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# A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region

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#### Abstract

The South East Asian Melanesian (SEAM) region contains the world's largest deposits of nickel lateritic ores. Environmental impacts may occur if mining operations are not adequately managed. Effects data for tropical ecosystems are required to assess risks of contaminant exposure and to derive water quality guidelines (WQG) to manage these risks. Currently, risk assessment tools and WQGs for the tropics are limited due to the sparse research on how contaminants impact tropical biota. As part of a larger project to develop appropriate risk assessment tools to ensure sustainable nickel production in SEAM, nickel effects data were required. The aim of this review was to compile data on the effects of nickel on tropical marine, estuarine, pelagic and benthic species, with a particular focus on SEAM. There were limited high guality chronic nickel toxicity data for tropical marine species, and even fewer for those relevant to SEAM. Of the data available, the most sensitive SEAM species to nickel were a sea urchin, copepod and anemone. There is a significant lack of high quality chronic data for several ecologically important taxonomic groups including cnidarians, molluscs, crustaceans, echinoderms, macroalgae and fish. No high quality chronic nickel toxicity data were available for estuarine waters or marine and estuarine sediments. The very sparse toxicity data for tropical species limits our ability to conduct robust ecological risk assessment and may require additional data generation or read-across from similar species in other databases (e.g. temperate) to fill data gaps. Recommendations on testing priorities to fill these data gaps are presented.

#### Disciplines

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# A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region

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#### Capsule abstract

Nickel toxicity data is lacking for tropical marine biota; data is required for key taxa including cnidarians, molluscs, crustaceans, echinoderms, macroalgae and fish.

#### Highlights

South East Asia and Melanesia contains the largest deposits of nickel lateritic ores Environmental impacts may occur if mining operations are not adequately managed Risk assessment tools for nickel in the tropics are limited due to lack of data We compiled nickel data for aquatic tropical ecosystems and identified key gaps Nickel data is required for corals, molluscs, crustaceans, echinoderms, macroalgae, fish

#### Keywords

Mining, tropical ecotoxicology, ecological risk assessment, bioassay, species sensitivity distribution.

#### Abstract

The South East Asian Melanesian (SEAM) region contains the world's largest deposits of nickel lateritic ores. Environmental impacts may occur if mining operations are not adequately managed. Effects data for tropical ecosystems are required to assess risks of contaminant exposure and to derive water quality guidelines (WQG) to manage these risks. Currently, risk assessment tools and WQGs for the tropics are limited due to the sparse research on how contaminants impact tropical biota. As part of a larger project to develop appropriate risk assessment tools to ensure sustainable nickel production in SEAM, nickel effects data were required. The aim of this review was to compile data on the effects of nickel on tropical marine, estuarine, pelagic and benthic species, with a particular focus on SEAM.

There were limited high quality chronic nickel toxicity data for tropical marine species, and even fewer for those relevant to SEAM. Of the data available, the most sensitive SEAM species to nickel were a sea urchin, copepod and anemone. There is a significant lack of high quality chronic data for several ecologically important taxonomic groups including cnidarians, molluscs, crustaceans, echinoderms, macroalgae and fish. No high quality chronic nickel toxicity data were available for estuarine waters or marine and estuarine sediments. The very sparse toxicity data for tropical species limits our ability to conduct robust ecological risk assessment and may require additional data generation or read-across from similar species in other databases (e.g. temperate) to fill data gaps. Recommendations on testing priorities to fill these data gaps are presented.



#### Graphical abstract

#### Introduction

Anthropogenic activities including industry, urbanisation and land development (agriculture and mining) are having a major impact on tropical coasts through an increase in sedimentation and input of nutrients and chemical contaminants (Peters et al. 1997). Tropical ecosystems are valuable and unique, with greater than 80% of the global ecological diversity found in these regions (Biodiversity Futures, JCU 2015). Tropical seas occupy 36% of the global ocean and hold high cultural and economic value (Crame 2000).

Risk assessment tools (e.g. toxicity tests, bioavailability models, water quality guidelines (WQGs)) are used by policy makers, governments, industry and environmental agencies to protect aquatic environments from contaminants (Wang et al. 2014). These are well established for use in Europe, North America, Australia and New Zealand, but it may not be appropriate to apply these same tools to tropical regions. Effects data are required to understand the sensitivity of tropical biota to contaminants, and this together with appropriate exposure data can aid risk assessment of contaminants in tropical systems.

Limited studies using species sensitivity distributions (SSDs) have shown that tropical marine species are no more or less sensitive to contaminants than their temperate counterparts and that it is difficult to predict sensitivity between different climatic regions (Chapman et al. 2006). Wang et al. (2014) found only small differences in the acute toxicity of chemicals between tropical and temperate marine biota. Based on the acute data compiled in that study, the authors found that tropical species were more sensitive to copper, mercury, zinc, phenol and pentachlorophenol than temperate species, while temperate species were more sensitive to nickel, chromium, lead, TBT and unionised ammonia than tropical species. These conclusions were based on limited tropical acute data and therefore these generalizations cannot be made for all species, all endpoints, or all chemicals. From their SSDs, hazardous concentrations for nickel to 10% of species (HC10) were determined, giving values of 658 (557-767, 95% confidence limits, CL) μg Ni L<sup>-1</sup> for temperate species, and 1560 (366-3060) μg Ni L<sup>-1</sup> for tropical species (Wang et al. 2014). While this suggests that tropical species may be less sensitive to nickel than temperate species, there is considerable uncertainty (overlap of the 95% confidence limits). Similar comparisons with chronic nickel data have not been published and the toxicity of nickel to key unique tropical taxa such as corals and seagrasses has not been studied.

The Indo-Pacific region is thought to exhibit measures of biodiversity and occurrences of geographically unique taxa that are among the highest of any region on earth (Roberts et al. 2002; Barber et al. 2006). Within this region, South East Asia and Melanesia (SEAM), comprise many sensitive habitats, unusual taxa and unique biodiversity. It is also a region of developing countries and the small island nations of SEAM rely heavily on their tropical coasts as a major supplier of their dietary protein and as the main driver of their economy through tourism and fishing (Reichelt-Brushett 2012). Many of these countries also have poorly implemented regulatory frameworks and limited environmental monitoring data (Reichelt-Brushett 2012).

In recent years, the SEAM region has seen an increase in the development of lateritic nickel deposits. The shallow enrichment of nickel laterites over large surface areas has resulted in large open-cut mining (Brand 1998). In 2014 some of the highest production of nickel from lateritic ores (both mining and smelting) occurred on small island nations in

SEAM including the Philippines (26%), Indonesia (14%) and New Caledonia (10%) (U.S. Geological Survey 2015). Metal contaminants can be introduced into the aquatic environment through tailings spills, dam seepage or direct discharge (Franks et al. 2011; Reichelt-Brushett 2012). Environmental impacts may occur through direct exposure to nickel, as well as co-existing metals (such as manganese, cobalt and chromium), and anions (such as sulfate and chloride). There is also a risk of erosion, sedimentation and increased turbidity in freshwater and marine ecosystems if mining operations are not adequately managed.

Ecologically relevant risk assessment tools are needed to assess risks associated with nickel mining and use among tropical marine and estuarine ecosystems of SEAM. It may not be appropriate to apply tools developed for temperate regions to the tropics may due to the vast differences in the evolutionarily distinct habitats and biota, which may have different inherent sensitivities to nickel, as well as different geochemical and climatic conditions (temperature, rainfall, seasonality) of tropical regions which can influence the bioavailability of nickel. There is a lack of understanding of both exposure and effects of nickel on tropical marine species, especially those in key habitats such as mangroves, seagrasses and coral reefs. While it is well known that water quality parameters such as pH, salinity and dissolved organic carbon influence nickel bioavailability in temperate systems, these findings have not been validated in tropical ecosystems. It is also difficult to uncouple the effects of temperature versus the intrinsic differences in sensitivities between tropical and temperate species. To date, no comparative data for chronic nickel toxicity between temperate and tropical marine species have been reported.

This paper focuses on the need to develop an appropriate ecotoxicity database to support the determination of effects thresholds for nickel in tropical coastal and marine systems. This review summarises currently available nickel effects data and toxicity tests for tropical marine and estuarine species in aquatic and sediment compartments, with a particular focus on those relevant to SEAM. Once data gaps were identified, recommendations are made to fill these data gaps, either by developing and applying appropriate new tests to generate new data, or by using read-across methods from temperate data.

The objectives of this study were to firstly compile and critique existing nickel toxicity data for tropical marine and estuarine species, specifically tropical species that are relevant to the SEAM region, and to determine their quality and therefore their applicability to water quality guideline development. We also reviewed existing tropical marine and estuarine aqueous and whole-sediment toxicity tests, with a particular focus on species relevant to the SEAM region. Following this the additional nickel data and toxicity tests that may be required to help decrease uncertainty when assessing risks of Ni exposure in SEAM was identified. The effects of water chemistry parameters on nickel toxicity to tropical marine biota was also reviewed. This is important in respect to development of water quality guidelines and also in determining the applicability of bioavailability-based models such as the biotic ligand model (BLM) to risk assessment in the tropics.

#### Methods

For the purpose of this review, tropical biota were defined as test species isolated from tropical regions, and/or having a natural geographical distribution between the Tropic of Cancer and the Tropic of Capricorn. Tropical toxicity tests were those that were conducted at temperatures ≥ 25°C. Throughout this paper, toxicity tests refer to laboratory bioassays carried out under controlled conditions.

Studies were classified as marine if the test species was predominantly found in marine habitats and the toxicity test was conducted at salinities  $\geq 28\%$ , and estuarine if the test species was predominately found in estuarine habitats and the toxicity test was conducted at salinities < 28‰ (USEPA, 2013).

For the purpose of this review, sediment toxicity tests refer to whole-sediment toxicity tests which are designed to study contaminant impacts on sediment-dwelling organisms and those in close association with the sediment and allow for both pore water, overlying water and dietary exposure pathways (Simpson et al., 2013).

The SEAM region, as described by the United Nations (UN 2013), is outlined in Figure 1. The relevance of the toxicity test species and nickel data to SEAM was based on the geographical distribution and origins of the test species using literature searches or through the use of databases including Ocean Biogeographic Information System (OBIS), FishBase, and AlgaeBase.

#### Nickel toxicity data compilation

Compiled nickel toxicity data were screened for reliability, i.e. quality, and then for relevance to SEAM. Databases searched for nickel effects data included Web of Science, Scopus and Google Scholar. Nickel effects data included statistical estimates of toxicity such as EC/IC10 (the concentration that causes a 10% effect or inhibition in the population relative to the control), EC/IC50 (the concentration that causes a 50% effect or inhibition in the population relative to the control), NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration). Additional data were compiled including species and life-stage of test organisms, exposure duration and test type (acute or chronic), endpoint (e.g. survival, growth, fertilisation), the type of nickel salt used and key water quality parameters including test temperature, pH, dissolved organic carbon (DOC) and salinity. Sediment quality parameters also included particle size, acid volatile sulfide and organic matter. Definitions of acute and chronic tests were those defined for Australia/New Zealand by Batley et al. (2014). Acute toxicity is an adverse effect that occurs as the result of a short exposure period to a chemical, relative to the organism's life span. Chronic toxicity occurs as the result of exposure to a chemical for a substantial proportion of the organism's life span or an adverse sub-lethal effect on a sensitive early life stage (Batley et al 2014).

#### Tropical toxicity test compilation

Toxicity tests for tropical marine and estuarine species, with a particular focus on species relevant to SEAM, were compiled. This was done to identify tests which may be used to fill the gaps in nickel toxicity data. Information on the test species and test method were compiled for each toxicity test.



Figure 1. Map outlining the region of South East Asia and Melanesia (SEAM)

#### Quality assessment of data

Compiled nickel toxicity values (e.g. EC10/50, NOEC, LC50, LOEC values) were assessed using a quality checklist modified from ANZECC/ARMCANZ (2000), Batley et al. (2014) and Warne et al. (2015). The checklist criteria and the scoring system used are presented in Table 1. Data were scored as high quality (QA1) if they answered yes to the two questions:

- Question A: Was the measured endpoint ecologically relevant (e.g. lethality, immobilisation, growth, development, population growth, or reproduction)?
- Question B: Were nickel concentrations measured?

and the data scored ≥80% (based on the remaining criteria identified in Table 1). Remaining data were scored as QA2 or QA3, or fail, depending on whether they met the above questions and based on their quality assessment score (for descriptions see Table 1). Data quality and quantity were visualised as pie charts to identify data gaps for specific taxa (Figure 2).

#### Species sensitivity distributions (SSDs)

Guidance exists in North America, Europe and Australia around the use of SSDs and hazardous concentration (HC) values. Typically the HC5 value is reported which is the hazardous concentration to 5% of test species, i.e. protective of 95% of species. The basic principles behind all guidance is that as the number of species (data) increases in the SSD, greater confidence can be placed around the HC value. In North America, a minimum of 15 species is recommended in SSDs (USEPA 2005). In Europe a minimum of 10 but preferably 15 species is recommended in SSDs (ECHA 2008; OECD 2011). In Australia the minimum is eight from at least four taxonomic groups (ANZECC/ARMCANZ 2000). Wheeler et al. (2002) found through a series of SSD analyses that 10 data points should be set as the minimum requirement to generate a reliable HC value or water quality guideline.

