2012-13 SURVEY OF AUSTRALIAN WILD-CAUGHT PRAWNS FOR ANALYSIS OF CADMIUM AND SELENIUM

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EXECUTIVE SUMMARY

1. Cadmium is a recognised contaminant in a wide range of foods; allowable concentrations are prescribed by food safety regulators internationally and in Australia. Some agencies monitor and regulate cadmium in crustaceans, including prawns.
2. We found 148 separate publications from across the world reporting cadmium levels in edible prawns, from both observational and experimental studies. Concentrations ranged from below detection to 31 mg/kg in whole prawns. Coral prawns (*Metapenaeopsis crassissima*) from Western Australia had cadmium concentrations that were amongst the highest ever reported.
3. Selenium is an essential micronutrient; seafood is generally viewed as a good source of dietary selenium.
4. This survey measured cadmium and selenium in 140 prawn samples, collected from the four principal Australian wild-capture fisheries: the Northern prawn Fishery, the Queensland east coast prawn fishery, the Spencer Gulf western king prawn fishery, and Shark Bay and Exmouth fisheries in Western Australia.
5. One hundred and thirty four of the 140 prawn samples (95.7%) complied with the European Commission regulatory limit of 0.5 mg/kg.
6. Six prawn samples (4.3%) had cadmium concentrations in de-veined muscle tissue that exceeded the European Commission regulatory limit. Two of these samples were *M. crassissima* from Shark Bay in WA; the other four were sampled from the Gulf of Carpentaria within the Northern Prawn Fishery. Three of these four were endeavour prawns (*Metapenaeus endeavouri*) and one was a sample of brown tiger prawns (*Penaeus esculentus*).
7. Statistical hypothesis testing showed that prawn species and fishery – in that order – were the strongest predictors of variability in prawn muscle cadmium. Endeavour prawns were found to accumulate higher concentrations of cadmium; western king prawns (*P. latisulcatus*) and banana prawns (*P. merguiensis*) were low cadmium accumulators. Prawns from the South Australian and Queensland East Coast fisheries were notably low in cadmium.
8. No clear relationship between prawn size and cadmium concentration was identified.
9. Experiments to determine whether or not the contents of prawn gut tubes affected measured prawn muscle cadmium concentrations found that de-veining prawns made no difference to the measurements.
10. Analysis of prawn hepatopancreas demonstrated higher cadmium concentrations in these tissues; this finding is consistent with previously published work from elsewhere in the world.
11. All prawn samples had selenium concentrations greater than 0.38mg/kg in muscle tissue. These levels meet formal requirements in Australia's Food Standard 1.2.7. on nutrition claims for Australian wild-caught prawns to be marketed as a good source of dietary selenium.

RECOMMENDATIONS:

To reduce the risks from non-compliance with EC cadmium import requirements, Australia's wild-capture prawn industry could consider the following strategies:

- Targeting particular prawn species: coral prawns, as represented by *M. crassissima*, and endeavour prawns are higher-risk species. Western king prawns and banana prawns are the lowest cadmium-risk species identified in this survey.
- Targeting particular prawn fisheries: the Spencer Gulf fishery, Queensland's east coast fisheries, the Western Australia fisheries – provided *M. crassissima* are excluded from consideration – and NPF fisheries west of the Gulf of Carpentaria were all found to have prawns with low levels of cadmium in muscle tissues.
- Australian wild-caught prawns should be marketed as good sources of dietary selenium.
- Industry-commissioned laboratory analysis of prawns to measure cadmium concentrations should specify which tissues are to be analysed. Analysis of whole prawns or head-only measurements will usually return cadmium levels that are higher than would be measured in muscle-only tissues. Decisions on which tissues to analyse should be made by the industry consigner, and should be determined by the purpose of commissioning the analysis.
- An industry-owned database to archive results of laboratory testing for cadmium and other contaminants in prawns may be a useful resource that would allow the industry to standardise testing and compare measurements across time and location. A comprehensive database on cadmium in prawns would be a robust and reliable longer-term resource that can capture trends and identify potential problem areas.
- Cadmium in Australian marine and coastal waters may arise from varying proportions of natural and anthropogenic sources. The ratio of natural to anthropogenic cadmium may vary across time and location; the various contributions of natural and anthropogenic cadmium that enter marine food webs – including prawns for human consumption – are poorly understood, but may be elucidated by future research investigations. A better understanding of the relative proportions of natural and anthropogenic cadmium in prawns and other seafood may identify strategies with potential for intervention to reduce cadmium levels in marketed product.

1.1. GLOSSARY

Adsorb: in chemistry, adhesion of molecules or atoms onto the surface of a solid (an adsorbent)

Aeolian: relating to wind actions

Alumina: an oxide of aluminium

Bioavailable / bioavailability: the amount or proportion of a substance (e.g. a toxin) than can be ingested, absorbed, reach target tissues and exert an effect on a living organism

Brachyuran: crab

Brown meat: common descriptor for decapod crustacean midgut or hepatopancreas tissues

Decapod: crustacean occupying the taxonomic level of the Order Decapoda. Includes prawns, crabs and lobsters

Euphotic zone: layer of water (e.g. coastal or marine water) that is infiltrated by sunlight such that photosynthesis can occur

Hepatopancreas: digestive gland of decapod crustaceans; incorporates both hepatic and pancreatic tissues and functions. Also acts as the storage organ for a range of organic and inorganic contaminants and toxins

Homarid: large-clawed lobster

Igneous: in geology, rocks formed as a result of volcanic activity, from molten lava

Lithosphere: the rigid outer layer of the Earth, comprising the crust and upper mantle

Metalloid: a non-metal that nevertheless has some chemical properties of metals (e.g. selenium)

Metallothionein: a family of low molecular weight metal-binding proteins

Metamorphic: in geology, a rock-form that has undergone transformation by extreme heat or pressure

Null hypothesis: in inferential statistics, the concept that there is no significant difference between observations or measurements from two or more groups under investigation. The principle here rests on the degree of confidence by which the statistical test employed can either succeed or fail to reject the null hypothesis, which is an important distinction between the commonly-accepted wisdom that such tests “prove” that a significant difference exists.

Organometal /organometallic: a very large group of compounds containing a metal atom bonded to one or more carbon atoms or groups

Penaeid: prawns taxonomically assigned to the Family Penaeidae

Phytoplankton: free-floating or drifting microalgae

Prawn mustard: common descriptor for prawn midgut or hepatopancreas tissues

Pseudoreplication: an error in experimental design whereby samples are falsely or incorrectly treated as independent of one another

Redox: in chemistry, pertaining to changes in oxidation state (**red**uction and **ox**idation)

Sedimentary: in geology, rocks formed from accumulated particles carried and deposited by the action of water, as in rivers, lakes and oceans

Selenoprotein: a family of proteins containing selenium; typically enzymes

Seston: particulates suspended in a water column, comprising both living and non-living material. Typically includes plankton, detritus and soil particles

Tomalley: common descriptor for decapod crustacean midgut or hepatopancreas tissues

Upwelling: in oceanography, a phenomenon whereby cold, deeper, usually nutrient-enriched water rises to the surface layers

Zooplankton: free-floating, drifting or weakly self-propelling animals, typically comprising very small crustaceans, molluscs and marine larval forms

1.2. LIST OF ABBREVIATIONS

AAA	Advanced Analytical Australia P/L	**AAS**	atomic absorption spectrometry
ABARES	Australian Bureau of Agricultural and Resource Economics and Sciences	**$Al(OH)_3$**	aluminium hydroxide
		ANOVA	analysis of variance
Bi	bismuth	**Cd**	cadmium
CSIRO	Commonwealth Scientific and Industrial Research Organisation	**EC**	European Commission
EU	European Union	**$Fe(OH)_3$**	iron (III) hydroxide
FSANZ	Food Standards Australia New Zealand	**Ho**	holmium
HP	hepatopancreas	**ICP-MS**	inductively-coupled plasma mass spectrometry
ICP-OES	inductively-coupled plasma optical emission spectroscopy	**In**	indium
Li	lithium	**MnO_2**	manganese oxide
NATA	National Association of Testing Authorities, Australia	**NPF**	(Commonwealth-managed) Northern Prawn Fishery
QA	quality assurance	**RDI**	recommended daily intake/s
Rh	rhodium	**RSD**	relative standard deviation/s
SARDI	South Australian Research and Development Institute	**Sc**	scandium
Se	selenium	**Tb**	terbium
USEPA	United States Environmental Protection Agency	**Y**	yttrium

2. AIMS AND BACKGROUND

The specific objectives of this study were to:

1. Investigate the occurrence and distribution of cadmium in Australian wild-caught prawns in 2012/13, in order to inform a formal risk assessment of cadmium in prawns
2. Evaluate selenium concentrations in Australian wild-caught prawns

The project team put out a call for results of cadmium analyses in Australian wild-caught prawns and received data from various government agencies and industry sectors. The dataset was then compiled by the project team. That dataset (referred to subsequently in this report and the accompanying *Risk assessment for cadmium in Australian wild-caught prawn muscle tissue* report as the "Historical prawns database") was collated from various sources, primarily the National Residue Survey within the Commonwealth Department of Agriculture, Forestry and Fisheries, and results of analyses commissioned privately by some fisheries industry operators. While the historical prawns database was useful both for provisional risk assessment simulations and for guiding sample size estimates for the survey outlined in this report, there are some limitations and constraints that apply to use of that information. The historical prawns database provides measured cadmium concentrations, sample collection dates (from 1991 through 2010 inclusive) and locations, and prawn common names, but does not provide information on sampling procedures, sample handling and storage conditions, analytical procedures, detection and reporting limits, and species identification methods. The historical prawns database also does not provide systematic information on all the sources of data, i.e. we do not know in all cases which industry groups did and did not contribute analytical results to the dataset.

In order to address these constraints and potential biases, we sought to conduct a systematic survey of Australia's wild-capture prawn fisheries, using a methodical strategy to deliver an unbiased sampling regimen, using a single accredited laboratory that employs standard analytical methods, and appropriate inferential statistical analysis of the results.

2.1. Cadmium concentrations in prawns from around the world

In order to place the current survey into a global context, a systematic review of published cadmium levels in prawn tissues was conducted. A review of the literature encompassing 149 publications on levels of cadmium measured in decapod prawns that are food items for humans is presented in Table 1. The bibliographic databases PubMed and Web of Science were searched with the string "(cadmium AND (prawn OR shrimp))" and abstracts were perused to identify original publications and reviews that reported analytical investigation of that metal. Further publications were identified from the bibliographies of papers identified by the search string. We did not systematically search the grey literature for conference proceedings, research theses, government reports and book chapters, although some of these are also presented in Table 1. We did not consider papers published in languages other than English.

