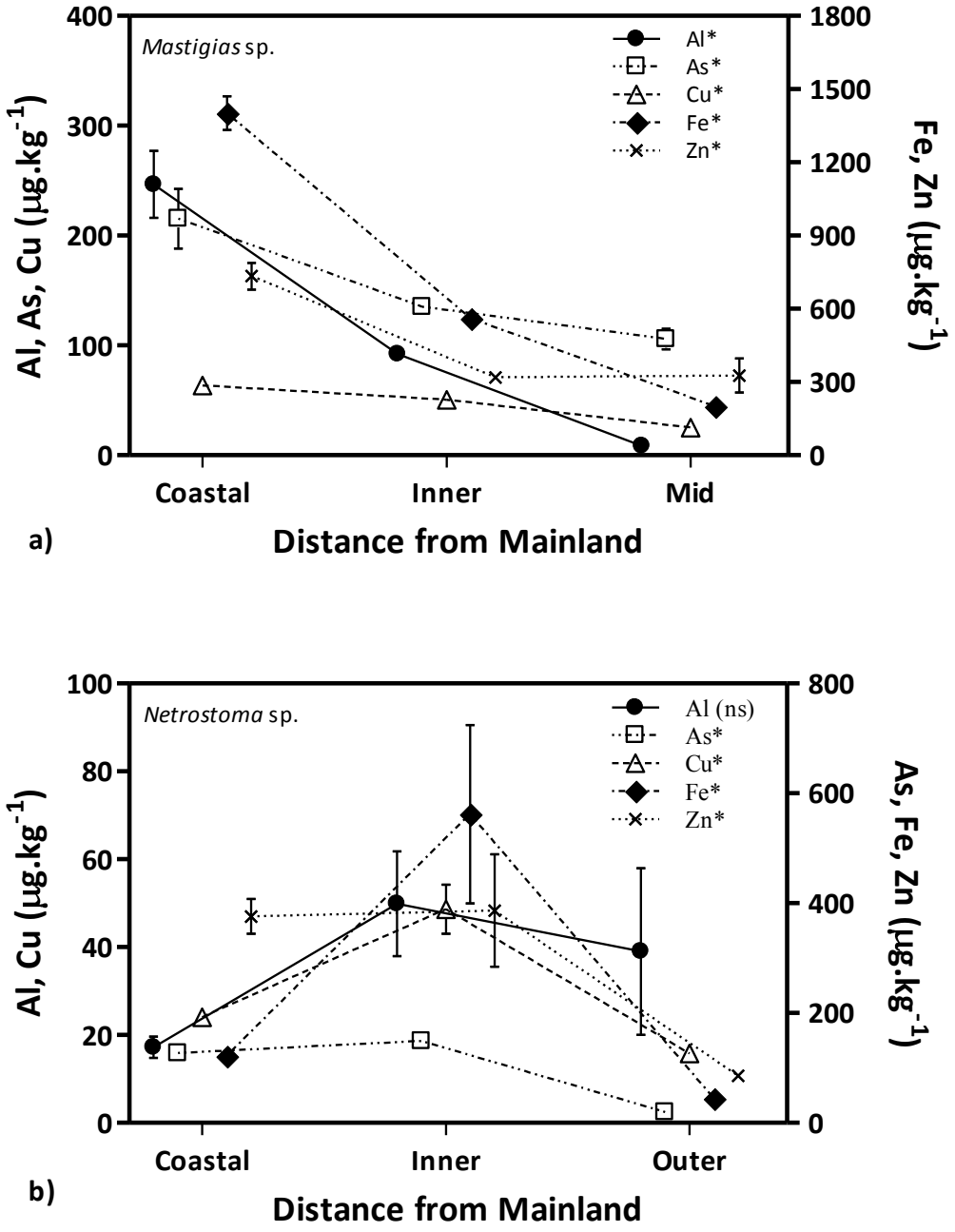


Figure 2.3: Variation in tissue concentrations of aluminium (Al), arsenic (As), copper (Cu), iron (Fe), and zinc (Zn) in µg.kg^{-1} wet weight with distance from shore for *Mastigias* sp. (a) and *Netrostoma* sp. medusae (b). Data pooled from all years. * represent significant differences ($p < 0.001$) among location for each element; ns = no significant difference. Data log n+1 transformed to meet ANOVA assumptions. Error bars represent standard errors.

3.4 Patterns in elemental fingerprints

Interpretation from the Principal Components Analysis (PCA) showed there was greater variation in elemental fingerprints among species than among years and locations (Figure 2.4). *Copula sivickisi* (B1-B3) and *Cassiopea* sp. (F1-F6) had different elemental fingerprints, but within each species there were no differences by location or time (Figure 2.4). In contrast,

elemental fingerprints of *Cyanea* sp. (E1-E3) differed by both location and time. The elemental fingerprints of both *Mastigias* sp. (C1-C5) and *Netrostoma* sp. (D1-D4) were more similar spatially, with distance from the mainland showing closer affinities than among locations in general (Figure 2.4). Despite having limited spatial association, *Aurelia* sp. (A1-A5) had a distinct temporal fingerprint, with 2007/08 samples (A1-A4) being more similar to each other than to other years (A5). Analyses of the ambient water collected with *Aurelia* sp. also showed temporal variation between 2007 / 08 and 2010.

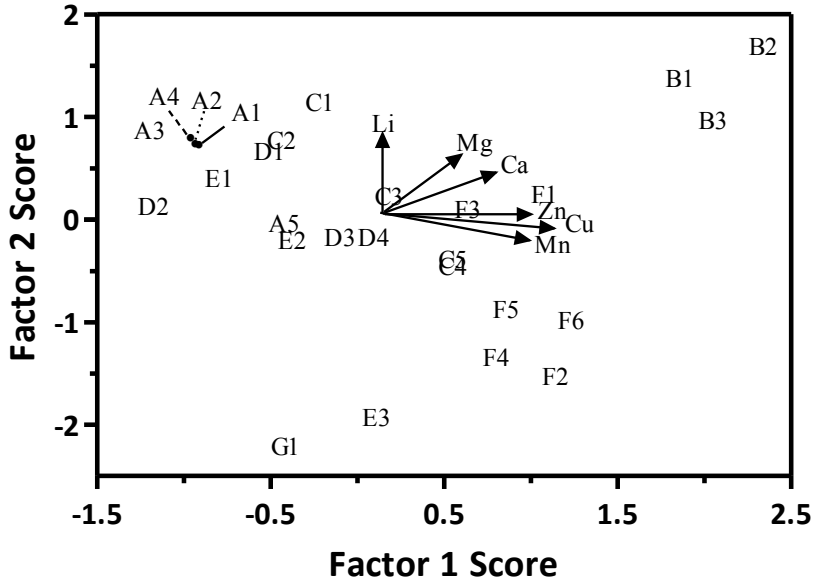


Figure 2.4: Results of multivariate Principle Components Analysis for multi-element signatures in $\mu g.kg^{-1}$ (wet weight) in jellyfish among locations and years. Percent of variation explained by Factor 1 = 58 % and Factor 2 = 16 %. Data logn+1 transformed prior to analysis to reduce contribution from elements with highest concentrations. Sample coding from Table 2.1.

Overall, total variance in the matrix was 74 %, with 58 % explained by Factor 1 and 16 % by Factor 2. Factor 1 was characterised by positive loadings for Cu, Mn and Zn (0.933, 0.915 and 0.894, respectively). Elements that were readily accumulated (i.e., Cu, Mn & Zn) influenced loadings on Factor 1. Factor 2 was characterised by positive loadings of 0.923, 0.732 and 0.516 for the osmoconforming elements Li, Mg and Ca, respectively. These results indicated that differences in salinity may be the driver for variations along the Factor 2 axis.

4. Discussion

Previous studies (e.g. Hanaoka et al. 2001; Fowler et al. 2004; Templeman & Kingsford 2010) demonstrated that jellyfishes and other gelatinous plankton are capable of absorbing trace

elements from the environment in measurable concentrations but most studies were limited in extent and species. This study demonstrated that both cubozoan and scyphozoan species accumulated elements above ambient water concentrations. The extent of accumulation varied among species depending on the element.

Accumulation of elements in tissues is dependent upon the species, speciation of the metal, uptake route, and organism sensitivity. For many marine fish, the bioconcentration factors for metals generally are less than 100 (Parametrix 1995), however, that is not typical for all marine organisms. Some invertebrates, including barnacles (Rainbow & Wang 2001) and the mussel *Mytilus edulis* (Talbot 1987), are very efficient bioconcentrators of metals, sometimes exceeding 1000 times ambient metal concentrations. The accumulative capacity was very high in two of the three symbiotic species (*Cassiopea* sp. & *Mastigias* sp.), and greater for some elements than others (Figure 2.2). *C. sivickisi* also readily accumulated Cd and Al above ambient water concentrations (Figure 2.2).

Typically, ideal biomonitor species should meet a number of criteria including behaviour, abundance, robustness, and bioaccumulative capacity (Luoma & Rainbow 2008). Sedentary behaviour is one criterion, as it can provide a time-integrated measure of bioavailable metals from a defined location (Rainbow & Phillips 1993). For this reason, biomonitoring research has often focused on sessile species like mussels and barnacles, although other species that are representative of the study location also can be used (Luoma & Rainbow 2008). As a group, jellyfishes are not sedentary, except for the upside-down jellyfish *Cassiopea* sp. Despite the patchy nature of jellyfish distributions, their ability to form large conspicuous blooms means they can constitute a significant portion of the biomass in local areas and may be the most visible representative at a given location (e.g. Graham et al. 2003; Dybas 2006). There is also evidence that many populations may be geographically constrained within bays and estuaries (e.g. Ishii & Bamstedt 1998; Pitt & Kingsford 2000; Purcell et al. 2000; Arai 2001). This aggregating behaviour, combined with the ability to accumulate metals, suggests that sedentary traits may not be a strong requirement for jellyfish to be considered useful biomonitors.

Size and age can be important in understanding the extent and intensity of metal accumulation (Rainbow & Phillips 1993). For many species, when age is unknown it can be inferred from size (e.g. Denney et al. 2002). Scyphozoan and cubozoans possess the capability to undergo degrowth (shrinkage) during periods of physiological stress and due to this behaviour size is not a reliable proxy for age. Therefore it was not possible to use size as an age proxy to infer time integrated measures of accumulation.

For some elements (e.g. Al, As, Fe and Zn), *Mastigias* sp. and *Netrostoma* sp. collected at coastal and inshore locations had greater variation in tissue concentrations than animals

collected from mid- or off-shore locations (Figure 2.3). This variation is typical of coastal locations because terrestrial inputs, both natural and anthropogenic, and riverine contributions can result in greater fluctuations in water quality and metal bioavailability (e.g. Lopes et al. 2007). Thus, the greater variability in tissue concentrations in animals from coastal locations implied they were probably reflecting local water quality variability and could potentially be useful in monitoring coastal water quality.

Water quality monitoring on the GBR has indicated that overall contaminant levels are low; however, areas adjacent to urban activity and intensive agriculture have elevated levels of contaminants (Haynes & Johnson 2000). In addition, the distribution of dissolved metals can be strongly influenced by the presence of suspended particulate matter (Balls 1988), which may be influencing metal concentrations in coastal regions (in particular) along the GBR. Seawater samples were filtered before analysis in this study and therefore the contribution of particulate matter was not assessed, although it may have influenced uptake in the medusae through ingestion. The typically higher concentrations of suspended particulate matter in coastal locations may have also affected the bioavailability of elements in these locations (Balls 1988). This is turn may have contributed to variations in elemental concentrations in the jellyfish collected from coastal locations. Due to the patchiness of sampling that is inherent with jellyfish collections, it was not possible to obtain samples at multiple distances from the mainland for most species. *Mastigias* sp. and *Netrostoma* sp. were the only species where spatial variation could be measured, but pooling of data from multiple years was required (Figure 2.3). With the exception of copper and zinc in *Netrostoma* sp., the tissue elemental concentrations did not reflect the seawater concentrations. This was in part, due to the low concentrations of the elements in the water but also indicated that elemental bioaccumulation was occurring. The change in tissue elemental concentration with distance from the mainland showed that animals may be either maintaining their position in given locations or drifting but maintaining exposure to a given water quality type. The higher concentrations of Al, As, Cu, Fe, and Zn at coastal and inner locations for these species indicated the presence of a general nearshore metal signal from either anthropogenic inputs or riverine plumes (Haynes & Johnson 2000; Haynes & Michalek-Wagner 2000).

As previously identified, some trace elements have an essential role in maintaining organism health and a minimum tissue concentration is necessary to maintain health (Chapter 1). Among other requirements, copper is utilised by symbiotic jellyfish, for inducing superoxide dismutase activity to defend against oxygen radicals produced by symbiont photosynthesis (Harland & Nganro 1990). Zinc is a component of another enzyme, carbonic anhydrase, which is particularly abundant in organisms with symbiotic zooxanthellae (Furla et al. 2000). Zinc was present in all species (Table 2.2), with the highest tissue concentrations found in two

(*Cassiopea* sp. and *Mastigias* sp.) of the three symbiotic jellyfish species, but not in *Netrostoma* sp. (Figure 2.2), however, other elements with no identified essential role (e.g. Al, Cd, and Pb) also were accumulated by multiple jellyfish species (Figure 2.2).

Jellyfish may use multiple routes to absorb essential trace elements to maintain health. Elements that were present in elevated concentrations in tissues but below detection in ambient seawater (e.g. As and Fe) may have alternate uptake routes, such as from surface-adsorbed particles, diet or, in the case of *Cassiopea* sp., from sediment. For example, Jantzen et al. (2010) found that *Cassiopea* sp. demonstrated active bioturbation of sediment, which may potentially expose them to elevated metal concentrations found in pore waters or adsorbed to sedimentary particles. Metal uptake from dietary sources has been identified as a potentially significant exposure route (e.g. Depledge & Rainbow 1990; Rainbow & Wang 2001) and may have been a source of accumulated metals measured in the jellyfish tissues.

The multi-element signature in both *Cassiopea* sp. and *C. sivickisi* discriminated them from other species and each other (Figure 2.4), however, there was minimal evidence of any temporal or spatial patterns in either of these two species. Spatially, this may be due to limited sampling locations because both species were only collected in two locations (Table 2.1). *Aurelia* sp. also displayed similarity among locations, although there was temporal separation between 2007 / 08 and 2010 collections (Figure 2.4). In contrast, *Mastigias* sp. and *Netrostoma* sp. showed spatial variation in elemental fingerprints. Because they were collected at a greater number of locations, this implied that tissue concentrations may have been reflecting local environmental exposure; however, spatial data are limited, and additional collections would be useful to elucidate this relationship further.

The extent of accumulation of elements in jellyfish should be considered important given the number of organisms that have been identified to prey on them including fishes, reptiles, birds, crustacean, cephalopods, etc (Pauly et al. 2009). Trophic transfer is an important route for accumulation of contaminants, although accumulation is dependent on how and where contaminants are stored in the prey. Caurant et al. (1999) demonstrated that jellyfish may be a major source of cadmium accumulation in the diet of leatherback turtles. There has been minimal other direct evidence of contaminant accumulation through jellyfish diets, however, given that nudibranchs selectively absorb and utilise the nematocysts from jellyfish (Arai 2005) and incorporate and concentrate pigments (Bayer 1963 cited in Arai 2005), the potential exists for contaminant accumulation.

The behaviour of metals in the environment and the organismal response are affected by both spatial and temporal factors. Climatic conditions, residence time of elements in the water column, and fluxes between water, air, and sediment can all change elemental load and

exposure. These changes can occur as short duration 'pulses' or more sustained long term 'press' events (Chapter 1), and the degree and duration of exposure affects metabolic function in different ways (Ives & Carpenter 2007). In marine systems, proximity to the coast can affect elemental loads with both biotic and abiotic factors cycling elements between the water, sediment, biota, and atmosphere (Dauer et al. 2000). Depending on the element, terrestrial inputs can strongly influence both the presence and persistence of dissolved metals (Balls 1988; Haynes & Michalek-Wagner 2000).

The ability to accumulate trace elements can be useful as it provides the opportunity to monitor pulse or press measures of contaminant loads in marine ecosystems. The extent and duration of accumulation is critical to establishing time-integrated measures of marine water quality. A previous study on *Cassiopea* sp. showed they were able to accumulate metals (both essential and non-esential) above ambient seawater concentrations (Templeman & Kingsford 2010). In addition, there was distinct spatial variation in elemental concentrations between populations at both small (< 1 km) and large (1000 km) distances (Templeman & Kingsford 2010). However, the duration of elemental retention was not assessed in this study. Uptake and retention of aqueous copper and zinc by *Cassiopea* sp. is discussed in Chapter 3. However, further experimental studies would be required to elucidate and fully characterise the extent and duration of the accumulative capacity for other elements and jellyfish species.

In conclusion, multiple elements were found in tissues of seven scyphozoan and cubozoan jellyfish species on the Great Barrier Reef. Except for the major osmoconforming elements (Ca, Mg, and Li), tissue concentrations of elements in all species exceeded that of ambient seawater. Species differed in their abilities to accumulate the various elements, and temporal and spatial variation in tissue concentrations were also species dependent. However, the results indicated that jellyfish did accumulate and retain elements.

Chapter 3 - Bioconcentration and Retention of Copper and Zinc in *Cassiopea* sp.

1. Introduction

Jellyfishes have been shown to accumulate metals (e.g. Fowler et al. 2004; Templeman & Kingsford 2010). There has been very limited quantitative studies on metal bioconcentration in jellyfishes though (Fowler et al 2004). To understand the importance jellyfishes as biomonitors and their role in trophic transfer of metals, quantitative assessment of the bioaccumulative capacity is necessary.

Increases in population and associated industry in coastal regions have increased risks to marine ecosystems. Coastal waters around the world have been subjected to increasing discharge of anthropogenic contaminants from many and varied sources with detrimental consequences including increased sedimentation, nutrient pulses and chemical pollution (e.g. Sadiq 1992; Peters et al. 1997; Zhou et al. 2008). This combined with habitat degradation and destruction has resulted in systems that are less robust and ecologically diverse (Peters et al. 1997). Chemical pollution derives from a broad spectrum of contaminants from hydrocarbons and other organic compounds (PCBs etc) through to elemental metals. Anthropogenic metal discharge derives from many sources including mining, transport, sewage effluent, household leachates, corrosion protection, engine exhausts, etc (e.g. Peters et al. 1997; Cohen et al. 2001). Both riverine and storm runoff facilitate transport of these contaminants into coastal marine waters.

Metal pollution in marine and estuarine environments can affect biota directly as a result of metal toxicity. However, metals also have the potential for indirect toxic effects through bioaccumulation and biomagnification within and up the food chain. Because of the complexity of metal uptake, detoxification and excretion processes within organisms, it is difficult to directly predict all mechanisms and routes of bioaccumulation (Walker 1990). It is necessary though, to recognise the key chemical and biological processes affecting contaminant fluxes within organisms as well as the surrounding external medium (Walker 1990). The effects of metal bioaccumulation are an important aspect of understanding the fate of pollutants within aquatic systems and their impacts on system health and biodiversity.

Bioavailability and accumulation of trace elements in marine systems is strongly linked to the presence and speciation of the element/s in question as well as their ability to cross biological membranes and barriers (Worms et al. 2006). Uptake is influenced by both external and

internal chemistry of the metal, uptake pathways, metal source and the physicochemical nature of the internal and external media (Phillips & Rainbow 1989; Worms et al. 2006). Overall, there is general agreement with respect to many of the key interactions of trace elements and aquatic organisms. Uptake of metals from the water only is defined as metal bioconcentration, while metal bioaccumulation is considered uptake from all external sources: e.g. food, sediment as well as water (e.g. Neff 2002; Luoma & Rainbow 2005; Worms et al. 2006).

The differing physico-chemical conditions in aquatic environments including complexation and disassociation of metal complexes in water mean that control of trace metal uptake and regulation in aquatic animals is different than for terrestrial species (Philips & Rainbow 1989; Worms et al. 2006). Relevant factors that aquatic organisms must cope with include: 1) low solubilities of some essential metals; 2) active uptake of adequate amounts of essential elements under low ambient conditions while regulating excessive uptake; 3) non-selectivity of element uptake via certain metabolic pathways (e.g. metal ligands across the plasma membrane); and, 4) the co-uptake of non-essential elements with essential elements e.g. Cd with essential elements e.g. Zn (Phillips & Rainbow 1989; Worms et al. 2006). All of these factors can cause adverse effects if not adequately managed, and aquatic organisms have evolved a number of features to both facilitate and control uptake. These include mechanisms to control both the accumulation and excretion of unnecessary or excess concentrations of metals (both essential and non-essential). Mechanisms to facilitate uptake, detoxification, storage and excretion of metals include: physical exclusion mechanisms, secretion of metal binding chelates, induction of metallothioneins and other proteins, and incorporation of metals into insoluble intracellular granules (Phillips & Rainbow 1989; Neff 2002; Worms et al. 2006; Rainbow 2007). For marine organisms, relationships between the environment, and concentration and accumulation of metals vary among and within species. In many marine species, there is generally an inverse relationship between water and tissue concentrations for many metals suggesting metal regulation mechanisms (DeForest et al. 2007). Furthermore, acclimated organisms frequently display a greater capacity for accumulation and metal tolerance than non-metal acclimated species (Wang & Rainbow 2005).

Historically, most studies into metal uptake have explored the difference in body loads of metals compared to the ambient environmental concentrations (e.g. Benson & Summons 1981; Hanna & Muir 1990; Esslemont 2000). The mechanisms of uptake, accumulation and excretion are generally complex and dynamic, making them difficult to predict. As a result research has focussed on predicting patterns of accumulation according to trophic pathways. This has resulted in development of theoretical kinetic models that try to predict uptake and retention of metals in both terrestrial and aquatic species.

The initial kinetic models were developed for pharmacology and radioecology in the 1950s to predict the accumulative effects of commercially important pharmaceuticals and radioisotopes in laboratory animals (Wang 2002). This work was later expanded upon by aquatic scientists to attempt to quantify pollutant accumulation in aquatic biota. Accumulation of organic compounds and metals from water and food was predicted using first-order kinetics (Thomann 1981; Spacie & Hamelink 1982; Landrum et al. 1992; among others). The dynamics and fate of chemicals in exposed organisms was further explored through the development of higher order kinetic models to establish key uptake and clearance constants and derive kinetic bioconcentration factors (BCF_{kin}) and maximum theoretical loads (Luoma & Rainbow 2005). The use of short term uptake and clearance studies with the application of a two compartment kinetic model allows the description of uptake and retention processes to be simplified to these key parameters although the underlying biotic processes remain complex (Wang 2002).

Other models of accumulation also describe the pattern of uptake and retention of metals. For example, for some metals at some aqueous concentrations, the hyperbolic model is a better predictor of bioaccumulation than the two compartment model (e.g. Kahle & Zauke 2002; Clason et al. 2004). Despite the limitations that exist with model predictions, evolution of these models has allowed better understanding of the accumulation of metals within aquatic biota (Luoma & Rainbow 2005; Zauke 2008).

The models describe the accumulative and retentive capacity of an organism to a known metal contaminant. They can also discriminate between aqueous and dietary accumulation (e.g. Ke & Wang 2001). Kinetic factors derived from these models are important criteria for determining the potential of a given species as a biomonitor of metal pollution.

The objective of this study was to quantify the bioconcentration of aqueous copper or zinc in the Upside-down Jellyfish (*Cassiopea* sp.) and use the outcomes to assess the biomonitoring potential of this species. The study aimed to: 1) quantify the bioconcentration capacity of *Cassiopea* sp. for copper or zinc from the aqueous phase; 2) establish the kinetic bioconcentration factors (BCF_{kin}) and biological half-life ($t_{1/2}$) of copper or zinc in *Cassiopea* sp.; 3) establish which of two kinetic models best predicted bioconcentration of aqueous copper or zinc in *Cassiopea* sp.; and, 4) evaluate the potential of using *Cassiopea* sp. as a metal biomonitor.

2. Materials and methods

2.1 Summary

The Upside-down jellyfish (*Cassiopea* sp.) was used in this study. This jellyfish is found resting upside down in shallow tropical / sub-tropical estuaries and coastal marine waters. Bioconcentration and retention of copper or zinc was determined by measuring tissue concentrations of copper or zinc in animals that had been exposed to aqueous copper or zinc.

All medusae used in the tests were sourced from in-house collections. The parent stock of *Cassiopea* sp. polyps were obtained from spawning induction of medusae collected from Lake Magellan, an artificial marine lake located at Pelican Waters, Queensland. The culturing protocols for test organisms are detailed in Appendix A.

The study was conducted over a 28 day period with a 14 day uptake phase and 14 day clearance phase. This timeframe was selected to avoid confounding issues of jellyfish growth over the study period, and to avoid exceeding medusae loading limits (weight) in the experimental containers (ASTM 1997).

2.2 Test medusae

Approximately 130 *Cassiopea* sp. medusae with a mean size of 17 ± 2 mm were selected from a larger pool of animals. Pre-selected animals were placed in clean 10 litre holding containers of 20 µm filtered seawater and allowed to settle for 96 hours prior to the start of the experiment. Animals were fed with newly hatched *Artemia* sp. daily to ensure they were healthy and actively feeding. Only animals that looked healthy, were feeding well and showed no overt signs of deformity were used. All animals were approximately 8 weeks of age at test commencement. At test commencement, medusae were removed from the holding containers and randomly allocated to each of 4 replicate test chambers for the control and test treatments. The final number of 10 medusae were allocated to each replicate test chamber for the start of the study.

2.3 Cleaning and equipment preparation

Test chambers were prepared by initially calibrating 2 litre plastic ice-cream containers to 1 litre volume. The outflow from the test chamber was prepared by drilling 3 parallel holes at the 1 litre mark and screened with 500 µm plastic mesh held in place with aquarium safe silicon sealant. Tubing was prepared by joining 4 mm diameter food grade polyvinyl chloride tubing using 4 mm diameter plastic joiners and aquarium safe silicon sealant to Watson Marlow Marprene Double Manifold 1.02 mm diameter peristaltic tubing.

After all equipment was prepared, it was washed in phosphate free detergent, rinsed in tap water to remove any residual detergent and then soaked in 10 % AR grade nitric acid for a minimum of 12 hours. Acid soaked equipment was removed from the acid and rinsed three times with Milli-Q water and air dried in a Class 100 laminar flow unit. After drying, equipment was stored in clean plastic bags until required.

Holding drums, tubing and test chambers were equilibrated by pumping clean seawater through them for 48 hours prior to the commencement of the study. The holding drums were then emptied and refilled with the appropriate treatment or control water and solutions were pumped through the system for a minimum of 2 hours prior to start of the test. The pump was pre-calibrated to a flow of 3.5 $mL.min^{-1}$ which equated to approximately 5 litres per container per 24 hour period.

All tubing, containers and holding tanks were replaced with similar equipment that had been cleaned according to the method above at the end of the uptake phase of the study. This was to ensure there was no carryover of metals in the clearance phase of the study.

2.4 Control and test waters

The control / dilution water was sourced from collection ponds adjacent to the Australian Institute of Marine Science (AIMS) located approx 50 km south of Townsville, Queensland. The water was transported to the James Cook University (JCU) Marine and Aquaculture Facility (MARFU) using a contracted water tanker truck and stored in 60 000 L MARFU storage tanks. Water from the MARFU storage tanks was pumped to a 3000 L underfloor storage tank on demand. Water in the underfloor tank was filtered through a sand filter followed by a 20µm woven fibre cartridge filter before use and used as both culturing, control and dilution water. Control / dilution water was analysed regularly to determine background levels of trace elements. Some variability in background concentrations were measured when resupply to the storage tanks occurred, although elemental concentrations remained in the normal range for seawater.

A single experiment was conducted with a seawater control, a nominal 10 $\mu g.L^{-1}$ copper treatment and a nominal 50 $\mu g.L^{-1}$ zinc treatment. Concentrations were selected based on the outcomes of LC_{50} experiments conducted on *Cassiopea* sp. ephyra (Chapter 4). Test solutions were prepared by filling 3 x 100 litre black polythene holding drums with 80 litres of 20 µm pre-filtered seawater. Eight millilitres of the appropriately diluted stock solution (either $CuCl_2$ or $ZnSO_4$) was added to the copper and zinc treatment drums to provide the nominal 10 $\mu g.L^{-1}$ Cu or 50 $\mu g.L^{-1}$ Zn treatment solutions. The control treatment contained 20 µm pre-filtered seawater only. Each holding drum volume was sufficient for four days operation and drums were refilled every 96 hours for the duration of the uptake phase (14 days) of the study.

2.5 General experimental conditions

Lighting was provided by an Aqualina dual fluorescent reflector containing 2 x Dual CA PL-L 96W 10000K fluorescent tubes. The light regime was a 12:12 cycle. Light intensity was measured using a Li-COR meter for Photosynthetic Active Radiation (PAR) at the beginning of the experiment with the average PAR being 115 $\mu mol.m^2.s^{-1}$.

Air temperature in the laboratory was maintained at 25 ^{0}C using a reverse cycle airconditioning unit and monitored using a max-min thermometer to ensure the temperature did not fluctuate beyond the prescribed ±2 ^{0}C. Temperature of the control and treatment holding drums was monitored on days 0, 1, 2 and then every second day to ensure water remained within the range set out in the test acceptability criteria (Section 2.7). Due to damage sustained to the JCU MARFU facility during Cyclone Yasi (February 2011), the water temperature in the test chambers increased to 27 ^{0}C over the last 8 days of the experiment. Despite this increase in temperature there were no obvious indications of stress in the animals.

2.5.1 Jellyfish tissue collection and digestion

Animals were sampled on Day 0 (Test start), 1, 2, 4, 8, 14 (End of Uptake Phase & Day 0 of Clearance Phase), 15, 16, 18, 21, 28 of the study. At each sampling point, animals that were to be analysed were removed from the treatment and control tanks before feeding of the remaining jellyfish. This was done to ensure there was no confounding effect on the results from the presence of food in the gut. One animal from each replicate container was removed and placed in cleaned and acid washed 30 mL vials containing clean seawater to remove any weakly adsorbed copper or zinc. Individuals were placed into clean plastic 90 mm petri dishes containing seawater and bell diameter was measured to the nearest millimetre using a ruler. Animals were then removed from the seawater and accurately weighed using a Sartorius Genius ME analytical balance.

Individuals were placed in pre-cleaned acid washed 10 mL vials and stored at -18 ^{0}C until digested. The frozen jellyfish samples were digested within four weeks of collection. Tissues were digested using the nitric acid / hydrogen peroxide method from Templeman & Kingsford (2010). Due to the small size of the animals (<20mm diameter, <1 g wet weight), the final sample volume was 10 mL.

2.5.2 Physico-chemical and analytical measurements of water and tissues

Water quality parameters were measured on days 0, 1, 2 and then every second day on all treatment solutions for the duration of the study. The physico-chemical parameters measured were pH, salinity (ppt), temperature (^{0}C) and dissolved oxygen (% Saturation). Water quality measurements were performed using a pre-calibrated TPS WD-90 multi-parameter meter.

Water samples for analytical measurements were collected every second day and whenever holding tanks were refilled. Water samples were acidified immediately after collection with 20 % Suprapur grade nitric acid (HNO_3) and stored at 4 ^{0}C until analysed.

