TROPICAL MARINE TOXICITY TESTING IN AUSTRALIA: A REVIEW AND RECOMMENDATIONS

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ABSTRACT

Developmental pressure across Australia's northern coastal catchments will increase rapidly in the near future. These areas are important strongholds for marine biodiversity and contain some of the least impacted marine habitats in the world. Consequently, such development must take place in an environmentally sustainable manner and needs to be underpinned by sound scientific knowledge. A study was undertaken to 1) review the current state of the science for toxicity testing methods that have been developed for, or could be adapted to, Australian tropical marine species and environments and 2) use the information to identify the research and development needs to develop an appropriate suite of Australian tropical marine toxicity test methods. Sixteen taxonomic groupings (from 11 broad taxa groups) were reviewed and their suitability in routine toxicity testing protocols was assessed. The review revealed that there is a paucity of fully-developed, regionally-relevant marine toxicity testing methods for Australian tropical marine species. Currently, just two fully-developed routine sub-lethal/chronic test protocols exist, both of which are for tropical marine microalgae (*Nitzschia closterium* and *Isochrysis* aff. *galbana*), while sub-lethal tests using various tropical coral species have also been applied regularly. Numerous other Australian tropical marine species have been used for acute toxicity testing. In order to meet minimum requirements recommended by ANZECC/ARMCANZ (2000) for site-specific assessments, additional sub-lethal/chronic toxicity tests need to be developed. This review identified a number of different tropical marine species that may be suitable candidates in a suite of toxicity test protocols. The development of such methods will require a large R&D effort, and regulators, industries and community stakeholders should all have an interest in ensuring that these important knowledge gaps are addressed.

Key words: tropical; marine; ecotoxicology; Australia; review.

INTRODUCTION

In response to current and projected water shortages in southern Australia, the Australian Government established a taskforce in 2007 to examine land and water development in northern Australia and to investigate 'the new agricultural frontier' (Commonwealth of Australia 2007). In January 2008, the role of the taskforce was elevated to consider a range of development opportunities, including agriculture, tourism and mining (Commonwealth of Australia 2008). This initiative, combined with the current minerals 'boom' in Australia, is certain to increase development pressure across Australia's northern coastal catchments in the next 10–20 years and most likely beyond.

The coastal waters of northern Australia are recognised internationally as important strongholds for marine biodiversity and contain some of the least impacted marine habitats in the world (Hamilton and Gehrke 2005; Halpern et al. 2008), including the Great Barrier Reef Marine Park and World Heritage Area. Moreover, tropical marine ecosystems include numerous unique features, all of which necessitate careful consideration and management to preserve the unique aquatic flora and fauna. Increased urban, agricultural and industrial development will place added pressures on these important tropical coastal ecosystems through various means, including contamination by natural and anthropogenic chemicals. The Australian Government has emphasised that such development in the northern parts of Australia must take place in an environmentally sustainable manner and needs to be underpinned by sound scientific knowledge (Commonwealth of Australia 2007; 2008). Consequently, regulatory agencies and industries in the region will require access to assessment and monitoring tools for tropical ecosystems in order to acquire the necessary knowledge and ensure the environment is appropriately protected.

The use of toxicity testing for environmental impact and risk assessment and water quality guideline derivation is well established and forms a key component of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC/ARMCANZ 2000). Consequently, it stands to reason that as development increases in tropical Australia's coastal zones, there will be an increase in demand for toxicity testing methods using relevant tropical marine species.

Current developments across tropical Australia that represent existing or potential sources of contamination to marine environments include: urban development and intensive agriculture (metals, pesticides and nutrients) in the north-east (e.g. Haynes 2001); the oil and gas industry (petroleum hydrocarbons) in the north-west (e.g. Neff et al. 2000); and mining and minerals processing (metals and metalloids) across the whole region (e.g. Haynes 2001; Munksgaard and Parry 2002; ACIL Tasman and WorleyParsons 2005). To date, environmental regulators have accepted, and in some cases recommended, the use of temperate species and acute tests to assess contaminant toxicity in tropical marine environments (e.g. WA DOIR 2006). This practice

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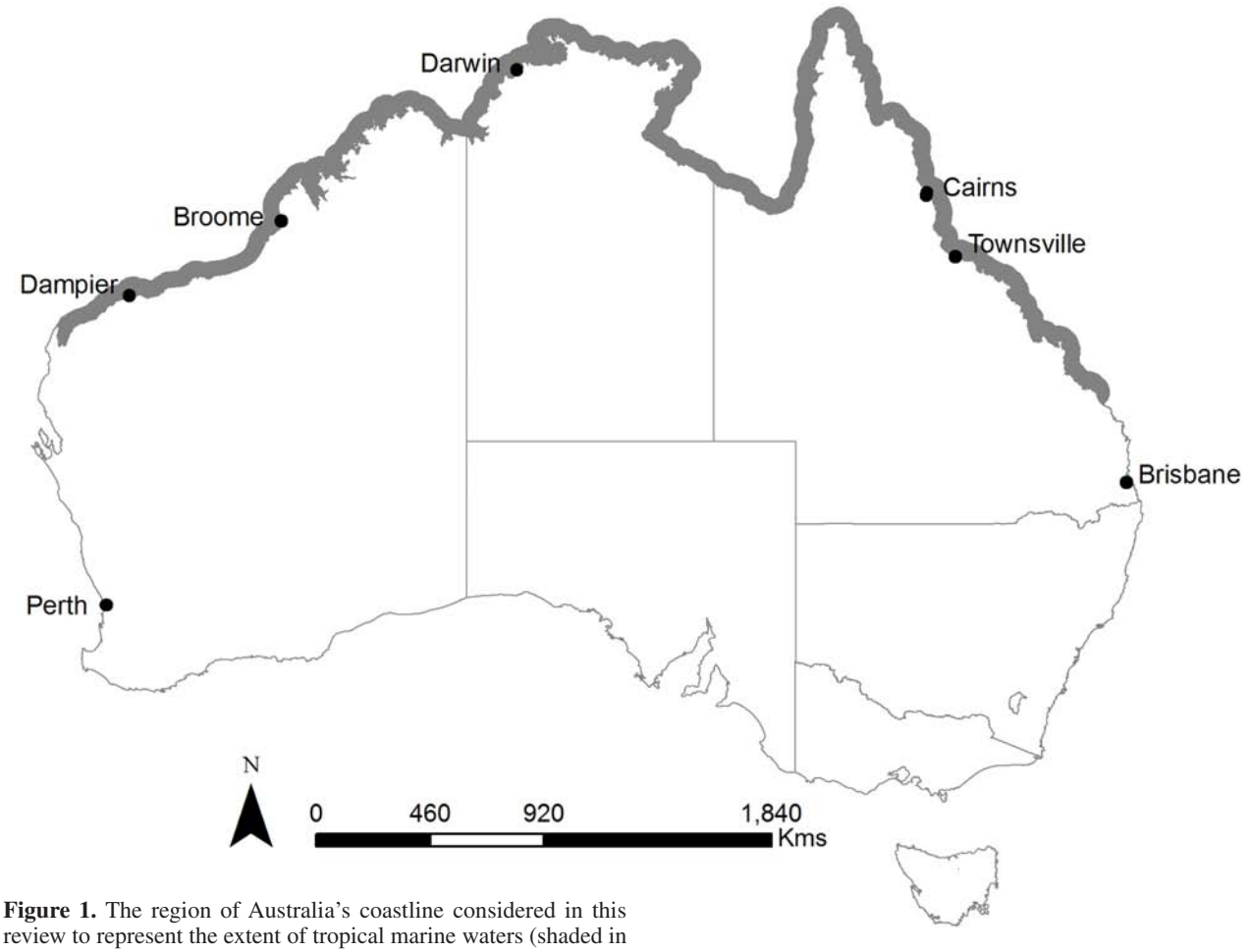

Figure 1. The region of Australia's coastline considered in this review to represent the extent of tropical marine waters (shaded in grey), as classified by IMCRA 4.0 (Commonwealth of Australia 2006).

is inconsistent with relevant national guidance (ANZECC/ ARMCANZ 2000) and the findings of recent research that has compared the sensitivity of temperate and tropical biota (Kwok et al. 2007).

The present review evaluated the current toxicity testing methods for water column contaminants using tropical Australian marine species. A review of Australian tropical (and temperate) toxicity testing methods for contaminants in whole sediments is provided by Adams and Stauber (this issue). For the purposes of the review, Australia's tropical marine waters were defined according to the Integrated Marine and Coastal Regionalisation of Australia (IMCRA) Version 4.0 (Commonwealth of Australia 2006). Of the 41 Provincial Bioregions around the Australian coastline, the ten 'Tropical waters' Provinces and six 'Transitions' zones amongst them were taken to incorporate the tropical marine waters of Australia. This area, which corresponds roughly to the Australian coastal waters north of the Tropic of Capricorn (Latitude 23.5°S), extends north from Exmouth Gulf in Western Australia (approx. Latitude 22°S), across northern Australia and southward along the Queensland coast to Bustard Bay (approx. Latitude 24°S) (Figure 1).

AIMS AND APPROACH

The aims of this study were to:

1. review the current state of the science for toxicity testing methods that have been developed for, or could be adapted to, Australian tropical marine species and environments; and
2. use the information to identify the research and development needs to develop an appropriate suite of Australian tropical marine toxicity test methods.

The review covered water column testing for marine plants and animals, at the 'whole organism' level, where the endpoints assessed were, preferably, sub-lethal, and considered to have some ecological relevance (e.g. growth, development, reproductive responses). This focus is consistent with the ANZECC/ARMCANZ (2000) preference for data based on such endpoints. In total, information is presented for 16 taxonomic groupings from 11 broad taxa groups: microalgae, macroalgae, vascular plants, microcrustaceans (amphipods, copepods, cladocerans), macrocrustaceans (crabs, prawns), bivalve molluscs (oysters, other bivalve molluscs), gastropod molluscs, echinoderms (sea urchins, holothurians), bryozoans, corals and fish. In reviewing literature for these groups, precedence was given to information about tests that have been developed using Australian tropical marine species, followed by tests using Australian temperate species that could

Table 1. The sensitivity of *Nitzschia closterium* and *Isochrysis galbana* to copper.

Species	Temperate or Tropical	Endpoint	Test duration	Cu EC50 (µg/L)	Reference
N. closterium	Temperate	Growth rate	72 h	20	Stauber et al. (2000)
				10	Stauber et al. (1994b)
				17	CSIRO (unpublished)
N. closterium	Tropical	Growth rate	72 h	50	Harford et al. (unpublished)
				35	CSIRO (unpublished)
				40	Johnson et al. (2007)
I. galbana	Temperate	Growth rate	72 h	1.6	Edding and Tala (1996)
I. galbana	Temperate	Growth rate	96 h	35	Ismail et al. (2002)
I. galbana	Tropical	Growth rate	72 h	4.4	Moreno-Garrido et al. (2000)
I. galbana	Tropical	Growth rate	5 d	910	Yap et al. (2004)

be adapted for use with tropical marine species, followed by less-detailed discussions of tropical marine toxicity tests developed and applied overseas. In describing and comparing the various toxicity tests, attention was paid to the following, where information allowed: the species used, and whether the species, or a similar species, is known or suspected to occur in northern Australia; the endpoints/responses measured and their sensitivity to key contaminants; the species' relevance and amenability to laboratory culture; and the extent to which tests have been developed, documented and, subsequently, applied.

MICROALGAE

Unicellular algae are essential to marine ecosystems because they are at the base of most aquatic food chains, they oxygenate the water and are important in the cycling of organic and inorganic substances (Walsh 1988). Consequently, impacts on microalgae have the potential to affect populations of higher organisms (Joubert 1983; van Coillie et al. 1983; Walsh and Merrill 1984).

Unicellular algal tests are typically chronic tests that measure sub-lethal effects rather than mortality. As such, they have often been shown to be more sensitive than many other test species (Walsh and Merrill 1984; Joubert 1983; Wong and Coulture 1986; Lewis 1995; Bailey and Young 1997). Unlike many larger marine species, unicellular algal cultures can be easily maintained in the laboratory all year round, over which time good reproducibility has been attained (Stauber 1995). Algal tests are simple, rapid and cost effective (Arensberg et al. 1995; Wong and Coulture 1986; Pun et al. 1995) and can be scaled down to mini-vial and microplate size so that very little sample is needed (Arensberg et al. 1995; Eisentraeger et al. 2003). This also makes them ideal organisms for large-scale testing with matrices of multiple toxicants or in Toxicity Identification Evaluations (TIEs) (Hogan et al. 2005). Algal tests have also been adapted for use in field studies where cells are contained inside dialysis tubes or alginate beads, which are deployed into the environment (Walsh 1993; Moreira dos Santos et al. 2002). These 'caged culture' experiments have not yet been widely used and have several limitations. However, with further development they have the potential to be a useful component of coastal marine monitoring programs.