For Table 1 we considered papers on decapod prawns that are or have been harvested commercially for human consumption. Therefore we did not include reports on cadmium measured in commercially insignificant forms such as some *Palaemonetes* species [1-4]. We also excluded studies investigating cadmium in mud shrimp *Callianassa* spp. and *Trypaea australiensis*, which are harvested as bait [5, 6].

Cadmium levels in mantis shrimp species, e.g. [7], these being crustaceans from the order Stomatopoda, were not considered for this review. We have, however, included in Table 1 a report of cadmium levels measured in decapod prawns sampled from hydrothermal vents [8]. While these particular and unusual ecological niches are not as yet trawled for commercial harvest, the capacity to bioaccumulate high metal loads by some species inhabiting the hot zones immediately adjacent to these natural sources of toxic metals suggests that food safety considerations may well impact on the economic value of this product, if and when hydrothermal vent fishing is evaluated for future exploitation.

Papers measuring cadmium concentrations in prawns were not included where results were pooled with those of other crustaceans such as lobster [9] or mantis shrimp [10]. Studies where cadmium concentrations in prawn tissues were not reported on a weight basis were not considered, e.g. [11-15], nor were reports of cadmium measured only in particular tissues such as gonads [16], gills [17] or hepatopancreas [18, 19].

Table entries are sorted in ascending order of the highest reported cadmium level (upper range, or highest mean value) in prawn muscle tissue, or in whole prawns if cadmium concentrations in muscle were not reported. Where analysed tissues were not specified, we assumed that whole prawns were tested.

Values reported as mg/kg cadmium dry weight have been converted to wet weight values for ease of comparison. Where tissue moisture contents are reported, those data were applied to convert cadmium dry weight values to wet weight values; for publications that did not report prawn tissue moisture contents, a factor of 0.25 was applied (i.e. 75% water content) for dry to wet weight conversion of muscle or whole prawns. A 0.20 conversion factor (80% moisture) was applied to reported hepatopancreas dry weights. These dry weight conversion factors (0.25 and 0.20) were derived from prawn moisture contents determined for this survey (see Tables 9 & 10). Reported dry weight concentrations of cadmium in prawn gills were not converted to wet weights in Table 1.

Table 1. Cadmium concentrations (mg kg^{-1}) measured in decapod prawn species that comprise human food items

TABLE 1 IS POSTED AS APPENDIX 1 AT THE END OF THIS REPORT

2.2. Cadmium in the environment: sources and sinks; uptake into aquatic and marine animals

Cadmium has a wide but mosaic distribution within and across the environmental compartments of air, water and soil. Anthropogenic sources reportedly account for over 90% of the cadmium in the surface environment, with principal vectors being rock phosphate fertilisers, fossil fuel combustion, metal refining, cement production, municipal refuse and sewage sludge [165]. Cadmium levels in soils from unpolluted areas can be less than 1µg/kg, whereas soils in residential, agricultural and industrial

regions have been measured between 400 and 660µg/kg; some rice paddies in Japan have concentrations up to 69mg/kg [166]. Cadmium in contaminated soil from the vicinity of a zinc smelter in Slovenia was measured at 289mg/kg [167], and up to 800mg/kg from soils in Shipham, England, the latter site being subject to legacy contamination from zinc mining between the 17th and 19th centuries [168]. Soils near bauxite mining areas in Jamaica can contain over 400mg/kg cadmium [169]. Allowable concentrations of cadmium in soils typically range from 1mg/kg in (for example) Japan, 2mg/kg in the USA through to 5mg/kg in Turkey and Canada. [167]. Average cadmium concentrations in soils across the globe (at 0.5mg/kg) are some five-fold higher than in the lithosphere, which implies that cadmium is a soil contaminant [170].

Volcanic activity represents an important natural release of cadmium into the atmosphere, with estimates of some 800 tonnes released annually [171]. However, this may be an irregular and difficult-to-measure input because of the intermittent nature of eruptions and the high degree of variability in cadmium enrichment of volcanic aerosols [172].

While some cadmium-bearing ores are known (greenockite and octavite), cadmium is found in close association with zinc, so cadmium for industrial use is recovered as a by-product of zinc processing, and may also be recovered from lead ores and some complex ores [171]. Sedimentary rocks have higher cadmium concentrations than igneous or metamorphic rocks [171, 173]. Phosphate rocks contain varying amounts of cadmium, ranging between 4 and >100mg/kg [174]. Fe-Al-P complexes in bauxite can immobilise cadmium and zinc [175]; indeed, so-called red mud, the waste product from extraction of alumina from bauxite, has a high affinity for cadmium and other toxic metals. Bauxite and red mud have been and continue to be investigated for their superior capacity to adsorb and therefore remove cadmium and other toxic metals from industrial combustion facilities [176, 177], sewage sludge [178], waste water [179-182] and contaminated soils [183, 184]. Bauxite is well-represented in Australia, with large deposits in Western Australia, Tasmania, NSW, Queensland and the Northern Territory. The longest-established bauxite mining and alumina processing facilities are centred on the Gulf of Carpentaria, at Weipa on the Cape York Peninsula, and on the Gove Peninsula. Bauxite mining began at Weipa in the early 1960s. Australia is the world's leading producer of bauxite, with some 60% of output coming from Western Australia, 30% from Queensland and 10% from NT. Australia exported 11.3 million tonnes in 2011 [185]. While bauxites all contain high concentrations of aluminium oxides, their mineralogy varies across deposits [186]. Jamaican bauxite contains high levels of cadmium, with an average of 35mg/kg [169]; red mud from Venezuela was measured at 19mg/kg [187], from Suriname at 20mg/kg and Pinjarra, Western Australia at 4.5mg/kg [188].

Bauxite mining generates dust pollution, and alumina processing creates considerable quantities of red mud which need to be stored in the vicinity of the processing plant. Red mud can also be a significant source of wind-borne pollution, with the majority of product from some plants being fine-grained particles less than 1µM in size [189]. In their review of aeolian distribution of metals from mining operations, Csavina *et al* note that airborne contaminant transport is poorly studied by comparison with soil and water, but "…contaminant transport by air and atmospheric particulates is…notable due to the potential speed, distance, and aerial extent in which contaminants can be transported in the environment." [190]. Of particular concern are the fine particles, which are more readily transported into the atmosphere and can travel long distances. Csavina *et al* note that 60% of all arsenic in the atmosphere is estimated to originate as point source emissions from mining operations, which are then transported and dispersed as airborne particulates [190]. Erosion by wind

and water of stored bauxite waste at mine sites is a recognised problem that requires short and long-term management strategies such as chemical dust suppression and revegetation [186]. Dust is also generated by mining processes and transport. However, the quantitative contribution of red mud to atmospheric and aquatic cadmium contamination in Australian air and water is poorly understood.

Atmospheric deposition into fresh and marine waters has been described as "…a major input of cadmium at the global level." [191]. However, global cadmium budget considerations are somewhat northern hemisphere-centric. Non-ferrous metal operations, particularly lead, copper-nickel and zinc-cadmium production, are the principal anthropogenic sources of atmospheric cadmium discharge on a global scale. Combustion of coal and oil are important secondary sources of atmospheric cadmium [192]. Industrial emissions of cadmium are calculated to exceed natural sources by a factor of five, again on a global scale [193]. While Australia does host lead, zinc, nickel and copper mining and smelting facilities, as well as coal-fired power stations, which will contribute to environmental cadmium pollution, our low population base and sparse geographic distribution of these heavy industries relative to developed countries in the northern hemisphere might suggest that cadmium fluxes by natural and human-derived sources may present a somewhat different picture here. In any event, the knowledge base for cadmium and other toxic metal pollution in Australian environmental compartments is relatively impoverished, again by comparison with that of the northern hemisphere.

The degree to which airborne and waterborne erosion of cadmium-contaminated particulates subsequently deposited onto estuarine and coastal sediments may be bioavailable and can be incorporated directly into prawn tissues via feeding is unknown but could be investigated by experimental feeding studies. Food web contamination is another potential route of entry into prawn tissues; microinvertebrates, including bivalve and gastropod molluscs, are an important food source for many prawn species, particularly some Australian penaeids, which as adults demonstrate feeding preferences for molluscs. Small juveniles eat microinvertebrates and plant-based detritus and epiphytes, then shift to larger invertebrates and less plant material as they develop [194]. Bivalve molluscs are highly efficient bioaccumulators of cadmium and other metals [195]. Much of the cadmium entering freshwater systems from industrial sources is rapidly adsorbed onto particulates, where it may be deposited onto sediment or remain suspended, as influenced by local variables [195]. The question of the relative bioavailability to decapods of cadmium adsorbed on inorganic particulates compared to organometallic compounds is likely to be a complex problem dependent on factors such as redox potential and desorption kinetics. Cadmium can be incorporated into marine biota from inorganic substrates; for example Wu *et al* demonstrated linear uptake of cadmium from $Fe(OH)_3$ (used as a model inorganic particulate complex) in a bivalve mollusc, whereas bioaccumulation of cadmium bound to a humic acid matrix appeared to plateau [196]. Follow-up work from the same team investigated the bioaccumulation efficiency of cadmium adsorbed to different mineral substrates: manganese delivered cadmium into bivalve tissues most effectively, with $Al(OH)_3$ intermediate between MnO_2 and $Fe(OH)_3$ [197].

Erosion and transport of cadmium into oceans by rivers represents a major flux of the global cadmium cycle [171]. Gradients of cadmium in marine and coastal waters are described, with higher levels seen in coastal waters and lower concentrations in the open ocean. Broadly similar findings of declining cadmium levels from estuarine to oceanic sediments are reported [198]. Vertical gradients and cycling of cadmium occurs in open waters; cadmium concentrations increase with depth below the euphotic zone. Cadmium is taken up from surface waters by phytoplankton and then zooplankton, from which

it can move further into marine food webs or otherwise be released at depths between 500 and 1000m by oxidative decomposition of planktonic wastes and dead cells [199]. Waters influenced by upwellings will have higher cadmium levels, as the metal is thereby redistributed into surface waters. Airborne and riverine deposition of anthropogenically-sourced cadmium is an important consideration in some coastal and oceanic regions [199].

Aquatic invertebrates take up cadmium and other trace elements from solution in their aquatic medium, and from their diet; the relative proportion from each source varies according to the taxonomic grouping and the bioavailability of the metal/s in water and food. [200]. Crustaceans do not regulate the uptake and excretion of cadmium, with newly acquired concentrations being added to total body stores, mostly bound to metallothionein [201]. Penaeid prawns can effectively assimilate cadmium from dietary sources, and may be able to slowly excrete it via cadmium-rich granules from the hepatopancreas [202].