Water and digested tissue samples were analysed using a Varian 820-MS Inductively Coupled Plasma Mass Spectrometer (ICP-MS) and a Varian Liberty Series II Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). Control water was analysed for a suite of elements and treatment concentrations were analysed for the test metals only. To determine the baseline concentration of elements in the jellyfish, a subset of digested tissue samples from the control treatment was analysed for the full suite of elements (Table 3.1). The remaining control animals and the copper and zinc exposed animals were analysed for copper and zinc only.

ICP-MS was used to determine aluminium (Al), arsenic (As), barium (Ba), copper (Cu), cadmium (Cd), chromium (Cr), lithium (Li), manganese (Mn), lead (Pb), strontium (Sr) and zinc (Zn), while ICP-AES was used to measure calcium (Ca), magnesium (Mg) and iron (Fe). Due to issues with signal suppression, it was necessary to dilute the water samples 1:10 (seawater : diluent) prior to analysis. The detection limits varied among elements and between the sample matrices (Table 3.1). Subsets of samples were spiked with known concentrations of all elements for quality control purposes and to determine recoveries in water (78-126 %).

Due to the lack of an appropriate standard reference material, subsets of digested jellyfish samples were spiked with known concentrations of all elements for quality control purposes and to determine recoveries in digested tissues (85-127 %). Indium, gallium and yttrium were used as internal standards to correct for potential instrument drift and matrix effects. Digested tissue samples were diluted 1:2 (tissue : diluent) to minimise issues of signal suppression. Analytical data was checked to ensure signal strength exceeded three standard deviations for all analyses. Digestion blanks were included with all jellyfish digestions to ensure integrity of the process. Digestion blanks had low levels of elements and tissue data was corrected for blank results before statistical analysis.

Table 3.1: Detection limits for elements analysed by ICP-MS/AES (in $\mu g.L^{-1}$).

Sample	Al	As	Ba	Ca	Cd	Cr	Cu	Fe	Li	Mg	Mn	Pb	Sr	Zn
Water	5	10	0.2	10	0.1	2	1	20	0.2	0.2	0.2	0.5	0.2	5
Tissue	1	2	0.2	10	0.1	1	1	2	0.2	0.2	0.2	0.1	0.2	5

2.6 Testing procedure

At the start of the experiment, all water used to equilibrate the system was emptied from the test chambers and polythene drums, and tubing was pumped dry. The pump setup comprised an

Altivar 31H variable speed drive pump with Watson-Marlow multi-channel micro-cassette peristaltic pump (1.02 mm diameter).

The test was set up with 4 replicate chambers each for the Control, 10 $\mu g.L^{-1}$ copper treatment and 50 $\mu g.L^{-1}$ zinc treatment. Each replicate chamber had 10 animals randomly allocated to it. The maximum animal loading was 5 grams per litre at test commencement with a flow-through rate of 5 litres per 24 hours. One animal was randomly removed from each replicate chamber at each sampling point. Test chambers were covered using cleaned, clear semi-rigid plastic sheets to minimise evaporation and potential dust contamination.

Animals were fed every second day with a pre-measured volume of freshly hatched *Artemia* sp. The amount of food supplied was adjusted according to the number of animals within each replicate container. Any uneaten food or debris that was not flushed from the containers via the flow-through apparatus was removed using a clean acid-washed pipette after 24 hours.

Fresh treatment and control solutions were prepared every 4 days to minimise loss of metals through surface adsorption onto the drums. Duplicate water samples were taken every second day from each of the treatment and control tanks for the duration of the study. Water samples were acidified with 300 µL 20 %Univar grade nitric acid and stored at 4 ^{0}C until analysed.

After 14 days of metal exposure, animals were transferred to clean containers. All tubing and the holding tanks were replaced with clean equipment for the duration of the clearance phase. On Day 7 of the clearance phase (Day 21), animals were sampled at 0630 hours due to the impending arrival of a severe Category 5 cyclone.

2.7 Test acceptability

Standard testing methods recommend certain criteria be monitored to ensure the integrity of the study and the quality of the results are not compromised (e.g. ASTM 1997). The key validation criteria that were monitored were designed to ensure variation in water quality was minimised and animal health maintained for the duration of the experiment. These criteria included: <2 ^{0}C variation in water temperature between consecutive measurements; <0.5 variation in pH units between measurements; <2 ppt variation in salinity over the duration of the study; <20 % mortality in control animals; and, >70 % dissolved oxygen saturation.

The physico-chemical parameters were stable over the duration of the study with the exception of temperature which increased to 27 ^{0}C from Day 23 due to cyclone damage (Chapter 3, Section 2.5). Despite the increase in temperature, the data was deemed acceptable as the temperature increase occurred over a period of 48 hours. pH varied by less than 0.2 units between sequential readings. Salinity varied by less than 0.5 ppt and dissolved oxygen exceeded

85 % for the duration of the study (Table 3.2). There was no mortality in any control or treatment replicates over the duration of the study.

Table 3.2: Summary of the water quality parameters for the study. Data is mean ± 1 S.D. *Temperature is mean ± S.D. for Days 14 – 20 only, as temperature increased to an mean temperature 27 ^{0}C after Day 23 due to cyclone damage (Section 2.5).

Treatment	Study Phase	pH	Salinity (ppt)	Oxygen (% satn)	Temperature (^{0}C)
Control	**Uptake**	8.17±0.03	32.8±0.3	92.4±2.3	24.8±0.9
Copper	**Uptake**	8.18±0.03	33.0±0.3	92.2±2.4	24.8±0.9
Zinc	**Uptake**	8.17±0.03	32.8±0.3	92.1±2.0	24.8±0.8
All Treatments	**Clearance**	8.17±0.03	32.7±0.4	92.6±2.4	24.5±1*

2.8 Data analysis

Variations in bell diameter were tested using a two-way ANOVA for independent data after testing for homogeneity using Bartlett's test. Comparisons between background tissue concentrations were analysed using a student's t-test.

The time course experiment was assessed using both the two compartment and the hyperbolic models (Kahle & Zauke 2002; Clason et al. 2004). The two compartment model can be described with water-metal concentration as compartment one and animal tissue concentration as compartment two (Clason & Zauke, 2000; Li et al. 2010; among others) (Figure 3.1). This type of model was originally developed for evaluating the toxicokinetics of organic chemicals in fish, etc (e.g. ASTM 1997). However, it has subsequently been successfully adapted for bioaccumulation of metals in aquatic invertebrates (e.g. Xu & Pascoe 1993; Wang et al. 1996; Zhang & Wang 2006).

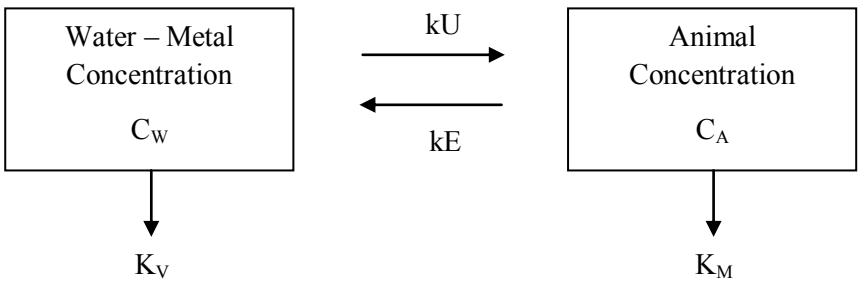


Figure 3.1: A simplified two compartment model with water as the first compartment and animal as the second compartment.

In the two compartment model, C_A is the mean metal concentration in the animal tissue ($\mu g.kg^{-1}$); C_W the mean measured metal exposure during the uptake phase ($\mu g.L^{-1}$); K_M the growth rate (d^{-1}); K_V the adsorption / volatilisation constant (d^{-1}); kU the rate constant for uptake (d^{-1}) and kE the rate constant for clearance (d^{-1}) (Figure 3.1). As both copper and zinc are essential metals, a background concentration was present in the animals. This is defined as C_O and is the mean concentration in animal tissue at t = 0 ($\mu g.kg^{-1}$). In this study, the growth rate (K_M) was considered insignificant while surface adsorption / volatilisation of the metals (K_V) was compensated for by regular replacement of the treatment and control waters over the duration of the study. All tissue metal concentrations were reported as $\mu g.kg^{-1}$ wet weight rather than dry weight due to the confounding presence of residual bound water of hydration remaining in jellyfish tissues after drying (Larson 1986; Arai 1997).

The model parameters kU and kE were estimated simultaneously for the uptake and clearance phases ([Eq 1] and [Eq 2]), using nonlinear iterative least square methods in Excel 2007 with Solver add-in. The uptake phase - $0 < t \leq t^*$, with t^* = end of uptake phase (days) was described by the equation:

$$C_A = C_O + C_W \, kU/ \, kE \, (1-e^{-kE \cdot t}) \qquad \text{[Eq 1]}$$

and the clearance phase ($t > t^*$) was described by:

$$C_A = C_O + C_W \, kU/ \, kE \, (e^{-kE \cdot (t - t^*) - kE \cdot t}) \qquad \text{[Eq 2]}$$

The kinetic BCF (BCF_{kin}) was then calculated using the equation:

$$BCF_{kin} = kU/ \, kE \qquad \text{[Eq 3]}$$

while the biological half-life was calculated by:

$$t_{1/2} = \ln (2)/ \, kE \qquad \text{[Eq 4]}$$

and the theoretical maximum tissue concentration at equilibrium as:

$$C_{A(max)} = BCF_{kin} * C_W \qquad \text{[Eq 5]}$$

In contrast, the hyperbolic model described the uptake phase as:

$$C_A = C_O + (C_{A(max)} \, t / \, t_{max/2} + t) \qquad \text{[Eq 6]}$$

where $t_{max/2}$ was the time taken to reach half of $C_{A(max)}$ (days).

The hyperbolic clearance phase ($t > t^*$) was described by:

$$C_A = C_{t^*} + ((C_O - C_{t^*}) \, (t - t^*)) / (t_{max/2} + (t - t^*)) \qquad \text{[Eq 7]}$$

where $C_{t^*} = C_A$ at the end of the uptake phase ($\mu g.kg^{-1}$) (from [Eq 6])

The hyperbolic model kinetic BCF (BCF_{kin}) was calculated using the equation:

$$BCF_{kin} = C_{A(max)} / C_W \quad [Eq\ 8]$$

The initial goodness of fit for both models were calculated using: $R^2 = 1- (SS_{res}/SS_{tot})$ with Excel 2007 Solver add-in. A linear regression of observed versus predicted model was then performed in Statistica Version 10.0 with each model to compare it with the measured data. A slope value of '1' and a constant value of '0' indicates complete agreement between the model and observed data sets. If the 95 % confidence intervals include these values, then it can be regarded as a good fit (Clason et al. 2004). All data were plotted in GraphPad Version 5.

3. Results

3.1 Copper uptake and retention

Copper was very rapidly accumulated in the jellyfish tissues (Figure 3.2). Accumulation of copper above background concentrations was measurable within 24 hours of exposure with an increase from 110 $\mu g.kg^{-1}$ wet weight to 808 $\mu g.kg^{-1}$. The accumulation followed a rapidly rising trend before reaching saturation after 7 days, indicating some level of regulation of copper (Figure 3.2).

Copper was rapidly purged from the animals when they were transferred to clean seawater. Copper concentrations in the copper exposed animals approached background after 14 days of purging (Figure 3.2). The calculated biological half-life ($t_{1/2}$) of copper was 1.68 days (Table 3.4). The two compartment model was a better fit to the data for copper accumulation and retention than the hyperbolic model, although the R^2 was similar (Figure 3.2).

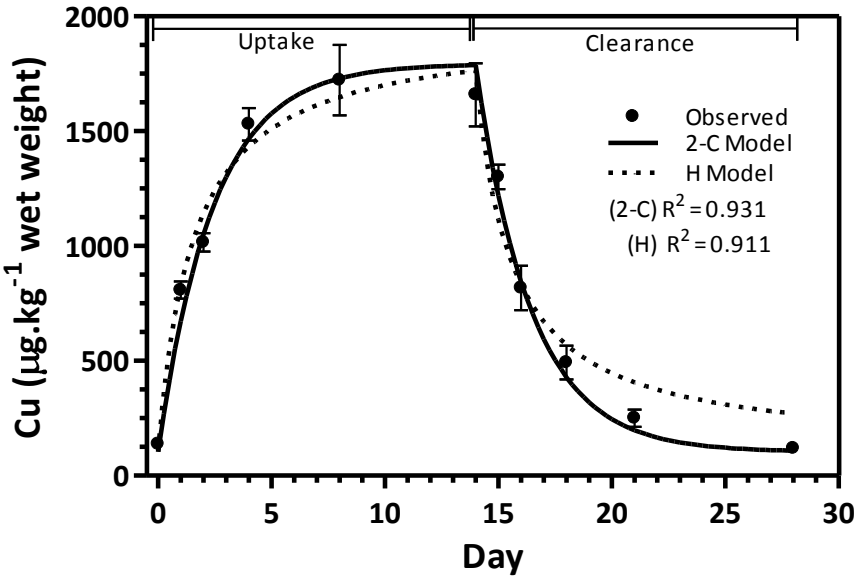


Figure 3.2: Bioconcentration of copper in *Cassiopea* sp. exposed to 10 µg.L^{-1}. Observed = Mean measured data ± SEM; 2-C represents fitted two compartment model; H represent fitted hyperbolic model. (2-C)R^2 indicates goodness of fit of two compartment model to measured data; and (H)R^2 indicates goodness of fit of hyperbolic model to measured data.

3.1.1 Copper concentrations in control and treatment waters

The average water quality and elemental concentrations of copper in the treatment solution was acceptable over the duration of the study. There was some variation in copper concentrations between sampling events in the treatments during the uptake phase and may have been due to surface adsorption of some copper to the holding drums (Table 3.3). The elemental concentration of copper in the control waters was always at least half the measured copper treatment concentrations at each sampling. The copper concentration in all treatments was less than the detection limit during the clearance phase of the experiment (Table 3.3).

Table 3.3: Summary of the mean water concentrations of copper and zinc for the bioaccumulation study (± SEM). SEM represents standard error of the mean. <D.L. below the reported detection limit (Table 3.1).

Treatment	Uptake Phase (Day 0 – 14)		Clearance Phase (Day 14 – 28)	
	Cu (µg.L^{-1})	Zn (µg.L^{-1})	Cu (µg.L^{-1})	Zn (µg.L^{-1})
Control	6.72 ± 2.13	11.38 ± 3.38	< 1	< 5.
10 µg.L^{-1} Cu	17.06 ± 2.90	12.94 ± 3.27	< 1	< 5
50 µg.L^{-1} Zn	7.33 ± 2.20	59.88 ± 1.90	< 1.	< 5

3.1.2 Baseline tissue copper concentrations

Tissue concentrations of copper were measured in both the control and zinc exposed medusae to establish a background level of copper exposure. Copper concentrations in both the control and zinc exposed medusae decreased over the 28 day study from a mean of 110 $\mu g.kg^{-1}$ to 69 $\mu g.kg^{-1}$ wet weight (Figure 3.3). The overall mean of background tissue concentrations for the study was 104 $\mu g.kg^{-1}$ wet weight, and this was used as the copper C_O constant for the kinetic models (Section 2.8). There was no significant difference in tissue concentrations between the control and zinc treatments (unpaired t-test, $t_{(18)} = 0.077$, $p > 0.05$). The similarity in tissue concentrations of copper between the control and zinc treatments indicated that zinc exposure did not affect the copper concentrations in the test medusae (Figure 3.3).

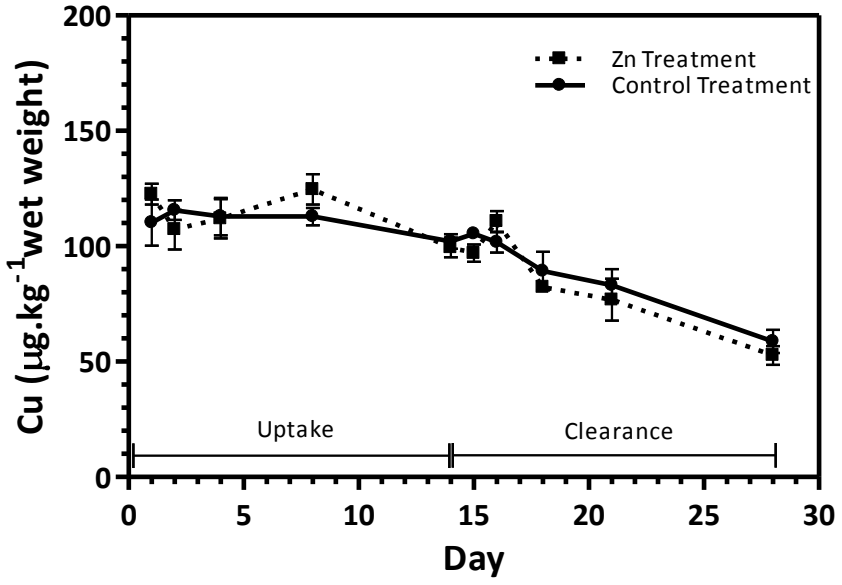


Figure 3.3: Background concentration of copper in control and 50 $\mu g.L^{-1}$ zinc exposed animals. Results are mean ± SEM and mass is wet weight.

3.2 Zinc uptake and retention

Zinc accumulation in the *Cassiopea* sp. was slower and more linear than copper accumulation (Figure 3.4). Zinc accumulation did not reach saturation within the 14 days and was retained for a longer period once the animals were transferred to clean seawater. There was a steady bioconcentration of zinc with an estimated time to half maximum concentration ($t_{max/2}$) of 10.6 days (from the hyperbolic model). The mean maximum measured bioconcentration after 14 days exposure was 5685 $\mu g.kg^{-1}$ wet weight (Figure 3.4). This was approximately three times the background concentration of zinc.

Clearance of zinc was also slower than copper with the mean tissue concentration after 14 days of 3166 $\mu g.kg^{-1}$ wet weight which was approximately double the background zinc concentration

in the animals (Figures 3.4 & 3.5). The biological half-life ($t_{1/2}$) for zinc in *Cassiopea* sp. was 9.11 days which was much longer than estimated for copper (Table 3.4). In contrast to the copper results, the prediction of zinc accumulation and retention was better represented by the hyperbolic model than the two compartment model (Figure 3.4 & Table 3.5).

Zinc was detectable in the control treatment on three occasions but was at low concentrations compared with the zinc treatment concentration (Table 3.3). The zinc treatment concentration was reasonably stable over the uptake phase (Table 3.3). Zinc was detected above the detection limit during the clearance phase on one sampling occasion and only just exceeded the detection limit of 5 $\mu g.L^{-1}$.

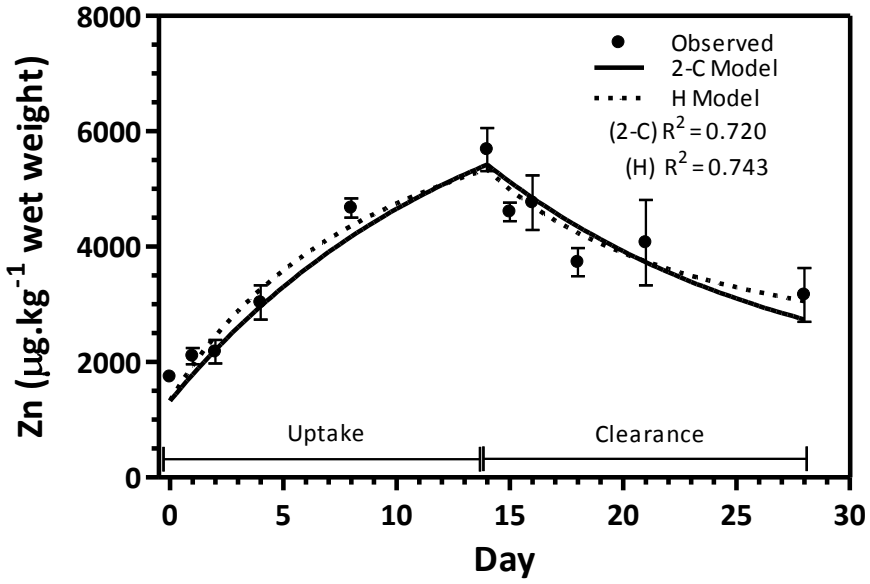


Figure 3.4: Bioconcentration of zinc in *Cassiopea* sp. Observed = Mean measured data ± SEM; 2-C represents fitted two compartment model; H represent fitted hyperbolic model. (2-C) R^2 indicates goodness of fit of two compartment model to measured data; and (H) R^2 indicates goodness of fit of hyperbolic model to measured data.

3.2.1 Zinc concentrations in control and treatment waters

As per the copper results, the average water quality and elemental concentrations of zinc in the treatment solution was acceptable over the duration of the study. There was little variation in zinc concentrations between sampling events in the treatments during the uptake phase. The elemental concentration of zinc in the control waters was well below the measured zinc treatment concentrations at each sampling. The zinc concentration in all treatments was less than the detection limit (except for one sampling event) during the clearance phase of the experiment (Table 3.3).

3.2.2 Baseline tissue zinc concentrations

Similar to the approach taken with copper, tissue concentrations of zinc were measured in the control and copper exposed medusae to establish a background level of zinc exposure (Figure 3.5). The background zinc concentrations were stable within the control animals over the duration of the study and likely reflect normal tissue concentrations for this species. There was no significant difference between the background tissue concentration of zinc within the copper exposed animals and the control animals indicating that copper exposure did not affect zinc accumulation (unpaired t-test, $t_{(18)} = 2.057$, $p > 0.05$). The background zinc concentrations in both the control and copper exposed tissues varied over the duration of the study but were still well below the accumulated concentrations of zinc exposed animals (Figure 3.5).

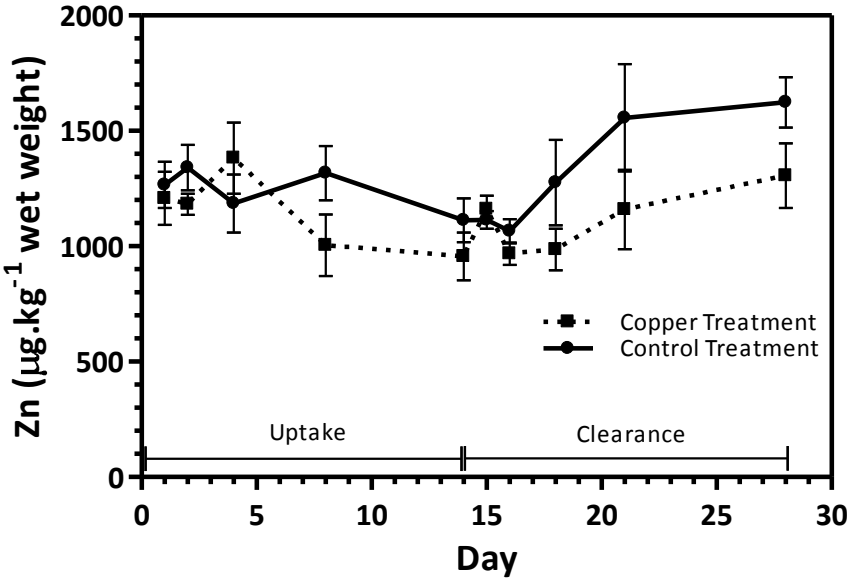


Figure 3.5: Background tissue concentrations of zinc in control and copper exposed animals. Results are mean ± SEM.

3.3 Kinetic models and parameters

The model that best described bioconcentration in *Cassiopea* sp. was different for the two metals. Bioconcentration of copper was best described by a two compartment model with the test solution as the first compartment and *Cassiopea* sp. as the second compartment. In contrast, zinc accumulation was better described by the hyperbolic model (Figures 3.2 & 3.4).

The estimated kinetic bioconcentration factor (BCF_{kin}) and the theoretical maximum concentration at steady state for both metals were higher using the parameters from hyperbolic model than the two compartment model, but were generally in good agreement between the two models (Table 3.4). The calculated theoretical maximum concentration of both metals was

also higher for both metals using the hyperbolic model than the two compartment model (Table 3.4).

Table 3.4: Kinetic parameters of copper and zinc accumulation in C*assiopea* sp. C_w = mean element concentration in water; C_O = mean element concentration in control animals; kU = uptake constant; kE = clearance constant; BCF = kinetic bioconcentration factor; $C_{A(max)}$ = maximum tissue concentration at steady state; $t_{1/2}$ = biological half-life of the element (days); (2C) = two compartment model estimate; (H) = hyperbolic model estimate.

Metal	C_w (µg.L^{-1})	C_O (µg.kg^{-1} wet weight)	kU (d^{-1})	kE (d^{-1})	BCF_{kin}	$C_{A(max)}$ (µg.kg^{-1} wet weight)	$t_{1/2}$ (d^{-1})	R^2
Cu	17.09	103.49	40.738	0.4114	99.01 (2C) 108.2 (H)	1689.1 (2C) 1845.7 (H)	1.68	0.931 (2C) 0.911 (H)
Zn	59.88	1327.2	7.942	0.076	104.37 (2C) 117.67 (H)	6249.6 (2C) 7046.0 (H)	9.11	0.720 (2C) 0.743 (H)

The goodness of fit between the observed data and the model predictions was assessed by linear regression (Table 3.5). There was better agreement between the observed data and two compartment model for copper with the 95 % CI of the slope bracketing 1 than for the hyperbolic model (see Chapter 3, Section 2.8). A slope value of '1' and a constant value of '0' indicates there is complete agreement between the model and observed data sets. If the 95 % confidence intervals include these values, then it can be regarded as a good fit (Clason et al. 2004). In contrast, the 95 % CI of the slope of linear regression of both models against measured zinc concentrations did not include 1 indicating some lack of agreement in the fit between the observed and predicted data. Notwithstanding this, the hyperbolic model showed slightly better agreement than the two compartment model for zinc accumulation (Table 3.5, Figure 3.4).

Table 3.5: Linear regression of observed metal concentrations in *Cassiopea* sp. against the two compartment and hyperbolic model predictions. 2C = two compartment model; H = Hyperbolic model.* indicates Constant significantly different from 0. t = t-value of slope, critical t-value (2 sided): $t_{42;0.05} = 2.02$.

Metal	Model	Constant	Slope	t	95 % CI slope	Corr R^2
Cu	2C	-5.976	0.982	24.47	0.901-1.063	0.933
	H	133.2*	0.870	20.81	0.786-0.955	0.909
Zn	2C	482.1	0.840	10.91	0.684-0.995	0.733
	H	752.4*	0.783	11.10	0.641-0.925	0.740

3.4 Effect of metals on bell diameter

The mean bell diameter of medusae at the start of the study was 17 ± 2 mm. The bell diameter of sampled medusae was also measured at each sampling event (Figure 3.6). Analysis of measured bell diameter (two-way ANOVA) showed no significant difference ($p > 0.05$) among treatments or time over the duration of the study (Figure 3.6).

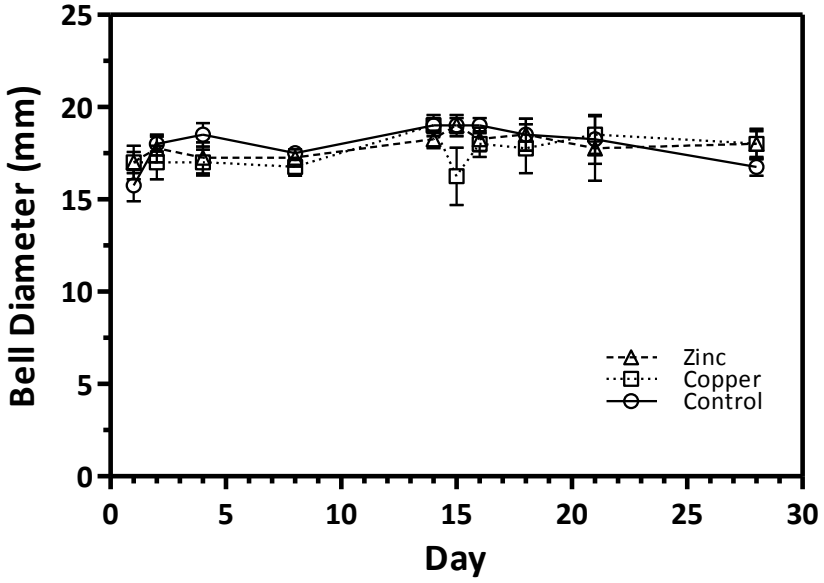


Figure 3.6: Measured bell diameter of sampled animals by treatment over duration of study. Results are mean ± SEM.

4. Discussion

This study demonstrated that *Cassiopea* sp. was able to readily accumulate both copper and zinc from seawater. The accumulation of copper was very rapid but retention times were short with a calculated half-life of 1.7 days (Table 3.4). Accumulation of zinc was slower and retention times were longer with a calculated half-life of 9.1 days (Table 3.4). Both the uptake constant and clearance constants were higher for copper than zinc, reflecting the greater mobility of copper both into and out of the animals (Table 3.4). There have been no previous studies assessing copper uptake from seawater in jellyfish and only one assessing zinc accumulation (Fowler et al. 2004), so direct comparisons with other jellyfishes for copper was not possible.

4.1 Comparison of observed data to kinetic models

The two compartment model was a better fit to the observed results for copper accumulation and clearance with an R^2 of 0.931 compared with the hyperbolic model with an R^2 = 0.911 (Figure 3.2). This indicated that use of the two compartment model with simultaneous estimation of the uptake and clearance constants (kU and kE) was an appropriate model for assessing copper accumulation in *Cassiopea* sp. Other uptake and retention studies using the oyster (*Saccostrea glomerata*) also indicated that the two compartment model provided a reasonable fit to measured data although inclusion of a temperature function did improve the fit (Richards & Chaloupka 2009). In this study, temperature was held constant so a temperature function was not necessary.

The overall goodness of fit from both models was lower for zinc with the hyperbolic model having a slightly better fit (R^2 = 0.743) than the two compartment model (R^2 = 0.720) (Figure 3.4). The overall goodness of fit of the two models (two compartment and hyperbolic) in this study was similar to zinc studies on the copepod *Calanoides acutus* (Kahle & Zauke 2002). This suggests that both models are useful for predicting patterns of zinc accumulation in *Cassiopea* sp., but the hyperbolic model may better reflect zinc accumulation. Notwithstanding that, the results from this study are comparable to the literature on zinc accumulation (e.g. Kahle & Zauke 2002; Richards & Chaloupka 2009).

4.2 Copper uptake and retention

A range of studies have investigated uptake and retention of copper in marine invertebrates (Table 3.6) Among these, molluscs have long been recognised as very effective accumulators of metals although a number of other taxa have also been identified as useful biomonitors (e.g. Phillips 1990; Rainbow 1995). The calculated copper BCF_{kin} for *Cassiopea* sp. in this study was lower than for the oyster, *Ostrea plicatula* (Table 3.6). The uptake constant (kU) for *Cassiopea* sp. was similar to *O. plicatula* (40.74 cv. 41.3 & 33.9) and intermediate to that reported for other species (Table 3.6). However, the clearance constant (kE) for *Cassiopea* sp. was much higher than *O. plicatula* (0.4114 cv. 0.0181-0.0252). This indicated that while the rate of uptake of aqueous copper was similar between the species, clearance was much more rapid in *Cassiopea* sp. compared with *O. plicatula* and therefore tissue bioconcentration in *Cassiopea* sp. was also lower. This was also reflected in the different biological half life ($t_{1/2}$) for retention of copper between the two species (Table 3.6).