A 72-h growth test using the diatom *Nitzschia closterium* is the most extensively-used marine microalgal test in Australia. *N. closterium* is both benthic and planktonic and is widely distributed in Australian coastal waters (Stauber 1995, Table 1). A temperate clone of this alga from Port Hacking, NSW, has been used in many ecotoxicological assessments and is sensitive to a wide range of metals, organic compounds and whole effluents (Florence and Stauber 1986; Stauber et al. 1994a, b; Stauber 1995; Hogan et al. 2005). A tropical clone, isolated from the Coral Sea, has been used to a much lesser extent, but has been shown to be similarly sensitive to metals (Florence et al. 1994; Johnson et al. 2007; Table 1). Furthermore, the *N. closterium* growth test has been adapted to TIE procedures (Hogan et al. 2005). *N. closterium* has been isolated from Darwin Harbour waters (Renaud et al. 1994) and identified in water samples from the Gulf of Carpentaria (Burford et al. 1995), although these strains have not been used for toxicity testing purposes.

Other marine microalgal tests used in Australia include a 72-h growth test using the tropical species *Isochrysis* aff. *galbana*, a planktonic golden-brown alga that has been used regularly for tropical issues, including the assessment of pollutants associated with the oil and gas industry in north west Australia (Evans et al. 1996; Tsvetnenko et al. 1996; SKM 2002). The comparative sensitivity of *N. closterium* and *I. galbana* to copper is shown in Table 1. Additionally, rapid (minute to hours) TIE procedures based on inhibition of photosynthesis [Y(II)] using Pulse Amplitude Moderated (PAM) fluorometry have been recently developed for this species (Strom et al. 2009). Although the strain of *Isochrysis* used in these studies was originally isolated from Tahiti, members of the genus have been isolated from northern Australian waters, namely Port Smith, near Broome, WA (Renaud et al. 1995) and Darwin Harbour, NT (Renaud 1994). A 2-h enzymatic inhibition test using a ubiquitous marine microalga, *Dunaliella tertiolecta*, was developed by Peterson and Stauber (1996) but was found to have similar or lower sensitivity to metals than *N. closterium* and lower sensitivity to organics.

A recent report compared the toxicity of agricultural herbicides to the tropical Australian strains of benthic diatom *Navicula* sp. and the green alga *Nephroselmis pyriformis* (Magnusson et al. 2008). This was the first study to compare the inhibition of photosynthesis [Y(II)] using PAM fluorometry with the more traditional sub-lethal endpoints such as biomass increase. This study reported that relationships between inhibition Y(II) and biomass increase were consistent and linear, validating the utility of PAM fluorometry as a rapid and reliable technique to measure sub-lethal toxicity thresholds of PSII-inhibiting herbicides in these microalgae. Although the use of PAM offers a rapid (e.g. minutes) endpoint for measuring the toxicity of some chemicals, the technique is not as sensitive for metals. Longer exposure durations (e.g. hours) are required due to a slower onset of metal absorption and action, and ultimately, the measurement of growth rate over 72 h provided a more sensitive indicator of metal toxicity (Strom et al. 2009).

Though it is reported to be sensitive to a range of toxicants, the diatom *Skeletonema costatum* has not been widely used as test species in Australia because it is a chain forming diatom that is difficult to enumerate using automated techniques (Stauber 1995). Recent work undertaken internationally to miniaturise microalgal tests by using chlorophyll *a* concentration, optical density, fluorometric and photometric measurements as a surrogate for cell counts (Ismail et al. 2002; Eisentraeger et al. 2003; Satoh et al. 2005) may be applicable to this species.

While marine microalgal tests using temperate species are quite common internationally (and are not included in this review), only two studies using tropical species could be found. Ismail et al. (2002) developed tests using *Chaetoceros calcitrans*, *I.* aff. *galbana*, *Tetraselmis tetrahele* and *Tetraselmis* sp., where optical density was used as a surrogate for cell growth. Each species was exposed to Cd, Cu, Mn and As for 96 h at 28°C. *I.* aff. *galbana* was the most sensitive species to Cd, Cu and Mn, with IC50s of 60, 40 and 7200 µg/L respectively. The overall sensitivity of all species to As, however, was quite low (lowest IC50 was 35.8 mg/L). Of these genera, *Isochrysis* is known to occur in tropical Australian marine waters. As unicellular algae are quite ubiquitous in their distribution, species of the two other genera also may exist across tropical Australia. Jensen et al. (2000) assessed the toxicity of Pb and Cd to three tropical marine microalgal species (i.e. *Chaetoceros calcitrans*, *Chlorella* sp. and *Dunaliella tertiolecta*) from Phuket, Thailand. They reported that *Chlorella* sp. was the most sensitive microalga and that Pb was more toxic than Cd. Specifically, the EC50s in natural seawater for Cd were 3.02, 0.32, and 34.6 mg/L, and for Pb were 0.18, 0.4 and 6.77 mg/L, for *C. calcitrans*, *Chlorella* sp. and *D. tertiolecta*, respectively.

Summary – Microalgae

Microalgae are highly relevant and suitable organisms to include as key components of a toxicity testing suite for northern Australia, for the following reasons:

- Marine algae are easily cultured in the laboratory, provide reproducible toxicity test results and generally have been found to be relatively sensitive to exposure to metals;
- The two marine microalgal species most widely used for toxicity testing purposes in Australia, *N. closterium* and *I.* aff. *galbana* are known to be present across tropical Australia; and
- The existence of suitable toxicity testing protocols for these algal species (including TIE procedures for *N. closterium*) minimises further technical development required to apply these tests to tropical Australian marine issues.

MACROALGAE

While unicellular marine algae are undoubtedly the dominant primary producers in the marine environment, marine macroalgae are responsible for a significant proportion of the primary production on a local scale in coastal regions (Walsh 1993). Furthermore, the complex structure of macroalgal communities provides important substrate and habitat for many other marine organisms (Burridge and Bidwell 2002; Eklund and Kautsky 2003).

Even considering their ecological significance, marine macroalgae have only recently, and are still quite rarely, used in ecotoxicological assessments and monitoring of discharges to the marine environment. As a result, there is still no international standardised toxicity test method using macroalgae and only one national standard (American Society for Testing and Materials – ASTM) for the red alga *Champia parvula* (Melville and Pulkownik 2006). A review of marine macroalgal testing undertaken by Eklund and Kautsky (2003) indicated that while tests have been conducted with 65 different species worldwide, only one chemical was tested on only one occasion for half of these species. Even for the most extensively used species, the green alga *Enteromorpha intestinalis*, toxicity data only existed for 27 different chemicals.

Several international and Australian field studies, reviewed by Burridge and Bidwell (2002), demonstrated the decline of macroalgae near sewage effluent and urban run-off sites, indicating the sensitivity of field populations to chemical contaminants. Notwithstanding this, single-celled algae have typically been used as representatives for all marine plants in ecotoxicological assessments (Eklund and Kautsky 2003). The delay in incorporating macroalgal tests in ecotoxicological assessments may be partly due to the complex life cycles of most species (Eklund and Kautsky 2003) and the resulting difficulty in culturing and determining the most sensitive life-stage of different species. In addition, macroalgae are, in general, very long-lived and slow growing (Haglund et al. 1996) so that test endpoints other than growth (the most common and often the most environmentally relevant endpoint used in chronic ecotoxicology testing) had to be developed.

Australian researchers have been using macroalgae as toxicity testing organisms since the early 1990s, with five species of brown algae, two species of red algae and one green alga having been used (Table 2). However, the most commonly-used macroalgal species for toxicity testing have been the temperate brown algae, *Hormosira banksii* (Stauber et al. 1994a; Myers et al. 2007) and *Ecklonia radiata* (Bidwell et al. 1998; Burridge et al. 1999). The most common endpoints

Table 2. Marine macroalgal species used for toxicity testing in Australia.

Division	Species	Test endpoint	Duration	Reference
Chlorophyta (Green Algae)	*Ulva lactuca*	Gametophyte development	72 h	AWT ES&T (1996)
Phaeophyta (Brown Algae)	*Durvillaea potatorum*	Fertilisation, germination and growth	24 h, 48 h and 4 d	Doblin and Clayton (1995)
	Ecklonia radiata	Zoospore germination	24 or 48 h	Bidwell et al. (1998)
		Germination tube length		Burridge (1999)
	Hormosira banksii	Fertilisation	2.5 h	Stauber et al. (1994a, b); Gunthorpe et al. (1995)
		Fertilisation, germination and growth	24 h, 48 h and 4 d	Doblin and Clayton (1995)
		Germination and growth	48 and 72 h	Myers et al. (2007)
	Macrocystis augustifolia	Germination	24 h	Burridge et al. (1996)
	Phyllospora comosa	Zygote and embryo mortality	96 h	Burridge et al. (1995)
		Germination	48 h	Burridge and Shir (1995)
Rhodophyta (Red Algae)	*Catenella nipae*	Net photosynthesis	3 h	Melville and Pulkownik (2006)
		Respiration rate	24 h	
	Caloglossa leprieurii	Net photosynthesis	3 h	Melville and Pulkownik (2006)
		Respiration rate	24 h	

used in these and other macroalgal tests have related to the ability of the algae to sexually reproduce (e.g. fertilisation and germination success, gametophyte development, spore motility, egg production and sexual fusion) (Walsh 1993; Burridge et al. 1995; Eklund and Kautsky 2003; Myers et al. 2007). The only growth test, reported by Walsh (1993), used an early macroscopic gametophyte stage of the brown alga *Macrocystis pyrifera* and, as such, could also be considered a reproductive endpoint.

Melville and Pulkownik (2006) questioned the relevance of reproductive endpoints in tests using red algae because although most species will reproduce sexually in the laboratory, many typically undergo asexual reproduction in the field (West et al. 2001). Considering that red algae (Rhodophytes) dominate tropical coastlines, while the brown algae (Phaeophytes) are more common in temperate regions (Eklund and Kautsky 2003), it is worth considering endpoints other than sexual reproduction for assessing discharges into tropical marine waters.

Melville and Pulkownik (2006) investigated the use of physiological endpoints (net photosynthesis and respiration rate) in routine ecotoxicological testing with two red algal species. They found that net photosynthesis, over a 3-h exposure to Cu, to be a more sensitive endpoint than respiration over 24 h. Even with such a short exposure period, the more sensitive species of the two (*Catenella nipae*) was found to be more sensitive (EC50 = 3.1 ± 0.2 µg/L Cu) than many of the species incorporated in the Australian/New Zealand marine water quality guideline for Cu (ANZECC/ ARMCANZ 2000).

Information on the distribution of macroalgae across northern Australia is very limited with records from only three studies being sourced for this review. Womersley (1958) described the marine macroalgae collected as part of the American-Australian Scientific Expedition to Arnhem Land as consisting of species that are widely distributed in most warm waters of the world and, that of those recorded, only a small group appeared to be restricted to tropical Australia. A total of 50 species of macroalgae was collected during the expedition, 20 of which were red, 18 were brown and 12 were green (Womersley 1958 with taxonomic review by Lewis 1984, 1985 and 1987). None of the species recorded have been used in ecotoxicology testing in Australia or overseas.

In a more recent survey, described by King and Puttock (1994), both of the red algal species used by Melville and Pulkownik (2006) were recorded as occurring in mangrove forests in northern Australian waters. While observations at specific sites were not described, both *C. nipae* and *Caloglossa leprieurii* were recorded between the South Alligator River (NT) and Wyndham (WA). However, only *C. leprieurii* was recorded further east, in the Gulf of Carpentaria between Weipa (Qld) and the Roper River (NT). Other surveys in the Gulf of Carpentaria have reported 113 macroalgal species (Phillips et al. 1999), with many of these also found in the tropical east-coast regions of Queensland (Phillips 1997).

Interestingly, the tropical/sub tropical red alga, *Champia parvula*, which is widely used as a test organism in the United States and Canada (US EPA 2002), has been recorded from both the Great Barrier Reef in north-east Australia (Lewis 1984) and the Dampier Archipelago and King Sound in north-west Australia (Huisman 2004).

Burridge et al. (1995) suggested that because members of the Order Fucales (Phaeophyta) share similar reproductive strategies, that the methods used for southern species from this order (e.g. *Phyllospora comosa, H. banksii*) may be applied at a broader scale. Up to 14 fucoid species were recorded in Arnhem Land and Gulf of Carpentaria waters by Womersley (1958) and Phillips et al. (1999).

Similarly, the endpoint of net photosynthesis, used by Melville and Pulkownik (2006), could be applied to other tropical red algal species, over 47 of which have been identified across tropical Australia (Womersley 1958; Phillips et al. 1999). However, the use of photosynthetic endpoints would need to be validated against other endpoints such as reproduction/ fertilisation and growth for various types of toxicants.

Summary – Macroalgae

A macroalgal species may be relevant to consider as part of a toxicity testing suite for tropical northern Australia, for the following reasons:

- The incorporation of macroalgal tests into toxicity testing suites increases the environmental relevance of ecotoxicological assessments of discharges into the coastal marine environment;
- A move in recent years to develop more macroalgae tests has resulted in a wide suite of available species and endpoints, although further standardisation of methods is needed;
- Two species of red algae, *C. nipae* and *C. leprieurii*, that have been successfully used in photosynthetic rate tests in New South Wales, are known to occur in northern Australian coastal waters; and
- Several procedures using southern Australian or internationally occurring species may also be applicable to macroalgal species of tropical Australia.