2.3 SELENIUM

2.3.1. Sources of selenium, bioavailability

Selenium is a non-metal, sometimes classified as a metalloid, in Group 16 of the periodic table together with sulfur and tellurium. Selenium is found in cereal grains, red meat, organ meat (particularly liver and kidney) and seafood; high concentrations occur in brazil nuts [203]. Dark bread, eggs and poultry are also important sources [204, 205]. The chemical form of dietary selenium is an important consideration for determining selenium status, and this topic is incompletely understood. Selenium forms a range of organic and inorganic compounds, and numerous metabolic pathways are involved in the formation of selenoproteins and excretion of excess selenium in methylated form [206]. Selenite and selenate – both forms being found in fish [207] – may be taken up from the human gut by different mechanisms: selenate appears to be absorbed more rapidly, by an active transport mechanism, whereas selenite may cross the gut wall by passive diffusion [208]. The principal forms of selenium in foods of animal origin are selenomethionine and selenocysteine, which are incorporated into muscle proteins, and seafoods may contain other selenoproteins [207]. Indeed, marine animals reportedly have a richer endowment of selenoproteins than do terrestrial animals; at 30 - 37 selenoproteins, the so-called selenoproteome of fish is one of the largest known [209]. A novel selenoprotein with strong antioxidant properties, named selenoneine, was recently found to be the major form of organic selenium in northern bluefin tuna (*Thunnus orientalis*) [210, 211]. While the selenoproteome of prawns is unknown, these discoveries in fish hint at unexplored opportunities to enhance the understanding of the nutritional benefits of other seafood products.

Selenium concentrations measured in over 700 food items procured mainly from the UK, but with some sourced internationally, range from low levels in foods such as sago (mean 0.001mg/kg), cornflour (0.004mg/kg), selected fruits (0.004mg/kg) and skim milk (0.01mg/kg) to high levels in kidney (up to 2mg/kg) and brazil nuts (up to 7mg/kg) [212]. A review of selenium in Australian foods showed that most fruit and vegetable items had mean levels below 0.01mg/kg; eggs had up to 0.4mg/kg, vegemite at 0.1mg/kg, and kangaroo meat at 0.3mg/kg. Australian seafood products

generally have a higher selenium content than other Australian foods, with 0.6mg/kg measured in morwong, 0.5mg/kg in trevally and 0.8mg/kg in oysters [213].

2.3.2. Selenium as an essential micronutrient, selenium toxicity

Twenty-five known selenoproteins serve important roles in various metabolic and sub-cellular processes: as constituents of glutathione peroxidases (antioxidant enzymes), iodothyronine deiodinases (regulate thyroid function), thioredoxin reductases (maintain intracellular redox potential), and others [214, 215]. Selenium is reported to possess anticarcinogenic properties, possibly through anti-oxidant mechanisms and redox changes in tumour cells having anti-proliferative and pro-apoptotic effects. However, the epidemiology of selenium sufficiency and deficiency is inconclusive with regard to cancer risk [205, 214, 215]. A large, multi-centre, randomised, placebo-controlled trial conducted in North America and Puerto Rico investigated the impact of selenium, vitamin E and selenium + vit E supplementation on prostate cancer and secondary outcomes of lung, colorectal and all primary cancers. The intervention study was terminated when no treatment effects could be demonstrated. Final follow-up could not show a protective effect of selenium on prostate or any other cancer type [216, 217]. Yet a recent systematic review and meta-analysis did reveal an inverse relationship between selenium status and prostate cancer over a narrow range of selenium concentrations [218]. A U-shaped curve has been posited whereby adverse health effects, including prostate cancer risk, may be increased at low and high body selenium stores, with a protective effect seen at an optimal intermediate concentration range [214, 219]. Selenium is also a focus of interest for putative protective effects against chronic neurodegenerative disease [220].

Selenium is an antagonist of several toxic metals, including mercury, cadmium, chromium and others [221]. Selenium interacts with cadmium *in vivo* to reduce its toxicity; selenium has been posited as a breast cancer protectant if present in the body at concentrations sufficient to antagonise the deleterious effects of cadmium, although such benefits may be negated in the event of a relative selenium deficiency whereby excess cadmium remains biologically active [222].

While selenium is an essential trace nutrient, it is toxic in excess; chronic selenosis occurs in some areas, particularly in China, where soils are naturally enriched with selenium [223]. Acute selenium intoxication, which may be fatal, is usually associated with accidental or deliberate ingestion of excess selenium in dietary supplements [224, 225].

Nevertheless, selenium deficiency is undoubtedly a public health problem in some parts of the world, with profound consequences arising from overt deficiency manifesting in neurological, cardiac, skeletal and thyroid dysfunction [226-230]. Sub-clinical selenium deficiency is reportedly widespread; estimates of the number of individuals experiencing sub-optimal selenium intake range between 50 million to 1 billion, including many in developed countries across Europe because of selenium-depleted soils [228].

2.3.3. Dietary selenium: recommended and maximum intakes

For their health risk assessment of selenium intake from contaminated Lake Macquarie fish, Dalton & Bird presented levels of 5 - 7 µg selenium per kg body weight per day as, variously, “maximal daily safe

intake", "reference dose" and "minimal risk level" as assessed by various public health and food safety authorities [231]. The US recommended dietary allowance is 55µg selenium per day for adult males and females, which represents a dose of 0.72 - 0.9 µg per kg body weight per day, based on the US reference weights of 61kg for adult females and 76kg for adult males [232]. Australian recommended dietary intakes are 60 µg selenium per day for adult women and 70 µg per day for men. Australian reference weights are as for the USA; Australian RDIs therefore represent doses of 0.92 - 0.98 µg selenium per kg per day [233]. Calls have been made for recommended intakes to be raised in order to achieve protective benefits against cancer, to 200 - 300 µg per day [221, 228]. Daily intakes of 300 µg selenium per day, equating to doses of 3.9 - 4.9 µg selenium per kg body weight per day based on the aforementioned national reference weights, would seem to lie within defined safe maximum exposure levels.

2.3.4. Selenium in seafoods

Seafoods, particularly fish, are often presented in the biomedical literature as "good" "excellent" or "rich" sources of selenium, see for example [228, 234, 235]. Some reported concentrations of selenium in seafoods are 2mg/kg (approx) dry weight in raw lobster muscle [236], 2mg/kg dry weight in fresh oysters [237] (2mg/kg dry weight = 0.5mg/kg wet weight, assuming 75% water content) and 0.09 - 0.26mg/kg in fat tissues of various fish [235]. A mean concentration of 1.2mg/kg selenium was measured across a range of whole fish tissue homogenates; this was reportedly an excess concentration of selenium, as the lake they were captured from – Lake Macquarie in NSW – was subject to metals contamination associated with industrial activities [231]. Subsequent studies have shown levels of up to 9.3mg/kg dry weight in muscle of dusky flathead (*Platycephalus fuscus*) from Lake Macquarie [116]. Assuming a 75% water content, this would equate to 2.3mg/kg wet weight. Selenium contamination in the Lake Macquarie system is thought to be caused by fly ash deposition from a nearby coal-fired power station [116].

Examples of reported concentrations in brachyuran and homarid crustaceans are: up to 1.3mg/kg wet weight in "edible tissues" of crab [212], and mean 1.8mg/kg wet weight in raw lobster hepatopancreas [236]. Selenium is apparently found in highest concentrations in lobster hepatopancreas [236]. Examples of reported levels of selenium in prawns are 0.16 - 0.23mg/kg in raw edible tissues of "regular" prawns, and 0.08 - 0.42mg/kg in tiger prawns [212]. Higher concentrations were reported in northern deep sea prawns (*Pandalus borealis*) captured off Greenland, with geometric means of up to 1.8mg/kg wet weight selenium in whole animals [152]. Bay prawns (*Metapenaeus bennettae*) captured from Lake Macquarie had mean concentrations of 3.6mg/kg dry weight measured in muscle tissue [116]. Assuming a 75% water content, this would equate to 0.9mg/kg wet weight.

3. METHODS

3.1. Australian wild-capture prawn fisheries

To determine the relative contribution of the various state and commonwealth managed prawn fisheries to the Australian wild-capture prawn fisheries, Australian fisheries production data published by ABARES was accessed in spreadsheet format: http://adl.brs.gov.au/data/warehouse/pe_abares20110830.01/AFS_Production_rev20110905.xls

Fishery production in tonnes was tabulated for each of ten years, for each of Australia's prawn fisheries from 2000-2001 to 2009-2010. The average production over the ten-year period was then determined for each fishery. These data are presented in Figure 1.

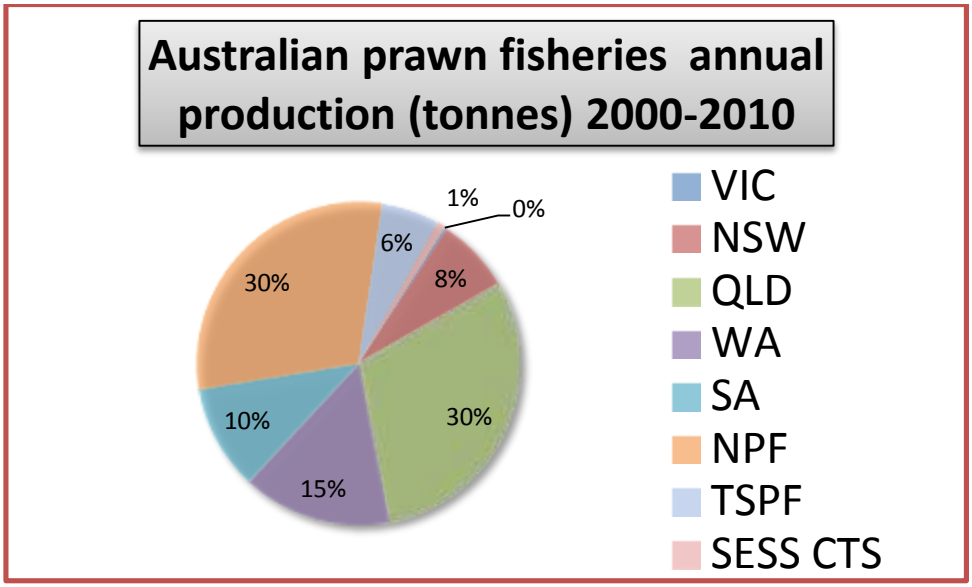


Figure 1. Relative prawn production by Australian fisheries. Graph comprises mean production in tonnes for each fishery, years 2000-01 to 2009-10 (NPF: Northern Prawn Fishery; TSPF: Torres Strait Prawn Fishery; SESS CTS: Commonwealth Trawl Sector of the Southern and Eastern Scalefish and Shark Fishery).