Although the clearance rate was higher than that reported for other species (Table 3.6), *Cassiopea* sp. can still be considered a net accumulator of copper rather than a regulator. Luoma & Rainbow (2005) describe "metal regulators" as typically having such high rates of excretion such that internal metal concentrations do not vary significantly with exposure. This

study demonstrated that although *Cassiopea* sp. could be considered to have a high clearance rate, the uptake rate was greater as they remained net accumulators of copper.

Field BCF measurements for *Cassiopea* sp. on the Great Barrier Reef (GBR) showed accumulation of copper 151 times seawater (Figure 2.2) which was higher than the reported BCF_{kin} in this study (Table 3.6). In addition, Templeman & Kingsford (2010) reported bioaccumulation of copper in the oral arms of *Cassiopea* sp. ranging from 23 to 84 times ambient seawater depending on location. These results demonstrated that *Cassiopea* sp. were efficient accumulators of copper at low ambient water concentrations.

The overall steady state tissue concentration for this study is lower than that reported *O. plicatula* (Li et al. 2010; Table 3.6). It is difficult to more widely compare the steady state tissue concentrations with the literature, due to both differences in units (ie wet weight in this study cv. dry weight reported for some others) and limited reporting of steady state concentrations in other studies (Table 3.6). However, using an estimated 95 % water content for *Cassiopea* sp. from previous work (Templeman, unpublished data), the dry weight steady state tissue concentration in this study for copper can be estimated as 33.78 $\mu g.g^{-1}$ dry weight. This estimated maximum concentration is intermediate between the amphipod, *Chaetogammarus marinus* (Clason et al. 2004) and copepod *Calanoides acutus* (Kahle & Zauke 2002; Table 3.6). However, in all these other studies the reported exposure concentrations were higher (Table 3.6). In the case of *O. plicatula*, the reported steady state tissue concentration doubled with an increased exposure concentration (Table 3.6; Li et al. 2010). This and other data, suggests that steady-state tissue concentrations are dependent on both exposure time and exposure concentrations (e.g. Harland et al. 1990; Xu & Pascoe 1993, Rainbow et al. 2009). In addition, Li et al. (2010) reported that they were unable to fit the two compartment model to animals exposed to the lowest concentrations of copper tested (10.45 $\mu g.L^{-1}$ Cu) as tissue copper concentrations did not change over the study. This suggests that there may be a threshold concentration required for accumulation in some species, but also reinforces the dependency of steady state tissue concentration on exposure conditions.

Table 3.6: Comparative studies of copper uptake from water in bioaccumulation and biokinetic models in selected marine animals. C_W is water concentration; kU and kE represent uptake and clearance constants in days; BCF_{kin} is the kinetic bioconcentration factor calculated according to Section 3.8; $t_{1/2}$ is the biological half-life of copper; and, $C_{A(max)}$ is the derived steady-state maximum of tissue copper. (2C) two compartment model; (H) hyperbolic model; ww = wet weight; dw = dry weight.

Species	**C_W ($\mu g.L^{-1}$)**	**kU**	**kE**	**BCF_{kin}**	**$t_{1/2}$ (days)**	**$C_{A(max)}$**	**Source**
Cassiopea sp. (Upside-down Jellyfish)	17.1	40.74	0.4114	99 (2C) 108 (H)	1.68	1.69 ($\mu g.g^{-1}$ ww) (2C) 1.85 ($\mu g.g^{-1}$ ww) (H)	This Study
Calanoides acutus (Copepod)	28	95	0.044	2179 (2C) 2700 (H)		76 ($\mu g.g^{-1}$ dw)	Kahle & Zauke 2002
Chaetogammarus marinus (Amphipod)	27	168	0.24	696 (2C) 889 (H)		24 ($\mu g.g^{-1}$ dw)	Clason et al. 2004
Saccostrea glomerata (Oyster)	2.7	1.43	0.008				Richards & Chaloupka 2009
Acanthopagrus Schlegeli (Black Sea Bream)	2 – 200	6.24	0.091				Dang et al. 2009
Ostrea plicatula (Oyster)	47.8	41.265	0.0252	1636.4	27.49	78.22 ($\mu g.g^{-1}$ ww)	Li et al. 2010
Ostrea plicatula (Oyster)	98.05	33.96	0.0181	1874.4	38.26	183.03($\mu g.g^{-1}$ ww)	Li et al. 2010

4.3 Zinc uptake and retention

The kinetic bioconcentration factor and biological half-life for zinc was lower in this study than that reported by Fowler et al. (2004) for *Cassiopea andromeda* or *Aurelia aurita* (Table 3.7). However, the BCF given in the literature includes the effects of dietary uptake, and it was reported that food may be a critical pathway for both bioaccumulation and retention in *C. andromeda* and *A. aurita* (Fowler et al. 2004). This study assessed uptake from water only, and the results reflect the accumulative capacity and retention of aqueous zinc. It is possible that dietary zinc may be retained in higher concentrations and for longer than water borne zinc however further work would need to be undertaken to confirm this hypothesis.

Mean accumulation of zinc in *Cassiopea* sp. on the Great Barrier Reef (GBR) was 221 times seawater (Figure 2.2). Other reported field BCFs for accumulation of zinc in *Cassiopea* sp. ranged from 190 to 756 times ambient seawater depending on location (Templeman & Kingsford 2010). These results were higher than the BCF_{kin} of 104 to 117 in this study but were comparable to that reported by Fowler et al. (2004) (Table 3.7). The range of measured accumulation suggests that zinc uptake and retention is influenced by multiple factors including

ambient seawater concentrations, bioavailability, dietary influences, etc (e.g. Rainbow & Wang 2001; Luoma & Rainbow 2005).

The patterns of zinc uptake and retention in this study were less predictable than for copper (Figures 3.2 & 3.4) but were similar to other reported literature for zinc (e.g Xu & Pascoe 1993; Kahle & Zauke 2002). Unlike copper, uptake of zinc did not reach saturation during the study and may have been a carrier-mediated process as is seen in some other aquatic species (Wang & Fisher 1999b). It is possible that there may also be other underlying processes of localised binding, storage and release of zinc that increases the complexity of the overall patterns of accumulation and retention.

The uptake constant (kU) for zinc in *Cassiopea* sp. was higher than that reported for most other species (except *Calanoides acutus*) but still comparable (Table 3.7). The clearance constant was also within the range reported for other species. This suggests that *Cassiopea* sp. is comparable to other phyla in terms of zinc accumulation and has potential as a biomonitor.

Table 3.7: Comparative studies of zinc uptake from water in bioaccumulation and biokinetic models in selected marine invertebrates. C_W is water concentration (in $\mu g.L^{-1}$ unless otherwise stated); kU and kE represent uptake and clearance constants in days; BCF_{kin} is the kinetic bioconcentration factor calculated according to Section 2.8; $t_{1/2}$ is the biological half-life of copper; and, $C_{A(max)}$ is the derived steady-state maximum of tissue copper. (2C) two compartment model; (H) hyperbolic model; (sym – symbiotic, asym – asymbiotic).

Species	**C_W ($\mu g.L^{-1}$)**	**kU**	**kE**	**BCF_{kin}**	**$t_{1/2}$ (days)**	**$C_{A(max)}$**	**Source**
Cassiopea sp. (Upside-down Jellyfish)	59.88	7.937	0.076	104.4 (2C) 117.7 (H)	9.12	6.25 ($\mu g.g^{-1}$ ww) (2C) 7.05 ($\mu g.g^{-1}$ ww) (H)	This Study
Mytilus edulis (Mussel)	0.5-300	1.044	0.020		44-66		Wang et al. 1996
Temora longicornis (Copepod)	154 nM	3.29	0.108		0.65		Wang & Fisher 1998
Saccostrea glomerata (Oyster)	2 – 100	1.206	0.003				Ke & Wang 2001
Crassostrea rivularis (Oyster)	2 – 100	2.050	0.014				Ke & Wang 2001
Calanoides acutus (Copepod)	61 (2C) 89 (H)	196	0.249	787 (2C)		68.2 ($\mu g.g^{-1}$ dw)	Kahle & Zauke 2002
Cassiopea andromeda (Upside-down Jellyfish)	0.5 Bq/mL			412 sym 281 asym	28 – 65		Fowler et al. 2004
Aurelia aurita (Moon Jellyfish)	0.5 Bq/mL			317	20 - 29		Fowler et al. 2004
Nereis diversicolor (Polychaete)	24 – 60	0.0173-0.1021	0.0235-0.0393		17.6-29.5		Rainbow et al. 2009

The dry weight steady state tissue concentration for zinc in *Cassiopea* sp. in this study (assuming 95 % water content as per Templeman & Kingsford 2010) was 125.07 $\mu g.g^{-1}$ dry weight. The reported $C_{A(max)}$ for *Calanoides acutus* was lower (68.2 $\mu g.g^{-1}$ dry weight) than for *Cassiopea* sp, which contrasted to the higher calculated uptake (kU) and clearance (kE) factors and overall BCF_{kin} in *C. acutus* (Kahle & Zauke 2002). The rate of accumulation and excretion was higher in *C. acutus*, but the net capacity of accumulation was lower (Table 3.7). In freshwater uptake studies, the reported steady state concentrations of zinc in the freshwater snail, *Gammarus pulex* was 430-450 $\mu g.g^{-1}$ dw, which is much higher than this study, however, exposure concentrations (410-2020 $\mu g.L^{-1}$) for *G. pulex* were also much higher (Xu & Pascoe 1993). Again, this supports the paradigm that tissue steady state concentrations are linked with both exposure concentration and exposure time (e.g. Harland et al. 1990; Xu & Pascoe 1993; Rainbow et al. 2009).

4.4 Metal regulation strategies

Although there are few studies on bioaccumulation of metals in jellyfishes, there exists a larger body of information on tissue metal concentrations for the related anemones and corals. Harland & Nganro (1990) found that symbiotic zooxanthellae play an important role in regulation of copper in *Anemonia viridis*, with the zooxanthellae preferentially accumulating the metals over the host animal. Asymbiotic animals accumulated higher concentrations of copper than animals with their symbionts intact (Harland & Nganro 1990). This suggests that zooxanthellae can not only act as an additional sink for trace elements due to their own metabolic needs but may also be self regulating. Field and laboratory studies have shown that anemones and corals are able to regulate tissue concentrations of copper and zinc despite higher environmental levels of these elements, with indications that zooxanthellae may play a key role in metal regulation processes (e.g. Bryan & Gibbs 1983; Harland et al. 1990; Esslemont et al. 2000; Ferrier-Pages et al. 2005). Given the symbiotic nature of *Cassiopea* sp., it is likely zooxanthellae have a key role in metal regulation within host tissues. Unlike other species, the symbiotic zooxanthellae in *Cassiopea* sp. are typically located in amoebocytes (Arai 1997), and this compartmentalisation may offer a storage / detoxification location somewhat isolated from the host tissues.

Zooxanthellae can also be very important in accumulation of zinc in sea anemones. Harland et al. (1990) estimated that about a third of the zinc accumulated by *A. viridis* was taken up by the symbiotic zooxanthellae when compared with the asymbiotic *Actinia equina*. This suggests that the zooxanthellae may play an important role in metal uptake in symbiotic species like *Cassiopea* sp.

They also reported that when exposed to increased aqueous zinc concentrations, a new tissue equilibrium was reached within approximately one week of exposure (Harland et al. 1990). The response in *A. viridis* contrasts with this study, as the symbiotic *Cassiopea* sp. had not reached equilibrium after 14 days exposure to zinc.

Another mechanism used by a number of aquatic organisms to regulate and/or exclude metal uptake is mucous production (Howell 1982; Langston & Spence 1995). Due to the variations in mucous chemical composition among species, it is has been suggested that mucous metal regulation / exclusion strategies may also be variable (Harland & Nganro 1990). There was no evidence of excessive mucous production among individuals during this study, however, *Cassiopea* sp. excrete mucous as part of their normal physiological processes. It is possible that copper (in particular) may have been accumulated within the mucous rather than within the tissues per se, as a method for detoxification by the jellyfish and this mechanism may be an important metal regulation strategy. However, this hypothesis would require additional work to validate.

Numerous studies have demonstrated that uptake and assimilation strategies also vary for different metals and environmental conditions (e.g. Wang et al. 1996; Bastidas & Garcia 1999; Ferrier-Pages et al. 2005). Field and laboratory studies on uptake of multiple metals (Cd, Cr, Cu, Co, Pb, Ni and Zn) showed the amphipod, *Chaetogammarus marinus* readily accumulated most metals but not zinc (Clason et al. 2004). Similarly, the oyster, *Ostrea plicatula* readily accumulated copper above 50 $\mu g.L^{-1}$ Cu exposure concentrations but did not accumulate at 10.5 $\mu g.L^{-1}$ Cu (Li et al. 2010). The results from this study reflected this same level of variability with accumulation rates, background tissue concentrations, clearance rates and maximum tissue concentrations varying between the two metals for *Cassiopea* sp.

This study demonstrated that *Cassiopea* sp. is a net accumulator of both copper and zinc although the uptake and clearance rates, and half-life differed between the two metals. The ability of *Cassiopea* sp. to rapidly accumulate copper from low ambient concentrations compared with other commonly used biomonitors (Table 3.6) make them a viable biomonitoring species in low–moderately impacted systems. Zinc uptake and retention in *Cassiopea* sp. was comparable to other biomonitoring species also (Table 3.6).

4.5 Biomonitoring potential of Cassiopea sp.

The primary criterion of a biomonitor is to be a net accumulator of the contaminant of concern. However, to be a useful biomonitor requires other prerequisites including: 1) a sessile or sedentary nature; 2) tolerance of variations in physico-chemical parameters; 3) adaptability to laboratory culture; 4) abundance, large size and easy identification; and, 5) the ability to correlate between ambient pollutant concentrations and tissue concentrations (Phillips 1990;

Rainbow & Phillips 1993). *Cassiopea* sp. is euryhaline and found in tropical / subtropical shallow coastal and estuarine systems. They are one of the most common scyphozoan jellyfish in laboratory culture and are readily identified in the field. Atypically for jellyfish they are sedentary, with the habit of resting upside down on the benthos and are easily collected by hand. As such, *Cassiopea* sp. meet all the associated criteria for biomonitors. Their advantage compared with many other biomonitors is the ability to accumulate high tissue load of metals under low ambient conditions. The high uptake rate but short retention time for copper in *Cassiopea* sp. suggests this species may have particular utility for monitoring short term pulse events.

5. Conclusions

This study demonstrated that *Cassiopea* sp. readily bioconcentrated both copper and zinc from the water, although the accumulation and excretion rates varied for the two metals. Copper uptake and excretion were very rapid and high tissue concentrations able to be measured despite low ambient exposure concentrations. The identified steady-state tissue concentrations, half-life and retention times were lower than reported for other species. However, uptake and bioconcentration was measureable at lower ambient concentrations than for other species. Given these results, the utility of *Cassiopea* sp. as a copper biomonitor would be optimised towards monitors of immediate conditions (rather than longer term press events) or in situations where low ambient concentrations of copper were the focus.

For zinc, the clearance constant, maximum tissue concentration and half-life were comparable to other taxa, and suggested that zinc was more readily retained in the tissues than copper. As a zinc biomonitor, *Cassiopea* sp. would likely have similar utility as other taxa, particularly in circumstances that utilise their sedentary behaviour and preference for shallow coastal and estuarine tropical waters.

Overall, both copper and zinc accumulation and clearance in *Cassiopea* sp. were able to be fitted to kinetic models. A two compartment model was a better predictor of copper bioconcentration while a hyperbolic model was a better predictor of zinc bioconcentration. The two compartment model of copper bioconcentration closely matched with observed data while the hyperbolic model of zinc bioconcentration provided a less accurate but still reasonable predictive capacity.

Chapter 4 - Determination of the LC_{50} and EC_{50} of Copper and Zinc to Multiple Lifestages of Three Species of Jellyfish

1. Introduction

Studies into the response of jellyfishes to metal exposure are very limited (Spangenberg 1984; Spangenberg 1986; Todd et al. 2006). The presence of jellyfishes in urban marine waters suggests that they may be useful indicators of water quality. Quantitative assessment of their response to metals would provide valuable information on their sensitivity / tolerance to contaminants.

Copper and zinc are important essential elements that are required for critical biochemical processes in all terrestrial, freshwater and marine organisms. However, above a threshold these same elements can rapidly become toxic to organisms unless they have a means to exclude, detoxify or excrete excess quantities (e.g. Phillips & Rainbow 1989; De Forest et al. 2007).

Both copper and zinc are found naturally in aquatic systems, although levels in uncontaminated systems are generally very low (Neff 2002). In contrast, coastal marine ecosystems downstream from urban centres or industry, often have elevated levels of copper and zinc which have resulted in decreased biodiversity and species abundance (Peters et al. 1997; Grosell et al. 2007). Elevated copper and zinc loads in estuarine and marine waters are largely terrestrial in origin with mining and refining activities, sewage discharges, urban stormwater runoff, and leaching of pesticide treated timbers contributing to the discharge (e.g. Cohen et al. 2001; Neff 2002). The use of sacrificial zinc anodes on watercraft and zinc and copper pyrithiones as antifoulants have also added to the load in coastal marine waters (Bao et al. 2008). As a consequence, copper and zinc concentrations have been increasing in many coastal systems, particularly in semi-enclosed waterbodies including estuaries, harbours and marinas (Chapter 1).

Despite their persistence in marine environments and potential for toxic effects on biota at high concentrations, both copper and zinc are essential elements in several enzymatic systems (Chapter 1). Copper acts as a co-factor for a number of important proteins and also has a key role in cellular respiration (Bury et al. 2003). Zinc has been identified as a co-factor in over one hundred enzymes including carbonic anhydrase which is of critical importance for those organisms with photosynthetic symbionts (Estes et al. 2003).

Environmental pollutants, such as copper and zinc have the potential to affect organisms at all lifestages. However, sensitivity varies among lifestages, and earlier life stages of organisms are generally more sensitive than adults to pollutant-related stressors (e.g. Chapman 1978; Ringwood 1990, Kingsford & Gray 1996; Kennedy et al. 2006). Other factors including salinity, temperature, dissolved organic carbon and metal speciation can also affect the toxicity of a pollutant to a particular species (e.g. De Boek et al. 2007). Among both genera and species there is a wide range of sensitivities to any given pollutant (e.g. McPherson & Chapman 2000; Grosell et al. 2007). The complexity of lifestage and interspecies sensitivities, combined with the variability in external factors makes derivation of realistic and robust environmental regulations for pollutants difficult (Grosell et al. 2007). Nevertheless, the use of organisms in standardised ecological toxicity programs is an important strategy for protection of ecosystem health with the outcomes increasingly being used to set water quality management criteria at local, regional, national and international scales (ANZECC & ARMCANZ 2000).

Integral to development and use of these standardised toxicity testing programs is the selection of suitable test species that meet a number of important criteria. To be considered a suitable test organism, a species must be: 1) amenable to culture under laboratory conditions; 2) sensitive to the element being tested; 3) ecologically relevant to the location where the outcomes will be applied; and, 4) the results should be reproducible (McPherson & Chapman 2000; ASTM 2007; Mohammed 2009).

Whilst there is a body of work on the toxicity of pollutants, including metals to anthozoan Cnidaria (e.g. Reichelt-Brushett & Harrison 1999; Grant et al. 2003; Mitchelmore et al. 2003b), there are few data on jellyfishes. This in part has been due to the perception that jellyfishes are very tolerant of marine pollution and thus considered unlikely to be useful as marine bioindicators (Arai 1997). However, the limited studies that have been conducted contradict this, with toxicity exhibited at relatively low contaminant levels (e.g. Spangenberg 1984; Spangenberg 1986; Todd et al. 2006). Cadmium has been shown to affect statolith formation in *Aurelia* sp. (Spangenberg 1986) while exposure to hydrocarbons significantly affected metamorphosis (polyp to ephyra) and ephyra development in *Aurelia* sp. (Spangenberg 1984). In contrast to this, exposure to herbicide concentrations that were lethal to fish in 16 hours did not cause mortality to the sea nettle *Chrysaora quinquecirrha* (Calton & Burnett 1981).

The jellyfishes used in this study comprised representatives of the classes Scyphozoa (*Aurelia* sp. and *Cassiopea* sp.) and Cubozoa (*Alatina mordens*). The genus *Aurelia* (Moon Jelly) has a cosmopolitan distribution and is found in both coastal areas and open water (Dawson 2004). *Cassiopea* sp. is a rhizostome species containing symbiotic dinoflagellates and is distributed across tropical and sub-tropical regions of the world (Holland et al. 2004). *Cassiopea* sp. is unusual in that it is generally sessile and found upside down on the seabed in

shallow coastal waters and embayments. *A. mordens* is an off-shore species of cubozoan jellyfish and is considered to be one of the species responsible for irukandji syndrome. The distribution of *A. mordens* is thought to extend across equatorial western and central Pacific regions, including both Australia and Hawaii (Bentlage et al. 2010).

All three species of jellyfish have a bipartite lifecycle with alternation of generation (Arai 1997). A small, cryptic but long lasting polyp phase occurs in each of these species, and is capable of asexual reproduction by multiple pathways. Both *Aurelia* sp. and *A. mordens* undergo lateral budding to form new polyps through elongation and differentiation (Arai 1997; Fischer & Hofmann 2004). In contrast, *Cassiopea* sp. polyps reproduce by releasing small ciliated planuloid buds that are free swimming. These planuloid buds are able to remain in the water column for several days before settling to a suitable substrate and developing into a polyp (Arai 1997).

Both *Cassiopea* sp. and *Aurelia* sp. also produce ephyra through the process of strobilation of the polyp disc. *Cassiopea* sp. is mono-strobilic (producing a single ephyra from each polyp strobilation event) while *Aurelia* sp. is poly-strobilic, producing up to twenty ephyra from a single polyp strobilation event. These ephyra grow to become the adult medusae. The polyp is then capable of regeneration back to a fully functional polyp with the ability to undergo future strobilation. The cubozoan *A. mordens* undergoes complete metamorphosis of the polyp to medusa, resulting in a single medusa from each polyp (Fischer & Hofmann 2004).

The field of ecotoxicology has evolved rapidly over the last half century. The initial programs put in place were designed to measure differences between impacted and non-impacted sites as a way of measuring the effect of contaminant exposure (Chapman 1995). The measurable responses to perturbations are many and varied (e.g. avoidance, change in reproductive status, settlement, lesion formation etc). At the organism level, one of the most commonly used responses is lethality (ie death). Use of this response has led to the development of standardised measures of toxicity. Part of this overall process was the development of laboratory toxicity tests and the determination of LC_{50}. An LC_{50} is defined as the concentration of a toxicant required to kill 50 % of a test population of animals. This is used in conjunction with a defined timeframe as the LC_{50} varies with exposure length. Examples of standard timeframes include 24 hours (1 day), 72 hours (3 days) and 96 hours (4 days). Tests reporting LC_{50} are typically considered acute toxicity tests.

An alternative to an LC_{50} is the EC_{50} which uses an endpoint other than death and is the concentration of a toxicant that affects 50 % of a test population. Examples of alternate endpoints include growth, feeding, reproduction, etc (Chapman 1995). Tests measuring sub-lethal endpoints and often, but not always, including key lifecycle aspects are considered

chronic toxicity tests. The results from acute and chronic toxicity testing are increasingly being used to set water quality management criteria at local, regional, national and international scales (ANZECC & ARMCANZ 2000).

The objective of this study was to determine the relative sensitivity of three species of jellyfish to copper and zinc and their potential as metal bioindicators. The specific aims were to: 1) determine the specific LC_{50} or EC_{50} of different lifestages of *Cassiopea* sp., *Alatina mordens* and *Aurelia* sp. to copper and zinc exposure; 2) compare the sensitivity of the different lifestages within species to determine the most sensitive lifestage to copper and zinc; 3) identify the most sensitive jellyfish species from the animals tested to copper and zinc exposure; and 4) assess the overall bioindicator potential of these species.

2. Materials and methods

2.1 Summary

It was hypothesised that the responses of *Alatina mordens*, *Aurelia* sp. and *Cassiopea* sp. to copper or zinc would vary by lifestage. Lifestages assessed were polyps from all three species; newly released medusa from *A. mordens* and newly released ephyra from *Cassiopea* sp.; and ciliated planuloid buds from *Cassiopea* sp. Despite intensive efforts, it was not possible to induce strobilation in *Aurelia* sp. and no ephyra were produced. Due to a lack of polyp substrates, *A. mordens* polyps were not tested against zinc in this study.

The methodology for all tests except for the planuloid bud tests was a standard 96 hour static-renewal toxicity test (e.g. OECD 1992). The planuloid buds were exposed for 72 hours (Chapter 4, Section 2.6.2), but followed the same methodology otherwise. The reported endpoints for the ciliated planuloid buds were survival or tentacle development, while survival was the endpoint for polyps and medusae / ephyra.

All animals used in the tests were sourced from in-house collections. The parent stock of *Aurelia* sp. and *A. mordens* polyps were obtained from Dr Jamie Seymour (James Cook University, Cairns Campus). The parent stock of *Cassiopea* sp. polyps were obtained from spawning induction of medusae collected from Lake Magellan at Pelican Waters, Queensland. The culturing protocols for test organisms are detailed in Appendix A.

2.2 Test organisms

The test organisms used in the toxicity studies were one of three identified species (*A. mordens*, *Cassiopea* sp. or *Aurelia* sp.). Polyps used in the experiments were collected by placing cleaned and acid washed small glass substrates (microscope slides) in culturing tanks and left to allow polyps to colonise them for a minimum of eight weeks. Substrates used in the experiments

were colonised by a minimum of twenty polyps. The day before tests commenced, 36 polyp substrates of the desired species were removed from the culture tanks and placed in approximately five litres of control water. Substrates were examined individually under a stereomicroscope to ensure polyps were healthy and feeding. Any substrates containing deformed or ill polyps were discarded. Polyp numbers were estimated and the back and sides of the substrates were cleaned of adhering polyps and extraneous material so that only one side of the substrate contained polyps. They were allowed to rest overnight before being used in the test.

Cassiopea sp. does not bud in the same manner as *Aurelia* sp. or *A. mordens*. Instead they produce small free swimming ciliated planuloid buds from the undersurface of the polyp calyx (Arai 1997). Planuloid buds from *Cassiopea* sp. were less than 24 hours from detachment from parent polyps at test start. The day prior to test commencement all free swimming planuloid buds were removed from containers of polyp colonies by carefully rinsing them with control seawater. The containers were then refilled with control seawater and left overnight. The following day, water from the polyp containers were decanted into clean containers. A minimum of 300 free swimming planuloid buds were collected by carefully pipetting them into clean 90 mm diameter plastic petri dishes containing 30 mL control seawater.

A. mordens and *Cassiopea* sp. medusae / ephyra were less than 10 days from release at test start. Animals were collected by monitoring polyp colonies daily for indications of strobilation / metamorphosis. Once medusae / ephyra had detached from the polyps, they were carefully captured using cut off plastic pipettes and placed into holding tanks of control seawater. Animals were fed daily with freshly hatched *Artemia* sp. until test start. The day prior to test commencement, approximately 40 newly released *A. mordens* medusa or *Cassiopea* sp. ephyra were removed from holding tanks and placed in approximately five litres of control seawater. Animals were examined individually under a stereomicroscope to ensure they were healthy and feeding. Any deformed or ill animals were discarded. All potential test organisms were randomly placed into clean, acid washed two litre containers of control water overnight. Due to the small number of available medusae / ephyra of the appropriate age, only five animals per replicate treatment were used in the experiments.

2.3 Cleaning and equipment preparation

All equipment was washed in phosphate free detergent, rinsed in tap water to remove any residual detergent and then soaked in 10 % AR grade nitric acid for a minimum of 12 hours. Acid soaked equipment was removed from the acid and rinsed three times with Milli-Q water and air dried in a Class 100 laminar flow unit. After drying, equipment was stored in clean plastic bags until required.

2.4 Control and test waters

Control / dilution water was sourced from the JCU MARFU facility and filtered through both sand and 20 µm filters before use (Chapter 3, Section 2.4). A sample of the control / dilution water was analysed using ICP-MS / ICP-AES to determine background levels of a suite of trace elements and ensure elemental concentrations were in the normal range for seawater (Table 4.1).

Table 4.1: Analytical results for seawater used for culturing, control and dilution waters in experiments.

Al ($\mu g.L^{-1}$)	As ($\mu g.L^{-1}$)	Ba ($\mu g.L^{-1}$)	Ca ($mg.L^{-1}$)	Cd ($\mu g.L^{-1}$)	Cr ($\mu g.L^{-1}$)	Cu ($\mu g.L^{-1}$)	Fe ($\mu g.L^{-1}$)	Li ($\mu g.L^{-1}$)	Mg ($mg.L^{-1}$)	Mn ($\mu g.L^{-1}$)	Pb ($\mu g.L^{-1}$)	Sr ($\mu g.L^{-1}$)	Zn ($\mu g.L^{-1}$)
11.0	<1.0	17.2	361	1.0	5.6	2.0	<1.0	154	1221	<0.1	0.32	6749	1.24

Test solutions were prepared 24 hours before the commencement of each test in one or two litre clean, acid washed plastic containers. Test solutions were prepared by diluting the required volumes of either $1 g.L^{-1}$ Cu as $CuCl_2.2H_2O$ or $1 g.L^{-1}$ Zn as $ZnSO_4.7H_2O$ to the necessary concentration with 20 µm filtered control seawater.

The test volume varied among lifestage with polyps of all species exposed to 50 mL of control or treatment solution. Medusae / ephyra were exposed to 40 mL of control or treatment solution. For the survival only test (Test 1), using planuloid buds, groups of 10 planuloid buds were exposed to 30 mL of control or treatment solution. The planuloid survival and development tests (Test 2) exposed individual planuloid buds to 3 mL of test or control solutions. All control and treatment solutions were renewed daily in all tests for all lifestages (ie Static-renewal).

2.5 General test conditions

The test facility was held under a light regime of 12 hours light: 12 hours dark with a grow-light double fluorescent lights suspended 45 cm above the testing bench. The laboratory temperature was maintained at 25 ^{0}C using a reverse cycle airconditioning unit and monitored using a max-min thermometer to ensure the temperature did not fluctuate beyond the prescribed ±2 ^{0}C.

A minimum of five replicates were used in each treatment concentration for all tests. For the polyp tests, each treatment comprised five replicate containers each with one substrate containing a minimum of twenty polyps. Individual planuloid buds (Test 2) and medusae / ephyra were allocated one per replicate container. With the exception of the planuloid bud (non-feeding lifestage) tests, all animals were fed daily (except for Day 0) with newly hatched *Artemia* sp. to ensure animals were in optimum condition.

For all tests, control and treatment waters were replaced daily. Once all test chambers had been cleaned and refilled, they were replaced back on the test bench using the random number generator to allocate position. Replicates were randomly allocated to new positions each day to avoid confounding effects of localised differences in temperature and light. With the exception of the pkanuloid tests, old test waters were analysed for pH, salinity, dissolved oxygen and temperature daily before being discarded.

The endpoint for each test was either 72 hour EC_{50} / LC_{50} (planuloid buds) or 96 hour LC_{50} (polyps and medusa/ephyra). A shorter time period was used for the planuloids buds as they were not fed for the duration of the study and the development of tentacles was used as a reference / end point for development into polyps.