VASCULAR PLANTS

Marine vascular plants, including seagrasses and mangroves, play a vital role in buffering the coast, providing habitat to a wide range of organisms and cycling nutrients through the production of large amounts of detrital material (Wightman 1989; Peters et al. 1997). Mangrove communities, in particular, are culturally important to traditional coastal people in that they provide food, medicine and materials for tools, along with habitat for many useful faunal species (Davis 1985). Seagrass beds are used as foraging grounds for culturally important species such as dugong (Roelofs et al. 2005).

The seagrasses of northern Australia are typically found in shallow waters, in or around inshore islands, small bays and inlets. While their distribution is quite disjointed and most meadows consist only of aggregate patches, they are a common feature of the northern Australian coastal environment (Roelofs et al. 2005). Mangroves are defined as any vascular plant that regularly occurs in areas subject to tidal inundation. They are more suited to the hot and humid conditions of the tropics, and as such the highest diversity of mangroves occurs in these regions. In Australia, they cover between approximately 9 000 and 11 000 km^2 of river and coastal regions, with over 90% of this located in the tropics (Robertson and Alongi 1995; Duke 2006).

In many areas of the world, coastal plant communities are showing the effects of increasing pollutant concentrations. A decline in the health of seagrass beds has been associated with contaminant exposure, particularly in areas receiving agricultural run-off (Peters et al. 1997; Lytle and Lytle 2001). While mangrove communities have displayed signs of severe impact (including mass leaf and bud drop, malformed and discoloured leaves, reduced growth rates and mortality) after devastating events such as oil spills (Burns et al. 1993; Da Silva et al. 1997), lower concentrations of hydrocarbons from leaking marine engines are also considered a threat to mangrove communities in Australia (Mercurio 2002).

As with macroalgae, marine vascular plants are still rarely used in ecotoxicological testing, primarily because of difficulties in culturing and testing with such large, slow growing organisms. Ralph and Burchett (1998) commented on how large test volumes limited their ability to replicate in tests using the tropical seagrass species *Halophila ovalis.* To overcome culturing difficulties, and for greater environmental relevance, more recent seagrass studies have involved the measurement of photosynthetic endpoints (using PAM fluorometry) on wild plants in *in-situ* chamber experiments (Macinnis-Ng and Ralph 2002, 2003a and b, 2004a and b), though the complexity of the apparatus meant that the number of treatments and replication could not be improved. Although this methodology has been used successfully in Sydney Harbour to assess the impacts of herbicides, metals and petrochemicals on seagrass populations, the ability to conduct similar fixed chamber experiments in tropical waters may be hindered by factors such as large tides (although not all regions across tropical Australia experience large tidal ranges) and the presence of crocodiles. Other tropical seagrass species that have been used in toxicity studies in Australia include *Cymodocea serrulata*, *Halophila spinulosa*, *Halodule uninervis* and *Zostera capricorni*, with endpoints typically focusing on photosynthetic activity (Haynes et al. 2000; Prange and Dennison 2000).

Mangrove species appear to have even more complex culturing and testing requirements than seagrasses. Mercurio (2002) raised mangrove seedlings in customised 'tidal troughs' (where water was pumped in and out according to the tidal cycle) for twelve months prior to undertaking a six month experiment on the effects of petrochemicals. MacFarlane and Burchett (2001) germinated propagules of *Avicennia marina* and maintained them with daily manual watering for six months until the 8-week duration experiment was initiated. While similar tests would provide valuable information on the long-term response of mangroves to a particular contaminant or discharge of concern, unfortunately, such methodologies would not be suitable for incorporation into a routine toxicity testing program.

Although both seagrasses and mangroves have been shown to be highly sensitive to petrochemicals, only a small proportion of the response is likely to be due to chemical toxicity. Rather, physical (e.g. smothering and asphyxiation) and indirect impacts (e.g. light amelioration and destruction of habitat) are thought to contribute largely to the decline of exposed populations (Peters et al. 1997; Lytle and Lytle 2001).

Predictably, a number of herbicides have been shown to have direct toxic effects on vascular marine plants, due to their mechanisms of action (Ralph 2000; Lytle and Lytle 2001). In several cases reviewed by Lytle and Lytle (2001), marine vascular plants were found to have greater sensitivity to herbicides than both freshwater and marine algae.

Seagrasses and mangroves have been shown to be highly tolerant of heavy metal contamination. Recent laboratory and *in-situ* studies using the photosynthetic endpoint, quantum yield, have shown that concentrations of Cu, Cd, Pb and Zn in, and above, the 0.1 – 1 mg/L range, were required to reduce the photosynthetic activity of *H. ovalis* and *Zostera marina* (Ralph and Burchett 1998; Macinnis-Ng and Ralph 2002). These studies indicate that inhibition of photosynthesis may not be as sensitive to metals as growth rate inhibition by marine unicellular algae, which was significantly reduced at concentrations less than 10 µg/L Cu (Stauber 1995).

Seagrasses can accumulate metals to concentrations far greater than those found in the surrounding environment without exhibiting visible signs of stress (Peters et al. 1997; Lytle and Lytle 2001). Ward (1989) hypothesised that seagrasses possessed a mechanism to sequester metals into leaf tissue, thereby preventing them from affecting more sensitive metabolic processes. Consequently, the major potential toxicological impact of this bioaccumulation is believed to be the biomagnification of contaminants in grazing aquatic biota and higher trophic organisms.

The nature of mangrove sediments (i.e. fine particles, high organic content and low pH) is ideal for sequestering high concentrations of metals and preventing exposure, even at very polluted sites (Peters et al. 1997). In addition, the grey mangrove, *A. marina*, has been shown to prevent translocation of metals to the leaves by actively sequestering them in root tissue (MacFarlane and Burchett 2001). Even in cases where elevated concentrations of metals have been measured in mangrove leaf tissue (indicating a significant uptake by the plants), no adverse health effects have been observed (Peters et al. 1997).

Summary – Vascular plants

Considering the low sensitivity of marine vascular plants to metals and the difficulties associated with seagrass and mangrove testing, it does not seem practical or economical to incorporate a vascular plant test into a routine ecotoxicology testing program. However, marine vascular plants can be highly sensitive to some herbicides; laboratory based research into the effects on vascular plants could be considered if a particular contaminant of concern or a stable discharge requires a specific *de novo* risk assessment.

The value of seagrasses in a routine monitoring program may lie in their ability to accumulate metals and act as an integrated biomarker for heavy metal exposure, particularly in terms of a direct route for exposure to those organisms that use it as a food source. Also, field measurements of photosynthetic efficiency in both seagrasses and mangroves using PAM fluorometry (e.g. Macinnis-Ng and Ralph 2002) in impacted and unimpacted sites may also prove useful for monitoring plant health.

MICROCRUSTACEANS

Amphipods

Amphipods are extremely widespread and occur throughout the ocean and in freshwater and groundwater (Marsden and Rainbow 2004). Most are free-living and occupy an important position in the food chain providing a major source of food for predatory fish and other invertebrates (Ahsanullah and Palmer 1980; Marsden and Rainbow 2004). Many amphipods can burrow, constructing tubes out of sediment, and may also be exposed to toxicants through the sediment (Marsden and Wong 2001). However, the extent of sediment exposure can depend on the behaviour of a species, e.g. they way they feed and construct their dwellings (Simpson et al. 2005). Most amphipods are detritus feeders or scavengers but some are filter feeders (Ruppert and Barnes 1994). Reproduction occurs through internal fertilisation and there may be one or more broods (up to 750 eggs) per year with the maximum life span usually only one year (Ruppert and Barnes 1994).

Numerous toxicity tests have been developed for various species of marine amphipods, and being a sediment-dwelling organism, they have also been regularly used for sediment toxicity assessments (see Adams and Stauber, this issue). Studies have focused on temperate regions, and species that have been used for water column toxicity testing include *Allorchestes compressa*, *Paracorophium excavatum*, *Corophium colo* (formerly known as *C.* cf. *volutator*), *Ampelisca abdita* and *Gammarus locusta*. The majority of tests that have been developed are based on acute exposures (96 h to 10 d) and responses (e.g. Ahsanullah and Palmer 1980; Ahsanullah 1982; Ahsanullah and Florence 1984; Bat et al. 1998; Costa et al. 1998; Gulec and Holdway 1999; Marsden and Wong 2001; Hyne et al. 2002; ESA 2005), with very few having assessed chronic toxicity (e.g. Conradi and Depledge 1998; Gale et al. 2006; van den Heuvel-Greve et al. 2007). Chronic tests tend to have exposure durations ≥6 weeks, and measure endpoints such as growth and fertility. According to ESA (2005), *A. compressa* can be tested at water temperatures up to 25°C, however, this species is not found in tropical waters (Australian Faunal Directory 2006).

Overall, the marine amphipod faunas of northern Australia are very poorly known. Highlighting this, the first major amphipod survey in tropical Australian waters, on the Great Barrier Reef in February 2005, yielded around 180 species, 160 of which were new to science (J Lowry, Principal Research Scientist, Crustacea Section, Australian Museum, *pers. comm.* 2008). Additional surveys at other sites across tropical Australia have been underway in the past two years, however, results are yet to be published.

Summary – Amphipods

Given their presence at the surface water/sediment interface, amphipods may represent a relevant biotic group to consider as part of a toxicity testing suite for northern Australia. Amphipods could represent a useful test organism for assessing effects due to water column toxicants, pore water toxicants, sediment-bound toxicants and even physical smothering, and could potentially be used for *in situ* exposures. However, much more information is required on

the marine amphipod species of tropical Australia. Moreover, there needs to be substantially more effort put towards development of appropriate chronic tests with sub-lethal endpoints, although some advances in this area have been made in recent years.

Copepods

Copepods represent an important marine test species because of their wide distribution and position towards the base of the food chain (Forget et al. 1998). Most planktonic copepod species feed on phytoplankton and are the main link between phytoplankton and higher trophic levels in the marine food chain (Ruppert and Barnes 1994), making them the most important primary consumers in marine planktonic communities. Further, copepods are essential prey items for the larvae of many fish and larger invertebrates, and are used as a live food source in aquaculture (Kusk and Wollenberger 2005). Planktonic copepods live mostly in the upper 50 m of the water column but there are also benthic species (Ruppert and Barnes 1994). Further highlighting their significance, copepods constitute almost two thirds of mesozooplankton abundance on tropical continental shelves (Longhurst 1985).

Copepod reproduction occurs through transfer of a spermatophore to the female who then releases the fertilised eggs into the water either individually or together within an ovisac (Ruppert and Barnes 1994). Copepods grow and change body shape through a series of moults before they reach the adult stage (Rose 2004). Nauplii hatch from the eggs and progress through five or six nauplii stages and five copepodid stages before becoming adults, having a maximum life span of six to twelve months (Ruppert and Barnes 1994). Phytoplankton is usually the main component of the copepod diet; Rose (2004) noted that it has been found that algal species of *Isochrysis* are most suited for nauplii and small copepodid stages and the microalgae *Rhodomonas* and *Cryptomonas* sp. for adult copepods.

Copepods are known for their sensitivity to chemicals and suitability for toxicity testing (Nipper et al. 1993a; Kusk and Petersen 1997; Rose 2004). Copepod species are ideal for toxicity testing as they are suited to mass culture having a high reproductive potential, short turnover time (from egg to egg) and fast growth rate (Medina and Barata 2004; Rose 2004). Furthermore, the distinct copepod life stages facilitate the measurement of development and reproduction, which provide sensitive and ecologically-relevant endpoints that can be used to determine the potential sub-chronic or chronic toxicity of contaminants (Rose 2004; Kusk and Wollenberger 2005; OECD 2007). Numerous calanoid and harpacticoid copepod species have been used over the past 30 years to evaluate the acute and chronic toxicity of single chemicals and complex mixtures. Table 3 summarises tests that have been conducted with some marine copepods both in Australia and overseas (NB: Rose (2004) provides a more detailed summary of copepod species that have been used for toxicity testing). In addition, there is substantial information on appropriate culturing requirements and physico-chemical (e.g. salinity, temperature) tolerances for marine copepods (e.g. Kusk and Wollenberger 1999; McKinnon et al. 2000; Medina and Barata 2004; Kusk and Wollenberger 2005; Milione and Zeng 2007; OECD 2007). Researchers have been able to measure numerous sub-lethal endpoints to assess toxicity including: development rates; sex ratios; total viable offspring production; time to first clutch; and time interval between successive clutches (OECD 2007). Copepods have demonstrated sensitivity to metals such as copper (Bechmann 1994; Arnott and Ahsanullah 1979; Kwok et al. 2008), zinc and cadmium (Arnott and Ahsanullah 1979), organometallic compounds such as tributyltin (Kusk and Petersen 1997), and organic compounds such as pesticides (e.g. cypermethrin; Barata et al. 2002) and surfactants (e.g. linear alkylbenzene sulfonate; Kusk and Petersen 1997).