To identify sensitive taxa, all tropical data and SEAM-only data were fitted to SSDs using BurrliOz Version 2 software (Barry, 2014). SSDs were generated using chronic data only. It was not possible to generate SSDs for estuarine species or tropical sediments, due to the lack of toxicity data available. The preferred order of statistical estimates of chronic toxicity to use in an SSD is: No effect concentration (NEC); effect, inhibition or lethal concentration (EC/IC/LCx where x ≤10); EC/IC/LC 15-20; and NOEC (Warne et al. 2015). If insufficient chronic NEC, EC/IC/LC (≤10 to ≤20) and NOEC data are available, chronic LOEC and EC/IC/LC50 data can be converted to chronic NOEC values by dividing by 2.5 and 5 respectively. There were two instances where this was necessary. For the copepod Acartia pacifica, the chronic LOEC was converted to an estimated NOEC by dividing by 2.5. For the coral *Platygyra daedalea*, the chronic EC50 was divided by 5. Where there were multiple endpoints for one species, the data for the longest exposure duration were selected first and then the lowest effects concentration was chosen to be included in the SSD. If there was more than one effect concentration for the same species, same lifestage, endpoint and exposure duration, the geometric mean was used. There was one instance where this occurred, for the microalga Nitzschia closterium (Table 2, now known as Ceratoneis closterium).

Table 1. Data quality assessment checklist and scoring criteria (adapted from Batley et al. 2014 and Warne et al. 2015).

Ques	tion	Score
Α	Was the measured endpoint ecologically relevant <sup>a</sup> ? Note: If this is not met, data automatically fails	Pass/Fail
В	Were nickel concentrations measured? Note: if this is not met, data may still pass as QA3, if overall score is >80%.	Pass/Fail
	Data Quality Assessment	
1	Was the exposure duration stated?	10, 0
2	Was the biological endpoint (e.g. immobilisation or population growth) stated and defined (10 marks)? Award 5 marks if the endpoint is only stated	10, 5 or 0
3	Was the biological effect stated (e.g. LC or NOEC)?	5 or 0
4	Was the biological effect quantified (e.g. 50% effect, 25% effect)?	5 or 0
5	Were appropriate controls (e.g. a no-toxicant control and/or solvent control) used?	5 or 0
6	Was each control and chemical concentration at least duplicated?	5 or 0
7	Were test acceptability criteria stated (e.g. mortality in controls must not exceed a certain percentage) OR inferred (e.g. tests used USEPA, OECD, etc. methods which have validation criteria) (award 2 marks).	5, 2 or 0
8	Were the characteristics of the test organism (e.g. length, mass, age) stated?	5 or 0
9	Was the type of test medium used stated?	5 or 0
10	Was the type of exposure (e.g. static, flow-through) stated?	4 or 0
11	Was the experiment replicated?	4 or 0
12	Were parallel reference toxicant toxicity tests conducted?	4 or 0
13	Was there a concentration-response relationship either observable or stated?	4 or 0
14	Was an accepted statistical method or model used to determine the toxicity?	4 or 0
15	For NOEC/LOEC/MDEC/MATC data was the significance level 0.05 or less? OR	4 or 0
	For LC/EC/NEC/BEC data was an estimate of variability provided?	4 or 0
16	Was pH measured? Award 3 marks if measured during the test and values stated. Award 1 mark if measured but values not stated or if they are measured and values are stated for the dilution water only.	3, 1 or 0
17	For metals tested in freshwater (FW), were the following parameters	
	measured? (i) hardness	3, 1 or 0
	(ii) alkalinity and	3, 1 or 0 3, 1 or 0

	<ul><li>(iii) dissolved organic carbon concentration</li><li>(iv) conductivity</li></ul>	3, 1 or 0 3, 1 or 0
	Award 3 marks for each variable measured during the test and values stated. Award 1 mark for each parameter measured but values not stated.	
18	For marine and estuarine water (MEW), was the salinity/conductivity measured and stated?	3 or 0
19	For tests not using aquatic macrophytes and alga, was the dissolved oxygen content of the test water measured during the test?	3, 1 or 0
	Award 3 marks if measured during the test and values stated. Award 1 mark if measured but values not stated.	
20	For metals tested in sediments, were the following parameters measured? Particulate organic carbon, Particle size (e.g. <63 µm or silt/clay) Acid-volatile sulfide (AVS) and 1-M HCl extractable metals Dissolved ammonia and sulfide in pore water or overlying water Salinity and conductivity (overlying water, marine/estuarine sediments) Was the alkalinity, hardness or concentrations of Ca and Mg measured in the overlying water? Were known interacting elements on bioavailability measured (e.g. Cl for Cd?) Award 3 marks for each variable measured during the test and values stated. Award 1 mark for each parameter measured but values not stated or if they are measured and values are stated for the dilution water only.	3, 1 or 0 3, 1 or 0 3, 1 or 0 3, 1 or 0 3 or 0 3 or 0
21	Was the temperature measured and stated (3 marks)?	3 or 0
22	Was the grade or purity of the test contaminant stated (3 marks)	3 or 0
	Total Possible score i various types of dataTests with macrophytes and algaeFW = 103FW = 100Marine/estuarine = 97Marine/estuarine = 94Sediment = 112Sediment = 109Quality score = [Total possible score] x 100	
	<ul> <li>Final Scoring</li> <li>QA1 – Passes Q1 and Q2, achieves ≥80% in data quality assessment</li> <li>QA2 – Passes Q1 and Q2, achieves ≥ 50 % and &lt;80% in data quality assessment</li> <li>QA3 – Passes Q1, fails Q2, achieves &gt;80% in data quality assessment</li> <li>Fail – Fails Q1, passes Q2, achieves &lt;80% in data quality assessment</li> <li>Fail – Fails Q1</li> </ul>	

<sup>a</sup> Endpoints that are considered to be ecologically relevant include lethality, immobilisation, growth, development, population growth, and reproduction or the equivalent)

The type of distribution that is fitted to the data is automatically determined by BurrliOz, and will depend on the number of species and taxonomic groups (Barry 2014; Warne et al. 2015). Here, chronic toxicity datasets with eight or more data were fitted to a Burr Type III distribution. Smaller datasets were fitted to other distributions, e.g. a log-logistic.

#### Gap analysis

Data gaps were identified based on the existing spread of available data across taxa, with particular focus on ensuring that there were sufficient high quality chronic data which could be used to derive a WQG (Wheeler et al. 2002, ANZECC/ARMCANZ 2000, USEPA 2005), the ecological relevance to SEAM, and the sensitivity of particular taxa based on other nickel studies from temperate or tropical marine or freshwater studies (e.g. Niyogi et al. (2014) found a freshwater snail to be highly sensitive to nickel). A further literature search was undertaken to identify the ecological importance of key taxa to the SEAM region, based on endemism and biodiversity. This was challenging due to the lack of information on biodiversity of marine and estuarine species, particularly for those relevant to SEAM (Tittensor et al. 2010). Supplementary data Table 12 summarises information on the diversity and relevance of certain marine taxa to SEAM. This was used to prioritise future testing to obtain nickel toxicity data for tropical species from this region.

#### Results

#### Tropical nickel toxicity data

#### Marine

Nickel toxicity data (acute and chronic) were compiled for tropical marine biota including microalgae, crustaceans, echinoderms, annelids, cnidarians and fish. A total of 46 data were found for 24 different species (Tables 2-7). All toxicity tests used nickel chloride salt and results are expressed as dissolved (measured) nickel, except where otherwise stated. All data presented in these tables are below the solubility limit for nickel in seawater, which was found to be 150 mg L<sup>-1</sup> (tested in the range of 20-450 mg L<sup>-1</sup>) (Krauskopf 1956). More recent investigations by Apte et al. (CSIRO, Pers Comm) have shown nickel to have a solubility limit of 90 mg L<sup>-1</sup> in seawater. There is one test with the fish *Priopidichthys marianus* where the EC50 for 96-h juvenile survival was 100 mg Ni L<sup>-1</sup>, test concentrations were around the solubility limit for nickel (Denton and Burdon-Jones 1986; Table 7). This study failed the data quality assessment.

Based on acute endpoints the most sensitive taxa were crustaceans, snail, anemone, and polychaete. Shrimps and mysids were the most sensitive taxa, with LC50 (50% lethality) values ranging from 7 - 150  $\mu$ g Ni L<sup>-1</sup>, following acute 24-96-h exposures (Lussier at al. 1999; Asadpour et al. 2013; Table 3). Juvenile snails, *Babylonia areolate* were the next most sensitive to nickel; in acute 96-h survival tests the EC50 was 200  $\mu$ g Ni L<sup>-1</sup>, compared to and EC50 of 36000  $\mu$ g Ni L<sup>-1</sup> for adults (Vedamanikam and Hayimad 2013; Hajimad and Vedamanikam 2013; Table 4). For the anemone, *Aiptasia pulchella*, the acute endpoint of tentacle retraction was measured over 12 h and the calculated EC50 values ranged from 1400-3300  $\mu$ g Ni L<sup>-1</sup> (Howe et al. 2014 a; Table 6). *Hydriodes elegans*, a tropical polycheaete, was also found to be relatively sensitive to nickel, with 50% inhibition of adult survival over 96 h (EC50) of 1500  $\mu$ g Ni L<sup>-1</sup> (Gopalakrishnan et al. 2008; Table 5).

When assessing chronic endpoints, the most sensitive taxa (in order from most to least sensitive) were the anemone, sea urchin, copepod, coral and microalgae. The chronic 28d reproduction of *A. pulchella* was inhibited by 10% (IC10) at 65  $\mu$ g Ni L<sup>-1</sup> (Howe et al. 2014 c; Table 6). The next most sensitive species was the sea urchin *Diadema savignyi*, in which fertilisation and development after 48-h exposure was inhibited by 50% (IC50) between 72-120 μg Ni L<sup>-1</sup> (Rosen et al. 2015; Table 5). The copepod Acartia pacifica, showed similar sensitivity to nickel, with a LOEC for 10-d egg production of 100 μg Ni L<sup>-1</sup> (Mohammed et al. 2010; Table 3). The polychaete H. elegans was again one of the most sensitive species based on chronic larval settlement. After 96-h exposures, the EC50 was 160 μg Ni L<sup>-1</sup> (Gopalakrishnan et al. 2008; Table 5). The coral *Platygyra daedalea* was relatively insensitive to nickel; the 5-h fertilisation success was inhibited by 50% (EC50) at 1420 μg Ni <sup>-1</sup>L (Reichelt-Brushett and Hudspith 2016; Table 6). Reichelt-Brushett and Harrison (2005) also assessed the toxicity of nickel on fertilisation success (5 h exposure) to a different species of coral, Goniastrea aspera. In this study, toxicity estimates were not provided and nickel concentrations in test solutions were not measured, however fertilisation success was inhibited by 60% at 2000 μg Ni L<sup>-1</sup>. For two species of microalgae and one cyanobacterium, the IC10 values (10% inhibition in population growth rate) ranged from 340 to 6100 μg Ni L<sup>-1</sup> over 72-h exposures (Florence et al. 1994; Alqueza and Anastasi, 2013; Gissi et al. Unpub; Table 2).

Twelve of the 24 species were confirmed to be relevant to the SEAM region. The order of the SEAM-relevant species, from most to least sensitive, were sea urchin, anemone, snail, coral and microalgae.

#### Data quality assessment

Based on data quality assessment, eight data were scored as QA1, six as QA2, one as QA3, and nine failed. All prawn and fish data failed the QA assessment largely because nickel concentrations were not measured in the test solutions. Additional reasons included lack of replication of treatments in tests, lack of acceptability criteria and reference toxicant and no measurement of water quality parameters throughout the test. Of the SEAM relevant species, six were scored as QA1, five as QA2 and one as QA3.

Only six taxonomic groups satisfied the QA1 category, and half of these data were cyanobacteria and microalgae (Figure 2A), with only 12.5% each for crustaceans (mysid), echinoderms (sea urchin), annelids (polychaete) and cnidarians (anemone). Seven out of the eight data were chronic and all data except the crustaceans (mysid) and polychaete were relevant to SEAM. If all data graded in the quality assessment are included (QA1, QA2 and QA3), there were 12 data which represented seven taxonomic groups, and included the addition of a gastropod and coral. Of these data, 67% were chronic data and relevant to SEAM (Figure 2B).

#### Species sensitivity distributions

There were only a limited number of high quality data for tropical marine and SEAM relevant species that could be used in the SSDs. Using only QA1 data, there were six and five data for tropical and SEAM relevant species, respectively (Figures 3A and 3B). The inclusion of all data scored as QA1, QA2 or QA3 added two additional data points to both the tropical and SEAM SSDs (Figures 3C and 3D); these were the coral (QA2) and the copepod (QA3). The euryhaline copepod *Acartia pacifica* was included in the marine SSD, although this test was conducted at 25‰, because this species is known to have a coastal



Figure 2. Available number of data for use in tropical marine species sensitivity distributions for nickel toxicity using data scored as QA1 (A), and QA1, QA2, and QA3 (B). Note, there are two data for the same species of microalga, N.closterium, the geometric mean for these data was taken for input into species sensitivity distributions.

distribution. Given that increase in salinity is protective against nickel toxicity (Blewett et al. 2015), this value may be conservative for marine systems. In all cases, this is less than the minimum recommended requirement for at least 10 species from which to generate an SSD and a reliable HC5 value (Wheeler et al. 2002).