2.5.1 Physico-chemical and analytical measurements of water

Water quality parameters were measured daily on all treatment solutions for the duration of the study. Parameters measured were pH, Salinity (ppt), temperature (^{0}C) and dissolved oxygen (% Saturation). Water quality measurements were performed using a pre-calibrated TPS WD-90 multi-parameter meter.

Analytical chemistry was performed on duplicate water samples at the start of each test. Water samples collected in acid washed 30 mL plastic vials, acidified immediately after collection with 20 % Suprapur grade nitric acid (HNO_3) and stored at 4 ^{0}C until analysed. Water samples were analysed using the methodology set out in Chapter 3 (Section 2.5.2). Control samples were analysed for the full suite of elements while treatment waters were analysed for the relevant metal only (i.e copper or zinc) (Table 3.1). Subsets of samples were spiked with known concentrations of elements to correct for potential instrument drift and matrix effects and also to determine recoveries (72-116 %). Analytical data were checked to ensure the signal strength for results exceeded three standard deviations each element result.

2.6 Testing procedures

2.6.1 Polyp tests

At test commencement, replicate chambers (70 mL cleaned, acid washed urine specimen containers) were filled with 50 mL of test or control water. An additional 100 mL was set aside for physico-chemical analysis. Substrates were removed from the holding tank and the number of polyps counted. Each substrate was randomly allocated to a replicate test chamber and the number of polyps recorded on the data sheet. After all substrates were randomly allocated to test chambers, the chambers were randomly allocated (using a random number generator) to a position on the test bench. Animals were not fed on Day 0.

On Days 1 to 3, substrates were briefly inspected daily to determine if there were any obvious changes in animal health. Each polyp substrate was fed a 0.5 mL concentrated *Artemia* sp. suspension in control seawater daily. Polyps were allowed to feed and digest for 4 – 5 hours. After feeding, each replicate was cleaned to remove excess food and digested wastes and refilled with a fresh 50 mL volume of the appropriate test or control solution. Any polyps that were accidently dislodged during cleaning were pipetted back into their respective test chambers. On Day 4 animals were not fed. Each substrate was inspected under a stereomicroscope and the number of polyps counted. Other qualitative features (e.g. deformities, clubbing or partial tentacle retraction) were also documented.

2.6.2 Ciliated planuloid bud tests

A preliminary pilot study was undertaken to determine the length of time required for settlement and tentacle development in the buds. Three replicates of 10 planuloid buds were placed in 90 mm diameter plastic petri dishes containing 30 mL control seawater. The pilot continued until at least 90 % of planuloid buds had either died or settled and developed tentacles. Within 72 hours of release from the parent polyp, >90 % of planuloid buds had tentacle development. This was used as the test endpoint for the planuloid buds experiments.

Test 1 – Survival (Copper only)

The first planuloid experiment assessed planuloid bud survival to copper by exposing ten buds to 30 mL of control or test water in 90 mm plastic petri dishes. There were five replicates for each treatment or control solution. Once all animals had been allocated and trays were randomly assigned to a position on the test bench.

On Days 1 and 2, planuloids were inspected daily under a stereomicroscope to assess survival. The number of buds in each dish were counted and considered to be alive if they were free swimming or attached to the dish. Due to their small size and lack of obvious features, planuloids were only considered dead if there was complete tissue disintegration. Any buds not present were considered to be dead and disintegrated.

Each replicate was cleaned by carefully pipetting out approximately 20 mL of the old test solution without disturbing the planuloids and replacing it with fresh test solution. The test was terminated when all control animals were either dead or had visible tentacle development (72 hours).

Test 2 – Survival & Planuloid Bud Development (Copper and Zinc)

To assess planuloid bud development as well as survival the test protocol was modified slightly. One copper experiment and two zinc experiments were conducted to assess both development and survival. Ten 24-well Iwaki plastic microplates (16 mm diameter) were set up with each

row of 4 wells allocated to either a test or control water containing 3 mL of the appropriate solution. Individual planuloid buds were randomly allocated to each well so that each tray ended up with a 4 x 6 array of test and control water exposed animals. This was repeated for the remaining nine trays. Once all animals had been allocated and trays were randomly assigned to a position on the test bench.

On Days 1 and 2, planuloids were inspected daily under a stereomicroscope to determine if there were any obvious changes in animal health. Any animals that were dead were removed from the experiment. As already discussed, planuloids were considered dead if there was complete tissue disintegration.

Each replicate container was cleaned by carefully pipetting out 2.5 mL of the old test solution without disturbing the planuloids and replacing it with fresh test solution. Due to the small volume of water in each test chamber, it was not possible to measure the water quality parameters on the old test waters. The test was terminated when all control animals were either dead or had visible tentacle development (72 hours).

2.6.3 Medusa / ephyra tests

At the commencement of the experiment, individual *A. mordens* medusae or *Cassiopea* sp. ephyra were randomly allocated to a replicate test chamber containing 40 mL of control or treatment water. Each treatment and the control were allocated five replicate animals per treatment. After all animals had been randomly allocated to test chambers, the chambers were randomly allocated (using a random number generator) to a position on the test bench. Once all test chambers had been randomly allocated, the time was recorded as Day 0 of the test. Animals were not fed on Day 0. An additional 100 mL was set aside for physico-chemical analysis.

On Days 1 to 3, animals were inspected daily using a stereomicroscope to determine if there were any obvious changes in animal health. Any dead animals were removed from the experiment. Animals were considered dead if there were no visible signs of pulsing, or tissue contractions. Each surviving animal received 0.3 mL of a concentrated *Artemia* sp. suspension in control water. Medusae / ephyra were allowed to feed undisturbed for 4 – 5 hours. After feeding, each animal was gently removed from the old test water using a wide bore 3 mL plastic pasteur pipette that had its tip cut off. The old test water was poured into a 250 mL plastic container and the test container refilled with 40 mL of new control or test solution. The animal was gently replaced into the new solution. After ninety six hours, each replicate animal was inspected under a stereomicroscope and the health of the animal determined. Other qualitative features were also documented (e.g. any obvious deformities, poor pulse rates, etc).

2.7 Test acceptability

Each test was only considered to be valid if they met minimum criteria for acceptability. No test had a greater than 2 ^{0}C variation in temperature between measurements; >2 ppt variation in salinity; <1 pH unit between measurements; or <70 % dissolved oxygen saturation over the duration of the observations. All tests had 90 % or greater survival in the control animals.

2.8 Data analysis

All data was checked for anomalies and data were analysed using measured rather than nominal metal concentrations. The LC_{50} and EC_{50} values of copper and zinc for the different lifestages were determined using a non-linear regression with a four-parameter logistic equation with variable slope in GraphPad Prism version 5.00 (Motulsky & Christopoulos 2003; GraphPad Software, CA) (Equation 1). Normality of the residuals was checked to ensure data met the assumptions of the regression using D'Agostino-Pearson normality test.

$$Y = Min + (Max - Min) / (1+10^{(logLC_{50} - x) / Hillslope)}) \quad [Eq\ 1]$$

Where:

Min was the lowest response;

Max was the highest response; and,

Hillslope was the slope of the curve (also called the slope factor).

During curve fitting, some data provided ambiguous results due to a lack of sufficient data points at the bottom of the curve (0 % survival). Where this occurred, the minimum was constrained to 0 to improve the minimum plateau (Motulsky & Christopoulos 2003). The hillslope can be defined as the "steepness" of the slope of the curve (Motulsky & Christopoulos 2003). Due to the spread of data points in most experiments, hypothesis testing to derive the lowest-observed-effect-concentrations (LOEC) was higher than the point estimates of the LC_{50} / EC_{50} and thus has not been reported (Bruce & Versteeg 1992).

3. Results

3.1 Effects of copper exposure

Cassiopea sp. was the most sensitive of the three species to copper exposure (Table 4.2). There was decreased survival with increasing copper concentration for all lifestages of *Cassiopea* sp. exposed to copper. The planuloid bud was the most sensitive lifestage, followed by the ephyra with the polyp the least sensitive of the three lifestages in *Cassiope*a sp. (Table 4.2; Figures 4.1 to 4.3).

Planuloid bud survival was higher than planuloid bud development. The 72 hour LC_{50} for survival only (Test 1) was 22.7 $\mu g.L^{-1}$ Cu while the 72 hour EC_{50} for development (Test 2) was 11.3 $\mu g.L^{-1}$ (Table 4.2; Figure 4.1). Planuloid buds did not always attach to the sides or bottom of the test chamber before developing tentacles. The response of planuloid buds to copper exposure was variable with Test 1 having a higher sensitivity to copper than Test 2. The cause of this variability is unknown. As planuloid bud survival in the second test did not exceed 50 %, a reliable LC_{50} could not be determined (Figure 4.1). Planuloid bud development was not assessed in the planuloid Test 1 for copper.

The 96 hour LC_{50} for *Cassiopea* sp. ephyra was 24.3 $\mu g.L^{-1}$ Cu. This was only slightly less sensitive than the planuloid bud survival (Table 4.2). Due to only one data point falling between 0 % and 100 % survival in *Cassiopea* sp. ephyra, the 95 % confidence interval could not be determined. Animals exposed to the higher copper concentrations (38.8 $\mu g.L^{-1}$Cu and above) did not feed and showed no regular pulsing of the bell. Animals that were close to death were moribund and while they did not actively pulse, irregular tissue spasms were observed. Some ephyra in the intermediate copper treatments (24.4 $\mu g.L^{-1}$Cu) also everted their bell to form a "taco" shape, although this did not prevent them from feeding.

The *Cassiopea* sp. polyps were the least sensitive of the three lifestages to copper exposure with a 96 hour LC_{50} 44.6 $\mu g.L^{-1}$. *Cassiopea* sp. polyps exhibited qualitative responses to increasing copper exposure with tentacle clubbing and partial retraction in the higher concentrations (36.1 $\mu g.L^{-1}$ Cu and above). Polyps close to death completely retracted their tentacles and once animals died, the head of the polyp rapidly degraded leaving only the polyp stalk.

Table 4.2: Summary of results by species and lifestage to aqueous copper exposure ($\mu g.L^{-1}$). Values are calculated from the mean measured copper concentrations at start of tests. EC_{50} / LC_{50} – Effect or lethal concentration or copper ($\mu g.L^{-1}$) affecting 50 % animals; EC_{10} / LC_{10} – Effect or lethal concentration or copper ($\mu g.L^{-1}$) affecting 10 % animals; 95 % CI represents 95 % Confidence Interval; N.D. – not able to be determined.

Species	**Lifestage**	**EC_{50} / LC_{50}**	**95 % CI**	**EC_{10}/ LC_{10}**	**95 % CI**
Cassiopea sp.	Planuloid Bud – Survival (Test 1)	22.7	18.4-28.0	16.6	12.0-22.9
	Planuloid Bud – Development (Test 2)	11.3	9.52-13.4	7.1	5.4-9.2
	Ephyra	24.3	N.D.	23.5	N.D.
	Polyp	44.6	41.4-47.8	32.6	30.0-35.5
Alatina mordens	Medusae	38.8	33.3-45.5	29.4	19.4-44.8
	Polyp	>69.1	N.D.	N.D.	N.D.
Aurelia sp.	Polyp	107	103-110	99.5	N.D.

Alatina mordens medusae were more sensitive to copper than the polyps (Table 4.2). Prior to death in the higher copper treatments (40.6 $\mu g.L^{-1}$ Cu and above), medusae ceased to feed and showed erratic swimming patterns. Ill medusae also had very contracted and semi-everted bells prior to death. Although there was no mortality in *A. mordens* polyps exposed to copper concentrations as high as 69.1 $\mu g.L^{-1}$ (Figure 4.3), the polyps exhibited some behavioural responses at the higher (49 $\mu g.L^{-1}$ Cu and above) concentrations of copper with the tentacles remaining partially contracted. The tentacle retractions did not seem to interfere with feeding in *A. mordens* though, as polyps were observed to consume *Artemia* sp. despite the partially contracted tentacles.

The 96 hour LC_{50} for *Aurelia* sp. polyps was 107 $\mu g.L^{-1}$ Cu (Table 4.2). Healthy *Aurelia* sp. polyps fed very actively each day and were a light pink in colour. This feeding behaviour was modified in animals exposed to higher concentrations of copper. Polyp tentacles increasingly developed slight thickening at the tips of the tentacles (clubbing) and this response increased to partial tentacle contraction in the higher exposure concentrations. At the highest concentration (121.5 $\mu g.L^{-1}$ Cu), an initial response by the polyps was to continue to capture *Artemia* sp. prey but the polyps did not consume them. Colouration in the polyps faded to a pale white over the test period in sick animals and tentacle retractions increased until they were fully contracted. At the onset of death animals were white, completely contracted and the external tissue surface showed signs of degradation.

Cassiopea sp. polyp and ephyra lifestages were more sensitive than *A. mordens* and *Aurelia* sp. lifestages to copper (Figures 4.2 & 4.3). The 96 hour LC_{50} to *A. mordens* medusae was 38.8 $\mu g.L^{-1}$ compared with a 96 hour LC_{50} of 24.3 $\mu g.L^{-1}$ for *Cassiopea* sp. ephyra (Table 4.2). *Cassiopea* sp. polyps were more sensitive to copper than either *A. mordens* or *Aurelia* sp. polyps (Figure 4.3). As there is no equivalent lifestage to the *Cassiopea* sp. planuloid buds, it was not possible to compare the response with the other species.

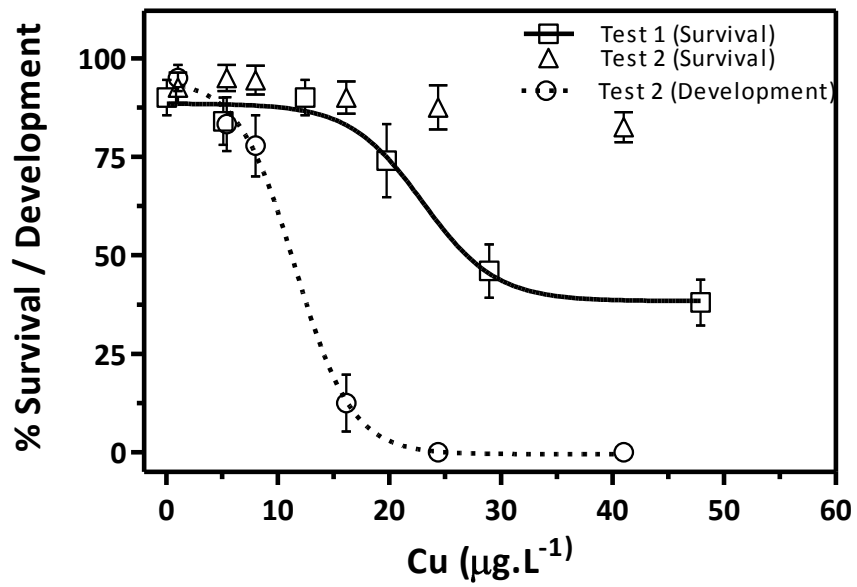


Figure 4.1: Survival and tentacle development in *Cassiopea* sp. planuloid buds exposed to measured aqueous copper with non-linear regression fitted. Curves represent results from individual experiments. No regression could be fitted for Test 2 (Survival). n=5 ± SEM for Test 1, n=10 ± SEM for Test 2 (Survival & Development).

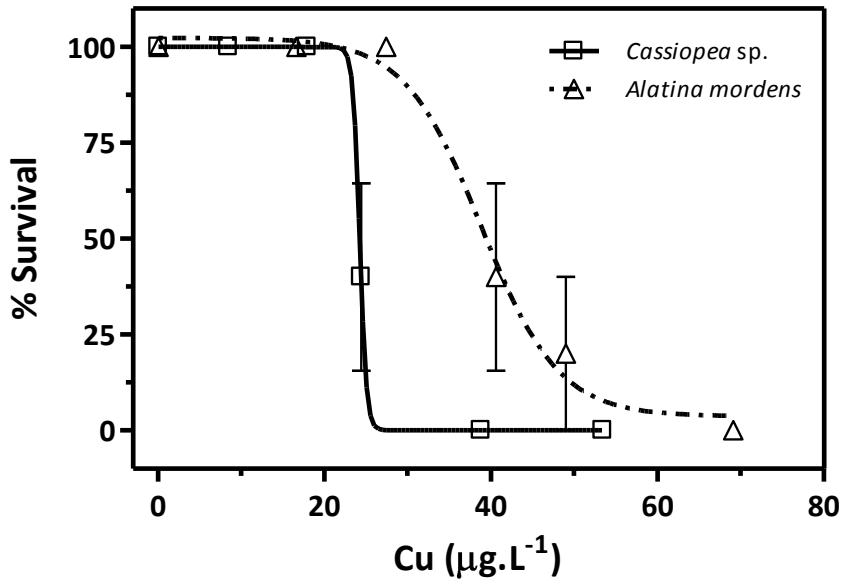


Figure 4.2: 96 hr survival of ephyra / medusae in *Cassiopea* sp. and *Alatina mordens* exposed to measured aqueous copper with non-linear regression fitted. Curves represent results from individual experiments. n=5 ± SEM.

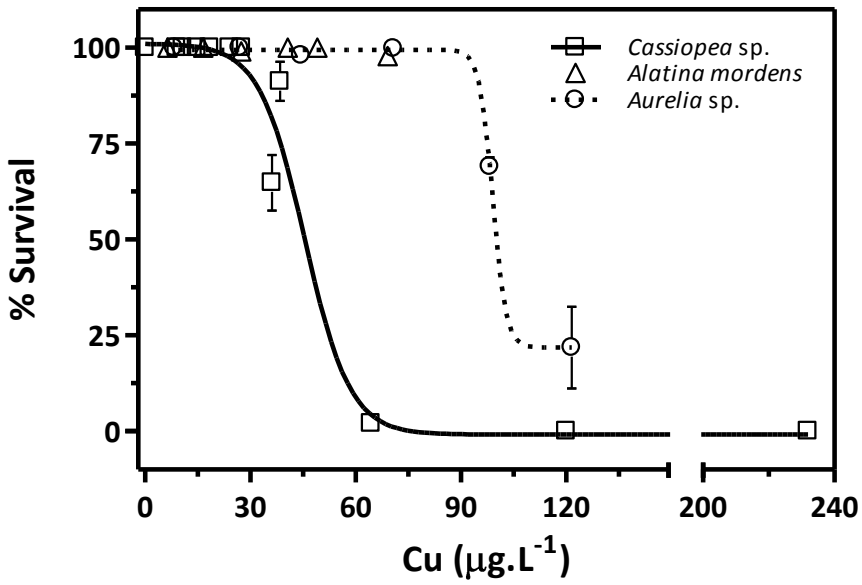


Figure 4.3: 96 hr survival in *Cassiopea* sp., *Alatina mordens* and *Aurelia* sp. polyps exposed to measured aqueous copper with non-linear regression fitted. Curves represent results from individual experiments. No regression could be fitted for *Alatina mordens* survival. n=5 ± SEM.

3.2 Effects of zinc exposure

All lifestages of all species were much less sensitive to zinc than to copper (Table 4.3). For most lifestages a reliable EC_{50} / LC_{50} could not be determined as mortality / effect did not exceed 50 % of the individuals. As for copper, *Cassiopea* sp. was more sensitive to zinc at all lifestages compared to *A. mordens* and *Aurelia* sp. The 96 hour LC_{50} for zinc in *Cassiopea* sp. ephyra was 1.84 $mg.L^{-1}$ (Table 4.3, Figure 4.4). The response of animals in the higher zinc concentrations (1.94 $mg.L^{-1}$ Zn and above) was similar to that seen in the higher copper treatments, i.e. lack of feeding, lack of pulsing and development of irregular spasms in the bell. Once the animals died, the tissue rapidly degraded. *A. mordens* medusae had decreased survival at 2.55 $mg.L^{-1}$ Zn but this was not significant. Medusae exposed to concentrations up to 0.86 $mg.L^{-1}$ Zn did not show any sub-lethal response. Individuals exposed to 2.55 $mg.L^{-1}$ Zn fed during the experiment but exhibited slower swimming behaviour which may have inhibited prey capture.

An EC_{50} / LC_{50} could not be derived for either the *Cassiopea* sp. polyps or planuloid buds to zinc as mortality did not exceed 50 % in either study (Figures 4.5 & 4.6). There was a decrease in survival in *Cassiopea* sp. polyps above 2 $mg.L^{-1}$ Zn but this was not significant. The polyps exhibited some sub-lethal responses at the higher zinc concentrations. Polyps exposed to 1.72 $mg.L^{-1}$ and 1.94 $mg.L^{-1}$ Zn had partial contraction of the tentacles. Polyp tentacles in the

2.40 mg.L^{-1} Zn and 2.75 mg.L^{-1} Zn concentrations were totally retracted and there was minimal feeding evident after three days.

Exposure to 0.34 mg.L^{-1} Zn was not lethal to the planuloid buds. There inhibition of planuloid development between 0.05 mg.L^{-1} and 0.2 mg.L^{-1} Zn but recovery at higher exposure concentrations (Figure 4.6). The response of the planuloid buds was similar between the two tests (Figure 4.6). In the highest concentration of zinc, many of the planuloid buds that underwent tentacle development to form polyps, however these were deformed with asymmetry in the tentacle distribution around the polyp head, and on occasion bifurcated tentacles. This effect was not seen in the control animals.

Despite exposure to concentrations as high as 5.47 mg.L^{-1} Zn, there was no mortality in *Aurelia* sp. polyps (Figure 4.5). Concentrations greater than 6 mg.L^{-1} Zn were not tested as there was the potential for significant precipitation / adsorption of the metal on the test equipment.

Table 4.3: Summary of results by species and lifestage to aqueous zinc exposure (mg.L^{-1}). Values are calculated from the mean measured zinc concentrations at start of tests. EC_{50} / LC_{50} – Effect or lethal concentration of zinc (mg.L^{-1}) affecting 50 % animals; EC_{10} / LC_{10} – Effect or lethal concentration of zinc (mg.L^{-1}) affecting 10 % animals; 95 % CI – 95 % Confidence Interval; N.D. – not able to be determined.

Species	**Lifestage**	**EC_{50} / LC_{50}**	**95 % CI**	**EC_{10}/ LC_{10}**	**95 % CI**
Cassiopea sp.	Planuloid Bud – Survival	>0.34	N.D.	N.D.	N.D.
	Planuloid Bud – Development	>0.34	N.D.	0.02	N.D.
	Ephyra	1.84	1.42 – 2.38	1.31	0.76-2.27
	Polyp	>2.75	N.D.	N.D.	N.D.
Alatina mordens	Medusae	>2.55	N.D.	N.D.	N.D.
Aurelia sp.	Polyp	>5.47	N.D.	N.D.	N.D.

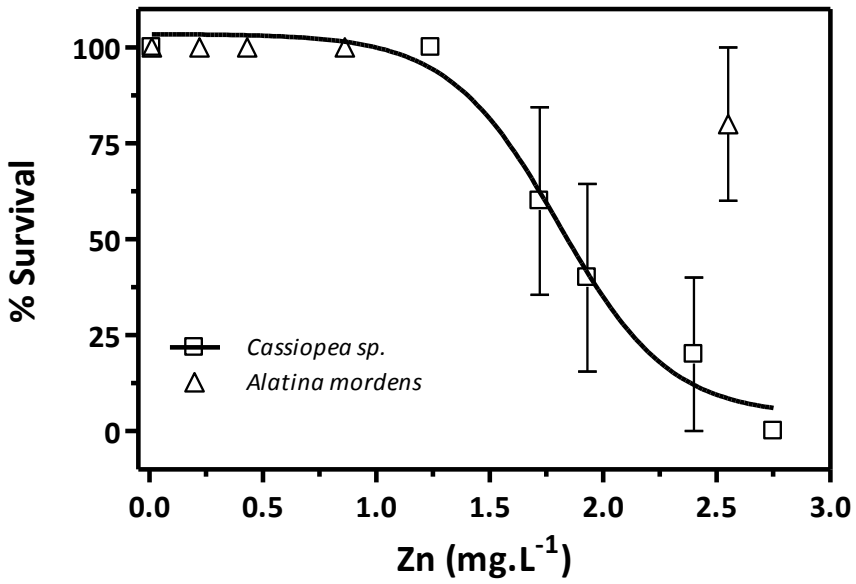


Figure 4.4: Survival of ephyra / medusae in *Cassiopea* sp. and *Alatina mordens* exposed to measured aqueous zinc with non-linear regression fitted. Curves represent results from individual experiments. No regression could be fitted for *Alatina mordens*. n=5 ± SEM.

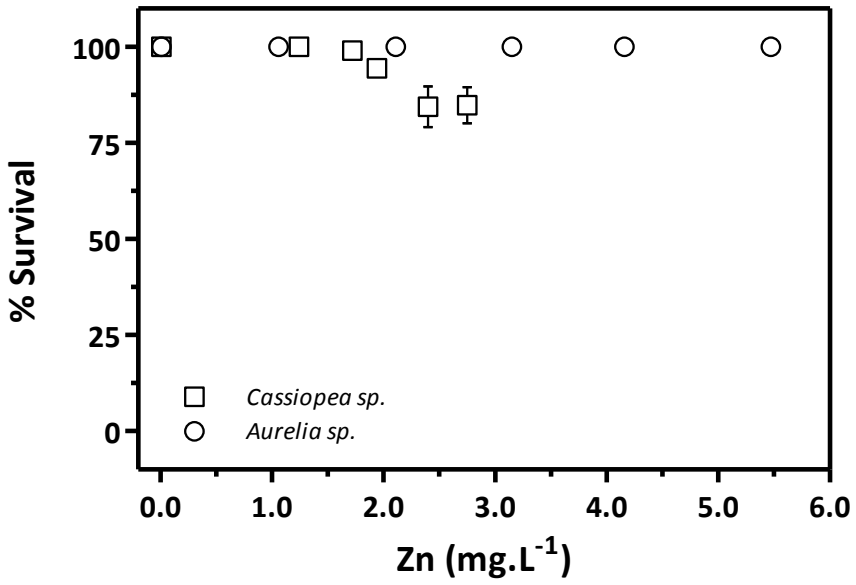


Figure 4.5: Polyp survival in *Cassiopea* sp. and *Aurelia* sp. exposed to measured aqueous zinc. No regression could be fitted to the data. n=5 ± SEM.

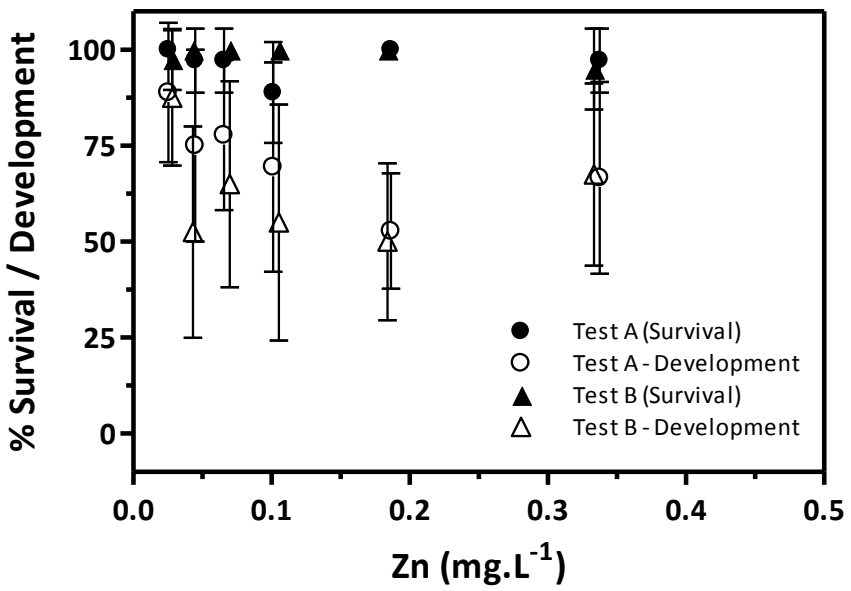


Figure 4.6: Survival and tentacle development in *Cassiopea* sp. planuloid buds exposed to measured aqueous zinc. Development - represents development in planuloid buds for each test. No regression could be fitted to the data. n=10 ± SEM.

Survival in the controls in all tests was 90 % or greater, validating one of the criteria for test acceptability (Section 2.6). The physico-chemical parameters held constant over the duration of the tests with pH varying by <0.5 unit, salinity <0.5 ppt and dissolved oxygen >80 % within each of the tests. Elemental results for both control and test solutions were typical for seawater.

4. Discussion

Historically, jellyfish have been assumed to be tolerant of pollution compared with other marine organisms (Arai 1997). However, there have been studies which demonstrated that exposure to pollutants caused morphological deformities and behavioural changes in *Aurelia aurita* polyps (Spangenberg et al. 1980; Spangenberg 1984; Spangenberg 1986). Although there are general descriptions of metal tissue concentrations in jellyfishes (e.g. Cimino et al. 1983; Fowler et al. 2004; Templeman & Kingsford 2010), there is only one study on the toxicity of metals in jellyfish (Spangenberg 1986).

This study investigated three jellyfish species and found that all three species were very sensitive to copper and by an order of magnitude less sensitive to zinc. *Cassiopea* sp. was the most sensitive of the three jellyfish species to both copper and zinc. The polyps of all three species were the least sensitive lifestage to copper and zinc exposure. Overall, the sensitivity of the different lifestages to both copper and zinc in this study was comparable with results for

other marine species (e.g. Calabrese et al. 1973; Frias-Espericueta et al. 2003; Bao et al. 2008; Langdon et al. 2009).

It was not possible to establish meaningful lowest-observed-effect-concentrations (LOEC) in this study due to the lack of intermediate data points bracketing the LC_{50} in many of the tests. This issue has been recognised by many researchers and has prompted debate on the most appropriate methods to derive ecologically relevant measures of effect (e.g. Bruce & Versteeg 1992; Moore & Caux 1997; Oris & Bailer 1997; Stephensen et al. 2000). For the purposes of this assessment, the determination of LC_{50} provided sufficient information to gauge relative sensitivities of the jellyfishes to metal pollution. However, further work would be useful to expand the results obtained here.

4.1 Response to copper

Copper concentrations are normally low in natural marine waters, but can be very high in polluted coastal waters (Xie et al. 2005). The use of copper based anti-foulants as an alternative to tributyltin is contributing to the increasing load in many systems (Bao et al. 2008). Given that aqueous copper can be toxic to many marine organisms at low concentrations, elevated copper levels can have a major impact on local biodiversity in coastal systems (Grosell et al. 2007).

All species tested in this study were sensitive to copper, although sensitivity varied among both species and lifestages. Of the three species, *Cassiopea* sp. had the greatest sensitivity to copper at all lifestages with the most sensitive lifestage being the planuloid buds. The newly released *Cassiopea* sp. ephyra were only slightly less sensitive than the planuloid buds. The polyps of all three species were the least sensitive of the lifestages to copper with polyp sensitivity *Cassiopea* sp. < *A. mordens* = *Aurelia* sp.

The LC_{50} of all lifestages in this study were lower than those reported for many other marine species indicating that these three jellyfish species and scyphozoan jellyfishes in general, may be quite sensitive to copper (Table 4.4). The *Cassiopea* sp. planuloid buds were very sensitive to copper with an LC_{50} similar to that of the flatworm (*Phrikoceros baibaiye*), which is among the lower LC_{50} reported in the literature (Table 4.4). Exposure concentrations inhibiting tentacle development in the planuloid buds were also similar to reported concentrations affecting mollusc embryo and egg development, which are considered very sensitive indicators of copper (e.g. Coglianese & Martin 1981; Gorski & Nugegoda 2006). The LC_{50} for *Cassiopea* sp. polyps was comparable to that reported for *Cancer magister* (Crab) zoeae, which is an often used test species (Table 4.4). The results demonstrated the jellyfishes tested in this study were very sensitive to copper exposure, and this sensitivity was at ecologically relevant concentrations.