Of the two copepod species that have been used for toxicity testing purposes in Australia, *Acartia sinjiensis* is found in tropical waters, including those of the Northern Territory, while *Gladioferens imparipes* is a temperate species (McKinnon et al. 2000; Rose and Carruthers 2006; Duggan et al. 2008). *A. sinjiensis* has been successfully cultured in northern Australia as a hatchery feed source for finfish (McKinnon et al. 2000; Milione and Zeng 2008). Rose (2004) found *A. sinjiensis* to be a useful organism for acute toxicity testing, and was confident that protocols measuring sub-lethal endpoints could be developed. However, it should be noted that *A. sinjiensis* does not grow optimally in full-strength seawater (Milione and Zeng 2008) and, due to its small size, can be difficult to work with in the laboratory (CSIRO, unpublished information). Therefore, investigation of alternative tropical copepod species may be beneficial.

The Organisation for Economic Co-operation and Development (OECD) has recently published Phase 1 results from a project that aims to validate chronic toxicity testing protocols using the calanoid species, *Acartia tonsa* and two harpacticoid species, *Nitocra spinipes* and *Amphiascus tenuiremis* (OECD 2007). In addition, a full life-cycle test using *Amphiascus tenuiremis* has been published recently by the American Society of Testing and Materials (ASTM 2004). Such protocols provide a sound basis for developing tests for other species.

Overall, the copepod fauna of northern Australia is reasonably well characterised (McKinnon and Klumpp 1998; McKinnon et al. 2005; Duggan et al. 2008; McKinnon et al. 2008), making an assessment of the potential suitability of species for toxicity testing moderately straightforward. For example, several species of another calanoid genus, *Pseudodiaptomus*, which is broadly distributed across northern Australia, have also been identified as being potentially suitable for toxicity testing (F Gusmao, Australian Institute of Marine Science, *pers. comm*. 2008).

Summary – Copepods

Copepods may represent a relevant biotic group to consider as part of a toxicity testing suite for northern Australia. This is particularly so because the copepods constitute the majority of the marine mesoplanktonic fauna, a group that is of great importance in marine trophic food webs, and that has been highly under-represented in ecotoxicological studies in Australia to date. In addition, the genus that has been most studied around the world, *Acartia*, is present

Table 3. Marine copepod species used for toxicity testing in Australia and overseas.

Species	Life stage	Test duration	Test endpoint	Temp (°C)	Salinity (ppt)	Reference
Australia – tropical						
Acartia sinjiensis	Adults (10–12 days old)	24 h and 48 h	Survival	27 ± 1	34-35	Rose (2004)
Australia – temperate						
Acartia simplex *Paracalanus parvus* *Scutellidium* sp.	Adults	24 h	Survival	17 ± 1	34.5-35.5	Arnott and Ahsanullah (1979)
Gladioferens imparipes	24 h neonates	96 h	Survival	28 ± 1	33	Evans et al. (1996); Tsvetnenko et al. (1996)
	24 h neonates	7 d	Survival, reproductive ability of female	27	20-35	J Woodworth, Geotech Services, *pers comm*. (2005)
Overseas – tropical						
Acartia lilljeborgi	Adults	48 h	Survival	25 ± 2	33.5 ± 1.5	Nipper et al. (1993a)
Temora stylifera	Adults	48 h	Survival	25 ± 2	33.5 ± 1.5	Nipper et al. (1993a)
Overseas – temperate						
Tisbe furcata	Newborn nauplii	96 h and 100 d	Survival, development rate, fecundity	15 ± 1	34 ± 2	Bechmann (1994)
Tigriopus brevicornis	Ovigerous females Copepodites, Nauplii	96 h	Survival	20 ± 1	34.5-35	Forget et al. (1998)
Tigriopus japonicus	Adult	96 h	Survival	25 ± 1	33	Kwok et al. (2008)
	Full life cycle	20-30 d	Development, time to 1st reproduction Intrinsic rate of increase			
Acartia tonsa	Full life cycle	21 d	Survival, development, sex ratio, length, onset/ stability of egg production	20	20	Kusk and Wollenberger (2005)
	Eggs/ newborn nauplii	8 d	Larval survival and development	17.5	18 and 28	Kusk and Petersen (1997)
	20 d old female	5 d	Adult/egg survival, egg production	20 ± 1	30	Barata et al. (2002)
	8 d old	48 h	Feeding rate			
	Newborn nauplii	32 d	Survival, egg production			
Acartia tonsa	Full life-cycle	25 d	Numerous developmental and reproductive endpoints	~20	~20	OECD 2007
Nitocra spinipes	Full life-cycle	40 d	Numerous developmental and reproductive endpoints	~22	~6.5	OECD 2007
Amphiascus tenuiremis	Full life-cycle	25 d	Numerous developmental and reproductive endpoints	~22	~30	OECD 2007
Amphiascus tenuiremis	Full life-cycle	25 d	Numerous developmental and reproductive endpoints	25	NR	ASTM 2004

NR: Not recorded

across tropical Australia, with the species most recently being studied in Australia, *A. sinjiensis*, being a tropical species. Finally, the culturing requirements of *A. sinjiensis* have been well researched and documented, and hence there is a solid knowledge base from which to develop appropriate protocols.

Cladocerans

Cladocerans are important freshwater microcrustaceans with about 600 different species recorded. However, only eight cladoceran species are known to populate marine ecosystems and these species are represented in two groups: the Podonidae, which includes the genera *Evadne, Pleopis, Podon* and *Pseudevadne*; and the Sididae, which consists of one species, *Penilia avirostris* (Cristescu and Hebert 2002). *P. avirostris* is known for its cosmopolitan distribution in warm waters and researchers have hypothesised that its increasing abundance in temperate oceans may be due to increasing sea surface temperatures (Johns et al. 2005). The marine cladocerans are abundant in tropical and subtropical regions and are found in both oceanic and coastal zones (Marazzo and Valentin 2000). At certain times of the year they dominant the mesozooplankton due to their ability to reproduce rapidly, although their importance in tropical marine ecosystems is under-appreciated (Rose et al. 2004).

Like their freshwater counterparts, marine cladocerans are able to reproduce by parthenogenesis when conditions are favourable (Turner et al. 1988). This trait makes them highly desirable for toxicity testing because a reproductive endpoint can be measured from one individual. Unfortunately, a continuous laboratory culture of marine cladocerans has not been maintained for longer than a few weeks, although researchers are currently working to resolve this issue. The difficult culture requirements are likely to be due to their complex dietary requirements, which are believed to consist of various microorganisms (Atienza et al. 2007). Consequently, no toxicity tests have used marine cladocerans, but this could change if researchers are able to establish their culturing requirements.

Summary – Cladocerans

Marine cladocerans appear to represent an important component of the mesozooplankton and are circumglobally distributed in the tropics and subtropics. Their ability to parthenogenically reproduce is likely to enable the measurement of a reproductive endpoint. However, before a toxicity test can be developed, a species needs to be isolated and identified and the culturing requirements need to be established, which would require a significant amount of effort.

MACROCRUSTACEANS

Crabs

Crabs are crustaceans of the Order Decapoda (Ruppert and Barnes 1994) and are related to prawns and amphipods (Class Malacostraca). Their early development consists of a series of moult stages being the zoea I-V, megalopa, crablet and juvenile before becoming an adult. The larvae (zoea) are planktonic until they begin to settle as megalopa, at which stage they have a pair of large claw-like arms. Most marine crabs mature and spawn in seawater, spend post-larval and juvenile stages in brackish water and then return to the sea as a sub-adult. The majority of crab species combine predatory feeding on small fish and invertebrates with scavenging of detritus (Ruppert and Barnes 1994).

Some toxicity tests that have been conducted in Australia and overseas using various crab species are listed in Table 4. Limited ecotoxicological testing using crab species has been done in Australia. Anderson (2003) assessed the effects of copper exposure for 10 weeks on carapace lesions in juveniles of the tropical mud crab, *S. serrata*. The exposures were generally 'one-off' experiments, with no well-developed methodologies resulting from the study. Black (2003) assessed the acute toxicity of whole sediments to the benthic scavenger hermit crab, *Diogenes* sp., although the test itself was based on an amphipod sediment toxicity testing protocol (US EPA 1994). This test is discussed in more detail by Adams and Stauber (this issue). In the most recent study, Neil et al. (2005) assessed the acute and chronic toxicity of ammonia to *S. serrata*. The chronic toxicity test lasted 19 days and assessed survival and moulting percentage over the complete larval cycle (i.e. all zoea stages through to megalopa). They reported that the tolerance of *S. serrata* larvae to ammonia did not increase during development, i.e. the megalopa stage was the most sensitive to ammonia (48-h LC50 = 21 mg/L total ammonia), while zoea 1 (48-h LC50 = 50 mg/L total ammonia) and 5 life stages (48-h LC50 = 47 mg/L total ammonia) were the most tolerant.

The international literature contains many toxicity studies for a range of species of marine crabs, however, the vast majority of these have assessed acute toxicity, measuring mortality over 48 to 96 h. Some studies have assessed the chronic toxicity of various toxicants to crab species (e.g. Caldwell et al. 1979; Kannupandi et al. 2001; López Greco et al. 2001b; Cripe et al. 2003). Exposure durations ranged from 20–180 days, and the types of endpoints assessed included growth and associated moulting variables such as intermoult periods and time to moult. Toxicity tests have been conducted using both tropical and temperate species (Table 4). Certainly, the crab zoea and megalopa stages lend themselves to the conduct of short-term tests for estimating sub-lethal toxicity using moulting/development variables as sensitive indicators of toxicity.

Studies using crab larvae have shown them to be sensitive to unionised ammonia (48-h LC50 = 1.35 mg/L for megalopa stage of *S. serrata*; Neil et al. 2005), copper (96-h LC50 = 80-170 µg/L; López Greco et al. 2001a, b; Ramachandran et al. 1997; Ahsanullah and Arnott 1978) and cadmium (96-h LC50 = 78-490 µg/L; Ramachandran et al. 1997; Ahsanullah and Arnott 1978).

There are four species of the genus *Scylla* that inhabit tropical to warm temperate waters, and which are important for commercial fisheries (Ruscoe et al. 2004). *S. serrata*, which is the most widespread of these species, occurs throughout the northern half of Australia (Gopurenko and Hughes 2002; Ruscoe et al. 2004), and has significant economic

Table 4. Marine crab species used for toxicity testing in Australia and overseas.

Species	Life stage	Test duration	Test endpoint	Temp (°C)	Salinity (ppt)	Reference
Australia – tropical						
Scylla serrata	Adult	10 weeks	Carapace lesions	26-28	NR	Anderson (2003)
	Zoea I-V and megalopa	48 h and 19 d	Survival and intermoult period	26.4-28.6	28-32 depending on larval stage	Neil et al. (2005)
Australia – temperate						
Paragrapsus quadridentatus	Larvae (less than 18 h old)	96 h	Survival	17 ± 1	34.5-35.5	Ahsanullah and Arnott (1978)
Overseas – tropical						
Scylla serrata	Zoea (4-6 days old)	48 h	Survival	NR	30	Ramachandran et al. (1997)
Tunicotheres moseri	Zoea I, II and megalopa	96 h	Survival Carapace width	25 ± 0.1	37 ± 1	López Greco et al. (2001a)
Overseas – temperate						
Cancer magister	Eggs and zoea	24 h	Egg hatching, early development	13 ± 1	28-30	Caldwell et al. (1979)
	Zoea	70 d	Survival, moulting	13 ± 1	28-30	
	Juvenile	80 d	Survival	13 ± 1	32-34	
	Adult	73-85 d	Survival	13 ± 1	23-34	
Macrophthalmus erato	Zoea and megalopa	NR (estimated at 21 d)	Survival, duration of moult cycle	27 ± 1	25 ± 1	Kannupandi et al. (2001)
Chasmagnathus granulata	Zoea I	96 h	Survival	20 ± 1	30 for zoea	López Greco et al. (2001b)
	Juvenile stages 5-7	180 d	Survival, time to moult, carapace width, size increment		12 for juveniles	
Rhithropanopeus harrisii	Zoea, megalopa, crablet	20 d	Survival, duration of moult cycle	25	20	Cripe et al. (2003)

NR: Not recorded

and cultural importance (Coleman et al. 2003; National Oceans Office 2004). In addition, given its importance as a fisheries/aquaculture resource, the culturing requirements of *S. serrata* have already been well researched, established and documented (Keenan and Blackshaw 1999; Allan and Fielder 2003), and they are currently cultured at a number of northern Australian aquaculture facilities. However, it should be noted that aquaculture facilities spawn their brood stock primarily during the warm wet season months (i.e. October to March), and testing would be restricted to this period unless methods for dry season spawning were developed.