#### Estuarine

Few nickel toxicity data were available for tropical estuarine species (test salinities of 20 to 24‰), most of which were not relevant to SEAM. Nickel toxicity data were found for two estuarine copepods, *Apocyclops borneoensis* and *Tigrious japonicus*, which were relatively insensitive to nickel, with acute (48-h adult survival) LC50 values of 13000 and 18000 µg Ni L<sup>-1</sup> (Mohammed et al. 2010; Table 3). These data were classified as QA3. The oyster, *Crassostrea virginica* with a temperate to sub-tropical distribution was also found to be relatively insensitive to nickel with an acute (48-h embryo survival) LC50 of 1200 µg Ni L<sup>-1</sup>, however this failed the QA assessment (Calabrese et al. 1977; Table 4). Two estuarine fish, *Leiostomus xanthurus* and *Menidia peninsulae* had acute (96-h adult survival) LC50 values of 70000 and 38000 µg Ni L<sup>-1</sup>, respectively (USEPA ecotox database; Table 7), although these also failed the quality assessment.

#### Marine and estuarine sediments

No tropical marine or estuarine sediment toxicity data for nickel were found to meet the quality criteria defined in Section 2.3 and Table 1 because of one or more of the following reasons; 1. Tests were conducted with sediment elutriate or pore water, rather than whole sediment; 2. Tests used field-collected sediment containing co-occurring contaminants; 3. Test temperature conditions were not ≥25°C; 4. Test endpoints were not based on a concentration-response relationship or not ecologically relevant; and 5. Nickel concentrations were not measured.

#### *Compilation of tropical toxicity tests Marine*

The main aim of this data compilation was to find established toxicity tests that are available to fill the gaps in the tropical nickel data. Of the SEAM-relevant species, two microalgae, three copepod, one barnacle, four bivalve, one gastropod, two sea urchin, one anemone, one fish and five coral tests are potentially available for use (Supplementary data Tables 1-9). Most tests are chronic, although some acute tests (acute copepod and fish survival) were also identified. Chronic toxicity tests are also available with four species of bivalve and two species of sea urchins which are endemic to New Caledonia (Jocelyn Senia, Pers comm.). We are aware of some research efforts to develop tests with other tropical marine species, relevant to SEAM, including a chronic copepod developmental test (Binet and Stone 2015), a chronic gastropod growth and survival test (Zheng Wang Pers comm.). Taxonomic groups missing that are an important component of tropical systems, particularly SEAM include macroalgae, seagrass, mangroves and fish.

#### Estuarine

Toxicity tests found in the literature for tropical estuarine species included a macroalga, a rotifer, a copepod, a crab, a seed shrimp, two prawns, two gastropods and six species of

fish. Of these tests, only the crab and two prawn species are relevant to SEAM. These tests are listed in full in Supplementary Tables 1, 2, 3, 5 and 9. Further test development is required with additional tropical estuarine biota.

#### Marine and estuarine sediments

There were five tropical whole-sediment marine toxicity tests in the literature representing five species including a copepod, two amphipods and two bivalves. However most of these tests were acute survival tests, with only the copepod toxicity test based on a chronic reproduction endpoint. The only tropical toxicity test relevant to the SEAM region was an acute survival and reburial test with a common marine bivalve, however, the bioassay was conducted at a sub-tropical/temperate temperature of 20°C (Supplementary Table S10).

There was a single tropical whole-sediment estuarine toxicity test conducted with the benthic harpacticoid copepod *Nitocra* sp. The endpoint was chronic reproduction rate over 10 days at 25°C and 17‰ salinity (Buruaem et al. 2013; Nilin et al. 2013; Krull et al. 2014). The data presented all came from South America and there are no records of this genus in SEAM (Supplementary Table S11).

#### Discussion

#### Marine

Only five high quality chronic data (five different species) from only four taxonomic groups (microalgae/cyanobacteria, cnidarian (anemone) and echinoderm (sea urchin)) relevant to the SEAM region were found (Figure 3B) to be suitable for use in deriving a WQG for nickel. Key structural habitats in SEAM are coral reefs, mangroves and seagrasses, all of which have high species richness in SEAM and are critical to protect ecosystem biodiversity (Hoeksema 2007). Important species relevant to these ecosystems include not only corals, mangroves and seagrasses, but other taxa such as crustaceans, molluscs (bivalves, gastropods and cephalopods), echinoderms (sea urchins, star fish, sea cucumbers), cnidarians (anemones), ascidians and fish. High quality chronic data exists for one species of sea urchin and anemone, both relevant to SEAM (Tables 5 and 6). Only acute data (scored as QA2) are available for one gastropod species found in SEAM. Currently, there are no high quality chronic nickel toxicity data available for corals, mangroves, macroalgae, seagrasses, crustaceans, molluscs (gastropods and bivalves), ascidians or fish.

Several options are available to address these data gaps; 1. Develop and validate readacross strategies using temperate databases, or 2. Target future testing with key taxa to generate more data. If a read-across method is implemented, this would have to be done with caution as the data does not confidently show that a species under temperate conditions will respond the same way to a contaminant under tropical conditions, due to differences in water quality parameters. In addition, the inherent sensitivity of different strains of the same species is likely to be different due to prior exposure to contaminants and adaptation to specific locales. For example, Florence et al. (1994) tested the toxicity of nickel to both a temperate and tropical strain of *Nitzschia closterium* (now known as *Ceratoneis closterium*). The temperate strain was more sensitive to nickel than the tropical strain with 72-h EC50 values (cell division rate) of 250 and 500 µg Ni L<sup>-1</sup>, respectively (Florence et al. 1994; Table 2).



Figure 3. Species sensitivity distributions using chronic nickel toxicity data, for QA1 data for (A) all tropical marine species and (B) species relevant to SEAM, and for all QA1, QA2 and QA3 data for (C) all tropical marine species and (D) species relevant to SEAM.

Toxicity data exists for two species of corals, *G. aspera* and *P. daedalea* (Reichelt-Brushett and Harrison 2005; Reichelt-Brushett and Hudspith 2016; Table 6), based on chronic 5-h fertilisation success. In the study by Reichelt-Brushett and Harrison (2005) with the coral *G. aspera*, nickel concentrations in test solutions were not measured and toxicity estimates were not provided, so this data failed the quality assurance assessment. In addition, this test was done twice across two years and significantly different results were obtained. The study by Reichelt-Brushett and Hudspith (2016) is highly relevant to nickel mining in SEAM, as it considers the toxicity of nickel, cobalt and mixtures of both to the coral *P. daedalea*. While measured nickel concentrations were used, water physicochemical parameters including temperature were not documented. Also due to the logistical difficulties of testing, possible only during the annual coral spawning event, this experiment could not be repeated, and this data was subsequently scored as QA2 in the quality assessment. These toxicity tests (Supplementary data Table S8) should be repeated to investigate the sensitivities of different species of corals to nickel.

Anemones and sea urchins were the most sensitive species to nickel, with toxic effects observed between 65 and 120  $\mu$ g Ni L<sup>-1</sup> (Howe et al. 2014 a, b, c; Table 6, Rosen et al. 2015; Table 5). Anemones are important species found on coral reefs of SEAM and are widely distributed throughout tropical and subtropical ecosystems (Howe et al. 2012). Sea urchins are also important inhabitants of coral reefs; high diversity of sea urchins has been recorded in the Caribbean, and, while there has been limited sampling in SEAM, asteroids (starfish) and holothurians (sea cucumbers) appear to dominate in the Northeast Pacific (Iken et al. 2010).

Mollusc diversity (bivalves and gastropods) is highest in the tropics, particularly in the Indo-Pacific coral reef environments including SEAM (Bouchet et al. 2002; Roberts et al. 2002; Wells 1990), and both gastropods and bivalves are an important food source for the tropical islanders in this region. The acute data available for one gastropod species, *Babylonia areolata* indicates that juveniles are sensitive to nickel (acute survival, 96 h, inhibited by 50% (EC50 at 200  $\mu$ g Ni L<sup>-1</sup>) (Hajimad and Vedamanikam 2013; Vedamanikam and Hajimad 2013; Table 4). The widely distributed snail *Lymnaea stagnalis* has been shown to be among the most sensitive species to nickel exposure (Nys et al. 2016, Niyogi et al. 2014, Schlekat et al. 2010), with chronic juvenile growth inhibited by concentrations as low as 1.3  $\mu$ g Ni L<sup>-1</sup>, following 30 days of exposure (Niyogi et al. 2014).

Crustaceans, including copepods, amphipods, barnacles, shrimps and lobsters have high species diversity in the Indo-Pacific, particularly living in association with coral reefs (Williams et al. 1988; Thomas 1993; Humes 1994). The life cycle of some crustaceans link the key habitats, mangroves, seagrasses and coral reefs, as they are used as breeding grounds and nurseries (Nemeth 2009). Amphidromous shrimp species, common in the tropics, are important because their life cycle crosses all aquatic systems; adults live in fresh or estuarine waters, and juveniles develop in marine waters before migrating back to freshwater (Kikkert et al. 2009). As well as osmoregulatory challenges, these species must cope with potential contaminants as they migrate between fresh, estuarine and marine waters. Mysids and shrimps were found to be sensitive to nickel, with acute mortality (24-96 h) observed between 7-150 µg Ni L<sup>-1</sup> (Lussier et al. 1999; Asadpour et al. 2013; Table 3), however none of these species have been reported in SEAM. Chronic data were available for one estuarine copepod relevant to SEAM. Following a 10-d exposure

the LOEC for this copepod was 100  $\mu$ g Ni L<sup>-1</sup> (Mohammed et al. 2010; Table 3). These few studies suggest that crustaceans may be sensitive to nickel.

Nickel data and toxicity tests are not available for tropical marine macroalgae. Macroalgae are not considered to be the main habitat providers in tropical systems; they are not as dominant and have a lower species diversity than in cooler temperate regions (Mejia et al. 2012). Nonetheless, macroalgae are still ecologically important species, particularly on coral reefs where crustose coralline algae play a significant role in limestone formation and consolidation of loose substrates providing a surface for coral larval settlement (Mejia et al. 2012). Macroalgae also play a key role in nitrogen fixation and are a food source for higher trophic organisms (Diaz-Pulido and McCook 2008; Chaves et al. 2013).

The richest fish diversity (globally) is found around Indonesia, New Guinea and the Philippines (Randall 1998). Fish are important components of food webs in coral reefs, seagrasses and mangroves and form a link between these three habitats, in the same way that some crustaceans do (Nemeth 2009). Amphidromous and catadromous fish are also unique to tropical systems. Catadromous species live in freshwaters and spawn in coastal marine waters, where juveniles develop before returning to freshwaters (e.g. barramundi). Coral reef fish play an important economic role as a food source and in tourism and recreational activities (Hoeksema 2007). Despite being ecologically important, tropical fish have been shown to be insensitive to nickel, although this is based on a limited dataset (Table 7). No chronic toxicity tests are available for tropical marine fish, and so further research and development is required to address this data gap.

Other important taxa in tropical marine environments include cephalopods, sponges and ascidians. However no toxicity test protocols exist for these taxa. Similarly, although mangroves and seagrasses are important tropical habitats, there are no laboratory-based toxicity tests currently available for use. Any further test development will likely be based on *in situ* measurement of photosynthesis in seagrasses (Macinnis-Ng and Ralph 2002), but such endpoints are not routinely used in the region to set water quality guidelines.

As discussed above, cnidarians (corals, anemones), molluscs (gastropods, bivalves), crustaceans and echinoderms (sea urchins, starfish and sea cucumbers) have high ecological importance in SEAM. Based on limited studies we know that one anemone, a gastropod, crustaceans and a sea urchin are sensitive to nickel. While toxicity tests with coral gametes have demonstrated that two species appear to be less sensitive to nickel (effects observed at >1 mg Ni L<sup>-1</sup>), it is recommend that further testing with the two species previously tested (*G. aspera, P. daedalea*) is repeated and the toxicity of nickel to other species of corals is investigated. It would also be valuable to consider other endpoints e.g. larval motility, settlement, adult survival, or symbiotic zooxanthellae.

This review has considered the sensitivities of taxa based on available data in the literature and the ecological importance of key taxa to the SEAM region, as well as the requirements for water quality guideline development. From this it can be recommended that, in order of priority, further targeted testing with nickel include the following taxa: cnidarians (corals and anemones), molluscs (gastropods, bivalves), crustaceans

(copepods, amphipods, barnacles, shrimps, prawns), echinoderms (sea urchins, star fish and sea cucumbers), macroalgae and fish.

#### Estuarine

There are some important features of tropical estuaries that distinguish them from temperate and sub-tropical estuaries including marked seasonality of river flow with concomitant salinity regimes, uniform temperatures and as nurseries for highly diverse marine fauna (mangroves) (Blaber 1980). There are no high quality nickel toxicity data available for tropical estuarine species, or species relevant to SEAM. Toxicity test protocols for SEAM relevant species were found for a crab and two species of prawn only.