The prawn fisheries of NSW, Victoria, Torres Strait and SESS Commonwealth Trawl Sector collectively accounted for 15% of production over the ten-year period; these minor fisheries were therefore not incorporated into this survey. The four main fisheries, which collectively represented 85% of production, formed the scope of the survey. The Commonwealth-managed Northern Prawn Fishery and the Queensland East Coast prawn fishery are Australia's most productive fisheries, each accounting for 30% of Australia's wild-capture output. Western Australia and South Australia, representing 15% and 10% respectively of Australia's catch, completed the fisheries to be surveyed for this project.

Relative production across the four surveyed fisheries is presented in Figure 2.

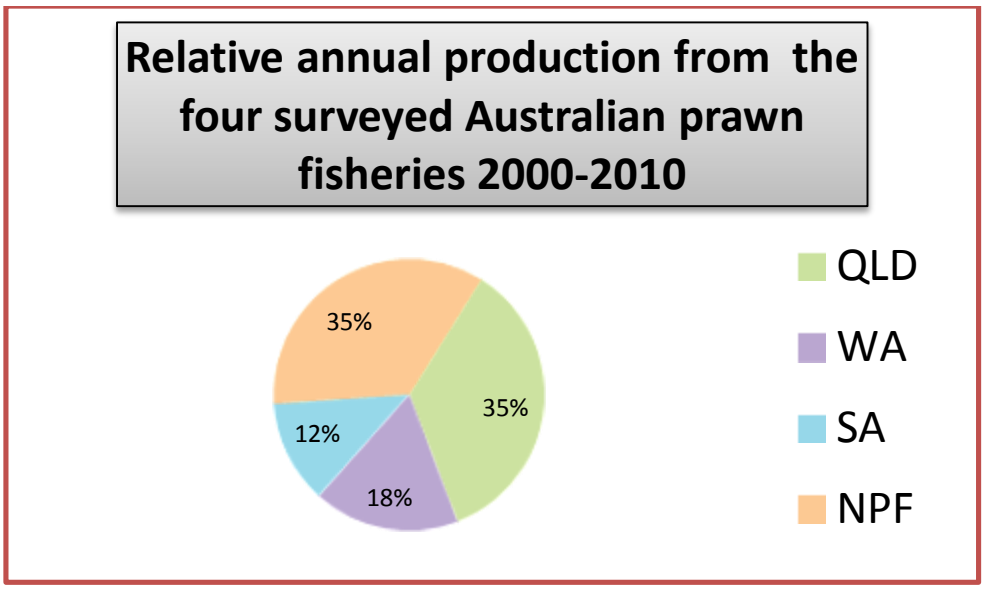


Figure 2. Relative production across the four surveyed Australian prawn fisheries.

3.2. Sample size estimates

In order to determine sample sizes for this survey, a large historical dataset was interrogated. This dataset comprised 2494 samples of Australian prawn tissues analysed for cadmium between 1991 and 2010. These analyses were conducted for a range of reasons on behalf of various organisations, including the National Residue Survey (Commonwealth Department of Agriculture, Fisheries and Forestry) and the Australian prawn fishing industry. The European Commission regulatory level of 0.5mg/kg cadmium in prawn tissue was adopted as the indicator level for sample size considerations. 10.1% (*n*=252) of samples were measured at greater than 0.5mg/kg cadmium.

Using the formula $n = \frac{t^2 pq}{d^2}$ in sampling for proportions, as described by Cochran [238], sample size estimates for the survey were calculated, where:

n = sample size estimate

t = abscissa of the normal curve that cuts off an area at the tails corresponding to the required level of confidence, i.e. *t* = z-score of 1.96 for 95% confidence; *t* = z-score of 2.58 for 99% confidence

p = estimated proportion of the outcome of interest in the population, expressed as a decimal

q = 1-*p*

d = maximum tolerable error, expressed as a decimal

Substituting for the requirements of this survey:

t = 1.96 (95% confidence)

p = 0.101 (10.1% of samples >0.5mg/kg from available dataset of prawns analysed for cadmium)

d = ±0.05

So for $n = (t^2 * p * (1-p))/d^2$

We get n = (3.84*0.101(0.899))/0.0025)

= 139

Applying this sample size estimate of n = 139 to the proportional production of the four fisheries to be surveyed, we arrive at the following distribution of samples to be collected from each fishery, as presented in Table 2.

Table 2. Sample numbers to be collected from each prawn fishery, as directed by calculated sample size estimates

FISHERY	Proportion of total productivity (%)	Number of samples
Northern Prawn Fishery	35.0	49
Queensland East Coast	35.3	49
South Australia	12.3	17
Western Australia	17.4	24
TOTAL	**100**	**139**

3.3. Random sampling program.

So that an unbiased sampling regime could be applied, a spreadsheet-based random sampling program was developed; the program was run separately for each of the four fisheries to be sampled. Each program was weighted for the mean fishery production over a five year period. Production linked to geographically-defined locations within each fishery was tabulated for each financial year between 2006/07 and 2010/11. Spatially-defined production data varied across the four fisheries; the various approaches are discussed below:

3.3.1. Northern Prawn Fishery:

This fishery is divided spatially by 1^O cells (111km), as shown in Figure 3.

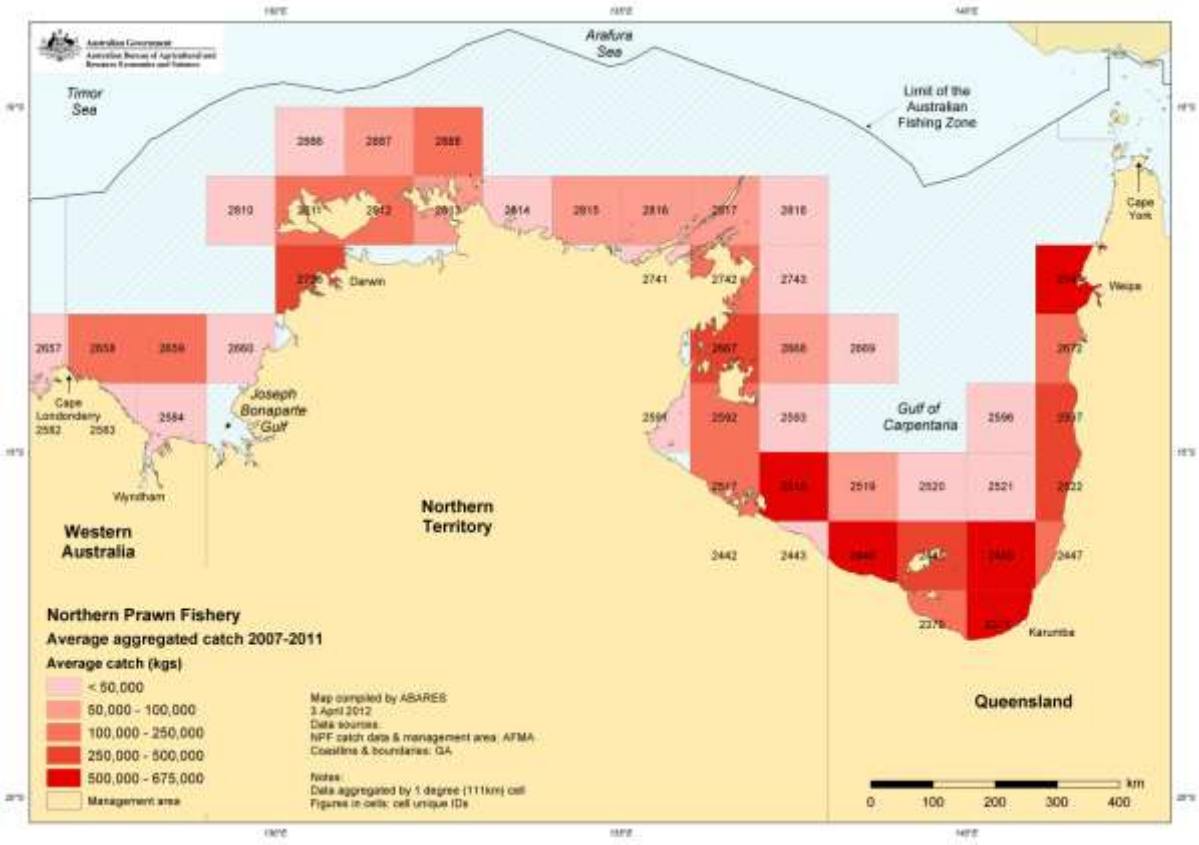


Figure 3. Northern Prawn Fishery grid map

A request was made to ABARES for spatially-defined prawn production data; ABARES provided a spreadsheet with average production (in tonnes) from each of the cells shown in Figure 3 for the period 2007-11. Those data were also presented to us graphically by ABARES in the map shown as Figure 3 above.

3.3.2. Queensland East Coast Fishery

Queensland's fisheries are represented spatially by a 30 nautical mile grid, as shown in Figure 4.