Summary – Crabs

A crab species would represent a relevant biotic group to consider as part of a routine toxicity testing suite for northern Australia. In particular, the mud crab, *S. serrata* represents a very promising test species for the following reasons:

- *S. serrata* is present across tropical Australia, and is a species of ecological, cultural and economic importance;
- Crab larval (zoea and megalopa) stages are planktonic while the juvenile and adults are generally demersal/ benthic, meaning that tests utilising different life stages can be used to assess water column and sediment contamination;
- *S. serrata* has already been found to be a suitable test organism for toxicity bioassays in tropical environments (Ramachandran et al. 1997; Neil et al. 2005), with the larval (zoea and megalopa) stages being particularly sensitive to toxicant exposure (Caldwell et al. 1979; Ramachandran et al. 1997); and
- The culturing requirements are well established and documented, and a number of aquaculture facilities are able to supply organisms on a consistent basis throughout the wet season.

Prawns

Prawns or 'shrimp' also belong to the Order Decapoda. Prawn larvae are an important food source for larger invertebrate and fish species in marine systems (Ruppert and Barnes 1994). The life cycle of penaeid prawns is relatively straightforward: following mating in the ocean, the eggs hatch and the larvae make their way to the shelter of estuarine nursery grounds to become juveniles and continue growing (Provenzano 1985). Penaeid prawns progress through a series of moult stages from nauplii (stages I-V or VI), to zoea (stages I-III), to mysis (stages I-III) and then to post larvae (PL). For the tiger prawn, *Penaeus monodon*, the larval stages to PL take approximately 12 days (Støttrup and McEvoy 2003) and PLs are then identified by the number of days they have been post-larvae. For example, a PL15 has been a post-larva for 15 days and is approximately 27 days old in total.

Penaeus species are the most important commercial shrimps throughout the world (Ruppert and Barnes 1994). *Penaeus merguiensis* (white banana prawn) is of high commercial importance within Australia (Denton and Burdon-Jones 1982) and *P. monodon* is also economically important and cultivable (Munshi et al. 1996; Das and Sahu 2005). The culturing requirements of *P. monodon* have, in recent years, been the subject of substantial investigation, with many aspects now well researched and documented (e.g. Glencross et al. 1999; McKinnon et al. 2000).

Post-larvae of *Penaeus* spp. have been chosen as test organisms in many toxicity tests for their sensitivity (Brecken-Folse et al. 1994; Das and Sahu 2005) and because they survive well under laboratory conditions (Denton and Burdon Jones 1982). In Australia, the vast majority of toxicity testing using prawns has relied on acute exposures of the tiger prawn, *P. monodon* and some other *Penaeus* species (e.g. Ahsanullah and Ying 1995; Manning et al. 1996; Pablo et al. 1997; SKM 2002; Vaughan et al. 2002; ESA 2005). At present, this 96-h survival test is offered by the small number of commercial ecotoxicology laboratories operating in Australia, particularly for tropical issues (ESA 2005). However, the availability of the test relies on the seasonal availability of appropriate stage post-larval prawns from various commercial hatcheries. This test is used in Australia primarily because it is relatively simple to perform, but also because a more appropriate test using this or another species of marine crustacean has not been properly developed. In fact, the key stimulus for the development of a marine copepod toxicity test by Rose (2004) was to develop a short-term chronic (sub-lethal) test that could replace the acute prawn test. Moreover, moves to include crustaceans within the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes – 2004* (NHMRC 2004), if implemented, would severely limit the use of all crustaceans in acute toxicity tests.

Notwithstanding the above, there have been a few chronic toxicity tests conducted in Australia using prawns. Florence et al. (1994) conducted a series of 30-d microcosm experiments, in which juvenile *P. monodon* (1.5–2.0 cm length) and two other species (polychaete, *Galeolaria caespitosa*, and gastropod, *Nerita chamaeleon*) were exposed to nickel ores. The endpoint for *P. monodon* was growth (i.e. length and weight), and although only slightly inhibited by the nickel ores, this was a slightly more sensitive endpoint compared to those used for the other species (i.e. total numbers). Ahsanullah and Ying (1995) assessed the effects of copper on the growth (net weight gain) of juvenile *P. monodon* (2–3 cm length) and *P. merguiensis* (1.5-2.5 cm length) over 14 days, with the latter species being more sensitive than the former. More recently, Anderson et al. (2002) assessed growth and survival of juvenile banana prawns, *P. merguiensis* (~12 weeks old at test commencement) exposed to fluoride for 28 d. All the above chronic tests were undertaken using juvenile prawns, presumably a consequence of having to source them from commercial hatcheries. Ideally, however, chronic toxicity tests using prawns should be conducted on earlier life stages than juveniles, such as the nauplii, zoea and PL stages.

Prawns are a common test species in overseas studies, but as with Australian experience, most of the studies have related to the assessment of survival over acute exposure durations (e.g. Denton and Burdon-Jones 1982; Chen et al. 1996; Clark et al. 1989; Baticados and Tendencia 1991; Brecken-Folse et al. 1994; Munshi et al. 1996; Sulaiman and Noor 1996; Chinni et al. 2002; Das and Sahu 2005; Overmyer et al. 2005). However, some studies have focused on chronic toxicity of

various toxicants (e.g. ammonia, cadmium, copper, mercury), over durations from 20 to 72 days (Wickins 1976; McClurg 1984; Chen and Lin 2001).

Summary – Prawns

Prawns may represent a relevant biotic group to consider as part of a toxicity testing suite for northern Australia. Benefits of utilising a prawn species include:

- Like the copepods, larval prawns represent the marine planktonic fauna, a group that is of great importance in marine trophic food webs;
- The black tiger prawn (*P. monodon*), which has been the most commonly used prawn species for ecotoxicity testing, is present throughout northern Australia (Brooker et al. 2000); and
- *P. monodon* is a commercially important cultivable species.

However, there are also some limitations of utilising a prawn species such as *P. monodon*, including:

- The vast majority of toxicity testing using prawns has involved acute exposures assessing survival only; of the few chronic toxicity studies that have been done, none have produced a fully documented, potentially routine test method; and
- The culturing requirements, whilst well-documented, would be difficult to reproduce in a research laboratory, meaning that test animals would almost certainly have to be sourced from commercial hatcheries, with availability being seasonally-dependent.

BIVALVE MOLLUSCS

Oysters

Oysters are largely sessile filter-feeding bivalve molluscs (Phylum Mollusca, Class Bivalvia, Family Ostreiidae), that feed on fine plankton, largely phytoplankton (Ruppert and Barnes 1994). In most oysters, fertilisation occurs in the water column after the male and female have released eggs and sperm into the current (i.e. broadcast spawning). A free-swimming trochophore then develops followed by a veliger larva which is bilaterally symmetrical and eventually becomes enclosed within two valves (Ruppert and Barnes 1994). After 2-3 weeks, the larvae settle and attach themselves to a surface where they continue to grow, as spat (Queensland Department of Primary Industries and Fisheries 2001).

Many oyster species are of great ecological and economic importance in Australia, in particular *Saccostrea commercialis* (Smith et al. 2004), *Pinctada maxima* (Negri et al. 2004) and *Saccostrea echinata* (Peerzada and Dickinson 1989). In northern Australian waters, the milky oyster (*Saccostrea cuccullata*) and the black-lip oyster (*S. echinata* – syn *Striostrea mytiloides*) are wild-harvested from rocky foreshore areas where they have settled and grown naturally and, while there is little interest in their aquaculture, small quantities have been cultured and at least one hatchery has produced spat (AFFA 2002). There has been a small amount of research conducted on the culturing/rearing requirements of the tropical species, *S. echinata* (Southgate and Lee 1998; Horpet and Southgate 2004). Apparently, *S. echinata* has the fastest development to the trochophore and veliger stages so far recorded for Ostreid larvae; trochophore larvae develop 5.5 hours after fertilisation while D-stage veligers first appear 12.5 hours after fertilisation (Horpet and Southgate 2004). The pearl oyster, *P. maxima*, is commonly farmed across northern Australia, and its hatchery culturing/rearing requirements, have been reasonably well established (Minaur 1969; Love and Langenkamp 2003; Barton and Schipp 2004).

Oysters have been demonstrated to be useful indicators of trace metal and organochlorine contamination of marine waters (Scanes 1996). Further, they have been used as toxicity testing organisms for many years (e.g. Wisely and Blick 1967; Calabrese et al. 1973). Table 5 lists some oyster species that have been used in toxicity testing both in Australia and overseas. The vast majority of toxicity studies using oysters have assessed larval development and/or growth, endpoints that have provided one of the most rapid and sensitive toxicity tests (Geffard et al. 2002). These tests involve exposing fertilised eggs from wild-caught or hatchery-reared adults to a toxicant(s) and assessing normal development to the D-veliger stage after 24 - 72 h (ESA 2005).

In Australia, there is little published work of studies using oysters as toxicity test species, although a large amount of commercial-in-confidence work utilising oysters has been carried out (e.g. SKM 2002; Vaughan et al. 2002). Denton and Burdon-Jones (1981) studied the tropical species, *S. echinata*, but looked only at metal uptake, distribution and depuration, not toxicity. Negri et al. (2004) studied the tropical pearl oyster, *P. maxima*, although the focus was on effects to juveniles exposed to populations of cyanobacteria, rather than a toxicity study *per se*. Most toxicity studies in Australia have used the Sydney rock oyster, *S. commercialis*, larval development test, which has been demonstrated to be sensitive to metals (Krassoi 1995; Wilson and Hyne 1997), ammonia, and petroleum hydrocarbons and dispersants (Smith et al. 2004). Although it is a temperate species, *S. commercialis* is regularly used for tropical toxicity testing, because the species can tolerate temperatures up to 25°C. However, the species is restricted to the east coast and is not found in the tropics, with its northern-most limit being around Gladstone, Qld (approx. Latitude 24°S). As *S. commercialis* is commercially grown, broodstock can be purchased from local fish markets or directly from growers. However, the availability of reliable broodstock, and therefore availability for toxicity testing, tends to be limited to the summer months, which is the breeding season for this species (Smith et al. 2004).

Many overseas studies using oysters as test species have been published. As with the Australian studies, the majority have focused on larval/embryo development over 24 to 48 h as the endpoint (e.g. Calabrese et al. 1973; Ramachandran et al. 1997; Geffard et al. 2002), although some have assessed longer exposure durations (e.g. His et al. 1996). The US EPA has documented a 48-h larval development test using the American oyster, *Crassostrea virginica* (US EPA 1995). Elfwing and Tedengren (2002) assessed effects of copper on three tropical oyster species, including *S. cuccullata*, although the study focused on the measurement

Table 5. Oyster species used for toxicity testing in Australia and overseas.

Species	Life stage	Test duration	Test endpoint	Temp (°C)	Salinity (ppt)	Reference
Australia – tropical						
Saccostrea echinata	2-year old	30 d	Metal accumulation	20 and 30	20 and 36	Denton and Burdon-Jones (1981)
Pinctada maxima	Juveniles	7 d	Survival Retraction of mantle Accumulation	26-30	Seawater (NR)	Negri et al. (2004)
Australia – temperate						
Saccostrea commercialis	Larvae	48 h	Development to the D-veliger stage	24-25	23-35	Krassoi (1995); Wilson and Hyne (1997)
Overseas – tropical						
Crassostrea iredalei	Larvae	48 h	Larval Development	NR	25	Ramachandran et al. (1997)
Saccostrea cucullata *Crassostrea lugubris* *Crassostrea belcheri*	Adults	12 h	Metabolism Oxygen consumption Ammonia excretion Absorption efficiency	26 ± 1	30 ± 2	Elfwing and Tedengren (2002)
Overseas – temperate						
Crassostrea virginica	Embryos	48 h	Development to the D-veliger stage	26 ± 1	25	Calabrese et al. (1973)
Crassostrea gigas	Embryos	24 h	Development of D-shape larvae and larval survival	24 ± 1	NR	His et al. (1996)
	Larvae	9 d	Size and survival			
	Embryos	72 h	Development of viable D-shape larvae	20	35	Worboys et al. (2002)
	Embryos	24 h	Larval development	24 ± 1	33	Geffard et al. (2002)
Pinctada fucata martensii	Adults	96 h	Survival	20 and 25	NR	Takayanagi et al. (1999)

NR: Not recorded

of physiological endpoints rather than embryo/larval development. Ramachandran et al. (1997) assessed the effect of exposure to copper and cadmium on larval development of the tropical estuarine oyster, *Crassostrea iredalei*.

Peerzada and Dickinson (1989) documented two oyster species, *S. echinata* (black-lip oyster) and *Saccostrea cucullata* (milky oyster), as existing in Northern Territory waters. Neil et al. (2003) identified an additional oyster species, mangrove oyster (*Isognomon ephippium*, also known as tree oyster; Rees et al. 2006) to be present in north-east Arnhem Land, in the Northern Territory. Finally, the pearl oyster, *P. maxima*, is known to be broadly distributed across northern Australia, from Carnarvon on the west coast to south of Cairns on the east coast (Love and Langenkamp 2003).