Similar to marine fish, sensitivity of tropical and temperate estuarine fish (both adults and juveniles) to nickel is low, with most studies demonstrating effects above 1 mg/L (Denton and Burdon-Jones 1986; Bielmyer et al. 2013). A few studies have investigated the toxicity of nickel to estuarine crustaceans, but these were scored as lower quality or failed the quality assurance assessment. Mohammed et al. (2010) tested the acute (48-h adult survival) and chronic (10-d reproduction) toxicity of nickel to two estuarine copepods (at 20‰), *Apocyclops borneoensis* and *Tigrious japonicus*. Adults were less sensitive to nickel, with 50% lethality (LC50) occurring at concentrations >13 mg Ni L<sup>-1</sup>. Reproduction was a much more sensitive endpoint for both species, with ~ 30% reduction in the number of nauplii per female observed at 10  $\mu$ g Ni L<sup>-1</sup>. While these species of copepods have been found in some areas of the tropics, there is no evidence for their distribution in SEAM. Based on the sensitivity of estuarine copepods and some marine crustaceans (Table 3) to nickel, it is recommended that future testing include this taxa.

#### Influence of water quality parameters on nickel toxicity

Metal bioavailability is well known to be influenced by both water quality parameters (temperature, pH, salinity, conductivity, DOC, etc.) and the metal-species interaction at the biotic ligand/receptor (Merrington et al. 2016). The specific mode of toxic action that a metal induces in an organism, and the organism's behaviour and metabolism (e.g. feeding behaviour, filtration rates and detoxification processes) will determine the subsequent toxic effects (Chapman 2008). Water quality parameters in tropical marine systems differ significantly from temperate systems due to natural variations in climate and geochemical factors such as temperature and increased seasonal rainfall, nutrient and DOC quality and concentration. Understanding the influence of water quality parameters on nickel toxicity will assist in further development of the nickel biotic ligand model (BLM) for tropical species and in predicting potential toxic effects in tropical environments.

There are very few studies that investigate the influence of water quality parameters on nickel toxicity to tropical marine biota. Several studies, however, have investigated the effects of different water quality parameters on nickel toxicity to temperate marine organisms. Ho et al. (1999) investigated the acute toxicity of nickel to the mysid *Americamysis bahia* and the amphipod *Ampelisca abdita*, commonly found on the east coast of Central to North America. Results in this study demonstrated that the effect of pH on nickel toxicity was dependent on the organism. There was no change in nickel toxicity to the amphipod at different pH values. A decrease in pH over the range pH 7-9 increased nickel toxicity to the mysid by a factor of two. This is inconsistent with results

for freshwater organisms where the pH dependency is reversed, i.e. nickel toxicity decreases with decreasing pH (Merrington et al. 2016).

In other studies with temperate species, salinity appears to significantly alter nickel bioavailability and toxicity, with increasing salinity decreasing nickel toxicity. Blewett et al. (2015) found that nickel accumulation in the euryhaline crab *Caricinus maenas*, was three-to-five times higher in 6‰ seawater than at 19‰ or 32‰, after a 24-h exposure. Blewett and Wood (2015) also found that nickel toxicity (accumulation and oxidative stress) to the euryhaline temperate fish, *Fundulus heteroclitus*, was significantly decreased in seawater (35‰) compared to freshwater (0‰). The decrease in nickel toxicity with increasing salinity is likely to be due to the competition between divalent cations and nickel for uptake (Blewett et al. 2015, Blewett and Wood 2015).

Lussier et al. (1999) investigated DOC and acute nickel toxicity to two temperate marine organisms, the mysid A. bahia and the mussel Mulinia lateralis. The mussel, similar to the mysid, is predominately located between Central and North America. The DOC concentrations in natural waters tested ranged from 1-11 mg L<sup>-1</sup>, and no effect on nickel toxicity was found over this DOC range. A recent study by Blewett et al. (2016) determined the effect of three different sources of natural organic matter (NOM) on the toxicity of nickel to a temperate marine sea urchin *Evechinus chloroticus*. They showed that one type of NOM exacerbated nickel toxicity as DOC concentration increased. The authors suggested that the composition of the NOM, rather than just the quantity, may play a key role in altering nickel toxicity to marine organisms. Mangroves are dominant habitats in the tropics and past studies have shown that mangrove-derived organic matter far exceeds that of terrigenic DOC in tropical coastal waters (Dittmar et al. 2001; Dittmar et al. 2006). It is recommended that further research investigate the influence of different sources of tropical marine DOC on relevant organisms to gain a better understanding of how this parameter determines the solubility and subsequent toxicity of nickel in tropical systems. It is possible that pH, salinity and DOC may play a similar role in influencing nickel toxicity to tropical marine species, however this cannot be confirmed without data.

Temperature also plays a key role in metal uptake and toxicity. While tropical species may be no more or less sensitive to a metal compared to their temperate counterparts, it is difficult to predict sensitivities based on climatic regions. Furthermore there is a lack of understanding of how higher temperatures will affect BLM predictions. It is recommended that future investigations consider how key water quality parameters (e.g. temperature and DOC) will influence the toxicity of nickel to tropical marine biota. This will assist in determining whether or not development of a tropical marine BLM is required.

#### Marine and estuarine sediments

There were very few nickel sediment toxicity data or whole-sediment toxicity tests for tropical marine and estuarine benthic biota. Factors that may modify the bioavailability of nickel in tropical sediments, particularly acid volatile sulfides and dissolved organic carbon in pore waters, would be expected to be similar to temperate systems (Chandler et al. 2014, Vangheluwe et al. 2013). Benthic organisms may be subject to higher levels of anthropogenic and environmental stressors, for example, extreme temperatures,

desiccation, low dissolved oxygen, excess sedimentation or erosion and climatic disturbances (cyclones, monsoons) (Alongi 1989). There are insufficient data to conclude whether tropical benthic species are more sensitive to nickel than temperate biota.

Nickel toxicity data, whole sediment toxicity tests and an understanding of nickel chemistry and bioavailability in tropical marine and estuarine sediments represent a significant data gap for the development of nickel sediment quality guideline values (SQGVs) in tropical regions and specifically SEAM.

#### Tropical versus temperate species sensitivities

There were insufficient good quality chronic data (QA1) on nickel toxicity to tropical marine biota to do a meaningful comparison of tropical versus temperate biota sensitivities or to derive a tropical water quality guideline value for nickel. The quantity of data required in SSDs and guideline development is jurisdiction-dependent (ANZECC/ARMCANZ 2000, USEPA 2005). In this study, data were found for six tropical species, of which five were relevant to SEAM. If all tropical chronic QA1, QA2 and QA3 data are used (eight species), then, based on Australian and New Zealand WQG approaches, a HC5 of 16 µg Ni L<sup>-1</sup> was estimated from the SSD (Figure 3C). Background concentrations of nickel in seawater have been reported in the range of 0.2-0.7 µg Ni L<sup>-1</sup>, while elevated concentrations have been recorded between the range of 100-2000  $\mu$ g Ni  $L^{-1}$  (Wood et al. 2011). Thus there is a risk that some tropical marine species will be adversely affected by nickel at these high exposure concentrations. The Australian and New Zealand 95% species protection HC5 value for nickel (based on temperate marine data) was 70 µg Ni L<sup>-1</sup>. Because this was insufficiently protective of some species the guideline was set at 7  $\mu$ g Ni L $^{-1}$  (99% species protection) (ANZECC/ARMCANZ, 2000). The USEPA derived a chronic nickel guideline for saltwater of 8.2 µg Ni L<sup>-1</sup>, similar to the Australasian guideline.

The European Union Environmental Risk Assessment of Nickel (NiPERA, 2012) derived an HC5 value of 17.2 µg Ni L<sup>-1</sup> for temperate marine waters. Later, DeForest and Schlekat (2013) did further toxicity testing with temperate marine species and provided an additional two data points to derive the HC5. In this study the most sensitive species to nickel was actually a tropical species of a long-spined sea urchin (Diadema antillarum) from the Caribbean region, which had an EC10 of 2.9  $\mu$ g Ni L<sup>-1</sup>. However, this toxicity test was carried out at 20°C, so was not included in our current tropical compilation. These authors derived a marine nickel HC5 of 3.9 μg Ni L<sup>-1</sup> (including this tropical sea urchin) and 21 μg Ni L<sup>-1</sup> (when the sea urchin data were excluded due to lack of relevance to European marine waters). This value is similar to that originally derived by NiPERA in 2012. Based on the data compiled in this review, the HC5 value calculated for nickel in tropical marine waters (16 µg Ni L<sup>-1</sup>) is similar to that derived by DeForest and Schlekat (2013) for European marine waters, but slightly higher than the Australian and U.S. HC5 for temperate marine waters. Acknowledging that this is a limited dataset, this suggests that temperate and tropical species have similar sensitivities to nickel (based on chronic data). However, these SSDs and HC5 estimates do not include amphidromous and catadromous species which are key components of tropical systems.

#### Conclusions

To support the sustainable development of lateritic nickel ores in the region of SEAM, bioavailability-based predictive models and toxicity tests with endemic species are required to develop ecologically relevant water quality guidelines for nickel.

Our data compilation and gap analysis identified that the most sensitive tropical marine species to nickel were echinoderms (sea urchins), anemones, crustaceans (copepod, mysid, and shrimp), gastropod (snail) and polychaetes, whereas corals and microalgae were less sensitive. Overall, very few high quality chronic nickel data were available for tropical marine species, and even less so for those relevant to SEAM. While corals, seagrasses and mangroves form key structural habitats in SEAM, toxicity tests are only likely to be available for corals in the near future. Early life stage tests based on fertilisation and coral larval development during the annual coral spawning event are currently underway to help fill this gap.

Based on their ecological importance to SEAM, sensitivity to nickel, and to meet the data quality requirements for water quality guideline development, it is recommended that high quality chronic nickel data are acquired for, in order of priority: cnidarians (corals and anemones), molluscs (gastropods, bivalves), crustaceans (copepods, amphipods, barnacles, shrimps, prawns), echinoderms (sea urchins, star fish and sea cucumbers), macroalgae and fish. Given the very limited nickel data and toxicity tests for tropical estuaries and marine and estuarine sediments, substantial further research is required. This may include use of read-across methods from temperate databases to fill data gaps, if resources are insufficient to support further testing. This will assist in the development of ecologically relevant nickel water and sediment quality guidelines for SEAM to support the sustainable development of nickel laterites in the region. This will also provide further information to determine whether current guidelines based on temperate datasets are sufficiently protective for unique tropical ecosystems.

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Table 2. Nickel toxicity data for tropical marine microalgae (rounded to 2 significant figures). Grey shading indicates relevance to SEAM; no shading indicates species is not relevant to SEAM<sup>a,b</sup>.

					Water quality parameters				Toxicity values (μg L <sup>-1</sup> )			
Species	Growth phase/initial cell density	Endpoint	Acute or chronic?	Test duration	Temp (°C)	рН	Salinity (‰)	DOC (mg L <sup>-1</sup> )	EC10	EC50	Data quality	Reference
					Cyanobad	teria						
Cyanobacteria Cyanobium sp.	6 x10^3 cells mL <sup>-1</sup>	growth rate	chronic	72 h	25	8	33	NR	3700	2300	QA1	Alqueza and Anastasi (2013)
					Microal	gae						
Nitzschia closterium <sup>c</sup>	log-phase. 3-5 x 10^4 cells mL <sup>-1</sup>	growth rate	chronic	72 h	27	~ 8.1	~ 35	NR	NR	>500	QA2	Florence et al. (1994)
Nitzschia closterium <sup>c e</sup>	log-phase. 1-3 x 10^3 cells mL <sup>-1</sup>	growth rate	chronic	72 h	27	8.1 ± 0.2	35	1±0.2	6100 (3600- 9300)	>9500	QA1	CSIRO. Gissi et al. Unpublished.
Nitzschia closterium <sup>d e</sup>	log-phase. 1-3 x 10^3 cells mL <sup>-1</sup>	growth rate	chronic	72 h	27	8.1 ± 0.2	35	1 ± 0.2	2900 (1500- 4200)	7600 (7060- 8200)	QA1	
lsochrysis sp.	log-phase. 1-3 x 10^3 cells mL <sup>-1</sup>	growth rate	chronic	72 h	27	8.1 ± 0.2	35	1± 0.2	340 (30- 510)	1700 (1600- 1800)	QA1	

<sup>a</sup> All toxicity values are measured, dissolved Ni, unless otherwise stated

<sup>b</sup> All tests used NiCl<sub>2</sub> or NiCl<sub>2</sub>6H<sub>2</sub>O, unless otherwise stated

<sup>c</sup> Now known as *Ceratoneis closterium,* grown in G2 medium

<sup>d</sup> Now known as *Ceratoneis closterium*, grown in F2 medium

<sup>e</sup> The geometric mean was taken for these two data and used in the species sensitivity distributions

Table 3. Nickel toxicity data for tropical marine and estuarine crustaceans (rounded up to 2 significant figures). Grey shading indicates relevance to SEAM; no shading indicates species is not relevant to SEAM<sup>a,b</sup>.