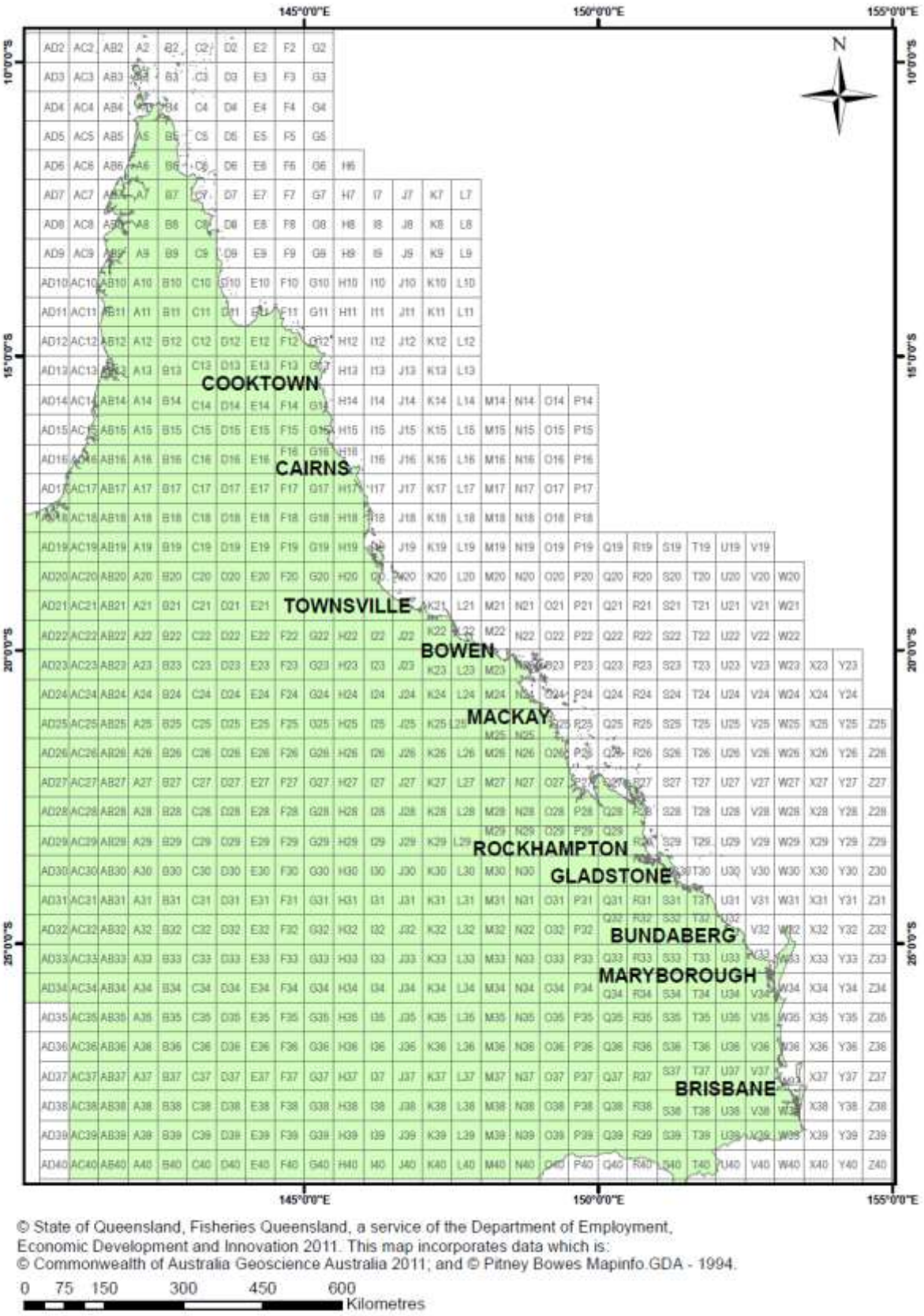


Figure 4. Queensland fisheries spatial grid map

A request was made to Fisheries Queensland, Department of Employment, Economic Development and Innovation (now Fisheries Queensland, Department of Agriculture, Fisheries and Forestry) for spatially-defined prawn production data. This was provided by Fisheries Queensland in spreadsheet form, from 2006 to 2010, and included production data for each commercial prawn species at each grid location.

3.3.3. South Australian Prawn Fishery

South Australia's prawn fisheries are represented spatially by a combination of square, rectangular, rhomboid and geometrically heterogeneous grids, as shown in Figure 5. The South Australian fishery is based on a single commercial species, the western king prawn (*Penaeus latisulcatus*). A request for spatially-linked prawn production data was made to SARDI Aquatic Sciences. These data were provided for the five years from 2006-07 to 2010-11.

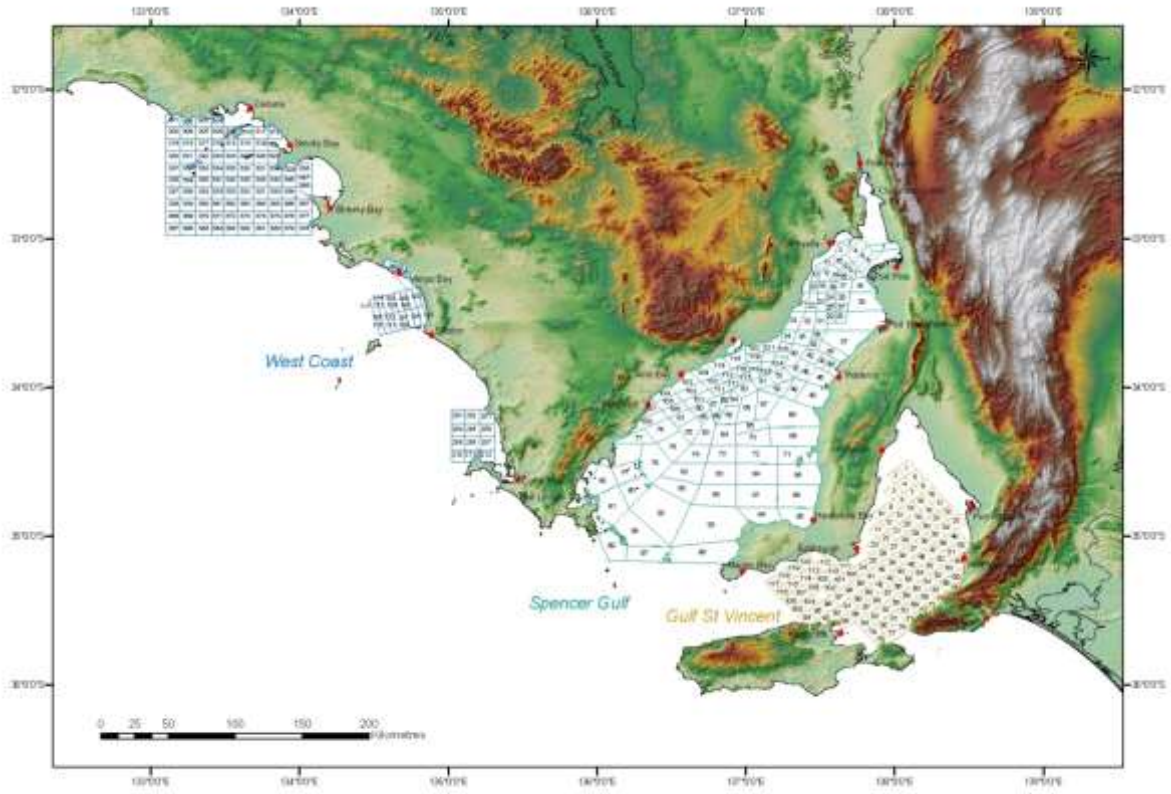


Figure 5. South Australian prawn fisheries

3.3.4. Western Australia prawn fisheries

Western Australia has seven commercial prawn fisheries, of which Shark Bay and Exmouth are the largest: http://www.fish.wa.gov.au/Species/Prawn/Pages/Prawn-Commercial-Fishing.aspx

A data request for spatially-delineated fishery production was made to the Western Australia Department of Fisheries. Production data for the various commercial species landed from the Shark Bay and Exmouth Gulf fisheries were provided for years 2006 to 2010 inclusive. Annual production from Shark Bay and Exmouth was subtracted from total annual production for WA, as reported in ABARES summary statistics; the differential annual catch figures were then designated to a category "Other" (representing the minor commercial prawn fisheries outside of Shark Bay and Exmouth) for the purposes of random sample allocation. Figure 6 shows the five-year average production by species from the Shark Bay and Exmouth fisheries, data for which initialised the WA random sampling program. As seen in Figure 6, the minor prawn fisheries outside of Shark Bay and Exmouth collectively accounted for only 18 tonnes of catch and were not selected for sampling by the random sampling program.

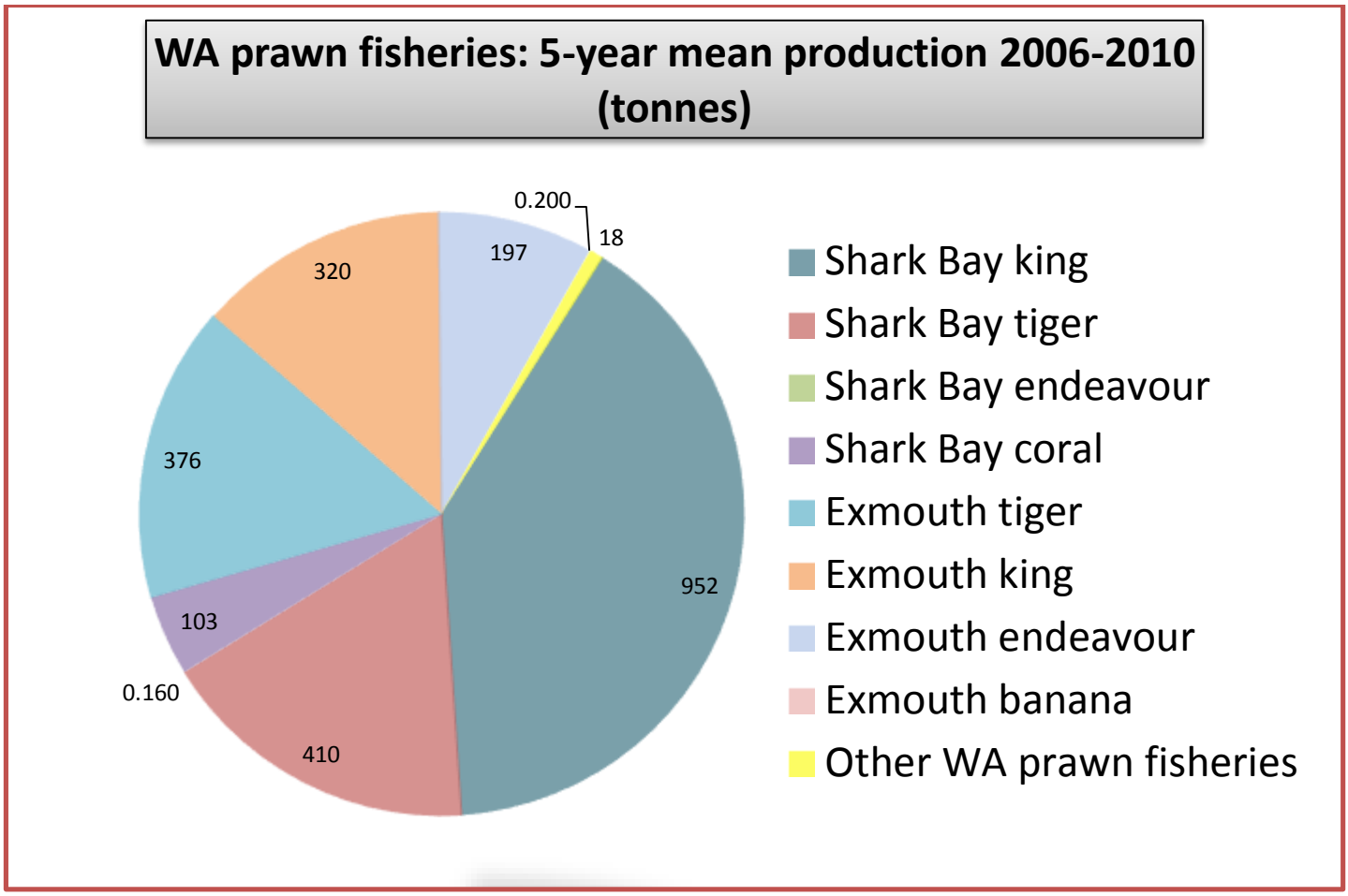


Figure 6. Western Australia prawn fisheries average annual production from 2006-2010

3.4. Random sampling program design

Random sampling programs were run separately for each of the four State and Commonwealth prawn fisheries. Sampling programs were designed in MS Excel spreadsheets using the random number generation feature in Excel's "Data Analysis Toolpak." Prawn species and/or geographic locations were

randomly allocated for sampling using a "sampling with replacement" strategy, with selection probability proportional to 5-year average production. This random sampling program weighted for prior production has the advantage of selecting an unbiased sample that is representative of recent production. Each previously-fished species and/or location had a non-zero probability of being selected for sampling.