Other bivalve molluscs

Other bivalves, such as scallops and mussels have also been used as toxicity testing organisms. To our knowledge, there have been very few, if any toxicity studies using tropical scallops. A standard scallop test is used in Australia, using the temperate doughboy scallop, *Mimachlamys asperrima* (Krassoi et al. 1996; ESA 2005), but this test is generally not run above temperatures of 18°C (ESA 2005). There have been some toxicity studies using tropical mussels, predominantly the Asian green mussel, *Perna viridis*, and the brown mussel, *Perna perna* (Watling and Watling 1982; Tan and Lim 1984; Mohan et al. 1986; Cheung and Cheung 1995). However, neither of these species is native to tropical Australia, and *P. viridis* is, in fact, a declared introduced marine pest (NIMPIS 2002a). There do not appear to have been any toxicity studies using Australian tropical marine mussels.

Summary – Bivalves

Bivalve molluscs may represent a relevant biotic group to consider as part of a toxicity testing suite for northern Australia. Given the common use of oysters as toxicity testing species and the fact that species previously used for toxicity testing are present in northern Australian marine waters, oysters may be the most appropriate bivalve group to target. Oysters have proven to be relevant and sensitive toxicity testing species. *Saccostrea* spp. have been regularly used as toxicity test species due to the ease of gamete generation and they adjust well to laboratory conditions (Ramachandran et al. 1997). What appear to be two of the major species of *Saccostrea* in northern Australia, the black-lip (*S. echinata*) and milky oyster (*S. cucullata*), both have economic importance as seafood and also significance to indigenous communities. In addition, the pearl oyster (*P. maxima*) is a commercially important species and is common across northern Australia. All the above species have been used previously in laboratory studies, albeit not to the extent of temperate species such as *S. commercialis*. In addition, the availability of broodstock, either wild or hatchery-sourced, would need to be ascertained.

GASTROPOD MOLLUSCS

Gastropods are the largest class of molluscs and are ubiquitous within the marine environment, existing from the tropics to the polar regions. They are distinguished by their large, flat, locomotive 'foot' and the asymmetric shell that many species, but not all, possess (Ruppert and Barnes 1994).

Several species of gastropod have been used for toxicity testing in Australia. The tropical snail, *Nerita chamaeleon* (collected from Cairns), has been used in a 30-day microcosm sediment study (with *P. monodon* and *G. caespitosa*) to assess the toxicity of nickel ores. However, the snails were insensitive to the ore with 100% survival reported at the end of the test (Florence et al. 1994). Australian researchers have also used the temperate marine snails, *Polinices conicus* and *Austrocochlea porcata*, to test the toxicity of crude oil, dispersants and dispersed oil (Gulec et al. 1997; Reid and MacFarlane 2003).

A few acute toxicity studies have been conducted by foreign research groups using tropical marine gastropods that are found in northern Australia to assess survival following exposure to metals and organometals, e.g. *Planaxis sulcatus*, *Trochus radiatus*, *Nerita albicilla, Nerita chamaeleon*, *Nassarius reticulates* (Kumar and Devi 1995; Kidwai and Ahmed 1999; Kulkarni et al. 2004; Sousa et al. 2005). Researchers in Florida investigated the use of the queen conch embryos, *Strombus gigas*, and reported that the control survival was good but the bioassay was not as sensitive as other species (Rumbold and Snedaker 1997). A Brazilian group exposed neonates from the tropical snail, *Pomacea lineata*, to effluents for up to 15 d but used survival as an endpoint (Lima Melo et al. 2000). It should be noted that the authors could find no studies that have used sub-lethal endpoints to assess the toxicity of environmental contaminants in the laboratory, although sub-lethal endpoints (specifically imposex) are commonly used in biomonitoring studies.

The vast majority of gastropod studies have used them as tools in biomonitoring, biomarker and bioaccumulation studies, due to their apparent sensitivity to tributyltin (TBT). The temperate marine snails, *Austrocochlea constricta*, *Bembicium auratum* and *Thais orbita*, have been used in Australia for monitoring the bioaccumulation of metals and organopollutants (Walsh et al. 1995; Taylor and Maher 2006) and in biomarker studies (Reid and MacFarlane 2003; Gibson and Wilson 2003). Internationally, the common (edible) periwinkle, *Littorina littorea*, and the dogwhelk, *Nucella lapillus*, have been used extensively in coastal biomonitoring programs, which have focused on the impacts of metals, and imposex and intersex induction following exposure to tributyltin (Ketata et al. 2008). These species do not occur in the tropics but a similar species of ubiquitous intertidal snails has been reported in northern Australia (e.g. *Littoraria* spp.; URS Australia and Alcan 2004). A limited number of studies have used *L. littorea* in laboratory exposure tests (Cajaraville et al. 1989), with the primary focus of most being the identification of biomarkers that can be applied in the field (Kwamla Atupra 2001).

Summary – Gastropods

Marine gastropods may be a useful addition to an ecotoxicological testing suite, as they are common in the marine environment and have been used extensively in biomonitoring studies. It is also likely that they will be easily cultured under laboratory conditions. However, a limited number of laboratory exposure studies have been conducted and none of these studies has used sub-lethal endpoints. Consequently, the development of a chronic sub-lethal snail test may involve significant developmental effort.

ECHINODERMS

Sea urchins

Sea urchins and other echinoderms make up a diverse and widely distributed group of marine organisms. The Phylum Echinodermata includes sea stars, sea urchins and sea cucumbers all of which are exclusively marine and mostly bottom-dwellers (Ruppert and Barnes 1994). For sea urchins and sea cucumbers, fertilisation of eggs by spermatozoa occurs in the water column. Notably, artificial spawning of sea urchins can be induced through the injection of 0.5 M potassium chloride into the body cavity (Nipper et al. 1997).

The use of sensitive life stages of echinoids for toxicity testing is relevant to assessing the health of reef communities, given the importance of some sea urchin species in maintaining healthy reef systems (Nipper and Carr 2001). Sea urchin tests are ideal for the detection of effects of low levels of pollution, due to the high sensitivity of sea urchin embryos and larvae (Nipper et al. 1997; Ozretic et al. 1998) and their specific morphological and physiological metamorphosis (Ozretic et al. 1998). Toxicity tests on echinoid sperm viability and embryo development are well established (e.g. US EPA 2002; ASTM 2003) and exposures of gametes have shown comparable or greater sensitivity to many contaminants than other marine species and life stages (ESA 2005).

Table 6 lists some tests that have been conducted on various echinoderm species in Australia and overseas. To our knowledge, no toxicity tests have been developed in Australia using tropical echinoids. However, the temperate sea urchin, *Heliocidaris tuberculata*, has become widely used in toxicity testing programs in Australia, with fertilisation (1-h exposure) and larval development (72-h exposure) being the major endpoints measured (as summarised by Smith et al. 2004). Both the fertilisation and larval development endpoints have been shown to be particularly sensitive to metals (Doyle et al. 2003). As there is currently no hatchery rearing of sea urchin species in Australia, it is necessary to collect broodstock for toxicity testing from wild populations.

Some toxicity testing using sub-tropical or tropical sea urchins, namely *Anthocidaris crassispina* (Vaschenko et al. 1999; Au et al. 2000), *Arbacia punctulata* (Nipper and Carr 2001), *Diadema setosum* (Kobayashi 1994; Ramachandran et al. 1997), *Echinometra mathaei* (Heslinga 1976) and *Lytechinus variegates* (Nipper et al. 1993b), has been undertaken overseas. Various life stages and endpoints have been measured, ranging from exposures of embryos and larvae for standard fertilisation and larval development tests to long-term (i.e. 4-week) exposures of adults and associated assessment of various reproductive parameters (e.g. sperm motility, egg morphology, fertilisation capability). Ramachandran et al. (1997) found *D. setosum* to be more sensitive to copper, but less sensitive to cadmium, than the oyster, *C. iredalei*, and the mud crab, *S. serrata*. It should also be noted that *D. setosum* is common throughout northern Australian coastal waters (Marsh and Morrison 2004; Australian Faunal Directory 2006).

Holothurians

Another group of echinoids, which has not been used for toxicity testing purposes, but which is relatively common and carries significant economic and cultural significance is the holothurians or sea cucumbers. Also known as trepang, holothurians are fished commercially across northern Australia, with the most common species being *Holothuria scabra* (or sand fish; National Oceans Office 2004). The fishery has been heavily exploited, and recent research has investigated conditions under which holothurians can be bred and reared for wild restocking purposes (Ramofafia et al. 2003; DBIRD 2004). Unlike the sea urchins, spawning in *H. scabra* cannot be induced by chemical stimuli. However, spawning can be artificially induced through exposure to short-term environmental stresses, e.g. temperature change, light intensity, photoperiod, salinity, tidal flux, food availability (Morgan 2000; Battaglene et al. 2002). *H. scabra* has a planktonic larval phase of 10-14 days during which they feed on microalgae. Following this they settle onto substrata and grow up to 40 mm in a month. However, during this first month of growth aquaculturists have reported high mortality rates (Battaglene et al. 1999). Given the above, holothurians represent relevant and potentially useful tropical marine toxicity testing species, although suitable laboratory culture conditions and sub-lethal endpoints need to be investigated.

Summary – Echinoderms

Echinoids, particularly sea urchins and potentially sea cucumbers, may represent a relevant biotic group to consider as part of a toxicity testing suite for northern Australia. Short-term, sub-lethal toxicity tests using sea urchin early life stages are well documented and established, and have been found to be sensitive to a range of toxicants. In addition, a species of tropical urchin that has been used in south-east Asia for toxicity testing, *D. setosum*, is known to occur throughout northern Australia. The sea cucumber, *H. scabra*, is also distributed across the region, is economically and culturally significant, and recent research has focused on hatchery breeding and rearing requirements. A key factor in determining the suitability of an echinoid species as part of a toxicity testing suite would be the ongoing need for field collection of broodstock or experimental adults. This latter requirement may be problematic given (i) the need for a conveniently located and abundant population of adults, and, possibly more importantly, (ii) the presence of estuarine crocodiles in the coastal waters of northern Australia.

Table 6. Echinoderm species used for toxicity testing in Australia and overseas.

Species	Life stage	Test duration	Test endpoint	Temp (°C)	Salinity (ppt)	Reference
Australia – temperate						
Heliocidaris tuberculata	Sperm	15 min exposure + 1 h post fertilisation	Fertilisation success	20 ±1	35 ± 1	Doyle et al. (2003); Ecotox Services Australasia (2005)
Overseas – tropical						
Echinometra mathaei	Sperm	10 min	Fertilisation success	28	33-35	Heslinga (1976)
	Embryos	110 min	Early cleavage, skeletal development			
	Larvae	96 h	Survival and no. swimming larvae			
	Adult	96 h	Survival			
Diadema setosum	Embryos		First cleavage, pluteus formation	27	NR	Kobayashi (1994)
	Sperm	1 h	Fertilisation success	NR	30	Ramachandran et al. (1997)
	Embryos	1 h	First cleavage			
		5 h	Gastrulation			
		48 h	Pluteus larvae, (% normal/abnormal development)			
Anthocidaris crassispina	Sperm	30 min	Fertilisation success	24-26	30	Vaschenko et al. (1999)
	Embryos	1.5 h	First cleavage Pluteus larval quality			
	Adult	4 weeks	Sperm motility Egg morphology Fertilisation rate First cleavage	25	30	Au et al. (2000)
Overseas – temperate						
Fellaster zelandiae	Sperm Embryos	10-60 min	Fertilisation success Embryo development	20 ± 4	34 ± 1	Nipper et al. (1997)
	Unfertilised eggs	30 min	Fertilisation success, cleavage rate	NR	'Sea water'	Ozretic et al. (1998)
	Sperm	30 min				
	Embryos	5 h and 30 h				
	Swimming blastulae	14 h				
Arbacia punctulata	Sperm	1 h	Fertilisation success	20	30 ± 1	Nipper and Carr (2001)
	Embryos	48 h	Developed pluteus			
Sphaerechinus granularis	Sperm	10-min exposure + 1-3 h post-fertilisation	Fertilisation success	NR	NR	Pagano et al. (2002)
	Embryos	72 h	Survival and normal development of pluteus larvae			
Paracentrotus lividus	Embryos	48 h	Fully developed pluteus	20	34	Bellas et al. (2005)

NR: Not recorded

Table 7. The sensitivity of corals to copper.

Species	Temperate or Tropical	Endpoint	Test duration	Cu EC50 (µg/L)	Reference
Acropora millepora	Tropical	Fertilisation	4 h	17	Negri and Heyward (2001)
Acropora millepora	Tropical	Metamorphosis	24 h	110	Negri and Heyward (2001)
Acropora tenuis	Tropical	Larval settlement	48 h	35	Reichelt-Brushett and Harrison (2000)
Goniastrea aspera	Tropical	Fertilisation	5 h	15	Reichelt-Brushett and Harrison (1999)

BRYOZOANS

Bryozoans are a group of invertebrates that form sessile colonies composed of small zooids approximately 0.5 mm in length (Ruppert and Barnes 1994). Colonies are formed by rapid asexual budding of these zooids which are physically and physiologically interjoined. Each zooid typically consists of a stationary trunk and a food-catching organ which encircles the mouth and bears tentacles (Ruppert and Barnes 1994). Each zooid possesses both male and female reproductive organs. Egg fertilisation and embryo development occur within the zooid (i.e. internal brooding) before the free-swimming larvae are released. Released larvae then settle to produce a colony (NIMPIS 2002b).