					W	ater qu	ality param	neters	Toxic	ity values	(µg L⁻¹)		
Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	рН	Salinity (‰)	DOC (mg L <sup>-1</sup> )	EC10	EC50	LOEC	Data quality	Reference
					Co	pepods	6						
Acartia pacifica	adult females	egg production	chronic	10 d	25	7.9- 8.25	25	NR			100	QA3	Mohammed et al. (2010)
	eggs	egg hatching success	acute	48 h	_				to calcula succes reduc	ox values r ated: egg h ss was sign ced at 10 μ	not latching ificantly g Ni L <sup>-1</sup>		-
	adult females	adult survival	acute	48 h	_				NR	2400 (2100- 2700)			-
Apocyclops borneoensis	adult females	reproduction (no. of nauplii /female)	chronic	10 d	30	7.9- 8.25	20	NR	tox v calcu بلا reduc prod	alues not lated: 10 g Ni L <sup>-1</sup> ed nauplii uction by 33%		QA3	Mohammed et al. (2010)
		adult survival	acute	48 h	_				NR	13000 (11000- 16000		-	
Tigrious japonicus	adult females	reproduction (no. of nauplii /female)	chronic	10 d	30	7.9- 8.25	20	NR	tox val calcula µg Ni I reduce produc 32%	lues not ated: 10 - <sup>-1</sup> ed nauplii ction by		QA3	Mohammed et al. (2010)
		adult survival	acute	48 h	_				NR	18000 (13000- 23000)		-	

					r	Aysids						
Americamysis bahia <sup>c</sup>	post- larval stage	survival	acute	96h	25 ± 1	NR	30 ± 2	<1-10	NR	150	QA1	Lussier et al. (1999)
					S	hrimps						
Artemia	< 24h old	survival	acute	24 h	27 ±	NR	35	NR	NR	7.2	QA2	Asadpour et al.
shrimp)	naupin	growth	chronic	5, 11, 17 d	- 1				Afte growth by ~50 N	er 11-d n reduced % at 3 μg Ni L <sup>-1</sup>		(2013)
	adult and nauplii	bioaccumulation	acute	24 h	-				At 3 concer naup μg g <sup>-1</sup> , 0.03	ug Ni L <sup>-1</sup> ntration in lii = 0.03 in adult = 5 μg g <sup>-1</sup>		
Artemia franciscana (brine shrimp)	< 24 h old nauplii	survival	acute	24 h	27 ± 1	NR	75	NR	NR	11		
		growth	chronic	5, 11, 17 d					Afte growtl by ~63 N	er 11-d n reduced % at 3 μg Ni L <sup>-1</sup>		
	adult and nauplii	bioaccumulation	acute	24 h	-				At 3 concer naupl μg/g, 0.05	μg Ni L <sup>-1</sup> htration in ii = 0.015 in adult = 5 μg/g		
Artemia <sup>d #</sup>	embryos	emergence, development and survival	chronic	72 h	28	NR	NR	NR	NR	>590	FAIL	MacRae et al. (1991)

Prawns												
Penaeus merguiensis (banana prawn) <sup>#</sup>	juvenile	survival	acute	96 h	20-35	8 ± 0.2	20 and 36	NR	NR	3500 (1800- 7100)	FAIL	Denton and Burdon- Jones (1982)
Metapenaeus	larvae	survival	acute	48 h	27 ±	8.7	NR -	NR	NR	1300	FAIL	Wong et al. (1993)
ensis <sup>#</sup>	PZIII				1		artificial					
	larvae						seawater		NR	9300		
	MII											
	PL3								NR	8900		
	postlarval											
	stage											

<sup>a</sup> All toxicity values are measured, dissolved Ni, unless otherwise stated

<sup>b</sup> All tests used NiCl<sub>2</sub> or NiCl<sub>2</sub>.6H<sub>2</sub>O, unless otherwise stated

<sup>c</sup> Previously known as *Mysidopsis bahia* 

<sup>d</sup> Tested NiCl<sub>2</sub> and NiSO<sub>4</sub>

<sup>#</sup>Nominal concentrations

Table 4. Nickel toxicity data for tropical marine molluscs (rounded up to 2 significant figures). Grey shading indicates relevance to SEAM; no shading indicates species is not relevant to SEAM<sup>a, b</sup>.

	Water quality paramete		meters	eters Toxicity values (μg L <sup>-1</sup> )								
Species	Life stage	Endpoint	Acute or	Test	Temp	рΗ	Salinity	DOC (mg	EC10	EC50	Data	Reference
			chronic?	duration	(°C)		(‰)	L <sup>-1</sup> )			quality	
						I	Bivalves					
Crassostrea	embryos	survival	acute	48 h	25 ± 1	NR	24 ± 2	NR	NR	1200	FAIL	Calabrese et al. (1977)
virginica (Eastern					_			_			_	
oyster) *	larvae	survival	acute	12 d					NR	12000		
						Ga	astropods					
Babylonia	adult	survival	acute	96 h	25	~8	~35	~8	NR	36000	QA2	Hajimad and
areolata										(35000-		Vedamanikam (2013)
										28000)		
	larvae	survival	acute	96 h	25	~8	~35	~8	NR	200 (110-	QA2	Vedamanikam and
										340)		Hayimad (2013)

<sup>a</sup> All toxicity values are measured, dissolved Ni, unless otherwise stated

<sup>b</sup> All tests used NiCl<sub>2</sub> or NiCl<sub>2</sub>6H<sub>2</sub>O, unless otherwise stated

<sup>#</sup>Nominal concentrations

Table 5. Nickel toxicity data for tropical marine echinoderms and annelids (rounded up to 2 significant figures). Grey shading indicates relevance to SEAM; no shading indicates species is not relevant to SEAM<sup>a,b</sup>.

					Wa	iter qu	ality para	meters		Toxicity value	s (µg L⁻¹)			
Species	Life stage	Endpoint	Acute	Test	Temp	рΗ	Salinity	DOC (mg	EC10	EC50	NOEC	LOEC	Data	Reference
			or	duration	(°C)		(‰)	L-1)					quality	
			chronic?											
						Echi	noderm							
						Sea	urchins							
Diadema	gametes	fertilisation and	chronic	48 h	25	8.1	34	0.8		Test 1: 120	24	37	QA1	Rosen et al.
savignyi		development								(100-140).				(2015)
										Test 2: 72				
										(63-80)				
						An	nelid							
						Poly	cheates							
Hydriodes	gametes	sperm	chronic	1 h	28 ±	8.1	34 ±	NR	NR	770 (590-	NR	NR	QA1	Gopalakrishnan
elegans		viability/fertilization			1	±	0.5			1100)	_			et al. (2008)
		egg	chronic	1 h		0.1				1200 (790-				
		viability/fertilization			_					2100)	_			
		embryo	chronic	2 h						2300 (1200-				
		development								2700)				
	"ripe"	larval release	chronic	20 h	_					410 (330-	_			
	worms,									520)				
	tube	larval settlement	chronic	96 h	-					160 (140-	-			
	length 5									190)				
	cm				_						-			
		adult survival	acute	96 h						1500 (1200-				
										1900)				

<sup>a</sup> All toxicity values are measured, dissolved Ni, unless otherwise stated

<sup>b</sup> All tests used NiCl<sub>2</sub> or NiCl<sub>2</sub>.6H<sub>2</sub>O, unless otherwise stated

Table 6. Nickel toxicity data for tropical marine cnidarians (rounded up to 2 significant figures). Grey shading indicates relevance to SEAM; no shading indicates species is not relevant to SEAM<sup>a,b</sup>.

					Wa	ater qua	lity parameters		Toxicity values (μg L <sup>-1</sup> )					
Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	рН	Salinity (‰)	DOC (mg L <sup>-1</sup> )	EC10	EC50	NOEC	LOEC	Data quality	Reference
							Anemones							
Aiptasia pulchella	juvenile (1-2 mm pedal	survival	acute	24 h	25± 1	7.90- 8.40	35-40	NR	NR	4700	NR	NR	QA1	Howe et al. (2014) a
	disc diameter)			48 h						3300 (3090-				
					_					5050)				
				96 h						2200				
										(1400-				
				1 -	-			ND	ND	3800)	ND	ND	0.4.1	-
		severe	acute	тn				INK	INK	3500- E100	NK	INK	QAI	
		rotraction			-									
		Tetraction		011						2500-				
				12 h	-					1400-				
				12.11						3300				
	lacerate	development	chronic	8 d	25± 1	8.0- 8.4	34-35	NR	NC	>490	NR	NR	QA1	Howe et al. (2014) b
				14 d	_	0			260 (40- 740)	>490				
		survival		8 d					NC	NC	NC	NC	QA1	
				14 d	-				NC	NC	NC	NC	_	
	reproductive adult, pedal	reproduction -total	chronic	28 d	25± 1	8.20- 8.50	NR	NR	260 (20- 300)	400 (310- 420)	NR	510	QA1	Howe et al. (2014) c
	of 3-4 mm	offspring												
		reproduction -total number juveniles							65 (10- 290)	370 (220- 410)	NR	510		

							Corals							
Goniastrea aspera <sup>c</sup>	gametes	fertilisation success	chronic	5 h	NR	NR	NR	NR	NR	>2000	NR	NR	FAIL	Reichelt- Brushett and Harrison (2005)
Platygyra daedalea	gametes	fertilisation success	chronic	5 h	NR	NR	NR	NR	NR	1420 (1160- 1800)	NR	NR	QA2	Reichelt- Brushett and Hudspith (2016)
Pocillopora damicornis <sup>d</sup>	planulae larvae	larval settlement	chronic	12-96 h	25-28	NR	NR	NR	NR	NR	NR	NR	QA2	Goh (1991)
		survival	acute	12 - 72 h					NR	9000	NR	NR		

<sup>a</sup> All toxicity values are measured, dissolved Ni, unless otherwise stated

<sup>b</sup> All tests used NiCl<sub>2</sub> or NiCl<sub>2</sub>.6H<sub>2</sub>O, unless otherwise stated

<sup>c</sup> Not included in SSD because a reliable toxicity value could not be calculated and nominal Ni concentrations were used

<sup>d</sup> Toxicity value not included in SSD because EC50 was derived during recovery period, following exposure to nickel

Table 7. Nickel toxicity data for tropical marine and estuarine fish (rounded up to 2 significant figures). Grey shading indicates relevance to SEAM; no shading indicates species is not relevant to SEAM<sup>a</sup>. All values reported in this table used nominal concentrations.

					Water quality parameters		Toxicity va	alues (µg L <sup>-1</sup> )				
Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	рН	Salinity (‰)	DOC (mg L <sup>-1</sup> )	EC10	EC50	Data quality	Reference
Priopidichthys marianus	juvenile	survival	acute	96 h	30	NR	36	NR	NR	100000 (80000- 125000)	FAIL	Denton and Burdon- jones (1986)
Liza klunzingeri (Mullet)	NR	survival	acute	71 h	25± 2	8.2	NR	NR	NR	4.2 (3.2-5)	FAIL	Bu-olayan and Thomas (2005)
Leiostomus xanthurus (Spot)	adult	survival	acute	96 h	25-26	NR	21	NR	NR	70000 (57000- 88000)	FAIL	USEPA ecotox database. Ref 3732
Menidia peninsulae (Tidewater silverside)	NR	survival	acute	96 h	25	NR	20	NR	NR	38000 (30000- 45000)	FAIL	-

<sup>a</sup> All tests used NiCl<sub>2</sub> or NiCl<sub>2</sub>.6H<sub>2</sub>O, unless otherwise stated

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# Supplementary Information

Table S1. Summary of tropical marine toxicity tests using microalgae and plants. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Growth phase/initial cell density	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference
	,	Cvanoba	acteria			. ,		
Cyanobacteria Cyanobium sp.	6 x 10^3 cells/mL	growth rate	chronic	72 h	25	33	8	Alquezar and Anastasi (2013)
		Mircoa	algae					•
Isochrysis galbana	log-phase. 10^3 - 10^4 cells mL <sup>-1</sup>	growth rate	acute	72 h	24	NR	NR	Moreno-Garrido et al. (2000)
	log-phase. 6.6 x 10^4 cells mL <sup>-1</sup>				20	NR	8	Debelius et al. (2009)
Nitzschia closterium <sup>a</sup>	log-phase. 3-5 x 10^4 cells mL <sup>-1</sup>	growth rate	chronic	72 h	27	NR	NR	Florence et al. (1994)
Odontella mobiliensis	4-5 d log-phase, 1.8 ± 0.23 x 10^4 cells mL <sup>-1</sup>	growth rate, cell morphology, size, nitrate reductase, antioxidant enzyme activity	chronic	72 h - 7 d	25 ± 1	30	8.0 ± 0.3	Manimaran et al. (2012)
Pyrocystis lunula (dinoflagellate)	x10^5 cells mL <sup>-1</sup>	bioluminescence	acute	24 h	25 ± 1	33 ± 0.5	8.1- 8.4	Bao et al. (2011)
Navicula sp.	log-phase, 3 x10^4 cells mL <sup>-1</sup>	growth rate, inhibition of photosynthetic efficiency and pigment concentrations	chronic	72 h	NR	NR	NR	Magnusson et al. (2008)
	log-phase, 1.5 x10^5 cells mL <sup>-1</sup>	photosynthetic efficiency	acute	4 h				Magnusson et al. (2010)
Nephroselmis pyriformis	log-phase, 3 x10^4 cells mL <sup>-1</sup>	growth rate, inhibition of photosynthetic efficiency and pigment concentrations	chronic	72 h	NR	NR	NR	Magnusson et al. (2008)
	log-phase, 1.5 x10^5 cells mL <sup>-1</sup>	photosynthetic efficiency	acute	4 h				Magnusson et al. (2008)
Cylindrotheca closterium	log-phase, 4 x10^5 cells mL <sup>-1</sup>	photosynthetic efficiency	acute	4 h	NR	NR	NR	Magnusson et al. (2008)
Chaetoceros gracilis	NR	growth rate inhibition	chronic	72 h	25	30	7.5	Zhen Wang. Pers comm.
Skeletonema costatum	NR	growth rate inhibition	chronic	72 h	20	NR	NR	Onduka et al. (2013)
		Plan	tae					
		Macro	algae					
Ulva reticulata	NR	growth rate	chronic	7 d	25	20 - 40	NR	Mamboya et al. (2009)
Ruppia maritima	leaves and roots, 5 cm	growth and chlorophyll a	chronic	7 d	25	12	NR	Castro et al. (2015)