3.5. Collection of samples from the Australian prawn fisheries

The randomly-selected sampling sites and species represented "ideal" sampling strategies from each of the fisheries. However, other considerations impacted on our ability to secure samples from each and every randomly selected location. These considerations broadly pertained to changes in fishing practices at specific locations, whereby the economics of the industry can change rapidly such that some intensively-fished areas in previous years can be temporarily vacated due to constraints such as cost of fuel and the Australian dollar exchange rate. Seasonal availability of particular prawn species also necessitated some deviation from our ideal sampling regimes. The particular variations in sampling from each of the four fisheries are discussed below:

3.5.1. South Australian prawn fisheries:

Samples were collected by SARDI Aquatic Sciences research workers, who conduct stock assessment surveys across the fisheries 2-3 times each year. Stock assessment staff undertake survey shots at pre-determined locations across the fisheries. Sites to be fished were compared with this survey's random sampling program, which had identified *n*=16 samples to come from Spencer Gulf, plus *n*=1 sample from Gulf St. Vincent. Two samples were randomly selected from map cell 57 in Spencer Gulf, but that site was not a routine stock assessment survey location. An adjacent cell (#56) was selected by spinning a pencil on a printed copy of the map. The sample requested from Gulf St Vincent could not be located in the SARDI Aquatic Sciences cold store following the April 2012 stock assessment survey, and that fishery was closed for 12 months commencing December 2012 for stock conservation purposes. Therefore a convenience sample of prawns from site 14 in Spencer Gulf comprised the final outstanding sample from the SA prawn fishery.

3.5.2. Western Australia prawn fisheries:

Random sample allocation identified samples from the Shark Bay and Exmouth Gulf fisheries only. Samples of all requested species and locations (*n*=24) were collected and shipped by officers from the Western Australia Department of Fisheries. Because we did not have spatially-defined production data from within the Shark Bay and Exmouth fisheries, we requested multiple samples of designated species from different locations within each fishery, at the discretion of WA Fisheries staff. Samples were provided to SARDI with latitude and longitude co-ordinates to designate exact capture locations. As with the South Australian fisheries, WA government fisheries workers conduct stock assessment surveys, so sample collection for the purposes of this survey was uncomplicated.

3.5.3. Northern Prawn Fishery:

Fishery stock assessment in the Gulf of Carpentaria is conducted by CSIRO Wealth from Oceans Flagship staff. Prawn samples for this project were provided by CSIRO from their routine survey work in the Gulf. Samples from the NPF outside of the Gulf, as well as some sites within the Gulf selected by the random sampling program that were not covered by the CSIRO stock assessment survey, were provided with assistance from the Northern Territory prawn fishing industry. No spatially-linked species-specific data was available from ABARES for the NPF; where multiple samples were requested from 1^{O} map cells, sample collectors were asked to take samples of different prawn species from the same location, or the same species from different locations within individual map cells, or different species from different locations within individual cells.

3.5.4. Queensland east coast prawn fisheries

Fishery-independent prawn stock assessment is not conducted in Queensland, therefore samples from the east coast fisheries were provided with industry assistance. Prawn samples were collected and shipped to survey headquarters at SARDI by a broad range of Queensland seafood industry operators, including large and small trawler operators, seafood distribution agencies, wholesalers and retailers.

3.6. Sample collection protocol

Samplers were asked to collect prawns for each sample according to the following instructions:

- Each sample to comprise 10 - 12 prawns, or approximately 200g in the case of small prawns
- Each sample double-bagged in zip-lock food-grade plastic bags
- Each sample labelled for date of capture, location and species
- Samples kept frozen until consignment ready for freighting to SARDI
- Samples wrapped in chill-wraps, shipped in styrofoam containers to SARDI by overnight air freight
- Samples to be collected, stored and shipped so as to avoid contamination by dust, sand or other extraneous material

3.7. Cooking of prawns in the laboratory

Prawn samples as received at SARDI were of both green (i.e. raw) and cooked types. Prawns sampled through the various State and Commonwealth stock assessment surveys were all of the green variety. However, samples provided through industry assistance were collected at various points of the supply chain (either direct from trawlers, or from wholesalers and retailers when the proprietors of those facilities were able to identify capture locations). Prawns marketed in cooked form are often prepared on trawlers immediately following capture. In order to reduce or eliminate variability in cadmium concentration due to cooking effects, all samples of green prawns were cooked in the SARDI laboratory prior to disassembly. Green prawn samples were cooked in the laboratory under conditions

that closely matched protocols for on-vessel processing, as described in an ISO Best Practice manual for onboard processing of prawns [239].

The laboratory cooking protocol was as follows:

- Cooking and cooling vessels, and all handling utensils were stainless steel
- 3L of tap water into each vessel, to which 105g food-grade NaCl was added to each (to approximate seawater at 3.5% NaCl). Water stirred to dissolve salt
- One vessel heated to vigorous boil
- Sample of green prawns ($n \leq 8$) added to boiling water
- Large prawns (average weight >10g) cooked for 60 seconds (using an electronic timer); small prawns cooked for 30 seconds
- Sample removed from cooking water with a stainless steel sieve, transferred to the second vessel containing mock seawater at ambient temperature
- Cooked prawns cooled for 60 seconds
- Cooled prawns placed on a tray prior to disassembly, vessels and utensils washed, rinsed and dried

3.8. Sample preparation

Analysis of prawns for cadmium and selenium was conducted on de-veined muscle meat, as per European Standard EN 13804:2002 which states that: “In crustaceans, the visible digestive tract shall be removed prior to analysis. The edible part prepared from the laboratory sample shall be free from remaining shell fragments or parts of exoskeleton.” [240].

Prawn sample preparation was conducted in the SARDI laboratories, according to the following protocols:

- Sample transferred from -20^{O}C to 4^{O}C approximately six hours prior to processing
- Each batch sample was defined as $n \leq 8$ prawns, as removed consecutively from the received laboratory sample. Prawns in excess of $n = 8$ for batch samples were returned to frozen storage. In the event that laboratory samples comprised small prawns such that $n = 8$ prawns weighed less than 100g, those batch samples were formed from all prawns received as the laboratory sample.
- The batch sample was arranged on an anodized tray with its unique identifier code, then photographed with a digital camera for subsequent confirmatory species identification.
- Individual prawns comprising each batch sample were weighed on a bench-top balance, rounded to 0.1g
- Prawns comprising each batch sample were disassembled and separated into five pooled compartments: carapace, including pleopods and telson; head, including pereopods, stomach and hepatopancreas; abdominal muscle; intestine and hindgut; liquid hepatopancreas. The latter was aspirated with a Pasteur pipette from the stainless steel receptacle after each batch sample disassembly. Care was taken to remove intestines *en bloc* whenever possible by making a superficial incision along the anterior midline and releasing transverse fasciae. When gut contents were freely mobile (i.e. not retained within the gut lumen on exposure) visible

gut material was carefully removed from the muscle compartment with a scalpel blade or stainless steel spatula and added to the intestine compartment of the batch sample. Figures 7 - 9 show removal of prawn intestinal tracts from muscle tissues. Figure 10 shows a completed specimen ready for homogenisation.

- Separated batch sample components were weighed.
- Abdominal muscle was homogenised to a paste consistency, using a domestic hand-held blender (Russell Hobbs 650W).
- Pooled batch sample compartments were placed in sealed containers (carapaces and heads in separate food-grade polyethylene bags; muscle homogenate, gut tubes and liquid tomalley into polypropylene containers). Approximately 20g of muscle paste from each batch was spooned into a 50mL polypropylene centrifuge tube to facilitate bulk shipment to the contracted analytical laboratory. All containers were stored at -20^{O}C.