Of the few toxicity studies that have been undertaken using bryozoans, the majority appear to be Australian. Between the 1940s and 1960s, substantial research was undertaken in relation to the effects of antifouling paints on marine fouling species, including bryozoans. Wisely (1958) and Wisely (1962a, b) assessed the effects of antifouling paints, with a focus on copper, to either *Bugula neritina* or *Watersipora cucullata*. Wisely and Blick (1967) assessed the survival of both the above two species and five other invertebrate species, following exposure to copper, mercury and zinc. Of the species tested, *W. cucullata* was the most sensitive to copper and mercury. More recently, Tania and Keough (2003) assessed the delayed effects of larval exposure to copper for *Watersipora subtorquata*, and Bennett (2006) assessed the effects of 24-h exposure to produced formation waters on various behavioural and physiological responses (e.g. attachment, metamorphosis, survival) of *W. subtorquata* and two populations of *B. neritina*, one of which was collected from Townsville. The Townsville-sourced *B. neritina* appears to be the only bryozoan used for ecotoxicological studies that has been collected from tropical waters, although the experiments were conducted at 20°C (Bennett 2006), which is more typical of temperate/sub-tropical waters.

B. neritina is a common marine species of Bryozoa (Ruppert and Barnes 1994), which is reported to be present in areas of tropical Australia (Arnold 2000). It is an upright, arborescent, red-purple-brown coloured, flexible species (Bennett 2006), which filter-feeds on microscopic plankton using its tentacles (NIMPIS 2002b). Along with *W. subtorquata*, *B. neritina* is considered an introduced species abundant in ports and harbours, growing well on pier piles, vessel hulls and other submerged surfaces (NIMPIS 2002b). Other Bryozoan species reported by Arnold (2000) to exist in northern Australia are *Bugula robusta, Zoobotryon verticillatum, Biflustra savartii* and *Savignyella lafontii.* Of these species, *Bugula robusta* is likely to be a naturally-occurring species in Australia (Arnold 2000).

Summary – Bryozoans

Bryozoans may represent a relevant biotic group to consider as part of a toxicity testing suite for tropical marine ecosystems. Previous studies have demonstrated that bryozoans can be used as toxicity testing organisms, although they have not been used routinely and there has not been a great deal of developmental work undertaken. Consequently, a suitable, and ideally native, species would first need to be identified, followed by a substantial amount of test development research.

CORALS

Of all the tropical marine biota groups, corals have probably received the most attention in terms of their health and the impacts of anthropogenic activities, including chemical contamination (Wilkinson 2004; Downs et al. 2005a). Scleractinian corals are generally composed of small colonial invertebrates that build hard calcareous skeletons, forming a variety of three dimensional colony types that provide habitat for hundreds of species of tropical invertebrates. Most corals in the tropics are host to symbiotic dinoflagellates of the species *Symbiodinium* which provide up to 95% of the energy required by the host animal colonies through the transfer of photosynthates across the cell walls of the alga (Muscatine 1990). Reproduction typically occurs via broadcast spawning, with many species ejecting gametes into the water column simultaneously at annual spawning events (Richmond and Hunter 1990). Fertilisation is usually external, with the planula larvae normally competent to undergo settlement and metamorphosis after five days of development. Coral spawning occurs annually (although at different times for different coral communities), which restricts the ability to undertake routine toxicity testing using early life stage forms such as gametes and larvae.

In Australia, a substantial amount of tropical coral ecotoxicology research has been undertaken, with studies focusing mostly on four endpoints: fertilisation; settlement and metamorphosis; responses of juvenile corals; and responses of adult colonies. The comparative sensitivity of these endpoints to copper is shown in Table 7. Coral fertilisation toxicity tests, which are usually of a short, 2–5 h duration, were first performed by Heyward (1988) to assess the effects of copper (Table 7) and zinc. Comparable studies have since assessed the effects of other metals as well as the antifoulant TBT, hydrocarbons, dispersants, herbicides and other toxicants on coral fertilisation (Reichelt-Brushett and Harrison 1999; Negri and Heyward 2000, 2001; Mercurio et al. 2004; Negri et al. 2005). Larval settlement and metamorphosis tests, which usually span 24–48 h, have also been used successfully for a range of coral species and toxicants, although exposure methods have varied (Reichelt-

Brushett and Harrison 2000; Negri and Heyward 2001; Negri et al. 2005). As noted above, it is difficult to use coral early life stages for routine toxicity testing purposes, because they are available only for short periods during the annual spawning events. The early life stages are, however, useful for research on specific toxicants, which can be timed to coincide with the spawning events.

Ecotoxicology experiments on adult coral colonies have been less common. They require larger experimental apparatus than those used for the early life histories, but this problem can be overcome by testing coral branchlets as an alternative to entire colonies. Numerous responses of coral branchlets to toxicant exposure have been assessed, including mortality, tissue retraction, tissue death, bleaching (loss of symbionts), and reproductive potential and output (Jones 1997; Jones and Hoegh-Guldberg 1999; Mercurio et al. 2004; Markey et al. 2007). In addition, several new methods have been developed including the measurement of stress proteins and enzymes, gene regulation (Downs et al. 2005b) and photosynthetic efficiency in the symbiotic algae (Jones and Hoegh-Guldberg 1999), although the ecological relevance of these remains unclear.

Overall, corals have been shown to be sensitive to a range of toxicants, including copper, TBT, petroleum products and herbicides such as diuron, atrazine and Irgarol 1051 (Harrison 1999; Negri and Heyward 2000; 2001; Jones and Kerswell 2003; Negri et al. 2005; Victor and Richmond 2005; Markey et al. 2007). Herbicides can affect coral symbiont photosynthesis at very low concentrations (e.g. <1 µg/L), however, these effects are largely reversible, and secondary effects such as bleaching and tissue retraction are not generally observed at environmentally relevant concentrations (Negri et al. 2005). Metals and organometals, however, are more likely to affect the coral tissue rather than the symbiont (Smith et al. 2003; Markey et al. 2007).

There have been numerous coral species used in Australia for toxicity testing purposes, with the most common being *Acropora tenuis*, *Acropora millepora*, *Pocillopora damicornis* and *Goniastrea aspera,* all of which are widely distributed across northern Australia (Veron 2000).

Summary – Corals

Corals represent a relevant biotic group to consider as part of a toxicity testing suite for tropical marine ecosystems, as they are important for reef and ecosystem integrity in tropical marine environments and have demonstrated sensitivity to a range of contaminants. Toxicity testing procedures have been developed to measure various responses, ranging from fertilisation to various responses of adult colonies. However, there are no formally documented test protocols for corals, with existing research using a variety of approaches. Fertilisation and metamorphosis toxicity tests are likely to be useful for research and to establish toxic thresholds for a variety of contaminants, but these tests must be timed to coincide with annual spawning events. The use of adult branchlets offers an alternative approach that can be applied to more routine testing year round.

FISH

Fish are the primary vertebrate component in aquatic systems and, as such, have comprised an integral part of toxicity assessments (Smith et al. 2004). Fish are ideal indicators of heavy metal contamination in aquatic systems because they occupy different trophic levels and are different sizes and ages (Burger et al. 2002). The early life stages of fish are generally considered to be the most sensitive to toxicant exposure (McKim 1977). It is important to note that in Australia, toxicity testing conducted on any vertebrate species, including fish, requires animal ethics approval, in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes – 2004* (NHMRC 2004).

This section reviews toxicity testing for tropical marine fish only, as the body of literature on temperate fish toxicity testing is far too extensive to enable a proper overview within the scope of this review. Thus, Table 8 lists some of the tropical marine fish species that have been used for toxicity testing in Australia and overseas. In Australia, several tropical species have been used to assess acute toxicity of chemicals, although only one with any regularity.

Denton and Burdon-Jones (1986) assessed the acute toxicity (96-h survival) of copper, cadmium, lead, mercury, nickel and zinc to juveniles of two species of tropical marine fish, the glass perch, *Ambassis marianus* (formerly known as *Priopidichthys marianus*), and the diamond-scaled mullet, *Liza vaigiensis*. However, neither of these species appears to have been used considerably for toxicity testing purposes since. In contrast, the barramundi, *Lates calcarifer*, has been used regularly for toxicity assessments, although most often as part of commercial-in-confidence studies, which are rarely published in the peer-reviewed literature. In Australia, *L. calcarifer* fry are available from specialist commercial hatcheries. These operations spawn their brood stocks regularly during summer (wet-season) but they are also able to provide fry during the 'off-season', although there is an increased cost involved. The predominant existing *L. calcarifer* test is a 96-h imbalance test, which measures the loss of swimming ability of juveniles, typically 20–30 mm in length, such that the fish can no longer remain upright (Smith et al. 2004; ESA 2005). The development of this sub-lethal endpoint over an acute exposure was in response to strict animal ethics legislation. It would be useful to understand the applicability of the 96-h imbalance response as a predictor of sub-lethal responses (e.g. on growth) to longer-term exposures, although there appear to be no data addressing this.

L. calcarifer has also been commonly used in overseas studies, particularly in Asia, where the species, known as sea bass, is an important aquaculture species (Shazili 1995). Most studies have assessed survival of juveniles over acute exposures (e.g. Perngmark and Tookwinas 1986; Shazili 1995; Krishnani et al. 2003), although some have investigated effects of contaminant exposure over sub-chronic exposures (e.g. Shazili 1995 – up to 23 days, survival; Thongra-ar et al. 2003 – 7 days, growth and survival). Thongra-ar et al. (2003) found growth to be a more sensitive indicator than survival following a 7-d exposure of *L. calcarifer* to mercury.

Table 8. Tropical marine fish species used for toxicity testing in Australia and overseas.

Species	Life stage	Test duration	Test endpoint	Temp (°C)	Salinity (ppt)	Reference
Australia						
Liza vaigiensis	Juveniles	96 h	Survival	20 & 30	20 & 36	Denton and Burdon-Jones (1986)
	Adults			20	36	
Ambassis marianus	Juveniles	96 h	Survival	20 & 30	20 & 36	Denton and Burdon-Jones (1986)
	Adults			20	36	
Lates calcarifer	20-30 mm juveniles	96 h	Imbalance	NR	NR	Smith et al. (2004); ESA (2005)
Amphiprion clarkii	Adults	96 h	Survival	NR	NR	Neff et al. (2000)
Overseas						
Lates calcarifer	Juvenile	24 h	Survival	28-29	31-32	Berngmark and Tookwinas (1986)
	20 d old	96 h - 16 d	Survival	24.5-28	20	Shazili (1995)
	4 months old	23 d	Survival	24.5-28	5, 15, 30	
	Juvenile (12 d old)	168 h	Survival, growth	27-30	23-29	Thongra-ar and Mukisa (1997)
	Fry (11 mm and 24 mm)	96 h	Survival, histopathology	28 ± 2	26 ± 1	Krishnani et al. (2003)
	Juvenile (10 d old)	168 h	Survival, growth	26-30	2, 10, 20, 30	Thongra-ar et al. (2003)
Cynoscion nebulosus	Embryo	24 h	Hatchability, head length, yolk diameter, body-depth at vent	29	36	Rumbold and Snedaker (1997)
Rivulus marmoratus	Juvenile (4-6 weeks old)	96 h	Survival	26-27	12-14	Lin and Dunson (1993)
Chanos chanos	Fingerling	24 h & 96 h	Survival, histopathology	27 ± 1	30	Cruz and Tamse (1989); Tamse and Gacutan (1994)

NR: Not recorded

Other tropical marine species that have been used for toxicity testing include the hermaphroditic fish, *Rivulus marmoratus* (e.g. Lin and Dunson 1993), clown fish, *Amphiprion clarkii* (e.g. Neff et al. 2000), spotted seatrout, *Cynoscion nebulosus* (e.g. Rumbold and Snedaker 1997) and milkfish, *Chanos chanos* (e.g. Cruz and Tamse 1989; Tamse and Gacutan 1994). However, all the above studies assessed acute toxicity.

In addition to its value as an aquaculture species, barramundi or sea bass (*L. calcarifer*), which is found throughout northern Australian waters (Keenan 1994), is also socially and economically important to commercial, recreational and indigenous fishers (Queensland Department of Primary Industries and Fisheries 2005). Being an estuarine fish, *L. calcarifer* can tolerate a wide range of environmental conditions; individuals move between fresh and salt water during various stages of their natural life cycle, with mature barramundi living in estuaries and coastal areas, the larvae and young juveniles in brackish swamps and older juveniles in the upper reaches of the river (Primary Industries and Resources South Australia 1999). Therefore, they potentially represent an adaptable test species for toxicity testing purposes.