Species	Growth phase/initial	Endpoint	Acute or	Test	Temp	Salinity	рН	Reference	
	cell density		chronic?	duration	(°C)	(‰)			
Seagrass									
Cymodocea serrulata	NR	photosynthesis	acute	5 d	20-35	32-39	8.1-	Haynes et al. (2000)	
							8.4		
Halophila ovalis	NR	photosynthesis	acute	5 d	20-35	32-39	8.1-		
							8.4		
Zostera capricorni	NR	photosynthesis	acute	5 d	20-35	32-39	8.1-		
							8.4		
Halophila ovalis	Single leaves	photosynthesis	acute	24 h	26	34 - 36	NR	Wilkinson et al. (2015)	

<sup>a</sup> Now known as Ceratoneis closterium

Tabel S2. Summary of tropical marine toxicity tests using microcrustaceans. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference			
				duration	( 0)	(,,					
			Rotifers					-			
Brachionus plicatillis	newly hatched nauplii	survival	acute	24 h	25	NR	NR	Martins et al. (2007)			
	0-2 h nauplii	survival	acute	48 h	24-26	15	7.3-	Arnold et al. (2011)			
							7.6				
	0-3 h nauplii	population intrinsic growth rate	chronic	96 h	24-26	15	7.3-				
							7.6				
Crustacea											
	Copepods										
Acartia lilljeborgi	adult	survival	acute	48 h	25 ± 2	33.5 ± 1.5	NR	Nipper et al. (1993)			
Acartia pacifica	adult	reproduction	chronic	10 d	25	25	NR	Mohammed et al. (2010)			
		survival	acute	48 h	25	25	NR	1			
Acartia sinjiensis	adult	survival	acute	24 and 48 h	30	35	8.1	Gissi et al. (2013)			
							±				
							0.2				
Gladioferens imparipes	adult females	reproduction	chronic	28+ d	20-25	35	8-	Geotech. Tristan Stringer, Pers			
							8.2	comm.			
	nauplii 1 and 2	development		5-7 d							
Temora stylifera	adult	survival	acute	48 h	25 ± 2	33.5 ± 1.5	NR	Nipper et al. (1993)			

Species	Life stage	Endpoint	Acute or	Test	Temp	Salinity	рН	Reference				
			chronic?	duration	(°C)	(‰)						
Tigrious japonicus	adult	survival	acute	96 h	24.5 ±	15 ± 0.5,	NR	Kwok and Leung (2005)				
					1.0, 34.5	34.5 ± 0.5,						
					± 1.0	45 ± 0.5						
	< 24 h nauplii	complete life-cycle, development	chronic	20-30 d (2	25 ± 1.0	30 ± 0.5	7.9-	Kwok et al. (2008)				
		and reproduction	·	broods)			8					
	< 24 h nauplii	complete life-cycle, development and reproduction	chronic	21 d	25 ± 1.0	33 ± 0.5	8.1- 8.4	Bao et al. (2014)				
	adult females	reproduction	chronic	10 d	20	25	NR	Mohammed et al. (2010)				
		survival	acute	48 h	20	25	NR					
Apocyclops borneoensis	adult females	reproduction	chronic	10 d	30	20	NR	Mohammed et al. (2010)				
		survival	acute	48 h	30	20	NR					
	Mysids											
Mysidopsis juniae	juvenile	lethality	acute	96 h	NR	NR	NR	Figueiredo et al. (2015)				
	juvenile and 1-d old adult	survival	acute	96 h	25 ± 2	33.5 ± 1.5	NR	Nipper et al. (1993)				
Americamysis bahia <sup>a</sup>	post-larval stage	growth and survival	chronic	96 h	25 ± 1	30 ± 2	NR	Lussier et al. (1999)				
	< 48 h old mysids	survival	acute	48 h	20 ± 1	30	NR	Ho et al. (1999)				
Mysidopsis intii	2-d old neonates	survival	acute	96 h	20	34	NR	Hunt et al. (2002)				
		life-cycle test: survival, growth, reproduction	chronic	28 h								
			Shrimps									
Artemia urmiana (brine	<24 h old nauplii	growth and survival	chronic	11-day	27± 1	35	NR	Asadpour et al. (2013)				
shrimp)	adult	bioaccumulation	acute	24 h	27± 1	75	NR					
Artemia franciscana	<24 h old nauplii	growth and survival	chronic	11-day	27± 1	35	NR					
(brine shrimp)	adult	bioaccumulation	acute	24 h	27± 1	75	NR					
Artemia salina (brine shrimp)	<24 h old nauplii	survival	acute	24 h	25	35	NR	Martins et al. (2007)				
Artemia	embryos	emergence, development and survival	chronic	72 h	28	NR	NR	MacRae and Pandey (1991)				
		Seed shr	imps (Ostracod	s)								
Cypris sp. (seed shrimp)	0.25 - 0.3 mm diameter	survival	acute	24 - 96 h	22-24.5	5	NR	Oyewo and Don-Pedro (2001)				

<sup>a</sup> Previously known as Mysidopsis bahia

Table S3. Summary of tropical marine toxicity tests using macrocrustaceans. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference	
		An	nphipods	1	,				
Elasmopus rapax	juvenile	survival	acute	96 h	25 ± 1	33 ± 0.5	8.1-8.4	Bao et al. (2011)	
	adult	survival	acute	48 <i>,</i> 96 h	24 ± 0.5	30	8.1 ±	Zanders and Rojas (1992)	
		bioaccumulation	chronic	240 h	-		0.05		
Ampelisca abdita	juvenile	survival	acute	24 h	20 ± 1	30	NR	Ho et al. (1999)	
Barnacles									
Amphibalanus amphitrite amphitrite	4-5 day old cyprids (larvae)	metamorphosis and settlement	chronic	48h	27	NR	NR	Afsar et al. (2003)	
	stage 2 nauplii	survival	acute	24 h	28	NR	NR	Rittschof et al. (1992)	
	cyprids	settlement	chronic	22 h	28	NR	NR		
Amphibalanus amphitrite	≤ 3 h old stage II larvae	larval development (inhibition of metamorphosis)	chronic	96 h	29 ± 1	30-35	7.8-9	Van Dam et al. (2015)	
Balanus amphitrite <sup>a</sup>	4d into cyprid larval stage	mortality	acute	24 h	27	33 ± 0.5	8.1-8.4	Bao et al. (2011)	
			Crabs	1					
Scylla serrata	instar 2 crablets (megalopa)	growth and survival	chronic	18 d	20-35	0-40	NR	Ruscoe et al. (2004)	
	zoea stages I-V, megalopa	survival	acute	48 h	26.4-	28-32	7.7-8.3	Neil et al. (2005)	
		survival and development from zoea I - megalopa	chronic	19 d	28.6				
Tunicotheres moseri	zoea I, II and megalopa	survival	acute	96 h	25	37	NR	Greco et al. (2001)	
Clibanarius africanus (Hermit crab)	0.48 g ex-shell	survival	acute	24 - 96 h	22 - 24.5	15	NR	Oyewo and Don-Pedro (2001)	
Hermit crab		Larval development	Chronic					Van Dam et al. (2015)	
		F	Prawns	1	•				
Penaeus merguiensis	juvenile (~ 6 weeks old)	survival	acute	96 h	27	20	NR	Ahsanullah and Ying (1995)	
		growth	chronic	14 d	27	20	NR		
Penaeus monodon	juvenile (~ 6 weeks old)	survival	acute	96 h	27	20	NR		
Penaeus merguiensis	juvenile	survival	acute	96 h	20-35	30, 36	NR	Denton and Burdon-Jones (1982)	
Penaeus monodon	juvenile	growth	chronic	14 and 30 d	27 ± 0.5	32 ± 0.1	NR	Florence et al. (1994)	
Penaeus merguiensis		survival	acute	96 h	20				
Litopenaeus vannamei <sup>b</sup>	NR	survival	acute	96 h	NR	NR	NR	Neff et al. (2000)	
	juvenile	accumulation and regulation	chronic	10 d	25	NR	NR	Nunez-Nogueira et al. (2012)	
Metapenaeus ensis	3 d postlarval (PL3) stage	survival	acute	48 h	27 ± 1	NR	8.1	Wong et al. (1993)	
		feeding behaviour		24 h					

<sup>a</sup> Now known as Amphibalanus amphiritite

<sup>b</sup> previously known as *Penaeus vannamei* 

Table S4. Summary of tropical marine toxicity tests using molluscs and bivalves. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference
		Bivalv	/es					
Crassostrea belcheri (oyster)	adult	metabolism, oxygen consumption, ammonia excretion	acute	12 h	26 ± 1	30 ± 2	NR	Elfwing and Tedengren (2002)
Crassostrea iredalei (oyster)	newly fertilised egg	development to larvae stage D- shaped veliger	chronic	48 h	25	NR	NR	Ramachandran (1997)
Crassostrea lugubris (oyster)	adult	metabolism, oxygen consumption, ammonia excretion	acute	12 h	26 ± 1	30 ± 2	NR	Elfwing and Tedengren (2002)
Crassostrea gigas (oyster)	embryo (newly fertilised eggs)	larval development to normal D- shape	chronic	24 h	24	35	NR	Libralato et al. (2007)
Crassostrea virginica (Eastern	embryos	survival	acute	48 h	25 ± 1	24 ± 2	NR	Calabrese et al. (1977)
oyster)	larvae	survival	acute	12 d				
Pinctada maxima (oyster)	4 mo old (25-40 mm)	feeding behaviour	chronic	7 d	26-30	NR	8.1- 8.2	Negri et al. (2004)
Saccostrea cuccullata (oyster)	adult	metabolism, oxygen consumption, ammonia excretion	acute	12 h	26 ± 1	30 ± 2	NR	Elfwing and Tedengren (2002)
Saccostrea echinata (oyster)	adult	metal accumulation	chronic	30 d	20 and 30	36, 20	NR	Denton and Burdon-Jones (1981)
	larvae	development	chronic	48 h	29 ± 1	NR	NR	ESA SOP 106
Pteria colymbus (Pearl oyster)	embryos	development to pluteus stage	chronic	24 h	27	36.6	8	Rumbold and Snedaker (1997)
Tridacna maxima (Small Giant clam)	fecundated egg	development to D larvae (veliger)	chronic	48 h	27 (summer)	34	8.4	Aquabiotech. Jocelyn Senia, Pers comm.
Saccostrea rhizophora (Mangrove Oyster)	fecundated egg	development to D larvae (veliger)	chronic	48 h	27 (summer)	34	8.4	
Mimachlamys gloriosa (scallop)	fecundated egg	development to D larvae (veliger)	chronic	48 h	24 (winter)	34	8.4	
Bractechlamys vexillum (seashell)	fecundated egg	development to D larvae (veliger)	chronic	48 h	24 (winter)	34	8.4	
Garfrarium tumidum (clam) Isognomon isognomon (oyster) Malleus regula (oyster)	adult shell width ≥ 35 mm. adult shell width ≥ 70 mm. adult shell width ≥ 70 mm.	Ni uptake and bioaccumulation Ni uptake and bioaccumulation	chronic	14 d	25 ± 1	35 ± 1	8.0 ± 0.1	Hédouin et al. (2007)
Musculus lateralis (mussel)	embryos	survival	acute	48 h	NR	NR	NR	Lussier et al. (1999)
Perna viridis (Green-lipped mussel)	spermatozoa	sperm motility and ultrastructure	sub- chronic	0, 30 and 60 min	22	30	NR	Au and Chiang (2000). Au and Reunov (2001)

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference
Haliotis rufescens (Abalone)	48 h old veliger larvae	normal shell development	acute	48 h	15	34	NR	Hunt et al. (2002)
	< 1 h old fertilised embryos	larvae/juvenile metamorphosis	chronic	14 d				
		juvenile shell growth		22 d				

Table S5. Summary of tropical marine toxicity tests using molluscs gastropods and cephalopod. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference	
		Gastro	opods			•			
Nerita senegalensis	7.5 mm shell length	survival	acute	24 - 96 h	22 - 24.5	15	NR	Oyewo and Don-Pedro (2002)	
Tympanotonus fuscatus	43 mm shell length	survival	acute	24 - 96 h	22 - 24.5	15	NR	Oyewo and Don-Pedro (2002)	
Strombu gigas (Queen conch)	embryos	development and survival	chronic	24 h	27	36.8	7.9	Rumbold and Snedaker (1997)	
Nerita chamaeleon (snail)	NR	growth and survival	chronic	30 d	26 ± 0.2	32 ± 0.1	NR	Florence et al. (1994)	
Nassarius dorsatus (Dogwhelk snail)	≤ 48 h old larvae	growth and survival	chronic	96 h	28	35	8- 8.2	AIMS. Mel Trenfield, Pers comm.	
Babylonia areolata	adult	survival	acute	96 h	25	~35	~8	Hajimad and Vedamanikam (2013)	
	larvae	survival	acute					Vedamanikamand and Hayimad (2013)	
Lithopoma americanum	adult	behaviour	acute	24 h	22 - 23	35	8.2	Fong et al. (2015)	
Cephalopods									
Sepia officinalis	1 month old	predatory behaviour and memory	acute	24 h	20	32	NR	Di Poi et al. (2013)	