Figure 7. Exposure of alimentary tract during dissection

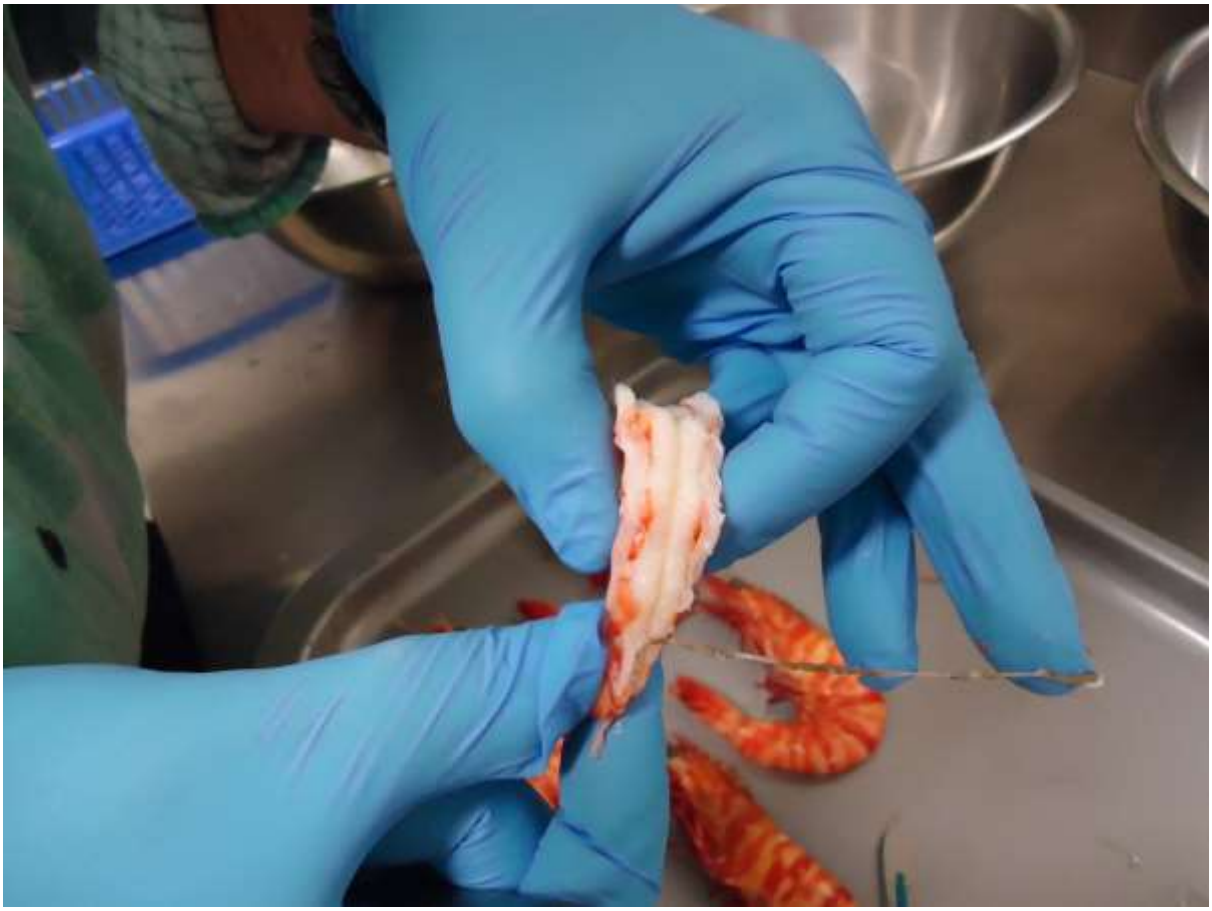


Figure 8. Removal of alimentary tract from muscle tissue

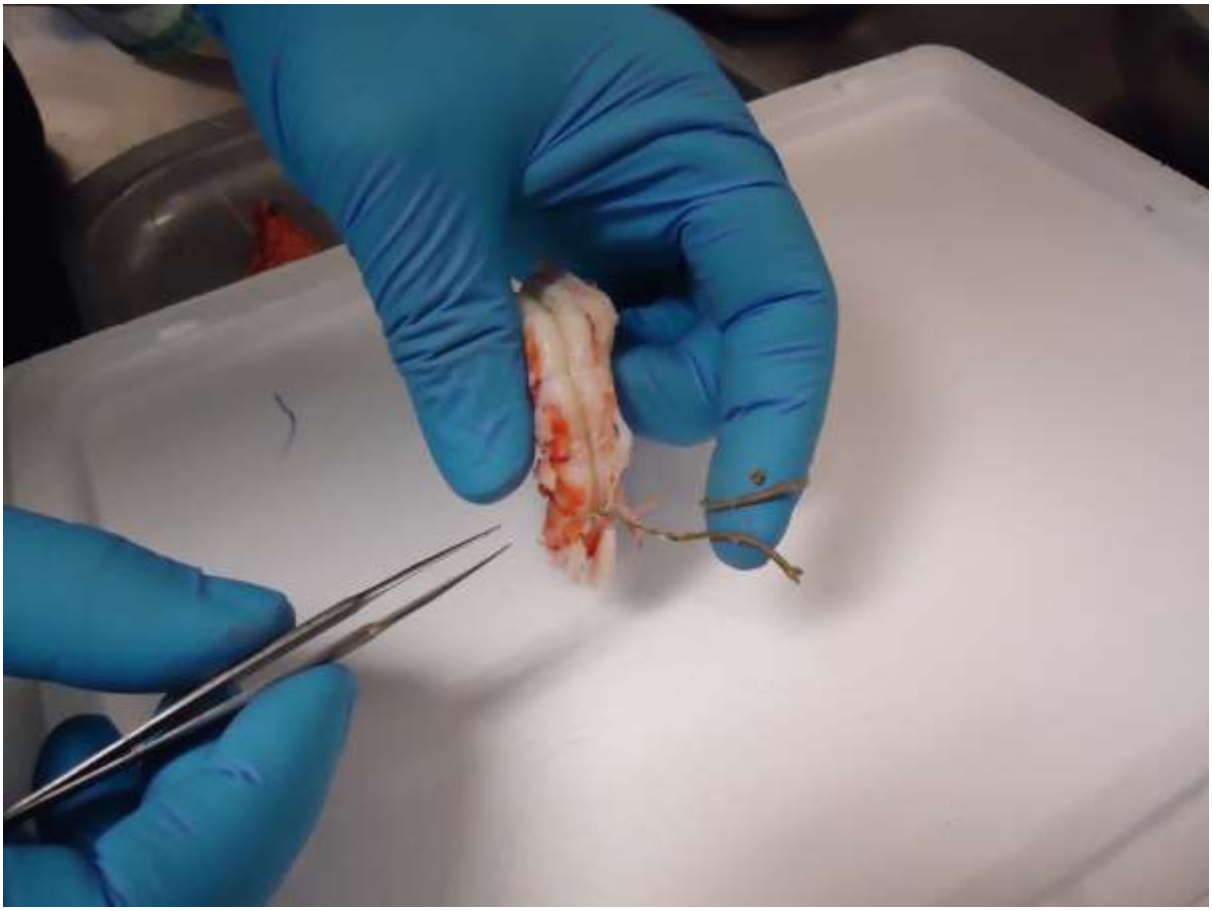


Figure 9. Removal of alimentary tract

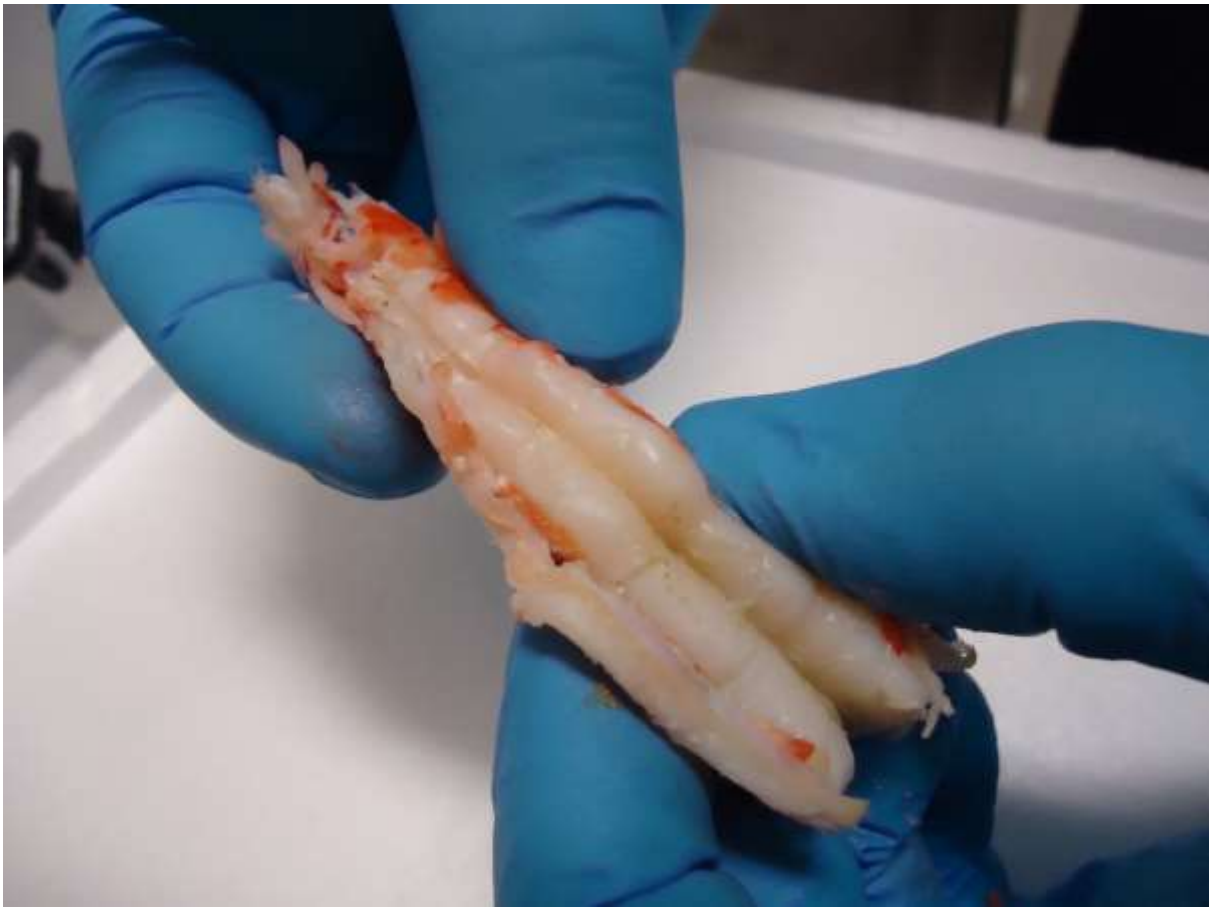


Figure 10. Prawn muscle with alimentary tract removed

3.9. Sample analysis for cadmium and selenium

A call for provision of analytical services was issued through SARDI's tender process, to conduct analysis of prawn tissues for cadmium and selenium. Providers were invited to address specifications related to analytical methods and instrumentation, quality assurance procedures and turnaround times, as well as general information regarding their laboratory's experience with metals analysis in biological matrices, chain-of-custody documentation, professional insurance covers and results reporting.

A contract for provision of analytical services was awarded to Advanced Analytical Australia P/L in February 2013. Advanced Analytical Australia (AAA) satisfactorily addressed the selection criteria, and also quoted the most cost-effective schedule of service. AAA's technique uses a method based on USEPA Method 200.8 for trace element analysis by acid digestion and inductively-coupled plasma-mass spectrometry (ICP-MS) [241]. AAA has a modern ICP-MS instrument (Varian 820-MS) and access to appropriate matrix-specific reference materials. Advanced Analytical Australia participates in a range of proficiency studies as a requirement of their NATA accreditation and ISO/IEC 17025 compliance.

Concentrations of cadmium and selenium in the samples were quantified as received on a wet weight basis, using an external standard method. ICP-MS instrument drift as well as suppression or enhancement of instrument response caused by the sample matrix was corrected for by the use of internal standards (i.e. using Bi, Ho, In, Li, Rh, Sc, Tb, and Y on ICP-MS), which cover the whole mass range of metals to be tested on ICP-MS. During sample analysis, quality of metals analysis was evaluated by assessing values of sample preparation blanks and laboratory control standards at a rate of 1 every 20 samples, sample duplicates at a rate of 1 every 10 samples, matrix spikes at a rate of 1 every 20 samples, and continuing calibration verification standards. AGAL-3 certified matrix reference material was tested with every batch of samples run on ICP-MS.

3.9.1. Supplementary tissues analysis and additional quality assurance

To investigate the contribution of alimentary tract contents to prawn muscle cadmium and selenium concentrations, experiments were conducted in which a sub-set of prawn muscle homogenates were re-analysed with and without the gut tubes. Cadmium and selenium concentrations were also measured in a sub-set of hepatopancreas tissues and (separately) complete alimentary tracts. These supplementary experiments were also designed to serve as additional quality assurance by being conducted in triplicate (or duplicate in cases where insufficient tissue was available for reanalysis in triplicate).