There is no reported data on copper toxicity in jellyfishes. However, studies with anthozoans and freshwater hydrozoans have demonstrated both that Cnidaria can be sensitive to copper and this response can be variable among species (e.g. Heyward 1988; Karntanut & Pascoe 2002; Reichelt-Brushett & Michalek-Wagner 2005). Adult sea anemones (*Actinia* sp.) exposed to copper had a reported 96 hr LC_{50} of between 182 $\mu g.L^{-1}$ and 347 $\mu g.L^{-1}$ (Hughes et al. 2005). This is 7.5 to 14.5 times less sensitive than the results reported here. The NOEC and LOEC reported by Hughes et al. (2005) for *Actinia* sp. were also higher than the LC_{50} for *Cassiopea* sp.

In comparison to other cnidarians, fertilisation success in the soft coral *Lobophytum compactum* was higher than in the hard coral, *Goniastrea aspera* exposed to copper (Reichelt-Brushett & Harrison 1999; Reichelt-Brushett & Michalek-Wagner 2005). Similar variation was also seen among species in this study with *Cassiopea* sp. polyps being twice as sensitive to copper as *Aurelia* sp. polyps (Table 4.4).

To date there have been no definitive studies on the mechanisms of copper toxicity in cnidarians. Generally, there is little information on the mechanisms of copper toxicity in marine invertebrates, although it is suggested that like freshwater species, the gill may be the main site for copper toxicity (Bianchini et al. 2004). It has also been suggested that copper may induce toxicity through a cytosolic reaction between copper and glutathione which is important in cell mitosis (Stauber & Florence 1987). This reaction can lead to a lowering of the reduced glutathione:oxidised glutathione ratio (GSH : GSSG) and consequential mitotic inhibition (Stauber & Florence 1987). For example, a high GSH : GSSG ratio has been associated with mitotic cell division in sea urchin eggs (Stauber & Florence 1987). This mechanism may explain the greater sensitivity of the newly metamorphosed lifestages to copper in the jellyfish species in this study compared with the morphologically mature polyps. As the ephyra / medusae had undergone energy intensive metamorphosis immediately prior to the experiment, it is also likely that metabolic reserves would have been very low, which could have also contributed to the higher sensitivity (Shilling et al. 1996).

Table 4.4: Acute toxicity of copper to selected marine species.

Species	Concentration ($\mu g.L^{-1}$)	Lifestage / Size	Response	Reference
Crassostrea virginica (Oyster)	103	Embryo	48 hr LC_{50}	Calabrese et al. 1973
Cancer magister (Crab)	49	Zoeae	96 hr LC_{50}	Martin et al. 1981
Litopenaeus vannamei (Whiteleg Shrimp)	37000	Post larvae	96 hr LC_{50}	Frias-Espericueta et al. 2003
Nephtys australiensis (Polychaete)	210	40 -50 mm	96 hr LC_{50}	King et al. 2004
Mysella anomala (Bivalve)	1500	4 – 5 mm	96 hr LC_{50}	King et al. 2004
Tellina deltoidalis (Bivalve)	150	15 – 20 mm	96 hr LC_{50}	King et al. 2004
Soletellina alba (Bivalve)	120	15 – 20 mm	96 hr LC_{50}	King et al. 2004
Phrikoceros baibaiye (Flatworm)	14 - 17	Adult	96 hr LC_{50}	Hughes et al. 2005
Alope orientalis (Shrimp)	54 - 128	Adult	96 hr LC_{50}	Hughes et al. 2005
Actinia sp. (Sea Anemone)	182 - 347	Adult	96 hr LC_{50}	Hughes et al. 2005
Squalus acanthias (Spiny Dogfish)	800-1000	0.79 ± 0.18 kg	96 hr LC_{50}	Deboek et al. 2007
Hydroides elegans (Polychaete)	120	Trochophore larvae	48 hr LC_{50}	Bao et al. 2008
Elasmopus rapax (Amphipod)	78	Juvenile	96 hr LC_{50}	Bao et al. 2008
Tigriopus japonicas (Copepod)	323 - 585	Adult	96 hr LC_{50}	Kwok et al. 2008
Spirorbis nordenskjoldi (Spirorbid Polychaete)	570	Healthy	10 day LC_{50}	Hill et al. 2009
Cassiopea sp. (Upside-down Jellyfish)	22.7 24.3 44.6	Planuloid Bud Ephyra Polyp	72 hr LC_{50} 96 hr LC_{50} 96 hr LC_{50}	This study
Alatina mordens (Cubozoan Jellyfish)	38.8 >69.1	Medusae Polyp	96 hr LC_{50} 96 hr LC_{50}	This study
Aurelia sp. (Moon Jellyfish)	107	Polyp	96 hr LC_{50}	This study

4.2 Response to zinc

Like copper, zinc is considered an essential element that is required in trace amounts for metabolic health. However, it can also be toxic at concentrations above that required for optimum metabolic function with toxicity varying depending on species and lifestage. There are currently no reported studies on zinc toxicity to any of the lifestages of schyphozoan or cubozoan jellyfishes.

Overall, this study found all jellyfish species at all lifestages to be less sensitive to zinc by an order of magnitude compared to copper. *Cassiopea* sp. ephyra were the most sensitive species lifestage tested with a 96 hour LC_{50} of 1.78 $mg.L^{-1}$ zinc. It was not possible to define an LC_{50} for the other lifestages and other species at the concentrations tested. This may be due to zinc being less toxic overall or that all three species were able to detoxify zinc in some manner.

For many species, zinc toxicity requires concentrations in the order of $mg.L^{-1}$ (Table 4.5). Sensitivity does occur, with early lifestages of the oyster, *Crassostrea virginica* and the crab *Cancer magister* found to be very sensitive to zinc (Calabrese et al. 1973; Martin et al. 1981). However, variation exists among similar species with the zoeae of the Southern King Crab (*Lithodes santolla*) an order of magnitude less sensitive than *C. magister* (Table 4.5). The *Cassiopea* sp. planuloid buds in this study were less sensitive than *C. virginica* but given limited toxicity at the highest exposure concentrations of 0.34 $mg.L^{-1}$ Zn it was not possible to determine exactly how tolerant they were to zinc exposure. The LC_{50} of the *Cassiopea* sp. ephyra was higher than that reported for early lifestage *C. virginica* and *C. magister* but lower than post larval *Litopenaeus vannamei* (Whiteleg Shrimp) and *L. santolla* (Table 4.5). It was also lower than the LC_{50} reported for many adult marine invertebrates (Table 4.5). As the LC_{50} could not be determined for the polyps of *Cassiopea* sp. and *Aurelia* sp., and the medusae of *A. mordens*, it was not possible to compare these lifestages to other species (Table 4.5). This suggests that these lifestages in the jellyfish species tested may not be sensitive to zinc exposure.

Table 4.5: Acute toxicity of zinc to selected marine species.

Species	Concentration ($mg.L^{-1}$)	Lifestage / Size	Response	Reference
Crassostrea virginica (Oyster)	0.31	Embryo	48 hr LC_{50}	Calabrese et al. 1973
Cancer magister (Crab)	0.456	Zoeae	96 hr LC_{50}	Martin et al. 1981
Lithodes santolla (Southern King Crab)	2.54	Zoeae	96 hr LC_{50}	Amin et al. 2003
Litopenaeus vannamei (Whiteleg Shrimp)	2.08	Post Larvae	96 h-LC_{50}	Frias-Espericueta et al. 2003
Nephtys australiensis (Polychaete)	>5.80	40 -50 mm	96 h-LC_{50}	King et al. 2004
Mysella anomala (Bivalve)	4.50	4 – 5 mm	96 h-LC_{50}	King et al. 2004
Tellina deltoidalis (Bivalve)	>0. 97	15 – 20 mm	96 h-LC_{50}	King et al. 2004
Soletellina alba (Bivalve)	2.90	15 – 20 mm	96 h-LC_{50}	King et al. 2004
Spirorbis nordenskjoldi (Spirorbid Polychaete)	>4.91	Healthy	10 day LC_{50}	Hill et al. 2009
Cassiopea sp. (Upside-down Jellyfish)	>0.34 1.84 >2.75	Planuloid Bud Ephyra Polyp	72 hr LC_{50} 96 hr LC_{50} 96 hr LC_{50}	This study
Alatina mordens (Cubozoan Jellyfish)	>2.55	Medusae	96 hr LC_{50}	This study
Aurelia sp. (Moon Jellyfish)	>5.47	Polyp	96 hr LC_{50}	This study

In freshwater species, zinc and calcium appear to compete for uptake sites, with elevated zinc concentrations inhibiting calcium uptake in fish, leading to hypocalcemia (Spry & Wood 1985; Santore et al. 2002). A similar response has also been shown in *Daphnia pulex* suggesting that competitive cation uptake effects are typical of zinc in freshwater and are a key means by which zinc can be toxic (Clifford & McGeer 2009). The higher concentrations of calcium in seawater may, therefore, confer a level of protection against zinc toxicity in marine biota. This effect has been seen in marine decapods with calcium concentrations modifying cadmium toxicity, and it is expected that competitive effects with zinc may be similar (Bjerregaard & Depledge 1994). Zinc toxicity is also thought to occur through the breakdown of respiratory and osmoregulatory processes, as demonstrated in the killifish (*Fundulus heteroclitus*) through desquamation of the mucosal epithelia (Crespo 1984). It is possible that zinc exposure may stimulate a similar process that interferes with mucosal production in jellyfish leading to sub–lethal or lethal effects, although there is no current research to support this. Mucosal production is important in both feeding and defence in scyphozoan jellyfish (Arai 1997), and any interference in production may increase inhibition of activity and health of the medusae.

4.3 Variations in metal response

Variation in LC_{50} among lifestages for the jellyfishes in this study is typical of many organisms (e.g. Williams et al. 1986; Medina et al. 2002; Hoang & Klaine 2007). The polyp is considered one of the two adult body forms in Cnidaria (Arai 1997) and is the only adult form in the Anthozoa (i.e. corals and anemones). In Scyphozoa, there is alternation of generation between the polyp and medusae (Arai 1997). Among the polyps tested in this study, *Aurelia* sp. and *A. mordens* were less sensitive to copper than *Cassiopea* sp. Overall, the polyps were less sensitive to copper than the other lifestages. This may have been due to its morphological maturity as many species exhibit decreasing sensitivity to toxicants with increasing maturity (e.g. Green et al. 1996).

Decreased sensitivity to metal exposure with increasing age is due to a number of factors including: change in developmental mechanisms, change in metabolic rate, change in reproductive state, increased lipid reserves and increase in overall size (e.g. Williams et al. 1986; Green et al. 1996; Xie et al. 2005). For example, copper sensitivity in larval stages of the polychaete *Hydroides elegans* decreased with increasing age and size (Xie et al. 2005). Williams et al. (1986) reviewed sensitivities of macroinvertebrates to toxicants and found that sensitivities could be 54 times greater in the youngest lifestages.

Metamorphosis is also a very energy intensive process (Shilling et al. 1996) and many studies have shown that copper exposure can affect metamorphosis in larval stages (e.g. Green et al. 1996; Xie et al. 2005). In the hard coral *Acropora millepora*, larval metamorphosis was inhibited (24 hour EC_{50}) at 110 $\mu g.L^{-1}$ Cu (Negri & Heyward 2001). Due to the increased energy demands associated with metamorphosis, animals potentially have a reduced ability to detoxify metals. In the polychaete *H. elegans,* settlement and metamorphosis of the trochophore was the most sensitive lifestage tested with a lower EC_{50} than other lifestages (Xie et al. 2005). This may be the reason for the increased sensitivity of both the ephyra / medusae in *Cassiopea* sp. and *A. mordens* to zinc and / or copper and also the increased sensitivity of *Cassiopea* sp. planuloid bud tentacle development to copper exposure.

Unlike other marine animals, Cnidaria do not seem to possess metallothioneins (Anderson et al. 1988), a metal binding protein that has a fundamental role in copper and zinc binding at both essential concentrations and in detoxification (Amiard et al. 2006). However, there may be alternative metal binding proteins that fulfil a similar role to metallothioneins (Anderson et al. 1988). For example, within sea anemones the production of metal binding anti-oxidants like glutathione is a common detoxification method (Mitchelmore et al. 2003a). Other detoxification mechanisms in invertebrates include the production of intra- or extra-cellular granules with varying chemical or cyto-chemical features. They include copper

containing granules (Cu detoxification) and calcium containing granules for zinc detoxification (Barka 2007). Further research is required to determine what detoxification mechanisms exist in the jellyfish lifestages to establish whether they utilise similar detoxification methods as other Cnidaria.

4.4 Bioindicator potential of jellyfishes

Acute toxicity tests and LC_{50} estimations are a common method for evaluating the toxicity of metals (Zhou et al. 2008). A range of species have been evaluated as standard toxicity species for assessing acute lethality of metals (e.g. Zhou et al. 2008; Langdon et al. 2009). However, there have been a number of knowledge gaps identified in these reviews, particularly with respect to limitations among metals and species (van Damm et al. 2008; Langdon et al. 2009). There has also been increasing recognition of geographical variability in organisms between polar, temperate and tropical regions (e.g. Kwok et al. 2007). While there are some data available on toxicity of metals to Cnidaria, it is both limited and largely restricted to anthozoans and hydrozoans (e.g. Negri & Heyward 2001; Hughes et al. 2005; Reichelt-Brushett & Michalek-Wagner 2005).

The outcomes of this study suggest that all three jellyfishes would be suitable candidates as bioindicator species for copper, although further work would be required to establish their utility as indicators of zinc pollution. The different lifestages were robust to handling in the laboratory and sensitive to the metal under consideration. Other studies have also shown that sub-lethal responses of *Cassiopea* sp. in particular, would be strong endpoints in toxicity testing programs (Chapter 5).

Cassiopea sp. has a wide distribution across the tropics and sub-tropics (Holland et al. 2004) and has been used experimentally for other studies. *Aurelia* sp. is cosmopolitan in distribution and is present in both temperate and tropical waters (Purcell et al. 2000), allowing them to be utilised across a wide geographic extent. It is probably the most widely studied and cultured jellyfish species (Purcell et al. 2000). *A. mordens* provided comparative data on the sensitivity between scyphozoan and cubozoan jellyfishes and is readily cultured under laboratory conditions. However, due to the uncertainty of distribution (Bentlage et al. 2010), generally low abundance and toxicity of its venom, *A. mordens* would not be as utilitarian as the other two species.

Overall, though this study demonstrates that jellyfish are sensitive to copper or zinc exposure. The ability to maintain and culture them under laboratory conditions supports the use of them as toxicity test species. Further work would be needed though to determine their relative sensitivities to other metals and toxicants.

5. Conclusions

The reported LC_{50} for the different species and lifestages in this study were similar to or lower than reported toxicities to copper for other marine species. Sensitivity to zinc was lower and the inability to establish a dose-response curve for many lifestages suggests that the jellyfish species tested here are less sensitive to zinc. The most sensitive lifestage to copper exposure was the *Cassiopea* sp. planuloid bud followed by the newly metamorphosed *Cassiopea* sp. ephyra and *A. mordens* medusa. Tentacle development in planuloid buds were a very sensitive sub-lethal response to copper exposure. These lifestages were sensitive to copper exposure at concentrations found in polluted waterways and thus fulfil the criteria for making a good indicator species. The *Cassiopea* sp. ephyra was the most sensitive lifestage to zinc. *Cassiopea* sp. was the most sensitive species to both copper or zinc exposure and *Aurelia* sp. the least sensitive. Species diversity, availability and ease of maintaining the different lifestages in laboratory culture indicate that both *Cassiopea* sp. and *Aurelia* sp. have utility as toxicity test species. Due to handling issues, uncertainties in distribution and lack of abundance, *A. mordens* would be less suitable as a test species.

Chapter 5 - Sub-lethal Responses of *Cassiopea* sp. and it's Endosymbiont *Symbiodinium* sp. to Copper and Zinc Exposure

1. Introduction

Cassiopea sp. is a jellyfish with zooxanthellae present as an endosymbiont. Like its anthozoan cousins the corals, it is capable of both autotrophy and heterotrophy. This offers the potential to assess the response of both the host and its symbiont to contaminant stress. This has been undertaken previously in both corals and sea anemones (e.g. Harland and Nganro 1990; Reichelt-Brushett & Harrison 2005) but not jellyfish.

Increasing inputs of anthropogenic contaminants into coastal marine environment are causing stress on marine assemblages (e.g. Hunter et al. 1997; Luoma & Rainbow 2008). In order to mitigate this pollution, the effects on biota need to be understood and warning signs of adverse impacts identified. The use of marine species as bioindicator and biomonitoring tools are recognised as effective methods for understanding the complexity of interactions between pollutants and the environment (Rainbow 1995; Peters et al. 1997; Luoma & Rainbow 2008). The first such studies undertaken to try and establish cause and effect used crude measures of effect (i.e. death). These acute toxicity responses are useful tools for deriving water quality criteria for broad measures of protecting ecosystem health. However, *in situ* and / or ongoing monitoring of ecosystem health requires identification of more sensitive biotic responses (Chapman 1995; Peters et al. 1997). It is also recognised that laboratory studies assessing the sub-lethal effects of pollutants can be very useful for establishing protective limits on discharges to ensure more effective protection of ecosystem health (ANZECC &ARMCANZ 2000). Development of these sub-lethal measures also allows the potential to monitor remedial actions to improve degraded ecosystems. The use of sub-lethal measures is now widespread and suites of species responses have been identified as integrated measures for protecting ecosystem health.

Many trace elements, particularly metals, have long been identified as pollutants that can impact marine biota (e.g. Peters et al. 1997). Typically, these elements can be separated into two biologically important groups: 1) non-essential elements and, 2) essential elements. Non-essential elements have no biological relevance to biota and can be toxic at very low levels, depending on exposure route (Luoma & Rainbow 2008). In contrast, essential elements are biologically important and are required by most organisms as part of their metabolic needs

(Lewis & Cave 1982; Sunda & Huntsman 1998). However, the required concentrations of essential elements are generally very low and for many elements, exposure to concentrations above their optimum requirements can rapidly lead to biotic toxicity. For many essential elements (e.g. copper) the difference between the essential and toxic concentrations in biota can be very small (Bury et al. 2003).

Copper and zinc are two metals that are essential trace elements (Lewis & Cave 1982; Bury et al. 2003). They are needed to maintain organism health in trace quantities and are co-factors in a number of enzymatic activities within the body (Chapter 1, Section 2.1). However, concentrations in excess of basal metabolic requirements can disrupt other biological processes and become toxic to the organism.

Jellyfish are widespread and increasingly conspicuous in coastal marine systems, with the ability to form large blooms under optimal conditions (Purcell et al. 2007). Although historically, they have been considered tolerant of polluted systems, other research is showing that they may be sensitive to some pollutants (e.g. Spangenberg 1984; Spangenberg 1986; Todd et al. 2006). Due to their perceived tolerance, however, the use of jellyfish as bioindicator species for pollution monitoring has been rarely explored. In the few studies that have been undertaken, exposure to cadmium or petroleum hydrocarbons inhibited statolith formation and strobilation in *Aurelia aurita* indicating that jellyfish do have some potential for ecotoxicological studies (Spangenberg 1984; Spangenberg 1986).

Like their close relatives, the hermatypic corals, many rhizostome jellyfish have a symbiotic relationship with the dinoflagellate *Symbiodinium* spp. (Arai 1997). These zooxanthellae are autotrophic and have the capacity to provide some of the nutritional requirements to their host jellyfish, while the host in turn provides the inorganic carbon required for photosynthesis (Verde & McCloskey 1998). It is estimated that under good light conditions zooxanthellae photosynthates can provide about 1.5 times the metabolic demand for organic carbon to the host (Verde & McCloskey 1998). The genus *Cassiopea* is one group possessing this symbiotic relationship. Unlike other jellyfishes, it is usually sessile, resting umbrella side down on the benthos. Consequently, the symbiotic zooxanthellae are mostly found in the lower surface of the umbrella (closest surface to sunlight) and within the oral arm tissues. In contrast with most other cnidarians symbioses, the zooxanthellae in *Cassiopea* sp. are contained in amoebocytes between the exumbrellar and subumbrellar epithelia rather than in gastrodermal or endodermal host cells (Arai 1997; Estes et al. 2003). These amoebocytes are motile within the host tissue and tend to cluster close to the epithelial surface in the animal tissue (Estes et al. 2003).

Given the symbiotic and sessile nature of *Cassiopea* sp., this study investigated the potential of using Pulse Amplitude Modulation (PAM) fluorometry to establish a sub-lethal measure of

pollutant exposure to the symbiont (*Symbiodinium* sp.). PAM fluorometry is a rapid and non-invasive technique used for investigating changes in photochemical efficiency. It has increasingly been used in recent years to measure photosynthetic inhibition in a range of photosynthetic organisms (e.g. Jones 2005; Magnusson et al. 2008; Bielmyer et al. 2010). In the photosystem II (PSII) process, a proportion of the absorbed light energy is not used to drive electron transport and is dissipated as heat or chlorophyll fluorescence (Genty et al. 1989; Baumann et al. 2009). The released fluorescence is measured by PAM fluorometry, and is used to derive the effective quantum yield which is a proportional measure of photosynthetic efficiency. A number of metals, including copper and zinc, have been identified as interfering with the PSII process by substituting the central Mg^{2+} ion on the chlorophyll molecule resulting in a shift in the fluorescence spectrum and a consequent lowering of the effective quantum yield (Baumann et al. 2009). Other studies have shown that copper can also interfere with the acceptor side of the PSII process including between the pheophytin and Q_A acceptors (Samson et al. 1988; Rouillon et al. 2006). This technology allows measurements of symbiont activity *in situ*, and also provides the opportunity to distinguish between host and symbiont responses to pollutant exposure (e.g. Elfwing et al. 2002; Bielmyer et al. 2010).

The objective of this study was to determine the sub-lethal sensitivity of *Cassiopea* sp. and associated zooxanthellae, *Symbiodinium* sp., to aqueous copper or zinc exposure, and the potential of these measures as standard toxicity test methods. The specific aims were to: 1) determine the response of the host *Cassiopea* sp. to copper or zinc exposure by measuring change in size; 2) determine the response of the symbiont *Symbiodinium* sp. to metal exposure by measuring photosynthetic activity; 3) isolate the cause of photosynthetic inhibition by evaluating zooxanthellae abundances in the tissues, post exposure; 4) compare the responses of both host and symbiont to determine most sensitive response to copper and zinc; 5) assess the ability of host and symbiont to recover from sub-lethal copper exposure; and, 6) assess the overall potential of these responses as endpoints in a standard toxicity method.

2. Materials and methods

2.1 Summary

Cassiopea sp. medusae of similar size and age were used for this study. All medusae used in the experiments were sourced from in-house stocks with parental material from Lake Magellan, Sunshine Coast, Queensland, Australia (Appendix A). The culturing protocols for test organisms are detailed in Appendix A.

Three separate experiments were conducted. Experiments 1 and 3 were run for 7 days measuring host and zooxanthellae response to aqueous copper or zinc exposure. Experiment 2

was run for 14 days with 7 days aqueous copper exposure and 7 days post-copper exposure which was designed to assess the ability and extent of recovery in the jellyfish and zooxanthellae (Table 5.1).

Table 5.1: Summary of sub-lethal experiments.

Experiment	Metal	Duration (Days)	No. Treatments (including Control)	No. Replicates per Treatment
1	Copper	7	6	5
2	Copper	14 (7 day exposure; 7 day recovery)	4	5
3	Zinc	7	6	5

2.2 Test medusae

Cassiopea sp. medusae were selected from a larger pool of animals that had been raised in the laboratory. Pre-selected animals were placed in clean 10 litre containers of 20 µm filtered seawater and allowed to settle for 96 hours prior to the start of the experiment. Jellyfish were fed daily with newly hatched *Artemia* sp. to ensure they were healthy and actively feeding prior to any manipulations. Only animals that looked healthy, were feeding well and showed no overt signs of deformity were used. Medusae were approximately 4 – 6 weeks post strobilation at test commencement.

2.3 Cleaning and equipment preparation

All equipment was washed in phosphate free detergent, rinsed in tap water to remove any residual detergent and then soaked in 10 % AR grade nitric acid for a minimum of 12 hours. Acid soaked equipment was removed from the acid and rinsed three times with Milli-Q water and air dried in a Class 100 laminar flow unit. After drying, equipment was stored in clean plastic bags until required. In Experiment 2, all containers and other test equipment were replaced with clean equipment at the end of the exposure phase (Day 7) of the experiment. This was to ensure there were no residual metals in the recovery phase of the study.

2.4 Control and test waters

Control / dilution water was sourced from the JCU MARFU facility and filtered through both sand and 20 µm filters before use (Chapter 3, Section 2.4). For this study, control and dilution seawater was then filtered through a 250 mm 0.5 µm Stefani cartridge filter to exclude free-swimming microalgae or zooxanthellae from potential recolonisation of the jellyfish before use.

Five litres of each experimental treatment solution was prepared by diluting the required volumes of either 1g.L^{-1} Cu as $CuCl_2.2H_2O$ or 1g.L^{-1} Zn as $ZnSO_4.7H_2O$ to the required concentration with filtered control seawater. Test solutions were prepared 24 hours before the commencement of the experiments in 5.5 L clean, acid washed plastic containers. The test solutions were stored at 25 ^{0}C for the duration of the study. Measured exposure concentrations for Experiment 1 were: control (2.8), 16.3, 19.8, 27.8, 37.4 or 51.3 µg.L^{-1} Cu. In Experiment 2 measured copper exposure concentrations were: control (2.8), 14, 23.8 or 30.8 µg.L^{-1}Cu for 7 days followed by 7 days in control water (recovery). The measured zinc exposure concentrations in Experiment 3 were: control (0.02), 0.44, 0.88, 1.34, 1.81 or 2.25 mg.L^{-1}Zn.

2.5 Testing procedure

Lighting was provided by an Aqualina dual fluorescent reflector containing 2 x Dual CA PL-L 96 W 10000 K fluorescent tubes. Lighting was on a 12:12 hour light cycle. Light intensity was measured using a Li-COR meter for Photosynthetic Active Radiation (PAR). The average PAR (400-700 nm) was 115 µE.$m^{-2}.s^{-1}$. Welsh et al. (2009) estimated that photosynthetic compensation (the point where respiration = photosynthesis) in *Cassiopea* sp. was approximately 50 µE.$m^{-2}.s^{-1}$ and saturating irradiance (the point where photosynthetic activity plateaus regardless of increasing irradiance) was approximately 400 µE.$m^{-2}.s^{-1}$. The irradiance in this study ensured that photosynthetic compensation was exceeded but saturating irradiance was not achieved to minimise any potential for confounding effects from photochemical damage to the animals (Furla et al. 2005).

At the start of each experiment, medusae were randomly selected and placed in containers with 120 mL of clean filtered seawater. The bell diameter of each individual and photosynthetic yield (triplicate reading) was measured and the medusae randomly allocated to a treatment replicate using a random number generator. Each medusa was then placed in 120 mL of control or treatment water in a clean, acid washed 250 mL clear polycarbonate jar.

Except for the start of each experiment (Day 0), medusae were fed daily with freshly harvested live *Artemia* sp. (<48 hours post hatch) and allowed to feed undisturbed for 4 to 6 hours. Approximately 6 hours post feeding, old treatment solution was removed from each experimental container by carefully pouring it out whilst avoiding excessive disturbance to the animal. This was immediately replaced with new treatment or control water.

Test solutions were chemically analysed at the start of each experiment and at Day 7 for Experiment 2 (Copper Recovery Experiment) only. Samples were analysed using ICP-MS / AES using the method described in Chapter 3. Control waters were analysed for a suite of elements (as per Chapter 3) while treatment solutions were analysed for copper or zinc only.

2.6 Photosynthetic yield measurements

Photosynthetic yield was measured at the start of each experiment and then daily for the duration of each experiment. Measurements were undertaken using a Heinz Walz GmbH Photosynthesis Yield Analyzer Mini-PAM Portable Chlorophyll Fluorometer (PAM). Photosynthetic yield measurements were undertaken 2.5 to 3 hours after the beginning of the light period to ensure all PSII sites were active. To minimise potentially confounding effects from *Artemia* sp. activity, all PAM measures were undertaken before feeding.

The PAM was pre-calibrated using a similar test container and volume of control seawater to compensate for incidental activity. PAM settings were optimised to ensure reliable and repeatable measurements for all experiments (Table 5.2). Measurements were taken by inserting the probe directly into the test container and held vertically 7 mm above each animal. The light adapted minimum fluorescence (F_0), maximum fluorescence (F_m) and effective photosynthetic (quantum) yield (Y II) were measured and recorded. Effective quantum yield was automatically calculated as $Y(II) = (F_m - F_0)/F_m$. The quantum yield of each animal was measured three times each morning with approximately 15 minutes between readings to allow recovery. Preliminary assessments indicated that yield recovery occurred within 1 to 2 minutes and the reproducibility of photosynthetic yield measurements was good.

Table 5.2: Summary of settings for mini-PAM fluorometry to optimise fluorescent measurements.

PAM Fluorometer Parameter	**Setting**
Measurement Frequency	Low
Measurement Intensity	3
Gain	3
Saturation Intensity	6
Outgain	3

2.7 Size of medusae

The bell diameter of each jellyfish was measured at the beginning and end of the experiment. In experiment one, the initial bell diameter was reported as the mean of all medusae at Day 0. For experiments two and three, the individual bell diameter for each medusa was reported for Day 0. For experiment two, the jellyfish were also measured on Day 7 also, during transfer to clean experimental containers. The diameters were measured using a ruler with millimetre graduations. Each animal was placed in a clean 90 mm diameter clean plastic petri dish

containing a small volume of control seawater. The ruler was placed under the petri dish and the bell diameter measured when the bell was completely relaxed between pulses.

2.8 Histological preparation and zooxanthellae abundance estimates

At termination of each experiment, the bell diameter of each individual was measured to the nearest millimetre using a plastic ruler. To inspect overall health of the animals and identify and obvious lesions, animals were photographed under a Leica SD6 Stereomicroscope with a DFC295 camera. Images were processed using Leica Application Suite Version 3.7.0.

After photographing and measuring the bell diameter, medusae were placed in clean, filtered seawater and 1 % formaldehyde was added to euthanize them. All specimens were bisected through the manubrium to provide a cross section through the entire animal. Due to their high water content, the animals were then fixed using a combination paraformaldehyde / glutaraldehyde / dimethylsulphoxide fixative in an actin stabilising buffer (ASB) to minimise shrinkage during histological processing (Traas et al. 1987).