Summary – Fish

Fish may represent a relevant biotic group to consider as part of a toxicity testing suite for tropical marine ecosystems. In particular, barramundi, *L. calcarifer*, would represent a relevant test species for the following reasons:

- *L. calcarifer* is present across tropical Australia and is of ecological, economic and cultural importance;
- *L. calcarifer* is the predominant species used for marine fish (imbalance) toxicity tests;
- Larvae or fingerlings are available year round from commercial and research hatcheries; and
- The use of *L. calcarifer* for toxicity testing would link well with programs of biomarker monitoring/assessment, which have been conducted using this species (see Codi King and Hassell this issue).

However, if a fish test were to be developed, it would be most appropriate to (i) investigate the usefulness of the currently used imbalance endpoint, and if necessary (ii) develop a more appropriate short-term, sub-lethal test for predicting chronic toxicity.

DISCUSSION AND SYNTHESIS

There are very few Australian tropical marine species for which routine, fully documented sub-lethal toxicity tests for assessing chronic toxicity have been established. One of the few examples, the diatom, *N. closterium*, is a well-established toxicity testing organism, is known to be sensitive to chronic exposure to metals (Table 1), and is widely distributed in northern Australian marine waters. Recent studies have further refined this protocol in terms of temperature tolerances of a tropical strain of *N. closterium* (Harford et al. unpublished data). Another tropical microalgal species, *I.* aff. *galbana*, has also been used regularly and test protocols are established (Table 1). Sub-lethal toxicity tests have also been developed and applied for early life stages of numerous coral species (e.g. *A. tenuis*, *A. millepora*, *G. aspera,* Table 7). However, they are less standardised than the microalgal tests and, due to the reliance on natural annual spawning events, cannot be conducted routinely.

There are several other marine species that are known to occur in northern Australia for which acute toxicity tests exist, but for which sub-lethal chronic test protocols have not been established. These include the copepod (*A. sinjiensis*), tiger prawn (*P. monodon*) and barramundi (*L. calcarifer*). For these species, moderate to significant developmental work would be required to develop sub-lethal chronic tests (see below).

In the past five years, the regulatory agencies of the three northern jurisdictions (i.e. Queensland, Western Australia, Northern Territory) have begun to recognise and utilise the framework provided by the ANZECC/ARMCANZ (2000) Water Quality Guidelines. Emphasis has been placed on site-specific assessment, and approaches such as toxicity testing have been incorporated into new waste discharge licences or at least environmental policy (Smith et al. 2004; Government of Western Australia 2005; EPA Northern Territory 2006). However, at present, there are insufficient ecotoxicity tests in existence for Australian tropical marine environments to meet the ANZECC/ARMCANZ (2000) preferred requirement for direct toxicity assessment of sub-lethal chronic toxicity tests for at least five regionally-relevant species from at least four taxonomic groups. In fact, this review has concluded that only the 72-h growth rate inhibition test using *N. closterium* or *I.* aff. *galbana*, and the sub-lethal tests for coral species such as *A. tenuis*, *A. millepora* and *G. aspera* could be considered to have such relevance.

Due to the lack of routine sub-lethal tests for tropical marine species, regulatory agencies have had to accept the use of (i) temperate species (e.g. the Sydney rock oyster, *S. commercialis*, and copepod, *G. imparipes*), and (ii) lethal acute tests (e.g. prawn 96-h survival) to supplement the existing sub-lethal chronic tropical algal tests when undertaking assessments of tropical marine contaminant issues. Whilst the use of a few established tests using a mix of non-local, tropical and temperate species with known sensitivity and reproducibility (with good QA) might be considered acceptable for preliminary toxicity studies in a tiered assessment approach, it still would not provide appropriate answers on the effects to *locally present* species under *local* conditions. Furthermore, such tests would not be appropriate to use for more specific research studies. Indeed, Kwok et al. (2007) found marked differences between the sensitivities of tropical and temperate freshwater species to a range of toxicants. Consequently, rather than continuing to accept and even recommend the use of temperate species and acute tests for tropical issues (e.g. WA DOIR 2006), relevant government agencies need to be more active in encouraging the development of appropriate test protocols for tropical marine species in line with the ANZECC/ARMCANZ (2000) rationale for more ecologically-relevant water quality assessment and monitoring.

Table 9 lists candidate species and summarises relevant attributes that could be used to help determine the relative worth of pursuing the development of particular toxicity tests. The categories in the column, *Developmental effort*, were based on the simple matrix of issues for candidate species and test methodology shown in Table 10. The information in Table 9 could be used to guide the final selection of a suite of tropical marine species for sub-lethal toxicity testing, with overall key criteria being:

1 Ecologically, economically and/or culturally relevant;
2 Readily obtainable/culturable;
3 A sub-lethal response can be measured, and the response is sensitive to key toxicants (although in many cases the sensitivity may not be known unless specific investigations are undertaken);
4 The suite of tests consists of at least five species from at least four taxonomic groups; and
5 The development of the suite of tests is cost-effective (e.g. the selection of one of two equally suited species could be based on predicted test development costs).

Based on these criteria, several species/species groups stand out as being good candidates for toxicity test development (in addition to the aforementioned microalgae and coral species):

- A microcrustacean, possibly a copepod (*A. sinjiensis* or other species). Copepod faunas across northern Australia are well known, some are readily culturable, and an existing sub-lethal test based on the temperate copepod, *A. tonsa*, could readily be adapted;
- The mud crab, *S. serrata*. This species is present across northern Australia, is of ecological, cultural and economic importance, its culturing requirements are well established, and several of its life stages may lend themselves to various types of toxicity testing. However, at present, toxicity testing would be restricted to the warmer months (October to March) when spawning takes place;
- The sea urchin, *D. setosum*, which almost certainly occurs across northern Australia and represents another trophic group, and for which there already exist well established protocols for sub-lethal toxicity testing (although the need for wild collection of adult broodstock may be problematic);
- Oysters, *Saccostrea* spp. and *P. maxima* are distributed across northern Australia, and are of ecological, cultural and economic importance. There already exist well-established protocols for oyster sub-lethal toxicity

Table 9. Tropical marine species/species groups and associated attributes that could be used to determine the relative worth of pursuing the development of water column toxicity tests.

Species/species group	Trophic description	Other candidate species	Species relevance[a]	Sub-lethal test already exists?	Developmental effort[b]	Summary of key developmental work required
Microalga, *Nitzschia closterium*	planktonic primary producer	*Isochrysis* sp.	2	Y	Minimal	Optimise existing short-term sub-lethal test.
Macroalga (various spp.)	benthic primary producer	*Catenella*, *Caloglossa*, *Champia* or *Enteromorpha*	2	Y	Significant	Identification and collection of appropriate species; development of short-term sub-lethal test; determination of temperature tolerance.
Amphipod	benthic detritivore/ scavenger (or filter feeder)	unknown	2	N	Major[c]	Identification and collection of appropriate species; determine culturing requirements; resolve availability and culturing issues; development of short-term sub-lethal test; determination of temperature tolerance.
Prawn, *Penaeus monodon*	planktonic/pelagic predator	*P. merguiensis*	3	N	Significant	Resolve availability and culturing issues; development of short-term sub-lethal test using early life stage; determination of temperature tolerance.
Copepod, *Acartia sinjiensis*	planktonic filter feeder	other *Acartia* spp., *Pseudodiaptomus* spp.	2	Y (for related *A. tonsa*)	Moderate	Collection of local strain; optimise culturing requirements; optimise existing short-term sub-lethal test using early life stage; determination of temperature tolerance.
Cladoceran, *Penilia avirostris*	Planktonic filter feeder	unknown	2	N	Significant	Identification of appropriate species; resolve culturing issues; develop short-term sub-lethal test; determination of temperature tolerance.
Crab, *Scylla serrata*	planktonic/ demersal predator	-	5	Y	Moderate	Resolve availability and culturing issues; optimise existing short-term sub-lethal test; develop additional tests using other life stages; determination of temperature tolerance.
Gastropod (various spp.)	Benthic/Intertidal Various food sources	unknown	3	N	Significant	Identification of appropriate species and determine availability; develop culturing conditions; develop short-term sub-lethal test; determination of temperature tolerance.
Bivalve mollusc, *Saccostrea* spp., *Pinctada maxima*	benthic filter feeder	various *Saccostrea* spp.	5	Y	Moderate[c]	Identification of appropriate species; resolve availability and culturing issues; optimise existing short-term sub-lethal test; determination of temperature tolerance.
Echinoderm (sea urchin), *Diadema setosum*	benthic invertebrate predator	other *Diadema* spp., possibly Holothurians (e.g. *H. scabra*)	2	Y (for sea urchins only)	Moderate[c]	Identification of appropriate species; resolve availability issues; optimise existing short-term sub-lethal test; determination of temperature tolerance.
Bryozoan *Bugula* spp.	sessile invertebrate predator	*B. neritina* or *B. robusta*	2	Y	Significant[c]	Identification of appropriate species; resolve availability and culturing issues; optimise existing short-term sub-lethal test; determination of temperature tolerance.
Coral (various spp.)	sessile invertebrate predator	*A. tenuis*, *A. millepora*, *P. damicornis*, *G. aspera*	5	Y	Minimal[c]	Optimise, standardise and document existing short-term sub-lethal tests
Fish (various spp.)	pelagic vertebrate predator	barramundi, clownfish or angelfish	3-5	N	Significant	Resolve availability and culturing issues; development of short-term sub-lethal test using early life stage; determination of temperature tolerance.

[a] *Species relevance:* Sum of perceived ecological (score = 2), socio-cultural (score = 2) and economic (score = 1) relevance.
[b] *Developmental effort:* See Table 10 for details of categories for developmental effort.
[c] The requirement to collect adult broodstock or test organisms from the wild may constrain the suitability of this species, due to (i) the need to find a conveniently located and abundant wild population, (ii) infrequent natural spawning events; and/or (iii) the presence of estuarine crocodiles in northern Australian coastal waters.

Table 10. Matrix to determine the extent of effort required to develop toxicity tests for local species.

Species issues	Testing issues		
	An appropriate sub-lethal test exists for the candidate species	An appropriate sub-lethal test exists for a related species and needs to be adapted	An appropriate sub-lethal test does not exist and one needs to be developed
There are **few** species identification, selection, availability and culturing issues to resolve	*Minimal*	*Moderate*	*Significant*
There are **some** species identification, selection, availability and culturing issues to resolve	*Moderate*	*Significant*	*Major*
There are **many** species identification, selection, availability and culturing issues to resolve	*Significant*	*Major*	*Major*

testing, and they may also serve as useful biomarker species (although the potential need for wild collection of adult *Saccostrea*, but not *P. maxima*, broodstock may be problematic); and

- Barramundi, *L. calcarifer*, is present across northern Australia, is of ecological, economic and possibly cultural importance, can be readily sourced from hatcheries and may also serve as a useful biomarker species. Test development should focus on early life stages, as acute tests with adult barramundi have been found to be insensitive to metals such as copper (J Stauber, CSIRO, *pers. comm.*). However, significant developmental effort would be required and the feasibility of the protocol may be compromised by animal ethics constraints.

As already noted, the need to regularly collect adult broodstock or test organisms from the wild, such as may be the case for amphipods, oysters, sea urchins, bryozoans and corals, may limit the suitability of the species, because of the potential need to rely on (i) a conveniently-located and abundant wild population of adults, and (ii) infrequent natural spawning events, and also the hazards posed by the presence of estuarine crocodiles across the region.

A substantial research and development (R&D) effort would be required to develop appropriate toxicity test methods for the above species. This effort would be greater for some protocols and species than others. In addition to the need to identify appropriate species and to develop culturing and testing methods (including selecting test conditions and endpoints), studies may be required to investigate the influence of key physico-chemical variables, such as temperature and alkalinity, and key contaminants, on test species, and how these variables interact with each other. Final decisions regarding species and protocol selection would need to take into account the relative costs and benefits of addressing the various R&D aspects.

SUMMARY

Toxicity testing methods are currently required as a tool for predicting and assessing the impacts of anthropogenic environmental stressors on tropical marine ecosystems. Bearing in mind the pristine nature of most tropical Australian ecosystems, and the expected expansion of industry and agriculture in these regions, such methods will also play a critical role in ensuring the ecologically sustainable development of future activities. However, this review found there is a paucity of fully-developed, regionally-relevant marine toxicity testing methods for Australian tropical marine species. Currently, just two fully-developed routine sub-lethal/chronic test protocols exist, both of which are for marine microalgae, while sub-lethal tests using various coral species have also been applied regularly. In order to meet minimum requirements recommended by ANZECC/ARMCANZ (2000) for site-specific assessments, additional toxicity tests need to be developed for at least four other tropical marine species representing at least three other taxonomic groups. This review identified a number of different tropical marine species that may be suitable candidates in a suite of toxicity test protocols. The development of such methods will require

a large R&D effort, and regulators, industries and community stakeholders should all have an interest in ensuring that these important knowledge gaps are addressed.

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