Species Life stage Endpoint Acute or **Test duration** Temp Salinity pН Reference chronic? (°C) (‰) Sea urchins 8 Anthocidaris crassipina gametes sperm fertilisation success chronic 30 min 24-26 30 Vaschenko et al. (1999) embryos first cleavage and 1.5 h - 48 h embryos chronic pluteus larval quality embryo first cleavage, chronic 27-30 h 26-28 NR NR Kobayashi and Okamura (2004) embryos pluteus formation Au and Chiang (2000). Au and sperm motility and sub-chronic 0, 30 and 60 22 30 NR spermatozoa ultrastructure Reunov (2001) min Diadema savianyi (Longfertilisation and 48 h 25 34 8.1 gametes chronic Rosen et al. (2015) spined sea urchin) development Diadema setosum (Long sperm fertilisation success sub-chronic 1 h NR NR NR Ramachandran (1997) gametes spined sea urchin) embrvos first cleavage sub-chronic 1 h 5 h embryos gastrulation sub-chronic 48 h pluteus larvae development sub-chronic fecundated egg development to pluteus chronic 48 h 27 34 8.4 Aquabiotech. Jocelyn Senia, Pers larvae comm. Heslinga (1976) Echinometra mathaei gametes sperm fertilisation success sub-chronic 10 min 28 32.6-35 8.2-8.5 (Rock boring sea urchin) embryo early cleavage, chronic 110 min skeletal development larval survival and no. chronic 96 h swimming adult adult survival acute 96 h fecundated egg development to pluteus chronic 48 h 27 34 8.4 Aquabiotech. Jocelyn Senia, Pers larvae comm. gametes fertilisation sub-chronic 1 h 25 35 8-8.2 Geotech. Tristan Stringer, Pers comm. 25 35 8-8.2 larvae larval development chronic 72 h Tripneustes gratilla 30 ± 1 fertilization, early mid and chronic 0.5, 3, 6, 9, 12 h 28 ± 2 7 ± 0.5 Edullantes and Galapate (2014) gametes (Collector urchin) late cleavage, blastulation Echinodermata lucunter embryos development to pluteus sub-chronic 24 h 26.8 36 8.1 Rumbold and Snedaker (1997) (Rock-boring sea urchin) stage Lytechinus variegatus embryos development to pluteus sub-chronic 24 h 27.3 35.7 8.2 (Variegated urchin) stage larvae (gastrula stage 6 dietary exposure - growth 23 - 25 Brix et al. (2012) chronic 7 - 18 d NR NR h) Arabica punctulata embryos development to pluteus chronic 60 h NR NR NR Neff et al. (2000) stage Star fish Amphipholis squamata juveniles (disc diameter mortality 96h 24-26 33-40 6.6-8.6 Black et al. (2015) acute (Brittle star) 0.9 - 1.5 mm)

Table S6. Summary of tropical marine toxicity tests using echinoderms. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark

grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or	Test duration	Temp	Salinity	рН	Reference
			chronic?		(°C)	(‰)		
	juveniles (disc diameter	behaviour - 1. ability to	sub-chronic					
	0.9 - 1.5 mm)	right 2. tube feet 3. curling						
		behaviour						
Asterias forbesi	adults	survival	acute	168 h and 96 h	20	20	7.8 ±	Eisler and Hennekey (1977)
							0.2	

Table S7. Summary of tropical marine toxicity tests using polychaetes. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference
					(-)	(****)		
			Polycha	etes				
Hydriodes elegans	gametes	sperm viability/fertilization	chronic	1 h	28 ± 1	34 ± 0.5	8.1 ± 0.1	Gopalakrishnan et al. (2008)
		egg viability/fertilization	chronic	1 h				
		embryo development	chronic	2 h				
	"ripe" worms, tube length 5 cm	larval release	chronic	20 h				
		larval settlement	chronic	96 h				
		adult survival	acute	96 h				
	trocophore larvae	survival	acute	48 h	25 ± 1	33 ± 0.5	8.1-8.4	Bao et al. (2011)
	eggs	survival	acute	48 h	NR	33-34	NR	Lau et al. (2007)
	2-cells	survival						
	trocophore larvae	survival						
	juvenile	survival						
	adult	survival						
	egg - juvenile	development	chronic	17 d				
	juvenile	growth and maturation	chronic	44 d				
	post-spawning females	survival and reproduction	chronic	60 d				

Table S8. Summary of tropical marine toxicity tests using cnidarians. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or	Test	Temp (°C)	Salinity	рН	Reference			
			chronic?	duration		(‰)					
			Anemone	es							
Aiptasia pulchella	adult	survival	acute	96-144 h	25± 2	NR	8.15-	Howe et al. (2012)			
	lacerate tentacle	development and survival (asexual reproduction)	chronic	28 d	25± 2	NR	8.48	Howe et al. (2014) A			
	lacerate tentacle	development and survival	chronic	14 d	25± 1	NR		Howe et al. (2014) B			
Aiptasia sp	larval and adult	survival	acute	96 h	25 ± 1	33 ± 0.5	8.1-8.4	Bao et al. (2011)			
Aiptasia pallidaª	adult	accumulation and enzyme activity and zooxanthellae density	chronic	21 d	20 - 22	20 and 25	7.8 - 8.1	Patel and Bielmyer-Fraser (2015)			
Corals											
Acropora millepora	gametes	fertilisation	chronic	4 h	28	NR	NR	Negri and Heyward (2001)			
	larvae	metamorphosis	chronic	24 h							
Acropora tenuis	5-d old larvae	larval settlement	chronic	48 h	NR (Ambient)	NR	NR	Reichelt-Brushett and Harrison (2000)			
	gametes	fertilisation	chronic	5 h	NR	NR	NR	Reichelt-Brushett and Harrison (2005)			
Acropora formosa	coral branches 4-6 cm long	photosynthesis (PAM)	acute	24 h	25 ± 1	35	NR	Jones et al. (2003)			
	coral branches 4-6 cm long	zooxanthellae loss	acute	48 h	22-25	NR	NR	Jones (1997)			
	adult coral branches	photosynthesis (PAM)	acute	48 h	NR	NR	NR	Mercurio et al. (2004)			
	4-5 cm long	growth (e.g. bleaching, mucous production) and survival	acute	48 h	NR	NR	NR				
		dinoflagellate density ( on adult corals)	acute	NR	NR	NR	NR				
Acropora longicyathus	gametes	fertilisation	chronic	5 h	NR	NR	NR	Reichelt-Brushett and Harrison (2005)			
Acropora tumida	larvae	survival	acute	24 h	25 ± 1	33 ± 0.5	8.1-8.4	Bao et al. (2011)			
Acropora microphthalma	gametes	fertilisation	chronic	4 h	NR	NR	NR	Mercurio et al. (2004)			
Lobophytum compactum <sup>b</sup>	gametes	fertilisation	chronic	30 min	NR	NR	NR	Reichelt-Brushett and Michalek-Wagner (2005)			
Goniastrea aspera	gametes	fertilisation	chronic	5 h	NR	NR	NR	Reichelt-Brushett and Harrison (1999)			
	4-6 d old larvae	survival	acute	72 h	NR	NR	NR	Reichelt-Brushett and Harrison (2004)			
	4-6 d old larvae	motility	acute	72 h	NR	NR	NR				
Goniastrea retiformis	gametes	fertilisation	chronic	5 h	NR	NR	NR	Reichelt-Brushett and Harrison (2005)			
IVIONTIPORA geguituberculata	gametes	fertilisation	chronic	4-6N	28	NK	NK	Negri et al. (2005)			
αεγαιταρειταίατα		metamorphosis	chronic	24 h	28	NR	NR				
		larval settlement	chronic	48 h	28	NR	NR				

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference
Montipora digitata	coral branches 4-6 cm long	photosynthesis (PAM)	acute	24 h	25 ± 1	35	NR	Jones et al. (2003)
Montipora capitata	gametes	fertilisation success	chronic	3 h	~22-25 (ambient)	NR	NR	Hédouin and Gates (2013)
Pocillopora	gametes	fertilisation	chronic	4 - 6 h	28	NR	NR	Negri et al. (2005)
damicornis		metamorphosis	chronic	24 h	28	NR	NR	]
		larval settlement	chronic	48 h	28	NR	NR	]
	planulae larvae	larval settlement	chronic	12-96 h	25-28	NR	NR	Goh (1991)
		survival	acute	12-72 h	1			
	coral branches 4-6 cm long	photosynthesis	acute	96 h	28	NR	NR	Negri et al. (2005)
Porites cylindrica	coral branches 4-6 cm long	photosynthesis (PAM)	acute	24 h	25 ± 1	35	NR	Jones et al. (2003)
Seriatopora hystrix	coral branches 4-6 cm long	photosynthesis (PAM)	acute	24 h	25 ± 1	35	NR	Jones et al. (2003)
Xenia elongata	coral colonies	zooxanthellae loss	acute	24 - 72 h	25	35	NR	Studivan et al. (2015)
Heteroxenia fuscescens (Soft coral)	larvae	planulae metamorphosis and settlement	chronic	8 d	25 ± 1	NR	NR	Kushmaro et al. (1997)
Montastraea faveolata (Mountain	planulae larvae	survival	sub-chronic	24 h	28	36	7.9 ±	Rumbold and Snedaker (1997)
coral)	fragments 5 - 10 cm	zooxanthellae loss	chronic	17 d	25 - 29	33.6	NR	Iovanovic and Guzman (2014)
Platygyra daedalea	gametes	fertilisation	chronic	5 h	NR	NR	NR	Reichelt-Brushett and Hudspith (2016)

<sup>a</sup> Now known as *Exaiptasia pallida* <sup>b</sup> Eggs and sperm exposed separately to Cu for 30 min, prior to fertilisation

Table S9. Summary of tropical marine toxicity tests using fish. Light grey shading indicates tests that are relevant to SEAM and currently available. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference
Acanthochromis polyacanthus (damsel fish)	larvae	imbalance and growth	chronic	21 d	28-29	NR	7.8-8.1	Munday et al. (2011)
Chanos chanos	fingerling	survival	acute	24 h and 96 h	27 ± 1	30	8.4	Cruz and Tamse (1989)
		survival and histopathology	acute	96	26-29	30-32	7.3-8.4	Tamse and Gacutan (1994)
Lates calcarifer	20-day old juvenile fish	survival	sub-chronic	96 h – 16 d	24.5-28	20	NR	Shazili (1995)
	4 month old	survival	sub-chronic	23d	24.5-28	30	NR	
	juvenile (2 sizes, 11 ± 3, or 24 ± 4)	fry survival, histopathology	acute	96 h	28 ± 2	26 ± 1	8.5± 0.2	Krishnani et al. (2003)
	young males (299 ± 39 g)	induction of enzyme activity (EROD)	chronic	96 h	27-28	35	NR	Mercurio et al. (2004)
	juvenile	juvenile imbalance, growth	acute	7 d	25 ± 1	NR	NR	ESA SOP 122
Liza klunzingeri (mullet)	NR	survival	acute	71 1	25 ± 2	NR	8.2	Bu-olayan and Thomas (2005)
Liza vaigiensis	juvenile	survival	acute	96 h	20 and 30	20 and 36	NR	Denton and Burdon-jones (1986)
Priopidichthys marianus	juvenile and adult	survival	acute	96 h	20 and 30	20 and 36	NR	
Rivulus marmoratus	juvenile	survival	acute	96 h	26-27	NR	NR	Lin and Dunson (1993)
Mullet ( <i>Mugil</i> sp.)	fingerling (70 mm)	survival	acute	24 - 96 h	22 - 24.5	15	NR	Oyewo and Don-Pedro (2001)
Tilapia guineensis	fingerling (65 mm)	survival	acute	24 - 96 h	22 - 24.5	15	NR	Oyewo and Don-Pedro (2001)
Oryzias javanicus (Java medaka)	fertilised egg	hatch success, development	chronic	15 d	NR	20	NR	Ismail and usof (2011)
	fertilised egg	survival hatch success, development	chronic	20 d	28-30	20	5.5-6.5	Yusof et al. (2014)
Oruzias melastiama	<24 h Januar	survival	acute	96 h	25 + 1	33 + 0 5	8 1 <sub>-</sub> 8 /	Bao et al. (2011)
oryzius meiustigmu	adult (2 months old)	growth and	chronic	28 d (dietary	25 ± 0.5	30	7.89 ±	Wang and Wang (2014)
Amphiprion clarkii (yellowtail clownfish)	NR	survival	acute	96 h	NR	NR	NR	Neff et al. (2000)
Atherinops affinis (topsmelt	9- 15- d old larvae	survival	acute	96 h	20	34	NR	Hunt et al. (2002)
fish)	early gastrula stage embryos	growth, development and survival	chronic	40 d				
Leiostomus xanthurus (spot)	adult	survival	acute	96 h	25-26	21	NR	