Tissues were fixed for a minimum of 24 hours. After fixation, tissues were placed in a 10 % ethanol solution. The ethanol concentration was increased in 5 % increments over 48 hours to a final 70 % ethanol : water mix. Tissues were embedded in paraffin using a Shandon Hypercenter Embedding Station using a standard fourteen hour embedding process. The embedding process involved the tissues being soaked in 70 % ethanol for 3 hours, followed by a second immersion in 70 % ethanol for one hour. They were then passed through a series of ethanol washes (80, 90, 95, 100, 100, 100 %) with immersion for one hour in each wash. This was followed by 2 one hour washes in xylene. All ethanol and xylene soaks were performed at room temperature (approximately 25 ^{0}C). The tissues were passed through two paraffin soaks for 2 hours each at 60 ^{0}C. Once the tissues were embedded in paraffin they were sectioned using a Leitz microtome at 5 µm thickness. A minimum of four tissue samples were taken from each animal. After sectioning, tissues were sequentially washed in xylene to remove paraffin and mounted using DPX mountant.

Jellyfish cross-sections were photographed under a precalibrated Leica DMLB Fluorescent microscope at 100x magnification and the images processed using Leica IM50 Image Manager software Version 4. Zooxanthellae abundances were estimated by counting five 100 µm linear sections of each bell tissue image. Only algal cells contained in the epithelial tissue on the surface closest to light were counted as it was expected that cells on the lower surface (aboral) would not have been contributing significantly to the PSII yield. These counts gave an overall zooxanthellae count / 500 µm per image. This was replicated on 5 separate images from the bell of each animal. An estimate of overall zooxanthellae abundance per animal was calculated using the mean linear count and measured bell diameter of each animal. Algal cells are

contained within the oral arms also. However, due to difficulties in obtaining reliable counts, algal cells were not counted in the oral arm tissue.

2.9 Data analysis

The bell diameter, photosynthetic yield, and zooxanthellae abundance data were analysed using one-way ANOVA (Statistica Version 10.0). Data were first tested to ensure they met the assumptions of normality and homogeneity of variance (Bartlett's) and data not conforming were log transformed before analysis. If data did not meet the assumptions after transformation, they were analysed using the non-parametric Krushal-Wallis Test. Significant differences from ANOVA analyses ($p < 0.05$) were post-hoc tested using Dunnett's multiple comparison against the control.

3. Results

Control medusae rapidly increased in size over the duration of each experiment with bell diameter increasing approximately 1 mm per day (mean 1.07 $mm.d^{-1}$) in controls. This is consistent with growth rates measured by Welsh et al. (2009), indicating that animals were growing normally and receiving more than adequate light and food over the duration of the studies. The zooxanthellae counts of the controls were similar among all experiments indicating that the initial density did not vary among animals used in the different experiments.

3.1 Experiment one - copper

Photosynthetic yield in *Cassiopea* sp. decreased after 7 days exposure to increasing copper concentrations (Figure 5.1). After 4 days of copper exposure, photosynthetic yields decreased slightly at higher copper concentrations, but this was not significant. At the highest copper concentration tested (51 $\mu g.L^{-1}$) photosynthetic yields were similar to control yields which may have indicated some possible ameliorating effect at the highest concentration. Although photosynthetic yields decreased at all copper concentrations after 7 days exposure results were not significant (Figure 5.1).

The number of zooxanthellae in the bell tissues varied with copper exposure (Figure 5.2). Exposure to increasing copper concentrations resulted in a higher density of zooxanthellae per unit area ($F_{5,24}= 18.90$, $p < 0.001$). There was also greater variation in counts among replicates in the highest concentration, although the overall algal abundance per animal did not increase significantly (Figure 5.2). Zooxanthellae abundance / 500 µm in medusae exposed to copper exceeding 19.8 $\mu g.L^{-1}$ were significantly different from control counts. However, when total zooxanthellae abundance per individual was estimated, while there was a trend for an increase

in overall zooxanthellae abundance in the higher copper concentrations, this was not significant ($F_{5,22} = 1.287$, $p > 0.05$).

Zooxanthellae distribution in the bell tissue was denser within the epithelial tissue in the higher copper concentrations. Some tissue cross sections from specimens exposed to higher copper concentrations had vertical densities of 6 to 10 cells (compared with control tissues with vertical densities of 1 to 3 cells) which may have contributed to reduced photosynthetic yields due to the effects of self-shading of cells.

Jellyfish growth decreased with increasing exposure to copper (Figure 5.3). Growth was inhibited at all copper concentrations tested ($F_{5,22} = 20.84$, $p < 0.001$). At the higher copper concentrations, growth was not only inhibited, the bell diameter of medusae shrank compared to their bell diameter at the start of the experiment (Figure 5.3). Visually, tissue lesions were observed in medusae exposed to the higher concentrations of copper (Figure 5.4a-f). In the higher copper concentrations, this tissue damage was more evident closer to the bell margin than the manubrium (Figure 5.4d-f). In addition, by Day 5 all jellyfish in the two highest concentrations (37 $\mu g.L^{-1}$ Cu and 51 $\mu g.L^{-1}$ Cu) looked unhealthy with tissue spasms and minimal pulsing activity compared to the control animals.

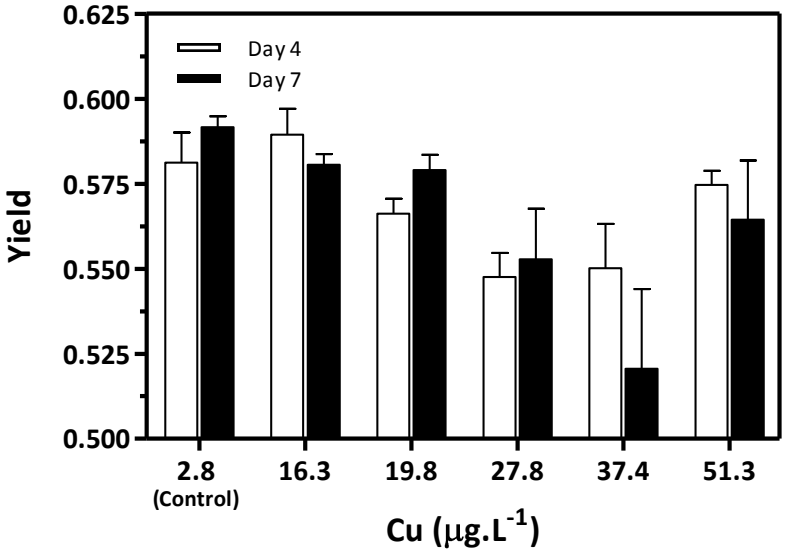


Figure 5.1: Effect of copper on photosynthetic yield in zooxanthellae from Experiment 1 after four and seven days exposure. Data was analysed using Kruskal-Wallis non-parametric test and yield was not significantly different among treatments ($p > 0.05$). n=5 ± SEM for all concentrations.

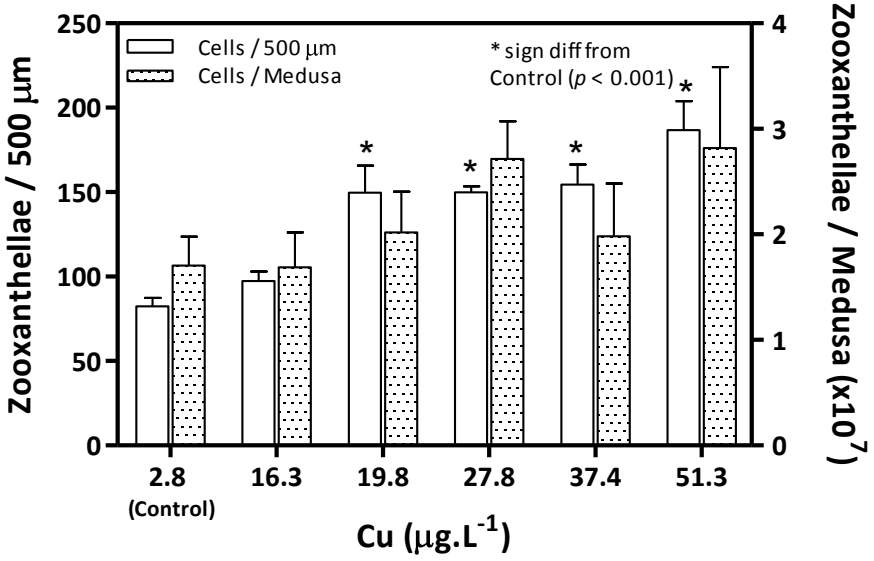


Figure 5.2: Effect of copper on zooxanthellae abundance in *Cassiopea* sp. bell tissue from Experiment 1 using one-way ANOVA. Treatments significantly different from control were post-hoc analysed using Dunnett's multiple comparison ($p < 0.05$). n=5 ± SEM for each concentration except 37.4 µg.L^{-1} and 51.3 µg.L^{-1} where n=4.

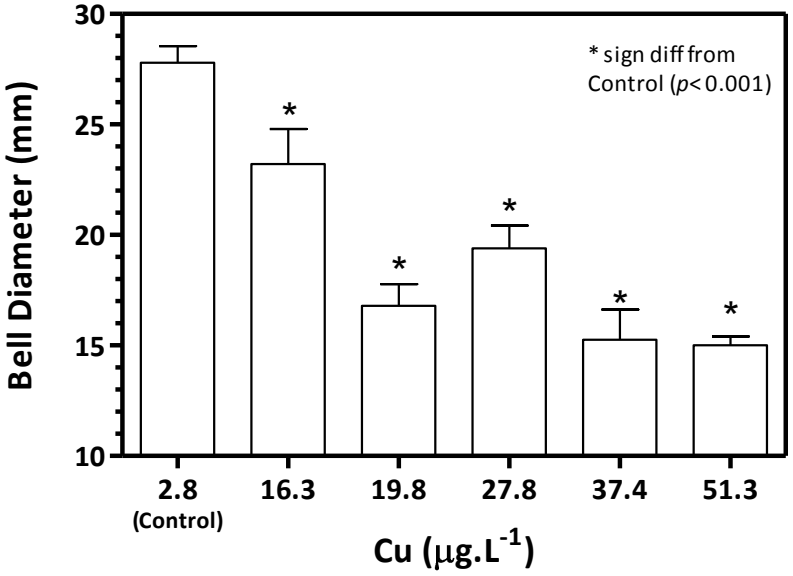
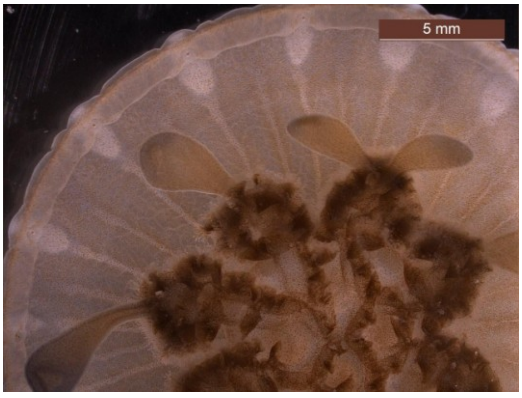
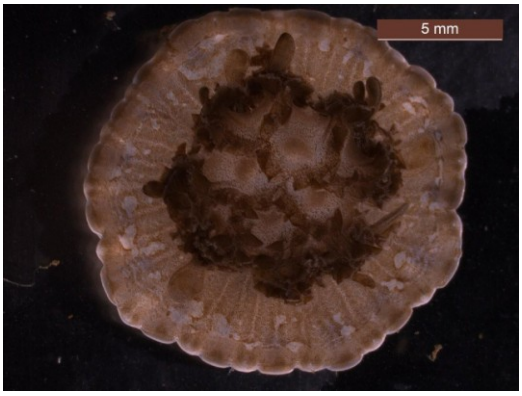


Figure 5.3: Effect of copper on bell diameter from Experiment 1 using one-way ANOVA. Treatments significantly different from control were post-hoc analysed using Dunnett's multiple comparison ($p < 0.05$). n=5 ± SEM for each concentration except 37.4 µg.L^{-1} and 51.3 µg.L^{-1} where n=4.



(a) Control Animal Cu

(b) 16.3 µg.L^{-1} Cu



(c) 19.8 µg.L^{-1} Cu

(d) 27.8 µg.L^{-1} Cu

(e) 37.4 µg.L^{-1} Cu

(f) 51.3 µg.L^{-1} Cu

Figure 5.4: *Cassiopea* sp. jellyfish after 7 days exposure to copper showing evidence of tissue necrosis and bell shrinkage with increasing copper exposure.

3.2 Experiment two- copper recovery

In Experiment 2 animals were exposed to control (2.8), 14, 23.8 or 30.8 $\mu g.L^{-1}$Cu (measured concentrations) for 7 days and then transferred to control seawater for a further 7 days to assess their capacity to recover from copper exposure. After 7 days exposure to copper there was a significant decrease in photosynthetic yield in the 23.8 $\mu g.L^{-1}$ and 30.8 $\mu g.L^{-1}$ exposure concentrations ($F_{3,16} = 5.304$, $p < 0.01$; Figure 5.5). However, on transfer to clean seawater, medusae in all copper concentrations recovered rapidly with no significant difference in yield among treatments 1 day post copper exposure ($F_{3,16} = 1.576$, $p > 0.05$; Figure 5.5). After seven days post copper exposure, the yield in the copper treatments was similar to the control yield although there was some variability in the results (Figure 5.5). For example, at Day 14, one individual in the 14 $\mu g.L^{-1}$ concentration had a low yield of 0.500. The response to copper exposure in this experiment was in contrast to experiment one where there was no significant difference in yield after seven days copper exposure at any concentrations.

Numbers of zooxanthellae in the copper exposed bell tissues from Experiment 2 did not vary significantly from the control (Figure 5.6). The counts / 500 µm did not vary greatly among treatments ($F_{3,15} = 1.008$, $p > 0.05$), however, when converted to density per animal there was a trend for decreased abundance of zooxanthellae with increasing copper concentration, but this was not significant ($F_{3,15} = 3.053$, $p > 0.05$) (Figure 5.6).

There was a significant difference in size in all copper exposed treatments after 7 days exposure ($F_{3,16} = 107.4$, $p < 0.001$). Animals exposed to higher copper concentrations were smaller relative to their starting diameter (Figure 5.7). Differences in size was still significant in the copper exposed animals after 7 days post exposure ($F_{3,16} = 42.92$, $p < 0.001$) but all treatments showed recovery compared to the end of the 7 day exposure period (Figure 5.7). Qualitative observations suggested that after 14 days, control animals may have started to become size constrained as they were approaching the diameter of the test containers.

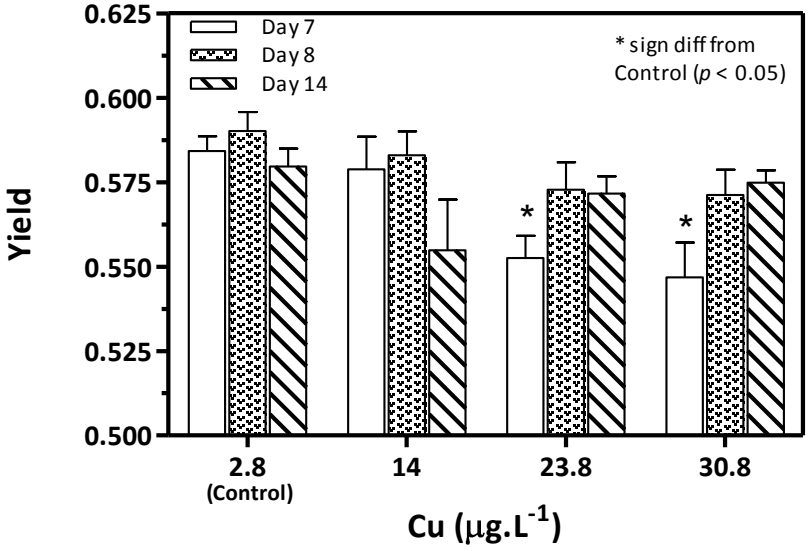


Figure 5.5: Effect of copper on photosynthetic yield in zooxanthellae from Experiment 2 using one-way ANOVA. Treatments significantly different from control were post-hoc analysed using Dunnett's multiple comparison ($p < 0.05$). Groups represent yield at Day 7 (final day of exposure), Day 8 (1 day post exposure) and Day 14 (7 days post exposure). n=5 ± SEM for all concentrations.

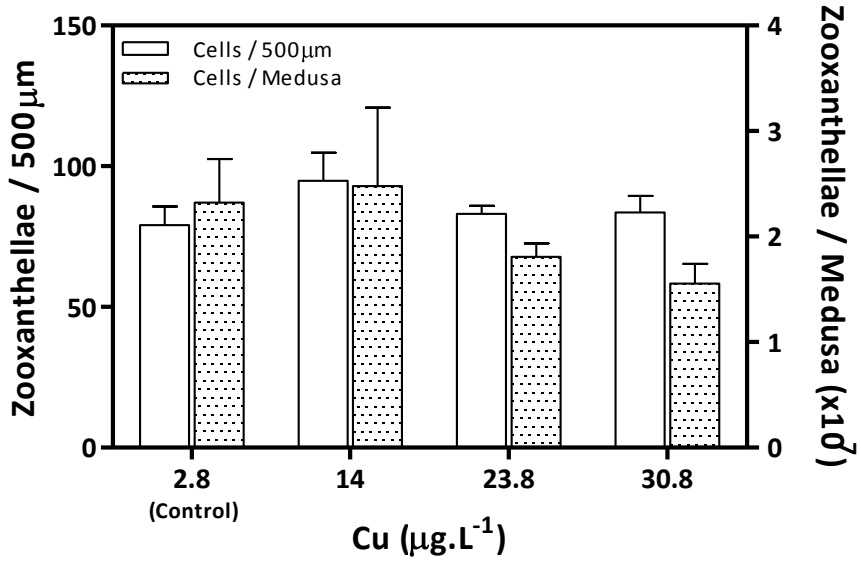


Figure 5.6: Effect of copper on zooxanthellae abundance in *Cassiopea* sp. bell tissue from Experiment 2 using one-way ANOVA. Treatments not significantly different at $p = 0.05$. n=5 ± SEM for each concentration except 14 µg.L^{-1} where n=4.

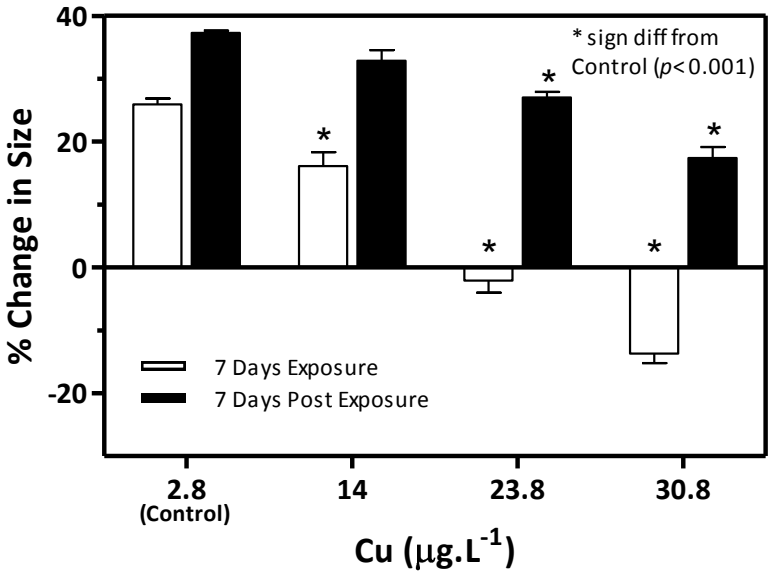


Figure 5.7: Effect of copper on bell diameter from Experiment 2 analysed using one-way ANOVA. Treatments significantly different from control were post-hoc analysed using Dunnett's multiple comparison ($p < 0.05$). % change represents difference in size (mm) from Day 0 for each time period. n=5 ± SEM for all concentrations.

3.3 Experiment three-zinc

Animals were exposed to control (0.02), 0.44, 0.88, 1.34, 1.81 or 2.25 mg.L^{-1}Zn (measured concentrations). Animals exposed to increasing concentrations of zinc showed a significant decrease in photosynthetic yield ($F_{5,24} = 13.40$, $p < 0.001$) from control animals at 0.88 mg.L^{-1} Zn and above (Figure 5.8). Increasing exposure resulted in decreased yield for all zinc treatment (Figure 5.8).

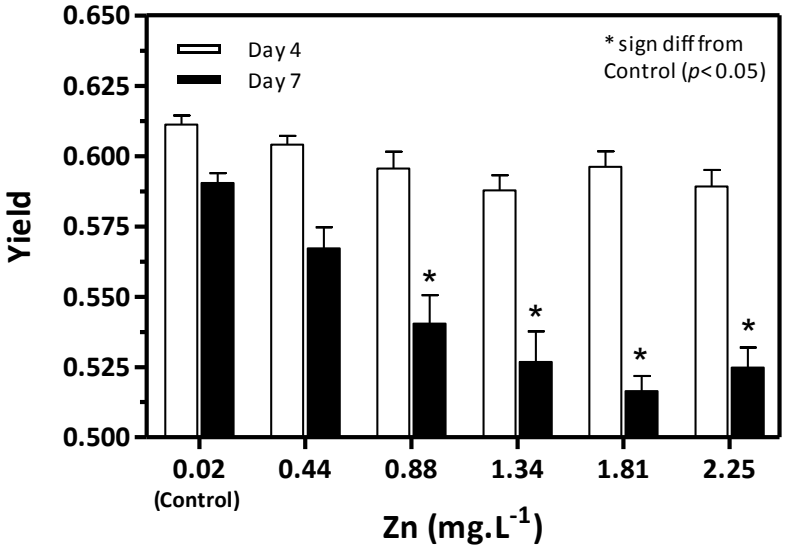


Figure 5.8: Effect of zinc on photosynthetic yield at days 4 and 7 from Experiment 3 analysed using one-way ANOVA. Treatments significantly different from control were post-hoc analysed using Dunnett's multiple comparison against the control ($p < 0.05$). n=5 ± SEM for all concentrations.

Zooxanthellae counts in the zinc exposed bell tissues did not vary significantly from the control (Figure 5.9). Zooxanthellae density per medusa were generally lower between 0.88-2.25 mg.L^{-1} Zn treatments than densities at 0.44 mg.L^{-1} Zn treatment but were not significantly different ($F_{5,23} = 1.445$, $p > 0.05$) (Figure 5.9). Overall, there was also greater variability in zooxanthellae numbers / 500 µm in the zinc exposed animals compared with the control animals but this difference was not significant ($F_{5,23} = 1.784$, $p > 0.05$). Cross-sections demonstrated that, not only was there variability in algal counts in the zinc exposed animals, but zooxanthellae were distributed throughout the entire cross section of bell tissue rather than being concentrated in the upper epithelial tissues only (Figure 5.10).

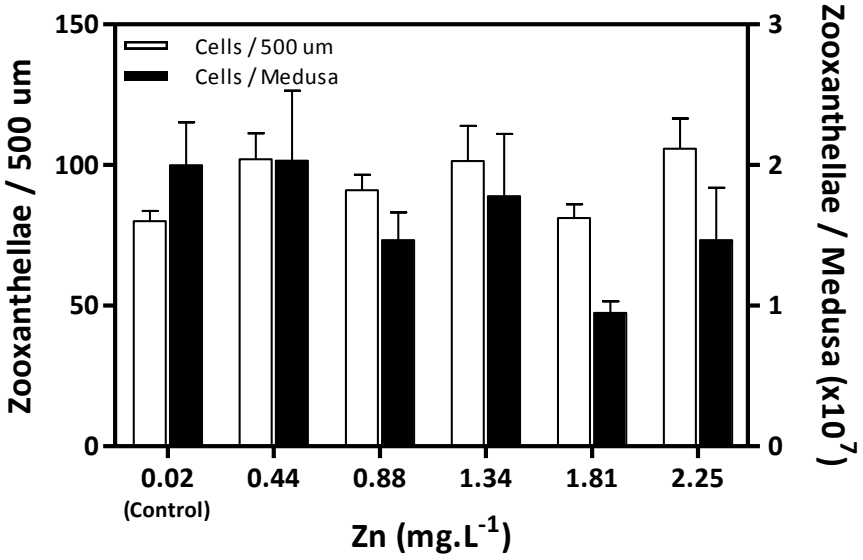
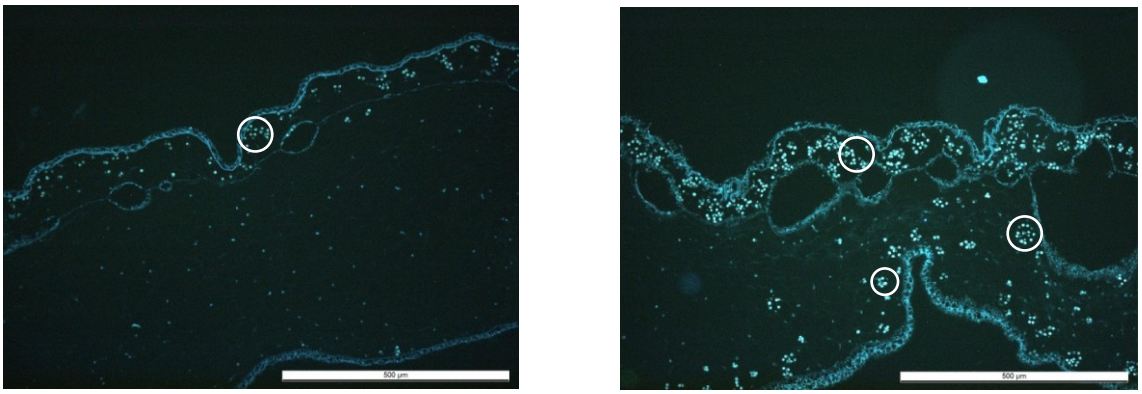


Figure 5.9: Effect of zinc on zooxanthellae abundance in *Cassiopea* sp. bell tissue from Experiment 3 analysed using one-way ANOVA. Treatments not significantly different at $p = 0.05$. n=5 ± SEM for each concentration except 2.25 mg.L^{-1} where n=4.



(a) Control **(b)** 2.25 mg.L^{-1} Zn

Figure 5.10: Distribution of zooxanthellae within *Cassiopea* sp. bell tissue in control and 2.25 mg.L^{-1} zinc from Experiment 3. Zooxanthellae are mainly constrained to the upper epithelial surface in the control tissues but are denser and more widely distributed through the mesoglea in the zinc exposed tissue. O indicates locations of zooxanthellae in tissue.

There was a significant decrease in bell diameter at all concentrations of zinc tested ($F_{5,24} = 15.44$, $p < 0.001$) (Figure 5.11). Over the period of the experiment (7 days) control animals typically increased in size from 23 mm to 31 mm diameter representing a 1mm diameter increase / day.

At higher zinc concentrations, medusae not only had a reduced growth but at concentrations higher than 1.34 mg.L^{-1} Zn, the animals shrank compared to their initial bell diameter at Day 0

(Figure 5.11). Unlike the copper exposed animals however, there was no obvious evidence of tissue lesions in zinc exposed animals (Figure 5.12a-f). Visually there was no evidence of bleaching or fading in the animals exposed to zinc which would have indicated possible zooxanthellae expulsion. Instead, the animals in the highest concentration of zinc tended to darken over the exposure period, suggesting that zooxanthellae were retained in the tissues despite the reduction in size (Figures 5.11, 5.12a-f).

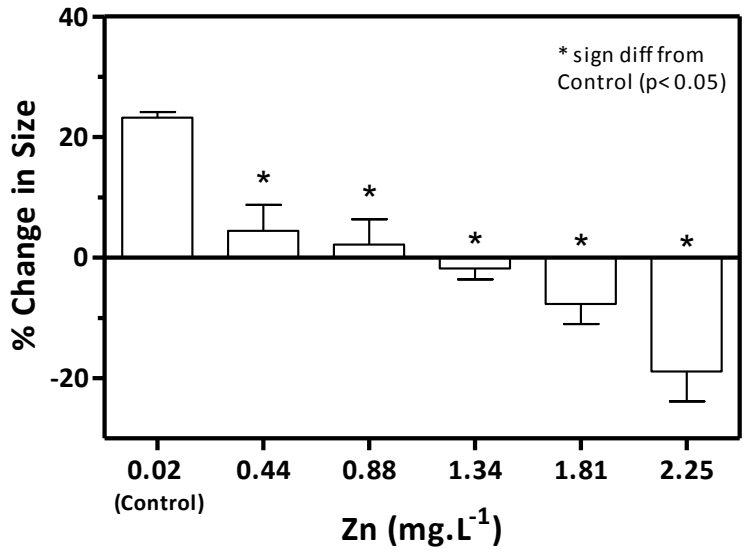
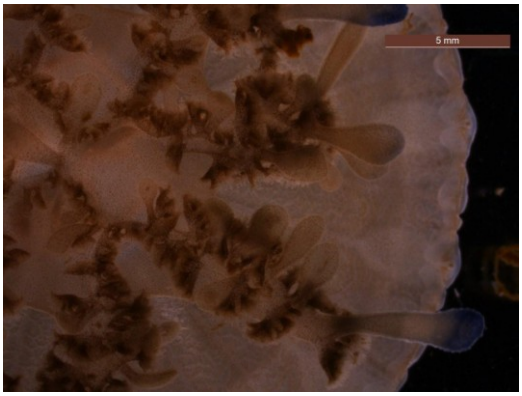
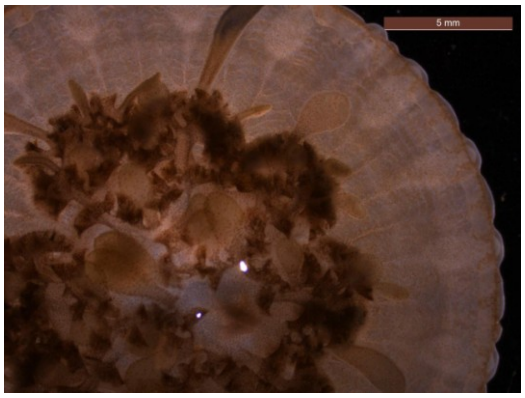


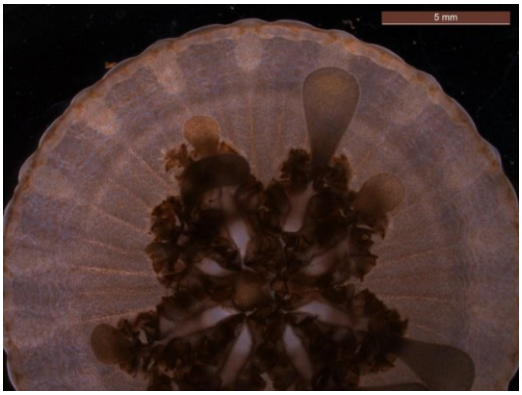
Figure 5.11: Effect of zinc on bell diameter from Experiment 3 analysed using one-way ANOVA. % change represents difference in size (mm) from Day 0. Treatments significantly different from control were post-hoc analysed using Dunnett's multiple comparison ($p < 0.05$). n=5 ± SEM for all concentrations.



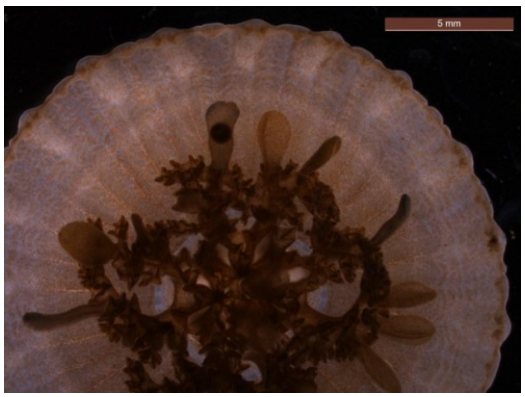
(a) Control Animal Zn



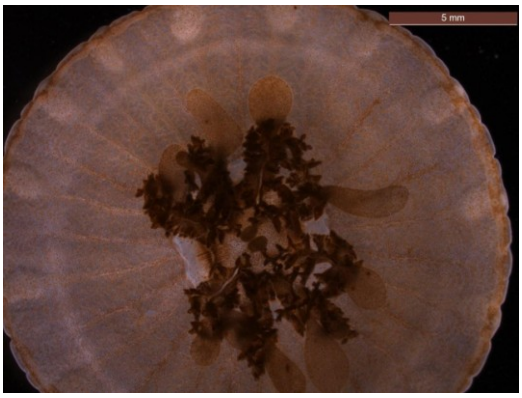
(b) 0.44 mg.L^{-1} Zn



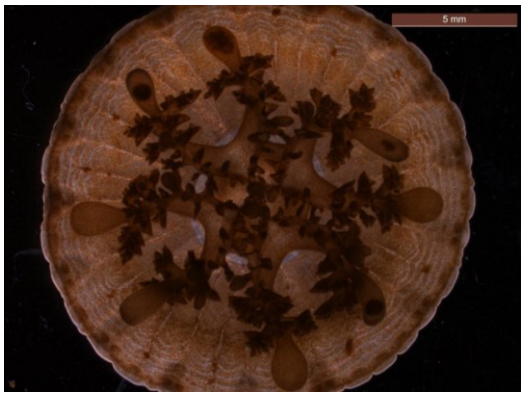
(c) 0.88 mg.L^{-1} Zn



(d) 1.34 mg.L^{-1} Zn



(e) 1.81 mg.L^{-1} Zn



(f) 2.25 mg.L^{-1} Zn

Figure 5.12: *Cassiopea* sp. jellyfish after 7 days exposure to zinc showing change in colour and indications of shrinkage with increasing zinc concentration but no evidence of tissue necrosis (as seen in copper exposed animals).

4. Discussion

This study was the first to investigate the response of a symbiotic jellyfish to metal exposure and one of few studies to utilise scyphozoan jellyfish as a toxicity test species (Spangenberg 1984; Spangenberg 1986). It was also the first study to measure the *in situ* zooxanthellae response to copper and zinc exposure in a jellyfish. Both the jellyfish *Cassiopea* sp. and their zooxanthellae symbiont (*Symbiodinium* sp.) demonstrated high levels of sensitivity to copper and zinc exposure at concentrations well below the measured lethal concentrations for all lifestages of *Cassiopea* sp. (Chapter 4). The photosynthetic yield response was variable between the two copper experiments (Experiments one & two) suggesting some differences in the response of individuals (Figures 5.1 & 5.5). Measurements of photosynthetic yield at Day 7 in experiment one were more variable in animals exposed to copper concentrations above 27.8 $\mu g.L^{-1}$ copper compared with the Day 7 photosynthetic yield measurements in the 23.8 and 30.8 $\mu g.L^{-1}$ Cu concentrations in experiment two (Figures 5.1 & 5.5). Overall, both size and photosynthetic activity decreased dose-dependently in response to copper and zinc.

4.1 Algal sensitivity to metals and detoxification strategies

Copper and zinc have the capacity to affect photosystem II (PSII) activity in a number of ways and at a number of sites. Copper has been found to interact with the oxidising side of the PSII process (Samson et al. 1988), but is also suspected of interacting at other sites including the reaction centre and the electron acceptor side (Rouillon et al. 2006). Zinc also affects the photosynthetic electron transport process through a Zn^{2+} inhibitory site on the donor side of PSII, although there is potential for additional effects on the acceptor side at the secondary plastoquinone electron acceptor site (Q_B) of the process as well (Rashid et al. 1991; Rouillon et al. 2006).

The zooxanthellae response to copper in this study showed a decreasing photosynthetic yield with increasing copper concentration up to approximately 40 $\mu g.L^{-1}$ Cu, but there was some indication of a reduced copper effect at the highest concentration. Counts of zooxanthellae abundance per unit area in the jellyfish showed increasing density at higher copper exposure, but total algal abundance among treatments was not significantly different (Figure 5.2). This lack of variation in algal abundance implied that copper interfered with PSII activity rather than decreased PSII yield being a function of decreased algal numbers. Examination of tissue cross sections of the jellyfish showed that in the higher exposure concentrations, algal cells were present in wide bands up to 10 cells deep, which may have caused self-shading and in part been responsible for the reduced PSII yield. The high numbers of zooxanthellae per unit area in the higher copper concentrations also suggested, that unlike some other cnidarians (e.g. corals),

Cassiopea sp. did not readily expel their symbionts as a stress response (e.g. Bielmyer et al. 2010).

Marine algal species have a wide range of sensitivities to copper and zinc exposure (Tables 5.3 and 5.4). Bao et al. (2008) reported a 96 hour EC_{50} of 970 $\mu g.L^{-1}$ Cu in the marine diatom *Thalassiosira pseudonana* but at lower concentrations (50-200 $\mu g.L^{-1}$ Cu) growth was stimulated. The reported EC_{50} for *T. pseudonana* demonstrated it was less sensitive to copper than other species (Table 5.3). It also indicated that *T. pseudonana* has a minimum metabolic requirement for copper for optimum growth (Bao et al. 2008).

The mechanisms for detoxification vary among algal species with some of the more common techniques including cellular exclusion and internal detoxification (Hall et al. 1979; Nielsen et al. 2003; Baumann et al. 2009). A study using multiple macroalgal species reported variations in sensitivity among species to copper and zinc exposure (Baumann et al. 2009). For copper exposed algae, photosynthetic yield was decreased in the red macroalgae, *Chondrus crispus* and *Palmaria palmata*. However, in the brown macroalgae (*Ascophyllum nodosum* and *Fucus vesiculosus*), the red algae *Polysiphonia lanosa*, and the green macroalgae *Cladophora rupestris* and *Ulva intestinalis*, photosynthetic yield was not affected (Baumann et al. 2009). In this study decreased photosynthetic yield did not necessarily correlate with high copper accumulation. *U. intestinalis* had no significant decrease in yield despite having the highest copper concentrations within its tissues (Baumann et al. 2009). In contrast, the decreased yield in *C. crispus* and *P. palmata* was associated with accumulated copper. This demonstrated that the efficiency of internal detoxification mechanisms varied among these species (Baumann et al. 2009). Similarly, in another study, the extent of copper accumulation in *A. nodosum* and *F. vesiculosus* varied, despite the same exposure conditions demonstrating differing uptake and internal binding mechanisms and sites (Connan & Stengel 2011).

In a review of toxicity of copper and zinc to Australasian marine algal species, Langdon et al. (2009) reported that sub-lethal copper toxicity varied both within and among diatom species with inhibition ranging from 0.6 $\mu g.L^{-1}$ Cu in *Minutocellus polymorphus* to 62 $\mu g.L^{-1}$ Cu in *Nitzschia closterium*. In contrast, dinoflagellates studies (which include the genus *Symbiodinium*), had a smaller reported range of 4.8 $\mu g.L^{-1}$ Cu to16 $\mu g.L^{-1}$ Cu (Levy et al. 2007 & Franklin et al. 2004, respectively). However, only two studies using this division were reported (Langdon et al. 2009). Goh & Chou (1997) reported that the growth rate of *Symbiodinium micoadriaticum* was inhibited at 40 $\mu g.L^{-1}$ Cu, which was similar to that reported in the current study, although the different study endpoints makes direct comparisons difficult (Table 5.3). Although the growth rate response of isolated zooxanthellae was not assessed in

this study, the range of sensitivities suggests that isolated cells would potentially be as sensitive as cells in hospice.

Table 5.3: Summary of copper toxicity to marine algal species.

Species	Concentration	Response	Reference
Symbiodinium micoadriaticum	40 $\mu g.L^{-1}$	Specific growth rate inhibition	Goh & Chou (1997)
Heterocapsa niei	16 $\mu g.L^{-1}$	72 hr IC_{50} (growth rate)	Franklin et al. (2004)
Heterocapsa niei	4.8 $\mu g.L^{-1}$	72 hr IC_{50} (cell division)	Levy et al. (2007)
Thalassiosira pseudonana	970 $\mu g.L^{-1}$	96 hr EC_{50}	Bao et al. (2008)
Phaeodactylum tricornutum *Tetraselmis* sp. *Dunaliella tertiolecta*	8.0 $\mu g.L^{-1}$ 47 $\mu g.L^{-1}$ 530 $\mu g.L^{-1}$	72 hr IC_{50} (growth rate)	Levy et al. (2008)
Ascophyllum nodosum, *Fucus vesiculosus*	5 $mg.L^{-1}$ Cu	Significant decrease in maximum quantum yield	Connan & Stengel (2011)

The response of micro- and macro- algae to zinc exposure varies among species (Table 5.4). Jensen et al. (1974) found sensitivity to zinc was very species dependent with growth of the marine diatom *Skeletonema costaturn* being inhibited at concentrations as low as 25 $\mu g.L^{-1}$ but growth of the marine diatom *Phaeodactylum tricornutum* was only inhibited at 25 $mg.L^{-1}$ Zn (Table 5.4). Growth of the diatom, *Nitzschia closterium* was inhibited at concentrations as low as 65 $\mu g.L^{-1}$ Zn (Stauber & Florence 1990). The cells readily accumulated zinc, however, most of the zinc was shown to be bound extracellularly. In addition, despite zinc exposure affecting growth of *N. closterium*, there was no significant effect on photosynthesis at 500 $\mu g.L^{-1}$ Zn although ultrastructural changes in the cells were evident (Stauber & Florence 1990).

The response of *N. closterium* is similar to that seen in the current study, with a significant decrease in photosynthetic yield occurring at higher Zn exposure concentrations compred to the change in size measured in the host jellyfish. It is possible that the symbiont may have preferentially bound zinc at the cell surface rather than intracellularly, thereby increasing the toxicity risk to the host animal. In addition, the presence of zinc binding sites on and within the jellyfish host would restrict exposure of the zooxanthellae to zinc until these sites were saturated thereby potentially reducing toxicity to the algae. This would require direct measures of zinc accumulation within both the jellyfish and zooxanthellae to confirm.

In contrast to the copper results already discussed in this section, Baumann et al. (2009) found that 10 µM zinc decreased photosynthetic yield in seven macroalgae species by Day 4. The corresponding tissue concentrations of zinc did not reflect the yield response as the tissue concentrations of zinc in the 10 µM exposure was lower than all other concentrations including the control (Baumann et al. 2009). This response was interesting as the decreased photosynthetic yield reflected the lowest internal tissue concentrations in the macroalgae despite the highest external concentrations (Baumann et al. 2009). Studies have shown that a combination of life history strategies and the number of binding sites related to biochemical composition play a major role in metal accumulation and effects on photosynthetic yield in algae (Stengel et al. 2004). These factors may also be important contributors to the ability of zooxanthellae to both accumulate and detoxify metals.

Table 5.4: Summary of zinc toxicity to marine algal species.

Species	Zn Concentration	Response	Reference
Skeletonema costatum	25 $\mu g.L^{-1}$	Growth inhibition	Jensen et al. 1974
Phaeodactylum tricornutum	25 $mg.L^{-1}$	Growth inhibition	Jensen et al. 1974
Thalassiosira pseudonana	500 $\mu g.L^{-1}$	Growth inhibition	Jensen et al. 1974
Amphidinium carteri, *Thalassiosira pseudonana*	400 $\mu g.L^{-1}$	Growth inhibition	Braek et al. 1976
Nitzschia closterium	65 $\mu g.L^{-1}$	96hr IC_{50} (cell division)	Stauber & Florence 1990
Nitzschia closterium	>500 $\mu g.L^{-1}$	Photosynthetic inhibition	Stauber & Florence 1990
Symbiodinium micoadriaticum	1.2 $mg.L^{-1}$	Specific growth rate inhibition	Goh & Chou 1992

4.2 Host-symbiont interactions in response to metal exposure

It is hypothesised that decreases in photosynthetic yield were due to metal toxicity, self-shading or a combination of effects. There was no significant decrease in overall zooxanthellae abundances in metal exposed animals compared with the controls but increases in algal densities at higher metal concentrations causing self-shading would result in overall decreases in photosynthetic yield even if there were no direct effects of metal toxicity on the algae. The rapidity of recovery in photosynthetic yield within 24 hours post-copper exposure suggests that there may have been some direct metal effects on the symbiont. The copper exposed jellyfish were still significantly smaller than the controls 7 days post-exposure which limited the ability of the zooxanthellae to avoid self-shading. However, without further work it is not possible to determine the relative importance of the two effects on decreased yields.

It is also hypothesised that metal effects on the host jellyfish were influenced by changes in symbiont activity, as decreases in photosynthetic yield would have cascading effects on the amount of photosynthate able to be transferred to the host, thereby potentially reducing the jellyfish's capacity to reduce toxic effects of the metal exposure. However, the significant change in size of the jellyfish with increased copper and zinc exposures suggests there were direct host metal effects occurring as well.

The average increase in bell diameter of *Cassiopea* sp. in the controls from all experiments was approximately 1 $mm.day^{-1}$. When increase in bell area was used as a proxy for increase in mass, the daily growth rate of control animals in this study was comparable with literature ranges (Arai 1997; Welsh et al. 2009). Studies have shown that under ideal growing conditions, the symbiont can contribute substantial amount of the carbon demanded by the host *Cassiopea* (Welsh et al. 2009). Under saturating light conditions *Cassiopea* sp. can achieve a growth rate of approximately 3 % per day on photosynthetically fixed carbon alone without any contribution from predation (Welsh et al. 2009). However, other studies on growth estimations in scyphozoans have shown that growth rates are variable and dependent on both age and assessment methodology (Arai 1997).

In the metal exposed animals in this study, the density of zooxanthellae per unit area increased with increasing metal exposure and this may have led to self-shading of the cells contributing to decreases in photosynthetic activity (Figures 5.2, 5.6 & 5.9). It was expected that due to reduced photosynthetic activity, the translocation of photosynthate from symbiont to host would be lower and may have contributed to slower growth in the metal exposed jellyfish although it is likely direct metal effects on the host were also occurring.

Xenobiotic induced reductions in growth, and shrinkage is likely to be ecologically detrimental to the animals, particularly if heterotrophically derived energy is also compromised. In this study, animals were fed daily ensuring unlimited access to food for the jellyfish. However, under stress conditions, bell and oral arm shrinkage would potentially impede prey capture and ingestion. If there was a reduced supply of nutrients from the symbiont as a consequence of metal exposure, the associated host response would compound the effect of diminished food supply.

Cassiopea sp. like other Scyphozoa is considered an osmoconformer, with the salinity of intracellular fluids conforming to the external environment (Arai 1997). Studies on euryhaline and marine organisms have shown that copper exposure can interfere with osmo- and iono-regulatory activity in animals (Weeks et al. 1993; Lee et al. 2010). Weeks et al. (1993) found that copper exposure impaired both osmoregulatory capacity and haemolymph calcium concentrations in the shore crab, *Carcinus maenas*. Copper toxicity has also been identified as a

cause of DNA damage and metabolic inhibition in marine osmoconformers (Lee et al. 2010). Although there were no direct measures of change in osmoregularity in this study, it is possible that for a soft bodied osmoconformer like *Cassiopea* sp., increasing copper exposure could also have contributed some of the observed changes in growth rate and size due to disruptions to osmoregulatory activity and metabolic inhibition.

Studies have shown that copper above essential requirements can cause oxidative damage to marine invertebrates and lead to inhibited physiological functions (Grant et al. 2003). The growth inhibition and tissue necrosis observed in the jellyfish may be a result of oxidative damage caused by direct copper exposure. It is hypothesised that due to the effects of copper on the jellyfish, carbonic anhydrase activity may also have been affected, resulting in secondary stress from the symbiont. Similar effects have been measured in the coral, *Plesiastrea versipora* where, despite variability in host signalling compounds, oxidative stress was evident in copper exposed coral tissues (Grant et al. 2003). However, further work would be necessary to elucidate the actual mechanisms occurring in the symbiotic relationship in *Cassiopea* sp.

In *Cassiopea* sp., zooxanthellae are typically contained in clusters in mobile amoebocytes rather than fixed in particular tissues (Estes et al. 2003). This mobility allowed increased algal cell numbers in the epidermal tissue in copper exposed tissues. There was also migration away from the oral (highest light exposure) regions in animals in the higher concentrations with zooxanthellae distributed through the mesoglea rather than being contained in the upper epidermis (data not shown). Although contained within the amoebocytes, the symbiont may still have bound the copper extracellularly to reduce toxicity to the algal cells. This form of binding could potentially contribute to toxic effects on the host if the copper is not bound in a detoxified form for the host animal.

As there has been minimal work investigating sub-lethal response of metals to jellyfish (Spangenberg 1986), direct comparisons are difficult. In one of the few studies, the effect of copper on the settlement of marine invertebrates showed that copper pulses significantly affected scyphozoan polyp settlement at high settlement times (Johnston & Keough 2000). The current status of metal toxicity investigations in Australasia has recently been reviewed, and while there is an increase in the number of marine and estuarine species for which toxicological data are available, no representatives of the scyphozoan or cubozoan jellyfishes were included (Langdon et al. 2009). Notwithstanding this lack of direct comparison, the sensitivity of response seen in *Cassiopea* sp. was similar to other symbiotic marine organisms globally (Table 5.5).

Studies using anthozoan Cnidaria have shown species-dependent variability in sensitivity to copper exposure (e.g. Harland and Nganro 1990; Smith et al. 2003; Bielmyer et al. 2010). In

corals, copper sensitivity has been shown to vary both within and among genera. Fertilisation of *Acropora longicyathus* was inhibited at 15.2 $\mu g.L^{-1}$ Cu (5.5 hr EC_{50}) while *Acropora tenius* was less sensitive at 39.7 $\mu g.L^{-1}$ Cu (Reichelt-Brushett & Harrison 2005). *Lobophytum compactum* (soft coral) was also less sensitive to copper with significantly reduced fertilisation at 261 $\mu g.L^{-1}$ Cu (EC_{50}) (Reichelt-Brushett & Michalek-Wagner 2005). Although reproductive effects were not assessed in this study, copper has been shown to inhibit planuloid bud development in *Cassiopea* sp. (Chapter 4) at concentrations similar to that seen in corals. Growth inhibition of the medusae also has the potential to inhibit gonad development and reduce reproductive capacity.

In a five week study comparing host and symbiont responses among corals to copper exposure, growth rates of the host corals *Pocillopora damicornis, Acropora cervicornis* and *Montastraea faveolata,* and photosynthetic yield of the respective symbionts were inhibited at different copper concentrations (Bielmyer et al. 2010). *P. damicornis* and its symbiont were both inhibited at concentrations as low as 4 $\mu g.L^{-1}$ Cu, while *A. cervicornis* and its symbiont were inhibited at 20 $\mu g.L^{-1}$ Cu (Table 5.5). In contrast, *M. faveolata* and the associated symbiont were not inhibited at any of the tested concentrations (Bielmyer et al. 2010). In part, this variation in symbiont response was identified as a consequence of the different algal symbiont clades present in the different corals (Bielmyer et al. 2010). The same study found that copper exposure caused coral bleaching in both *P. damicornis* and *A. cervicornis* but not *M. faveolata* indicating the symbionts had been expelled in the former (Bielmyer et al. 2010).

Unlike many other symbiotic species, *Cassiopea* sp. does not seem to readily expel zooxanthellae as a stress response. A number of studies, both laboratory and field, have shown that many symbiotic marine organisms use symbiont expulsion as a detoxification mechanism in response to chemical and physical stressors (e.g. Harland & Nganro 1990; Duquesne & Coll 1995; Bielmyer et al. 2010). However, this does not seem to be a universal mechanism for detoxification as other studies have found retention of zooxanthellae under metal stress (e.g. Gilbert & Guzman 2001). Smith et al. (2003) reported decreases in maximum photosynthetic yield in *Acropora formosa* branchlets with exposure to combined TBT / Cu / Zn contaminated sediment, but no corresponding decrease in zooxanthellae abundances. This is in contrast to the effects of copper stress on *A. cervicornis* where zooxanthellae expulsion was one of the responses to copper exposure (Bielmyer et al. 2010). This indicates that even within genera, algal expulsion is not a universal stress response. The rapid recovery of photosynthetic activity (PSII) in the zooxanthellae in post-copper exposure and minimal or no decrease in zooxanthellae numbers per animal with increased copper exposure demonstrated that zooxanthellae expulsion was not a stress response in *Cassiopea* sp. (Figure 5.5).

In the symbiotic clams *Tridacna gigas* and *Hippopus hippopus*, exposure to 5 $\mu g.L^{-1}$ Cu resulted in a significant decrease in gross production but no significant difference in quantum yield (Elfwing et al. 2002). These results indicated that copper exposure interfered with host metabolism, but not the PSII processes of the symbiont (Elfwing et al. 2002). Investigations of coral host and symbiont sensitivity to antifoulants has also shown that metal exposure may be more detrimental to the host than the zooxanthellae (Smith et al. 2003). Metal toxicity to the host over the symbiont may be common across many host-symbiont groups including *Cassiopea* sp. It is possible that the protective mechanisms imposed by the symbiont to protect itself from metal exposure may instead impair host health. The exact mechanisms for this have not been identified but may explain the expulsion of symbionts in some species.

Although there was both decrease in photosynthetic yield and growth in copper exposed *Cassiopea* sp., the recovery experiment demonstrated that this response was reversible when the animals were transferred to clean seawater after 7 days exposure to copper. This indicated that the jellyfish were able to either detoxify or excrete excess copper once it was no longer exposed to aqueous copper. It has previously been demonstrated that *Cassiopea* sp. is readily able to excrete copper when it is no longer exposed to aqueous copper and that this excretion is in the order of hours to days (Chapter 3). This ability to rapidly detoxify or excrete excess copper means *Cassiopea* sp. has the ability to recover from short-term exposure but may be less resilient to longer term exposure.

Table 5.5: Summary of selected symbiotic biota sub-lethal responses to copper or zinc exposure in marine ecosystems.

Species	Metal Concentration	Response	Reference
Tridacna gigas, *Hippopus hippopus* (Giant Clam)	5 $\mu g.L^{-1}$ Cu	Difference in stress index (Gross production:respiration ratio)	Elfwing et al. (2002)
Condylactus gigantea, Stichodactyla helianthus (Anemone)	10 $\mu g.L^{-1}$ Cu	Decrease in carbonic anhydrase activity	Gilbert & Guzman (2001)
Acropora cervicornis (Coral)	20 $\mu g.L^{-1}$ Cu	Decrease in effective quantum yield & decrease in growth	Bielmyer et al. (2010)
Pocillopora damicornis (Coral)	4 $\mu g.L^{-1}$ Cu	Decrease in effective quantum yield & decrease in growth	Bielmyer et al. (2010)
Cassiopea sp. (Upside-down Jellyfish)	16.3 $\mu g.L^{-1}$ Cu (Expt 1) 14 $\mu g.L^{-1}$ Cu (Expt 2) 23.8 $\mu g.L^{-1}$ Cu (Expt 2)	Decrease in size Decrease in size Photosynthetic inhibition	This Study
Anthopleura elegantissima (Anemone)	1.0 $mg.L^{-1}$ Zn	Decrease in algae number	Mitchelmore et al. (2003b)
Cassiopea sp. (Upside-down Jellyfish)	0.44 $mg.L^{-1}$ Zn 0.88 $mg.L^{-1}$ Zn	Decrease in size Photosynthetic inhibition	This Study

In contrast to data on copper exposure, available literature on sub-lethal effects of zinc to symbiotic species is more limited. In experiments with the scleractinian coral *Stylophora pistillata,* photosynthetic efficiency increased at low concentrations of zinc (10-100 nM Zn) but inhibited at higher concentrations (Ferrier-Pages et al. 2005). There was evidence of light stimulated zinc uptake which suggested that the zooxanthellae may be intimately involved in the zinc uptake process. It also suggested that under normal oligotrophic conditions, *S. pistillata* may be zinc limited (Ferrier-Pages et al. 2005), highlighting that zinc deficiency can also inhibit photosynthesis. In addition, whilst photosynthetic efficiency was inhibited at higher zinc concentrations under high light conditions, low light levels yielded protection of photosynthesis against zinc stress (Ferrier-Pages et al. 2005). This again demonstrates that symbiont / host interactions with metals are very complex.

In the symbiotic anemone, *Anthopleura elegantissima,* algal densities were reduced when exposed to 1 $mg.L^{-1}$ Zn but there was no decrease in mitotic index in the host

(Mitchelmore et al. 2003b). Post-exposure recovery of the anemone suggested the effects were not permanent (Mitchelmore et al. 2003b). The greater sensitivity of the symbiont over the host was atypical compared to responses by many other marine symbiotic species (Smith et al. 2003). Both the host and symbiont response in the current study was more sensitive than that reported by Mitchelmore et al. (2003b) suggesting that zinc may be more toxic to *Cassiopea* sp. than for other cnidarians.

4.3 Suitability as a toxicological species

The copper exposure concentrations used in this study were in the range of observed metal levels found in urbanised coastal marine systems (e.g. Chester & Stoner 1974; Bloom & Ayling 1977; Neff 2002). Zinc concentrations used in this study were higher than typically found in urban coastal marine systems although in highly polluted systems, zinc can exceed the highest concentrations tested here (Luoma & Rainbow 2008). The results from this study indicate that *Cassiopea* sp. is a sensitive monitoring species with potential to be used as an important bioindicator species. Interim size measures were not performed during the experiments, so it is unknown how rapidly size inhibition could be identified in the field. Notwithstanding this, a simple, reliable response like change in size is a robust tool that could be used in experimental field monitoring programs.

Cassiopea sp. is readily cultured in the laboratory. The bipartite lifecycle allowed for the maintenance of large populations of polyps in relatively small systems, with the ease of strobilation induction and rapid growth allowing test cultures to be established with relative simplicity. The outcomes of the study showed *Cassiopea* sp. was of comparable sensitivity to other symbiotic marine organisms (Table 5.5) and has potential to be used as a standard species in the toxicological suite.

5. Conclusions

Both *Cassiopea* sp. and the zooxanthellae symbiont (*Symbiodinium* sp.) were sensitive to sub-lethal concentrations of copper and zinc. Changes in size of *Cassiopea* sp. were a more sensitive indicator of copper or zinc exposure than change in photosynthetic activity in the zooxanthellae. Recovery in photosynthetic activity was very rapid in the zooxanthellae once copper exposure ceased and the jellyfish growth rates improved within 7 days post exposure to copper. There were indications that both copper and zinc exposure interfered with osmoregulatory behaviour in the host animals. The outcomes indicate that *Cassiopea* sp. can tolerate short-term (hours) pulse exposures to low concentrations of metals but sustained (days to weeks) exposure may result in significant impairment to the animals. The high degree of sensitivity to metal exposure and the ability to assess host and symbiont response independently

indicates there is potential for this species to be used for monitoring ecosystem health with experimentally deployed medusae.

Chapter 6 - Synthesis and General Discussion

1. Summary of outcomes

The objective of this project was to gain a better understanding of the responses of jellyfishes to aqueous metal exposure and the role they have in accumulating and cycling elements in the marine environment. The thesis sections were designed to assess both the biomonitoring and bioindicator potential to of jellyfishes for aqueous metals.

Chapter 2 described concentrations of selected elements in jellyfish tissues from multiple species across space and time on the Great Barrier Reef. These data were compared with ambient seawater concentrations to assess whether the different species were net accumulators or regulators of the different elements. For the major ions (Ca, Mg) all species were regulators with tissue concentrations mirroring ambient seawater. This was predicted given that jellyfishes are osmoconformers (Arai 1997) and thus likely to reflect ambient concentrations of these major ions. However, other metallic elements were accumulated at high levels in the tissues with some jellyfish species accumulating more than others. For example, *Cassiopea* sp. was a strong accumulator of Cu, Mn, Pb and Zn while *Copula sivickisi* strongly accumulated Al, Cd and Mn. In addition, some elements were accumulated to high levels despite water concentrations being below measurable detection limits (e.g. As, Fe).

Due to the patchy distribution of the jellyfish species across years, it was difficult to establish any strong patterns of temporal variation in elemental concentrations. However, *Aurelia* sp. collected in 2010 did have different elemental signatures to the individuals collected in 2007 / 08 suggesting variation in ambient elemental concentrations as have been found elsewhere (Romeo et al. 1992). To a lesser degree, *Cyanea* sp. also exhibited elemental variation in their tissues among years. In contrast, both *Mastigias* sp. and *Netrostoma* sp. had strong spatial separation in elemental concentrations in cross-shelf (latitudinal) comparisons, although there was little longitudinal variation (Chapter 2).

Having established that jellyfishes accumulated measurable concentrations of metals within their tissues, Chapter 3 investigated the uptake and biological half-life of two essential elements (copper and zinc) that were present in jellyfish tissues in high concentrations from the Chapter 2 data. Both metals are also considered priority pollutants and of concern in coastal marine systems (e.g. Peters et al. 1997; Neff 2002; Grosell et al. 2007; Zhou et al. 2008). *Cassiopea* sp. jellyfish was selected for testing as this species was easily maintained in laboratory culture and field data (Chapter 2; Templeman & Kingsford 2010) demonstrated it accumulated high levels

of trace elements. The study found that both metals were readily bioconcentrated from the water by orders of magnitude above background (Figures 3.2 and 3.4). Copper accumulation was very rapid and reached saturation in the tissues within seven days exposure. It was also excreted rapidly after transfer to clean seawater with a biological half life of 1.7 days. In contrast, zinc was accumulated more slowly and retained for longer.

In parallel with the uptake study it was necessary to establish toxicity thresholds of the target metals. Chapter 4 examined the toxic thresholds of copper and zinc to the different lifestages of three jellyfish species: *Cassiopea* sp., *Alatina mordens* and *Aurelia* sp. Of the three species, *Cassiopea* sp. was the most sensitive species to both copper and zinc, with the newly metamorphosed stages (ephyra and planuloid buds) the most sensitive lifestage. Newly metamorphosed medusae of *A. mordens* were only slightly less sensitive to copper than *Cassiopea* sp. The polyps of all three species were the least sensitive lifestage to both copper and zinc and *Aurelia* sp. polyps were the least sensitive. The lifestage sensitivity in the jellyfishes followed a similar pattern to other marine organisms (e.g. Williams et al. 1986; Medina et al. 2002; Hoang & Klaine 2007).

To assess more subtle effects of metal exposure, and explore the synergies of action between host and symbiont in a symbiotic jellyfish species, a series of sub-lethal toxicity tests were undertaken using *Cassiopea* sp. as described in Chapter 5. Exposure to increasing concentrations of aqueous copper or zinc inhibited growth in the jellyfish and at higher concentrations resulted in a decrease in size (shrinkage). Higher concentrations of copper or zinc also reduced photosynthetic yield of the symbiotic zooxanthellae but this response was not as sensitive as growth of the jellyfish host. These outcomes were similar to that seen in clams (Elfwing et al. 2002) and some corals (Smith et al. 2003). A recovery experiment showed that both the jellyfish and zooxanthellae recovered rapidly from aqueous copper exposure. Post-experiment algal counts showed that photosynthetic inhibition was due to impairment of activity rather than symbiont expulsion as zooxanthellae abundance did not change significantly between controls and treatments for medusae treated with either copper or zinc. The lack of zooxanthellae expulsion in this study was in contrast to the stress induced expulsion of algae seen in some anthozoans (Bielmyer et al. 2010).