Species	Life stage	Endpoint	Acute or chronic?	Test duration	Temp (°C)	Salinity (‰)	рН	Reference
Menidia peninsulae (Tidewater silverside)	NR	survival	acute	96 h	25	20	NR	USEPA ecotox database. Ref 3732 Hansen,D.J., 1983.
Epinephelus adscensionis (rock hind fish)	embryos	hatching success and survival	sub-chronic	24 h	27	36.5	8.2 ± 0.1	Rumbold and Snedaker (1997)
Epinephelus cruentatus (Graysby)					27	36.2	8.0 ± 0.1	
Cynoscion nebulosus (spotted sea trout)					28.8	36	8.1 ± 0.1	
Ocyurus Chrysurus (yellow tail snapper)					30	36	8.1 ± 0.1	
Terapon jarbua	NR	NR	NR	48 h	NR	NR	NR	Krishnakumari et al. (1983)
	NR	NR	NR	72 h				
	NR	NR	NR	96 h	1			
Kryptolebias marmoratus	7-9 d old	survival	acute	96 h	24	3 - 36	7.3 - 8.3	Bielmyer et al. (2013)

Table S10. Tropical whole-sediment marine bioassays. Dark grey shading indicates tests that are relevant to SEAM but availability unknown. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or	Test	Temp	Salinity	рН	Particle Size	тос	AVS	Reference		
			chronic?	duration	(°C)	(‰)			(%)	(µmol/g)			
Crustacea													
Copepod													
Tisbe biminiensis	ovigerous	reproduction	chronic	7 d	25 ± 2	35	NR	3 - 36% fine (silt +	1 - 10	NR	Buruaem et al. (2013)		
	females	rate						clay)					
Amphipod													
Tiburonella viscana	NR	survival	acute	10 d	NR	35	NR	3 - 36% fine (silt +	1 - 10	NR	Buruaem et al. (2013)		
								clay)					
	NR	survival	acute	10 d	25 ± 2	34 - 37	>7	3 - 92 % fine (silt +	2 - 25	NR	Nilin et al. (2013)		
								clay)					
Grandidierella sp.	adult	survival	acute	10 d	25 ± 2	30	7.8	≤ 0.5 mm	0.1 -	NR	Tsvetnenko et al.		
									0.3		(2000)		
					Molluso	a							
					Bivalve								
Paphies elongata	adult (18 mm	survival	acute	5 d	25 ± 2	30	7.8	≤ 1 mm	0.1 -	NR	Tsvetnenko et al.		
	shell size)								0.3		(2000)		
Ruditapes	adult (3 - 4 cm)	survival/reburial	acute	48 h	20	33	NR	8 - 70 % < 62 μm	NR	NR	Shin et al. (2002)		
philippinarum (short-													
neck clam)													

Table S11. Tropical whole-sediment estuarine bioassays. White shading indicates not relevant to SEAM

Species	Life stage	Endpoint	Acute or	Test	Temp	Salinity	рН	Particle Size	тос	AVS	Reference		
			Chronic?	duration	(°C)	(‰)			(%)	(µmol/g)			
	Crustacea												
Copepods													
Nitocra sp.	ovigerous females	reproduction rate	chronic	10 d	25 ± 2	17	NR	2 - 69% fine (silt + clay)	4 - 17	NR	Buruaem et al. (2013)		
	ovigerous females	reproduction rate	chronic	10 d	25 ± 2	17	NR	1 - 94 % fine (silt + clay)	NR	NR	Krull et al. (2014)		
	ovigerous females	reproduction rate	chronic	10 d	25 ± 2	17	>7.0	3 - 92 % fine (silt + clay)	2 - 25	NR	Nilin et al. (2013)		

## TableS12. Gap analysis for high quality (QA1) chronic nickel toxicity data for marine waters in the SEAM region

	Current	Additional	Potential for read across from alternative database (e.g. temperate)	Chronic test available to generate QA1 data?			Sensitivity range for nickel from other	Potential	Feasibility of toxicity testing?	Chronic test method development
Таха	QA1 Ni data	recommend ed		Available	Unknown	Notes on ecological significance in SEAM	data (based on temperate and freshwater studies)	from acute protocols?	(i.e. easy, moderate, difficult)	required, and target species (if known).
Bacteria	1	Yes	Yes	0	1	Limited information on diversity or specific ecological importance in SEAM. Live in association with other important key species, mangroves, seagrasses, corals, anemones and fish.	Low	0	Easy	Low priority
Protozoa	0	Yes	No	0	0	Important grazers of microbes, most species ubiquitous globally. High diversity of benthic marine flagellates in tropical systems (Larsen and Pattersen 1990)	Unknown	0	Moderate	Low priority, based on sensitivity of other microorganisms to Ni
Microalgae	3	Maybe	Yes	2	3	Limited information on diversity or specific ecological importance in SEAM. Primary producer, basis of food chain, most species ubiquitous globally. Dinoflagellate algae have important symbiotic relationship with cnidarians (Howe et al. 2012).	Low	0	Easy	Medium priority, but should consider growth inhibition tests with isolated coral zooxanthellae
Macroalgae	0	Yes	Maybe	0	1	Low diversity in tropical systems, but important structural function in coral reef building, nitrogen fixation and food source for higher trophic organisms as well as epiphytes growing on seagrass and mangroves (Diaz- Pulido and McCook 2008, Chaves et al. 2013). Red coralline algae (corralinales) and algae of the order Bryopsidophyceae reach peak biodiversity in the tropics (Kerswell 2006, Hoeksema 2007).	Unknown	0	Moderate	Medium priority
Seagrasses	0	Yes	No	0	4	Philippines, New Guinea and Indonesia are considered to be the centre of global seagrass biodiversity (Spalding et al. 2003). Form a key structural habitat for fish and invertebrates, act as nurseries for fish and crustaceans (Nagelkerken 2009).	Unknown	4 (in-situ studies measuring photosynth esis with PAM)	Difficult	Medium priority
Mangroves	0	Yes	No	0	0	Important structural habitat in tropical systems, form important buffering component	Unknown	0	Difficult	Medium priority

Таха	Current SEAM	Additional chronic data	Potential for read across	Chronic test available to generate QA1 data?			Sensitivity range for nickel from other	Potential test species	Feasibility of toxicity testing?	Chronic test method development
	QA1 Ni data	recommend ed	alternative database (e.g. temperate)	Available	Unknown	Notes on ecological significance in SEAM	data (based on temperate and freshwater studies)	from acute protocols?	(i.e. easy, moderate, difficult)	required, and target species (if known).
						between rivers and coral reefs, and act as nurseries for fish and crustaceans (Nagelkerken 2009). Provide high productivity, abundant detritus and high levels of organic carbon (Cavalcante et al. 2009). Mangrove species diversity is believed to be highest in the Indo-West Pacific (Hoeksema 2007).				
Rotifers	0	Yes	Maybe	0	0	Very limited information on species diversity and relevance to SEAM, or marine systems in general, predominately freshwater organisms (Fontaneto et al. 2006). Popular food source in aquaculture (Hagiwara et al. 1995). Common strain Brachionus is a tropical marine rotifer that has been collected around Thailand and Fiji (Hagiwara et al. 1995). Rotifer diversity expected to be much higher than what is currently known (JMBA Global Marine Environment).	Unknown	0	Difficult	Low priority
Crustaceans	0	Yes	Yes	4	5	Crustaceans are an important food source for higher trophic organisms and humans. High copepod diversity in tropical Asian region – particularly due to the high association of copepods with corals (Humes 1994). Copepods are a major food source for tropical fish, form the link between primary producers and higher trophic organisms (Williams et al. 1988). Peracarid crustaceans, mainly amphipods are believed to be the most dominant group of crustaceans in shallow waters including those of the tropics (Thomas 1993). Barnacles, particularly those associated with corals are believed to have a high species richness in Indo-Malayan region (Hoeksema 2007). Shrimps and lobsters have also been shown to have high species diversity in Indo-Pacific (Hoeksema 2007, Roberts et al. 2002).	Broad depending on species.	5	Moderate	High priority

Taxa C	Current	Additional	Potential for nal read across	Chronic test available to generate QA1 data?			Sensitivity range for	Potential	Feasibility of	Chronic test method
	QA1 Ni data	chronic data recommend ed	trom alternative database (e.g. temperate)	Available	Unknown	Notes on ecological significance in SEAM	data (based on temperate and freshwater studies)	from acute protocols?	(i.e. easy, moderate, difficult)	development required, and target species (if known).
						Life cycle of many crustaceans link key habitats – mangroves, seagrasses and coral reefs (Nemeth 2009). Importance of amphidromous shrimp species, live as adults in freshwater/estuaries, juveniles develop in marine waters before migrating back to freshwater (e.g. Macrobrachium). Common in tropical systems (Kikkert et al. 2009).				
Molluscs	0	Yes	Yes	5	10	Mollusc diversity is the highest in the tropics, particularly in the Indo-Pacific in coral reef environments, they are also a major human food source in the region. Extremely high number of bivalve molluscs have been found in New Caledonia. (Bouchet et al. 2002). The highest diversity of gastropod molluscs have been found in Nth Australia, New Guinea, Indonesia and the Philippines (Wells 1990, Roberts 2002).	High	4	Moderate	High priority
Cephalopods	0	Yes	No	0	0	No information on diversity or ecological importance of cephalopods in tropical marine environments, particularly those of SEAM. Mostly large pelagic organisms, highly developed, key predator in marine systems and also prey of larger fish species (Boyle and Rodhouse 2008). One nautilus species is endemic to New Caledonia and has been shown to accumulate Ni (Bustamante et al. 2000).	Unknown	1	Difficult	Low priority
Echinoderms	1	Yes	Yes	2	3	Important component of coral reefs, high diversity in the tropics, more so the Caribbean than SEAM. Echinoids (sea urchins) dominate the Caribbean. Limited sampling sites in SEAM, but asteroids (starfish) and holothurians (sea cucumber) dominate in Northeast Pacific (lken et al. 2010).	High	2	Moderate	High priority
Cnidarians	1	Yes	No	4-5	7	Anemones distributed throughout sub-tropical and tropical ecosystems (Howe et al. 2014). Not of commercial importance but hosts for	Anemones high sensitivity. Corals	6	Moderate	High priority

	Current	Additional chronic data recommend ed	Potential for read across from alternative database (e.g. temperate)	Chronic test available to generate QA1 data?			Sensitivity range for nickel from other	Potential	Feasibility of storig?	Chronic test method development
Таха	QA1 Ni data			Available	Unknown	Notes on ecological significance in SEAM	data (based on temperate and freshwater studies)	from acute protocols?	(i.e. easy, moderate, difficult)	required, and target species (if known).
						other important species, symbiotic dinoflagellates, bacteria, fish and invertebrates. Species diversity peaks in higher latitudes, rather than the tropics (Fautin et al. 2013). Coral diversity is highest in the tropics. Scleractinian corals, Fungiidae and Acropora corals have highest diversity in Indo-Pacific including SE Asia and West Pacific (Hoeksema 2007). Key structural habitat in tropical systems (Nagelkerken et al. 2000). Importance economically in tourism and recreational activities (Hoeksema 2007).	Medium – Iow sensitivity			
Sponges	0	Yes	Maybe	0	0	Limited information on sponges in SEAM. Primary consumer, benthic organism. Filter feeders, therefore at high risk of dissolved Ni exposure. Also provide microhabitats for all trophic level taxa from bacteria to fish, important microhabitat for tropical shrimps (Hoeksema 2007). High regional diversity of marine sponges in the Carribbean related to adaptability to different habitats and abiotic factors (Wulff 2005).	Unknown	0	Difficult	Medium priority
Ascidians	0	Yes	No	0	0	Sedentary filter feeders, accumulate metals, good indicators of pollution and recently exploited for medicinal properties. High diversity in warm tropical waters, particularly on coral reefs of New Caledonia (Monniot et al. 1991).	Unknown	0	Difficult	Medium priority
Insects	0	Yes	No	0	0	Heteroptera and Gerromorpha have high species diversity in estuaries and mangroves in the Indo-West Pacific region, predominately live on the surface of the water (Andersen 1999).	Unknown	0	Difficult	Medium priority

Таха	Current SEAM QA1 Ni	Additional chronic data recommend	Potential for read across ta from alternative database (e.g. temperate)	Chronic test available to generate QA1 data?		Notes on ecological significance in SEAM	Sensitivity range for nickel from other data (based on temperate and freshwater	Potential test species from acute	Feasibility of toxicity testing? (i.e. easy,	Chronic test method development required, and
	data	ea		Available	Unknown		studies)	protocols?	moderate, difficult)	known).
Annelids	0	Yes	Yes	0	0	Limited information on diversity or ecological relevance in SEAM. Marine worms play critical roles in trophic interactions and affecting biogeochemical cycles (Kicklighter and Hay 2006). Believed to be the most important taxon in benthic marine communities and sensitive indicators of environmental pollution (Dean 2008). Predominately benthic organisms.	Broad range depending on endpoint	0	Moderate	Medium priority
Fish	0	Yes	Yes	1	4	Richest fish diversity found around Eastern Indonesia, New Guinea and the Philippines. Indonesia has the highest concentration of rare and endemic fishes (Randall 1998). Life cycle of many tropical fish link key habitats – mangroves, seagrasses and coral reefs (Nemeth 2009). Common in tropical systems are amphidromous fish- adults live in freshwater/estuaries, larvae develop in marine waters before migrating back into freshwater to grow as adults (e.g. goby). Catadromous fish are born in marine waters, then migrate into freshwaters to develop into adults (e.g. eel). Anadromous fish are born in freshwaters, migrate to the ocean as juveniles where they grow into adults before returning to freshwater (Fievet et al. 2001) Important economically as a food source and in tourism and recreational activities (Hoeksema 2007).	Low	4	Difficult	Medium priority

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