The outcomes of this research showed that jellyfishes do accumulate metals from the environment and that this accumulation can be significant. Both bioconcentration and biological half-life could be readily modelled in *Cassiopea* sp. providing a predictive capacity for uptake and excretion of metals. Toxicity responses demonstrated that jellyfishes were sensitive to metals although sensitivity varied depending on metal, species and lifestage. Knowledge gaps still exist with respect to the metal uptake from diet, acute / chronic toxicity to

other pollutants, uptake from sediment, etc, but this work establishes baseline information on effect of exposure to dissolved metals, and provides a framework for further investigations.

2. Biomonitoring potential of jellyfishes

The use of biomonitors is widely recognised as a means of assessing pollutant fluxes in the marine environment. They provide not only a time integrated measure of exposure but also a measure of the extent and partitioning of the bioavailable fraction (Rainbow 1995). However, it is also recognised that full utility in any given environment requires a suite of biomonitor species to encompass the range of different uptake pathways that may occur (Rainbow & Phillips 1993).

Jellyfishes have rarely been considered as potential biomonitors in the literature on the assumption they do not possess the key traits useful for biomonitoring (e.g. Table 6.1). This, combined with minimal information on their accumulative capacity, has lead to them being overlooked for that purpose despite concerns regarding the potential for increasing blooms as a consequence of anthropogenic inputs (e.g. Purcell et al. 2007). However, assessment of the life history traits of jellyfishes suggests they meet many of the criteria for biomonitors even though definitive information on their bioaccumulative capacity has been lacking (Table 6.1). This lack is slowly being addressed but data on intensity and extent of metal bioaccumulation among jellyfish species is still scarce (Templeman & Kingsford 2010).

Table 6.1: Key criteria for good metal biomonitors and the extent to which jellyfishes from this project were able to meet them. Criteria derived from Rainbow & Phillips (1993) and Rainbow (1995). ND – No data.

Biomonitoring Criteria	***Cassiopea* sp.**	***Alatina* sp.**	***Aurelia* sp.**
Sedentary	Y	Y (polyp)	Y (polyp)
Abundant	Y	N	Y
Readily identified & sampled	Y	ND	Y
Large enough for analysis	Y	ND	Y
Resistant to handling stress	Y	N	Y
Tolerant of changes in physico-chemical parameters	Y	N	Y
Net accumulators of metal/s	Y	ND	Y
Available Year Round	Y	ND	Y
Able to be cultured in the laboratory	Y	Y	Y

The dataset on existing biomonitoring species is extensive and prompts the question as to what jellyfish can contribute to the existing knowledge base. With reference to the criteria from Rainbow & Phillips (1993) the potential for jellyfishes to improve on existing biomonitoring capabilities is summarised in the following sections.

Net accumulators of metals

The most important criterion for biomonitors of metal pollution is they must be net accumulators of the metal of concern. Measurement of tissue concentrations among jellyfish species on the GBR (Chapter 2) demonstrated that there is species, temporal and spatial variation in elemental tissue concentrations. These results are supported by other studies which have shown that many scyphozoan jellyfishes are net accumulators of metals (e.g. Romeo et al. 1987; Hanaoka et al. 2001; Fowler et al. 2004; Templeman & Kingsford 2010).

This research also provided the first quantitative assessment of bioconcentration of copper and is one of only two studies of zinc bioconcentration in jellyfish (Chapter 3; Fowler et al. 2004). *Cassiopea* sp. readily accumulated both elements from low ambient concentrations (17 $\mu g.L^{-1}$ Cu and 60 $\mu g.L^{-1}$ Zn) and uptake rates were comparable with that seen in other biomonitoring species (Li et al. 2010). The ability of *Cassiopea* sp. to accumulate metals from low ambient concentrations was better than that seen for many other species (e.g. Richards & Chaloupka 2009; Li et al. 2010) and would permit monitoring of low level pollution or sensitive ecosystems.

There are a number of studies that have identified high turnover and clearance rates of prey and nutrients in jellyfishes (e.g. Morand et al. 1987; Olesen 1995; Hansson et al. 2005). Although there is some variability in the estimated rates, they support the hypothesis that jellyfish can act as biological filters to concentrate low ambient concentration of contaminants. There is also the potential that these measures could be utilised to estimate ambient bioavailable concentrations in the water from tissue concentrations in the animals. The rapid bioconcentration of copper in *Cassiopea* sp. (Chapter 3) demonstrated that measurable accumulation occurred at timeframes that could be useful in monitoring pulse events. However, further work would be necessary to incorporate this strategy into biomonitoring programs.

Another, quite specialised behaviour in some jellyfish is their ability to absorb dissolved organic matter directly from the water (Arai 2001; Pitt et al. 2009). Both symbiotic and heterotrophic jellyfishes possess this capacity (Arai 1997). This ability adds to their utility as biomonitors as this ability is generally restricted to only a few groups e.g. algae (Rainbow & Phillips 1993).

Sedentary / Sessile Behaviour

The polyps of jellyfishes are sessile and attach to hard surfaces (Arai 1997). For *Cassiopea* sp., the medusal phase is also sedentary with medusae resting upside down on the benthos. Thus,

this species is geographically constrained at local spatial scales. Similar sedentary behaviour may be more widespread among jellyfishes with *Mastigias* sp. also observed resting upside down on the benthos although it returned to the pelagic realm at night (Dawson 2000).

While the medusae of most jellyfishes are pelagic, research has demonstrated that individual populations of jellyfish may often be restricted to very specific locations with minimal or no genetic mixing between adjacent bays / estuaries (e.g. Ishii & Bamstedt 1998; Pitt & Kingsford 2000; Purcell et al. 2000; Arai 2001). These findings have been supported by phylogenetic studies (Dawson & Martin 2001; Dawson 2004) showing that speciation in *Aurelia* sp. and other scyphozoan jellyfish may be more extensive than previously determined from morphological studies only. Certain species also exhibit vertical stratification that is linked with specific water quality parameters (Lucas 2001; Lindsay et al. 2004; Barz & Hirche 2005; Raskoff et al. 2005). In a biomonitoring context, this lack of mixing means that many jellyfish species will be present as populations of restricted geographic extent. It would also allow these species to be used as biomonitors of pelagic waters; a capacity that is lacking with many of the current biomonitors.

Other General Biomonitoring Characteristics

Jellyfish polyps are persistent in marine systems year round. Although the medusae are seasonal for many species (Kingsford et al. 2000), some e.g. *Cassiopea* sp. and *Mastigias* sp. may be present year round (Dawson 2000). The alternation of generation between polyp and medusal stages provides the additional potential for concurrent monitoring of the pelagic and benthic phases, although there is no data currently available on the bioaccumulative capacity of polyps, which is a key area still needing assessment.

The medusae stage of many jellyfishes seasonally form blooms with high biomass and densities (Kingsford et al. 2000; Purcell et al. 2007; Dong et al. 2010). With suggestions that these blooms are occurring more regularly, and are linked with anthropogenic disturbance (e.g. Hay 2006; Purcell et al. 2007) the potential exists to use blooms as effective biomonitors of pulse events. In addition, blooms are generally of single species cohort which reduces confounding effects of exposure length among ages (Arai 2001; Dong et al. 2010).

For many scyphozoans, identification of medusae to genus level is relatively straightforward, although there remains some confusion with respect to species distributions (Dawson 2004). Taxonomy of Cubozoa is more complex and regularly under review (Dawson 2004; Bentlage et al. 2010), and polyp taxonomy is challenging with the polyp phase not described for many species (Arai 1997).

Aurelia sp and *Cassiopea* sp. have been readily maintained in culture both for this project and many others. *Aurelia* sp. is probably the most widely studied jellyfish species

(e.g. Groat et al. 1980). Dawson (2000) provided details on maintenance of both *Mastigias* sp. and *Aurelia* sp. in variegated mesocosms that allowed manipulation of physico-chemical characteristics, demonstrating the robustness of these species to culture and handling. The alternation of generation in jellyfishes also allows large numbers of animals to be maintained in small culture facilities as polyps. Induction of strobilation using appropriate triggers then provides the capacity to provide medusae of defined cohorts and size for experimental manipulations, including deployment for field studies (e.g. Dawson & Martin 2001).

Studies on species with cosmopolitan or wide geographical distribution (e.g. *Aurelia aurita*) have demonstrated that despite large geographical (e.g. temperate vs. tropical) and genetic differences they exhibit similarities in feeding rates, growth, respiration and swimming (Dawson & Martin 2001), so biomonitoring responses are likely to be similar. This extent of generalist behaviour is only present in a few other biomonitoring groups (e.g. oysters and mussels) (Rainbow & Phillips 1993).

Many scyphozoan species of jellyfish are tolerant of large temperature variations, particularly cosmopolitan species like *Aurelia* sp. (Dawson & Martin 2001; Purcell et al. 2000). Tropical and sub-tropical species (e.g. *Cassiopea* sp. and many Cubozoa) are less tolerant of low temperatures (Fitt & Costley 1998) but still have a wide geographic distribution. Tolerance to variations in salinity is also species specific, however many scyphozoans (e.g. *Cassiopea* sp., *Aurelia* sp, and *Catostylus mosaicus*) are considered euryhaline (Fitt & Costley 1998; Pitt & Kingsford 2000; Dong et al. 2010). Cubozoa are considered to be less tolerant of changes in salinity although there is little in the way of definitive data.

3. Bioindicator potential in jellyfishes

Although there has been limited use of jellyfishes in toxicity testing historically, the outcomes from this research indicate they have potential be used more extensively in the future. The acute toxicity results demonstrated that for both copper and zinc, the early life stages of *Cassiopea* sp. and *Alatina mordens* (Chapter 4) were of comparable sensitivity to other more routinely used species (e.g. Calabrese et al. 1973; Martin et al. 1981; King et al. 2004; Bao et al. 2008). They also contradict the literature reports inferring jellyfish are pollution tolerant organisms (e.g. Calton & Burnett 1981; Arai 1997).

This work demonstrated that all three species investigated (*Cassiopea* sp., *Aurelia* sp. and *A. mordens*) were amenable to laboratory culture for ecotoxicological studies (despite the inability to induce strobilation in *Aurelia* sp.). Cultures of the first two species are also readily available from the field and / or aquarium wholesalers thereby providing a reliable source. Polyp cultures can be maintained with minimal equipment and their reproductive behaviour

means that ephyra can be produced almost on demand to meet experimental requirements. It also means that age and size specific cohorts can be generated for experimental purposes.

The paucity of toxicology data in tropical compared to temperate environments has been documented and extrapolation is not always viable (e.g. Peters et al. 1997; McPherson & Chapman 2000; van Dam et al. 2008). It is also recognised that whilst shallow tropical marine environments may be under increasing anthropogenic stress, there is a paucity of relevant ecotoxicological data available to better manage these environments (e.g. Peters et al. 1997; McPherson & Chapman 2000; van Dam et al. 2008). Results from this research with all three species of jellyfish contribute additional information for copper and zinc to help address this deficit. *Aurelia* sp. and to a lesser extent *Cassiopea* sp. also provide the potential to provide direct comparisons between climatic regions, given their wide distribution (e.g. Dawson 2000; Purcell et al. 2000).

The use of sub-lethal measures is widespread and a number of specific biomarkers have been identified as standard measures for monitoring pollution. Biomarkers have previously been defined as a quantifiable response to contaminant exposure but the ecological relevance of many biomarkers has been questioned (Van Gestel & Van Brummelen 1996; McCarty et al. 2002; Goodsell et al. 2009). It has been recommended that good biomarker responses should be linked to changes in ecologically relevant behaviours in a robust manner (McCarty et al. 2002; Goodsell et al. 2009). In the sub-lethal studies (Chapter 5), both change in size of the host jellyfish and effect on photosynthetic yield in the symbiont can be considered robust biomarker responses. Both effects are ecologically relevant and would affect the ongoing viability and survival of *Cassiopea* sp. exposed to metals in the field. In addition, these endpoints can be measured easily and provide better ecological measures than physical or chemical water quality assessments alone (Goodsell et al. 2009).

In addition to the measured responses of *Cassiopea* sp. and its symbiont to metal stress, identification of their capacity to recover from metal exposure was an important outcome. This demonstrated that not only could response to toxicant loads be assessed, but decreases in toxicant loads could also be monitored. Such recovery phase research has been identified as an important but is an often overlooked aspect of field toxicology (Ralph et al. 2007). These types of recovery responses can also be important in assessing efficacy of remediation strategies for reducing toxicant loads.

Other indicators of response to environmental change in jellyfishes also have potential utility. In some species, recruitment (strobilation) has been linked with salinity and food changes (Purcell et al. 1999; Kingsford et al. 2000). Given that strobilation may also correspond to changes in water quality, strobilation induction and increased recruitment could be explored as a

means of monitoring water quality. This is further supported by studies linking water quality changes with jellyfish blooms (Purcell et al. 2007) which in turn may have economic effects (e.g. impacts on commercial fisheries, tourism), particularly in coastal regions.

This research has contributed some important findings on the relative sensitivities of jellyfish lifestages to copper and zinc. In particular, both the acute and sub-lethal responses of *Cassiopea* sp. offer direct value as bioindicator tools. However, there are still significant knowledge gaps with respect to the extent of metal sensitivity in jellyfishes. There is also minimal data on the toxicity of other pollutants (e.g. hydrocarbons, pesticides) and water quality conditions to jellyfish. For example, although jellyfish are considered to be tolerant of eutrophic conditions (e.g. Arai 2001), Todd et al. (2006) found phosphate uptake was inhibited in *Cassiopea xamachana* exposed to increasing phosphate concentrations, suggesting this tolerance may not be universal within the Scyphozoa. Given the identified sensitivities of *Cassiopea* sp., it is likely that further investigations of jellyfishes will yield other useful bioindicating potentials.

4. Conclusions

Jellyfishes have a chequered reputation, largely as a result of both direct (e.g. stings) and indirect (e.g. fisheries competition) detrimental interactions with humans. There is also the perception in some quarters that they are trophic dead ends (e.g. Verity & Smetacek 1996), rather than organisms of ecological significance (e.g. Benovic & Lucic 2001; Jantzen et al. 2010). Their complex life cycles are often obscured by their most obvious form as medusae, and this too has been undervalued in their potential utility as environmental monitoring and management tools.

This research aimed to establish the biomonitoring and bioindicator potential of scyphozoan and cubozoan jellyfishes to trace element pollution. The outcomes of this study indicated that jellyfishes have significant potential as both biomonitoring and bioindicating taxa although some species (e.g. *Cassiopea* sp.) have more utility than others. Given the currently available data, cubozoan jellyfish would have limited potential due to the combination of taxonomic uncertainties, variations in abundances, seasonality, health and safety issues of handling acutely toxic species and difficulty in maintaining many species in culture.

Among the scyphozoans, *Cassiopea* sp. and to a lesser extent *Aurelia* sp., would both offer benefits as biomonitors. In particular, the life history traits, algal symbiosis and bioaccumulation characteristics of *Cassiopea* sp. suggest that it would be a prime candidate to be included in the biomonitoring suite for metals. Given the rapid accumulation and half life of

copper from water by *Cassiopea* sp., they may be ideally suited for monitoring short term pulse effects (on scales of days) and low concentrations of metal pollution.

Notwithstanding these outcomes, there still exists a large volume of research that could further develop and enhance the utilisation of jellyfishes as biomonitors. These include: 1) establishing the importance of diet in contaminant accumulation; 2) assessing uptake of contaminants from pore water and sediment in *Cassiopea* sp.; 3) measuring the bioaccumulative capacity for other metals and pollutants; 4) measuring synergistic effects among pollutants; and, 5) exploring the wider potential of other jellyfish species as biomonitors.

As bioindicators, jellyfishes lend themselves to being assessed for regional species sensitivities to metals, particularly given the dose-response to copper exposure and wide geographic distribution of many species. Given the sensitivity of *Cassiopea* sp. to both acute and sub-lethal copper and zinc exposure, and the simple and easily measured sub-lethal responses, there is merit in continuing work to establish it as a standard species for metal toxicity.

Chapter 7 - Bibliography

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Appendix A – Jellyfish Culture and Maintenance

1. Summary

Scyphozoan and cubozoan jellyfishes have a bipartite life cycle alternating between a small benthic polyp stage and pelagic medusal stage. Maintenance and culture of both polyps and medusae can be readily achieved, however the lifestages have slightly different requirements. Jellyfishes have been cultured for display purposes for many years and a number of techniques for rearing and maintenance have been developed (e.g. Lange & Kaiser 1995; Caughlan 1984; Pierce 2005). Many of these techniques are suitable for display facilities and were adapted to suit small laboratory culture for this study.

2. Jellyfish sources

All parent material used for culturing and experimental purposes in this study was sourced from in-house cultures (James Cook University) or obtained from the field. *Aurelia* sp. and *Alatina mordens* polyps were generously supplied by Dr Jamie Seymour (JCU - Cairns) from his collections. *Cassiopea* sp. medusae were obtained from Lake Magellan, an artificial urban marine lake located at Pelican Waters, Sunshine Coast, Australia.

Polyp stocks of *Cassiopea* sp. were obtained by spawning induction of the adult medusae which readily broadcast spawn on a daily basis. Ciliated planulae were obtained by placing between six and nine sexually mature medusae of both sexes in approximately six litres of seawater in a bucket overnight. The following morning, the medusae were removed and the remaining seawater placed into a 15 litre plastic aquarium to a final volume of approximately 10 litres. Due to the high mucosal production from the medusae, any obvious pools of mucous were siphoned out of the bucket prior to placing the seawater in the aquarium. Subsamples of the water were checked microscopically for the presence of ciliated planulae. The first polyps appeared within 3 to 5 days of fertilisation.

3. Polyp culture

Polyps are readily reared under laboratory conditions and culturing techniques were the same for all three species. All cultures were maintained at 25 ^{0}C on a 12 : 12 light : dark cycle. The control / dilution water was sourced from collection ponds adjacent to the Australian Institute of Marine Science (AIMS) located approx 50 km south of Townsville, Queensland. The water

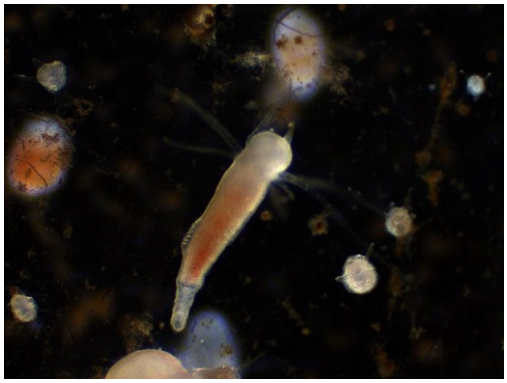
was transported to the James Cook University (JCU) Marine and Aquaculture Facility (MARFU) using a contracted water tanker truck and stored in the main MARFU 60 000 L storage tanks. Water from the MARFU storage tanks was pumped to a 3000 L underfloor storage tank on demand. Water in the underfloor tank was filtered through a sand filter followed by a 20 µm woven fibre cartridge filter before pumping to 2 x 25 L holding drums in the laboratory. Salinity and pH checks were conducted weekly on the underfloor tank and all holding drums. If the salinity exceeded 36 ppt in either the underfloor tank or holding drums it was adjusted to 33-35 ppt using deionised water.

Polyps were maintained in either 20 litre plastic aquariums or 30 L Biorbs®. Due to their small size several thousand polyps could be held in a single tank, although three tanks of each species were maintained to reduce the possibility of losing a culture. To avoid the potential for cross contamination between species tanks, each species had separate cleaning and feeding equipment. Polyp morphology varied among the three species. This made identification of the polyp stage relatively straightforward (Figure A.1).

Polyp cultures were fed with newly hatched *Artemia* sp. (<48 hours old). Cultures being held under a maintenance regime were fed three times weekly. Cultures undergoing strobilation induction or being prepared for experimental work were fed daily.

Polyp cultures being induced or prepared for experimental purposes were cleaned weekly by gently agitating the tank to resuspend any regurgitated food, removal of 30 % of the water by bailing and immediately replacing with clean seawater. Polyp tanks in the maintenance regime were cleaned every 2-3 weeks with a similar 30 % water change. Growth of biofilms and algae were the main issues with tank maintenance. Every two months each tank was completely emptied of water and approximately 25 % of the adhering biofilm was removed by carefully peeling it from the tank surface. The 'cleaned' tank was then rinsed and refilled with clean seawater. There were no indications that the polyps found this maintenance stressful and the cleaned areas were recolonised with new polyps within a few weeks.

To increase the surface area available for colonisation by polyps, 90 mm diameter plastic petri dishes were added to the tanks. Small holes were drilled in one edge of the petri dish and they were tied off to glass rods with fine nylon fishing line allowing them to be suspended in the place. In addition to the increased surface area, this also allowed small colonies of polyps to be removed for strobilation induction as needed.



(a)

(b)

(c)

Figure A.1: Examples of the three polyp species in culture. (a) *Alatina mordens* (b) *Aurelia* sp. (c) *Cassiopea* sp. with attached planuloid bud. Speckled colour in *Cassiopea* sp. is indicative of endosymbiotic zooxanthellae (Images: J. Hopf).

4. Strobilation induction

The polyps of most scyphozoan and cubozoan undergo either asexual strobilation or asexual metamorphosis to produce ephyra. In Scyphozoa, the polyp can regenerate after strobilation back to a fully functional polyp, while in Cubozoa the polyps completely metamorphoses to a medusae (Arai 1997).

There are a number of strobilation cues that have been identified in the literature (e.g. Hofmann et al. 1996; Liu et al. 2009). Strobilation in *Cassiopea* sp. and metamorphosis in *A. mordens* was induced by halo-shocking the polyps. This involved removing approximately 20 % of the seaweater in a culture tank and replacing it with deionised water to reduce the salinity from 33-35 ppt to 25-27 ppt. Strobilation generally occurred within 7 to 10 days after halo-shocking.

The first sign of successful strobilation or metamorphosis was characterised by the head of the polyp changing colour from a pale pink to orange-brown. In *Cassiopea* sp. the polyp head also became flattened and the tentacles were reabsorbed (Figure A.2).

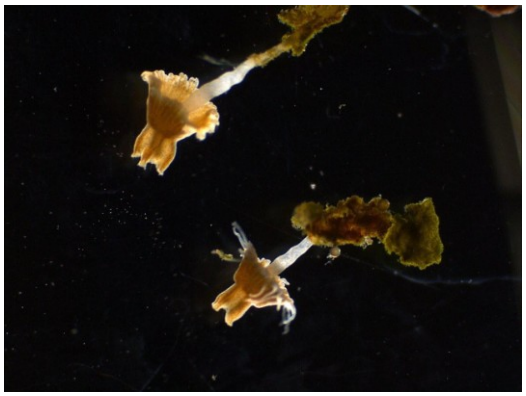


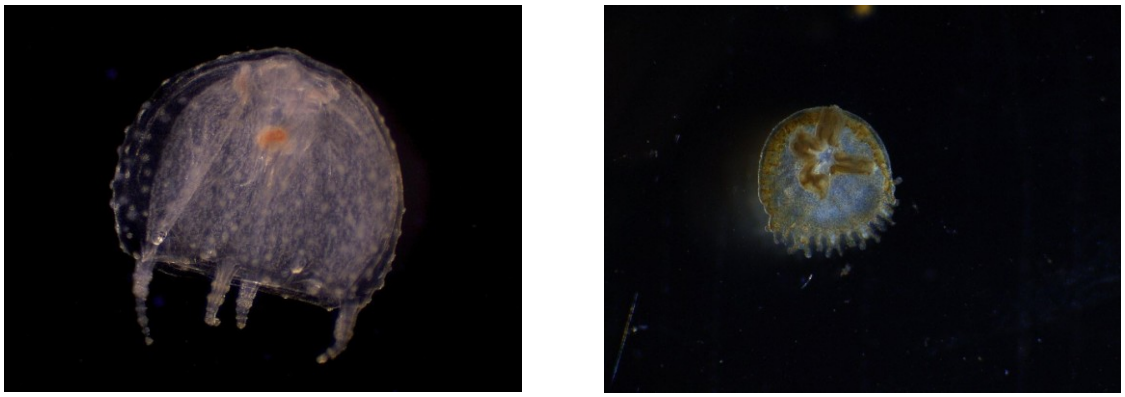
Figure A.2: *Cassiopea* sp. polyps mid strobilation. Lower polyp still has partial tentacles present, whilst upper polyp has reabsorbed tentacles completely (Image: J. Hopf).

Despite many attempts successful induction of strobilation in *Aurelia* sp. was unable to be achieved. A variety of techniques outlined in the literature were used, including thermal stress (Lucas 2001; Lo & Chen 2007), feeding regime (Purcell et al. 2009) and iodine induction (Olmon & Webb 1974), however no ephyra were produced. Discussions with other researchers indicated that this issue can occur in polyp colonies that have been maintained in laboratory culture for long periods although the specific cause is unknown.

5. Medusae culture

Once polyps had completely metamorphosed or strobilated and were free swimming, they were captured using a 3 mL plastic pasteur pipette with its tip removed. The newly released medusae / ephyra were placed in grow out tanks of seawater at 33-35 ppt salinity and held under a 12 : 12 light : dark regime.

Alatina mordens medusae were grown out in two litre ice cream containers (Figure A.3(a)). An airline in a glass pasteur pipette was placed in each container with a gentle airstream to maintain a slight current. Medusae readily fed on freshly hatched *Artemia* sp. and could be maintained for up to one month. To avoid toxic build up of nitrogenous wastes, any uneaten food or digested waste as pipetted out daily. Grow out tanks received a 50 % water change every second day to minimise increases in dissolved nutrients.



(a) **(b)**

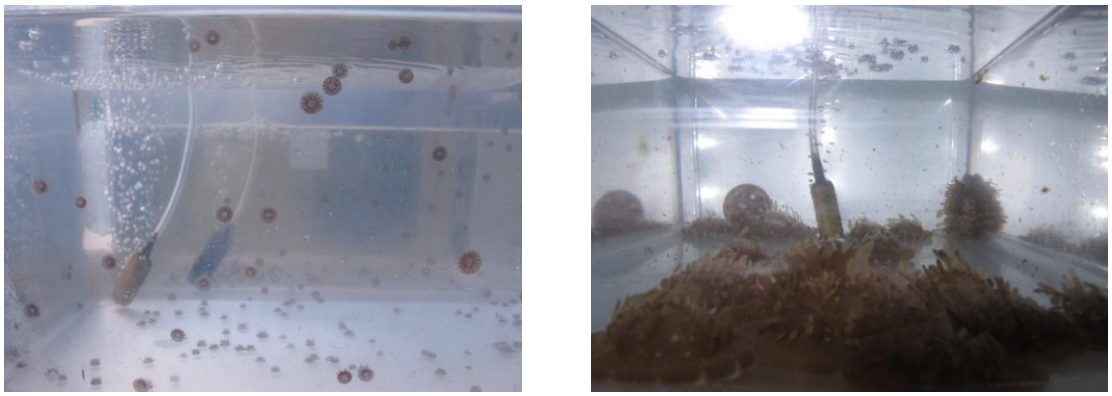
Figure A.3: Newly released ephyra / medusae. (a) *Alatina mordens* less than 4 days post metamorphosis with *Artemia* sp. present in gut. (b) *Cassiopea* sp. newly released ephyra less than 3 days from release. Brown colouring in tissue is endosymbiotic zooxanthellae (Image: J. Hopf (a) S. Templeman (b)).

Cassiopea sp. ephyra were relatively easy to grow out after strobilation (Figure A.3 (b)). Due to their sessile nature, there were no special requirements for within tank circulation to maintain jellyfish buoyancy as is needed for other pelagic species (Lange & Kaiser 1995). Ephyra were initially raised in either ice cream containers or 10 litre plastic containers (Figure A.4(a)).

Ephyra were fed with *Artemia* sp. with supplementary rotifers on occasion. The ephyra were fed daily with this until they reached approximately 25 mm diameter when they were classified as medusae. Once they reached medusae size, *Cassiopea* sp. were placed in shallow plastic containers (400 x 300 x 150 mm) in water to 100 mm depth. This was to achieve maximum surface area with minimum light attenuation.

Medusae on a maintenance regime were fed 4 times weekly. Due to the presence of endosymbiotic zooxanthellae, *Cassiopea* sp. recycle much of the nutrients produced in feeding (Welsh et al. 2009) and frequent water changes were not as critical as for asymbiotic species. Undigested food or excreted waste was pipetted out daily and a partial water change was performed 2 times weekly.

The *Cassiopea* sp. medusae were amenable to being cultured in high density provided good water quality and food are present (Figure A.4(b)). Air stones were placed in each grow out tank to provide some water circulation and to ensure dissolved oxygen levels remained above 70 % saturation.



(a) **(b)**

Figure A.4: Mass culture of *Cassiopea* sp. ephyra / medusae. (a) *Cassiopea* sp. ephyra in mass culture in 10 L container. (b) Medusae (>25mm) in mass culture in shallow containers. Brown colouring in tissue is endosymbiotic zooxanthellae (Images: J. Hopf).

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Glossary of Terms

BCF – the bioconcentration factor calculated as the tissue concentration of an element divided by the element concentration in the surrounding water. Also termed the "field BCF" (Sadiq 1992).

$BCF_{kinetic}$ – calculated bioconcentration of elements from water. It is calculated using parameters derived from kinetic models. The two compartment model uses the kinetic uptake constant (k_U) and kinetic clearance constant (k_E) to define the kinetic BCF. The hyperbolic model defines the kinetic BCF from the theoretical maximum tissue concentration ($C_{A(max)}$) and the water concentration (C_W).

Bioaccumulation – the net uptake and retention of an element into an organism from all external exposure sources including water, food, sediment, air, etc (Neff 2002).

Bioavailability – the extent to which an element can be incorporated into an organism by either active or passive mechanisms (Neff 2002).

Bioconcentration – net uptake and retention of an element from the aqueous phase.

Bioindicator – a quantifiable change in biochemical, physiological, toxicological or ecological process or function that has been correlated or causally linked to effects at one or more of the organism, population, community or ecosystem levels of organization (McCarty et al. 2002).

Biomonitor – organisms capable of accumulating trace elements within their tissues, which in turn can provide a relative measure of the total amount of trace element taken up from all routes in a preceding time frame by the individual (Luoma & Rainbow 2008).

Contaminant – a chemical or physical substance with a concentration that is elevated above normal background in the environment.

EC_{50} – the concentration of a metal or compound that will affect (but not necessarily kill) 50 % of a test population in a defined timeframe.

LC_{50} – the concentration of a metal or compound that will kill 50 % of a test population in a defined timeframe.

LOEC – Lowest-observed-effect-concentration. The lowest measured concentration showing a significantly different response from the control treatment.

Metal Detoxification – a mechanism or function that either reduces or eliminates a metal-induced toxic effect (Mason & Jenkins 1995).

Metal Toxicity – impairment of cellular or physiological function due to metal uptake (Mason & Jenkins 1995).

NOEC – No-observed-effect-concentration. The highest concentration not showing a significantly different response from the control treatment.

Photosynthetically Active Radiation (PAR) –spectral range of the light spectrum from 400 to 700 nanometres that photosynthetic organisms use for photosynthesis

Pollutant – a harmful or undesirable (usually anthropogenic) discharge into the air, soil or water that results in change to the physical, chemical or biological state of that system.

Trace Element – defined as Class B metals using the trace metal definition of Nieboer & Richardson (1980) but in this project also including aluminium (Al) which has Class A Lewis acid properties.