From the initial cadmium analyses conducted on prawn muscle homogenates without alimentary tracts, three groups of samples were identified:

1. Low cadmium: initial measurement ≤0.1mg/kg
2. Intermediate cadmium: initial measurement >0.1mg/kg to 0.5mg/kg
3. High cadmium: initial measurement >0.5mg/kg

Seven samples from each of the low and intermediate cadmium groups were randomly selected for muscle + gut tube analysis, using the "RAND" pseudo-random number function in Excel. As only six prawn samples were detected with cadmium levels greater than 0.5mg/kg, those six samples were to comprise the "High cadmium" group for this experiment. The actual experiment was conducted with eight samples in the "Low cadmium" group, seven "Intermediate cadmium" samples and five "High cadmium" samples. The discrepancy was due to an internal mis-communication, whereby the sample ID number for the intended 6th "High cadmium" sample was read incorrectly, and the actual sample retrieved from freezer storage for preparation of homogenates was in the "Low cadmium" group.

The procedure for each of the *n* = 20 prawn samples undergoing muscle + gut tube analysis was as follows:

- Muscle homogenates, alimentary tracts and hepatopancreas tissues were removed from freezer storage.
- 30.0g of muscle homogenate was weighed.
- Alimentary tract tissue was accurately weighed according to the proportional weight of alimentary tracts from the whole sample, as determined at the initial sample disassembly and preparation.

EXAMPLE: a sample of eight prawns weighing 200.4g (mean prawn weight = 25.05g) had 80.4g of muscle and 0.70g alimentary tracts recovered after sample disassembly. For preparation of muscle + gut tube homogenates, 30.0g of muscle homogenate was weighed, and (30/80.4)*0.7 = 0.26g alimentary tracts was weighed.

- Weighed gut tube components were oven-dried at 60^{O}C for 3 - 4 hours
- Dried gut tubes were powdered with a glass mortar & pestle
- Gut tube powder was added to the 30g muscle homogenate, taking care to fold the powder into the homogenate with a spatula, ensuring that powder was not retained on the walls of the receptacle
- Muscle homogenate with added gut tube powder was then thoroughly re-homogenised.
- Some samples required added water in order to produce a consistent, homogenous paste. Milli-Q water in 2.0mL aliquots was added; samples requiring additional water required either 4mL or 6mL in order to produce the required consistency. Added water was accounted for in subsequent calculations of tissue weight concentration of cadmium, whereby 1mL water equates to 1g tissue.

In order to determine the contribution of added alimentary tract tissues to cadmium concentrations, muscle homogenates from each sample were also sent for re-analysis at the same time (i.e. muscle homogenate free of alimentary tracts, as prepared during initial prawn disassembly and sample prep).

Wet hepatopancreas (HP) tissues were weighed, then oven-dried at 60^{O}C until stable weights were achieved. Dried HP was powdered in a mortar and pestle.

To determine the cadmium concentration of isolated alimentary tracts, seven prawn samples were randomly selected and the entire gut tube component was oven-dried and powdered as described above.

For additional quality assurance considerations, the following procedures were undertaken:

- Muscle + gut tube homogenates, and muscle-only homogenates were prepared in triplicate at the SARDI laboratory. In some cases where insufficient tissues were available for triplicate analysis, duplicates were prepared. Samples were allocated a randomly-generated three digit code before being shipped to the analytical laboratory. Therefore all analyses of SARDI-prepared replicate homogenates were analysed blind by AAA, such that the sequence of replicates was disrupted within the entire batch. Altogether, n = 103 samples were sent for blinded replicate analysis, comprising n = 32 samples analysed in triplicate, n = 3 samples analysed in duplicate, and n = 1 sample re-analysed as a single muscle-only homogenate (due to insufficient remaining tissue) for comparison with that sample's triplicate muscle + alimentary tract homogenates.
- To test the consistency and homogeneity of SARDI-prepared replicate tissue samples, seven samples had an additional prawn muscle-only sub-sample prepared, in which 20 - 30g of homogenate was sent to the analytical laboratory, with instructions for their laboratory to run those samples in triplicate.

  Results of replicate analyses for cadmium and selenium were separately compiled for calculation of relative standard deviations (RSDs). RSDs were calculated only for samples in which all replicate cadmium levels were greater than the detection limit (in order to avoid bias

in favour of lower RSDs in the case of replicate values below detection limit being assigned the same value). To compare the effectiveness of homogenate preparation by the two laboratories, RSDs of replicate samples prepared by SARDI (i.e. blinded allocation of random codes to muscle + gut tube homogenates and muscle-only homogenates) were compiled as a single group. These were compared to the RSDs of muscle homogenates prepared and analysed in triplicate by AAA. RSDs for replicates (duplicate and triplicate) of samples grouped into a single category (e.g. triplicate analysis of *n* = 7 samples by AAA, i.e. 21 measurements) were calculated and presented as single values. The results are denoted by $r_{ij}$for sample $i = 1, \dots, s$ and replicate $j = 1, \dots, n_i$ where s is the total number of samples and n_i is the number of replicates for sample i.

We calculate the formula:

$$R_{ij} = \frac{r_{ij}}{\frac{1}{n_i}\sum_{j=1}^{n_i} r_{ij}}$$

and the RSD is then given by the formula:

$$RSD = 100 \times \sqrt{\frac{1}{N}\sum_{\substack{i=1,\dots,s \\ j=1,\dots,n_i}} (R_{ij} - \mu)^2}$$

where

$$\mu = \frac{1}{N}\sum_{\substack{i=1,\dots,s \\ j=1,\dots,n_i}} R_{ij} \qquad N = \sum_{i=1}^{s} n_i$$

and $\mu = 1$

3.10. MOISTURE CONTENT OF PRAWN MUSCLE TISSUES

In order to accurately determine the moisture content of prawn muscle, homogenates from three prawn species (*P. indicus*, *P. latisulcatus* and *P. esculentus*) were accurately weighed in triplicate, then refrozen and lyophilised for five days in an industrial freeze-dryer (Cuddon 1015FD, Blenheim, NZ).

Prawn hepatopancreas moisture content was determined by oven drying, as described above (Section 3.9.1).

3.11. Statistical analysis of cadmium and selenium in prawns

Summary statistics, charts and inferential tests were conducted and prepared using MS Excel 2007, GraphPad Prism v5.04, and the statistical program R (R Core Team, v.3.0.2, 2013).

The outcome variables of cadmium and selenium in prawn muscle were tested separately against the following explanatory variables:

- Fishery (SA, QLD, WA, NPF)
- Genus
- Species
- Season: we used a two-season approach applicable to the tropical Top End (dry season from May to September, wet season from October to April) for prawn sample collection dates.
- Mean prawn weight: average weight of prawns in each sample prior to disassembly was used as a measure of prawn size.

Preliminary investigations were conducted with correlation coefficients to explore statistical dependence, and a series of two-group and multi-group hypothesis tests on specific variables and variable sub-sets. Tested values were submitted to D'Agostino-Pearson omnibus normality tests in order to assess the assumption of normality and hence determine the most appropriate statistical test. Several variable sub-sets, particularly for cadmium / species comparisons, failed normal distribution tests. Cadmium data were then $\log_{10}$ transformed, and resulting residuals were plotted to examine their distribution. Residuals were found to be approximately normally distributed (see Figures 22 & 30), so all subsequent hypothesis tests were conducted on $\log_{10}$-transformed data. Statistical significance was set at the 95% confidence level (p <0.05). Unless stated otherwise, all p-values are from two-tailed tests.

Unless otherwise indicated in figure legends and y-axis scales, box & whisker plots and correlation plots are presented with untransformed cadmium and selenium concentration data, for ease of visual interpretation, but all associated test statistics and p-values were derived from hypothesis tests on $\log_{10}$-transformed data.

A multiple regression model was then developed to determine the contribution to the variability in measured cadmium and selenium prawn muscle concentrations attributable to each explanatory variable. Stepwise AIC (Akaike information criterion) was applied in order to determine the most suitable regression model.

One-tailed paired t-tests were conducted to test the null hypothesis that prawn cadmium levels are not significantly higher when muscle tissue homogenates have the alimentary tracts intact. To investigate the effectiveness of replicate re-analyses, two-way paired t-tests were used to compare mean cadmium and selenium levels measured on homogenates prepared with and without alimentary tracts, and prepared by both laboratories (AAA and SARDI).

Twenty-eight samples from the survey (i.e. 20%) were found to have cadmium concentrations below the analytical reporting limit of 0.02mg/kg. We considered the various approaches discussed by Lubin *et al* [242] for the statistical evaluation of measurement data affected by detection or quantification limits. We populated these variables with fill-in values of zero (which would tend to bias results towards lower concentrations – a "best-case" scenario) and ran the regression model. We then

repeated the analysis with fill-in values of 0.02mg/kg (the "worst-case" scenario). No changes in inference and statistical significance were observed, so the final model was run with 0.5*LOQ fill-in values of 0.01mg/kg cadmium. This 0.01mg/kg fill-in value was also adopted for the two-group and multiple-group hypothesis tests, and the data that populated box & whisker and correlation plots. No prawn samples were found to be below reporting limits for selenium in muscle homogenates or hepatopancreas tissues. Two of seven analyses of isolated prawn alimentary tracts were below the LOQ of 0.1mg/kg selenium. Those results were populated with 0.5*LOQ values of 0.05mg/kg selenium for determination of correlation coefficients.

3.12. IDENTIFICATION OF PRAWN SAMPLE SPECIES

State and Commonwealth fishery stock assessment workers and seafood industry contacts were instructed to identify the species of prawns in each sample that they sent to us for this survey. Most of the samples received from fisheries staff were identified by Linnaean binomial nomenclature, whereas the majority of samples from seafood industry sources had common name identifiers. One hundred and thirty-three samples had either binomial or common name identification provided; seven samples were sent to us with no species identification details.

For confirmatory identification, author RK from CSIRO Wealth from Oceans in Brisbane provided expert assistance. Photographs of all 140 prawn samples taken at the sample processing step were edited to remove sample collector species identification details from the ID labels. These photos were then sent to RK, together with capture location information, so his initial identification was done as a blind procedure. RK was asked to rate his species identification from photographs with a three-tier classification:

1: confident

2: less confident

3: impossible to identify

After reviewing author RK's initial identifications and comparing them with sample provider identifications, some samples were re-photographed in order to better capture particular diagnostic features, after which RK was able to review and revise – still as a blinded investigator – his species identifications. The taxonomic keys published by Grey, Dall and Baker [243] were consulted to assist with confirmatory identifications. For tiger prawns, additional photographs facilitated observation of the presence or absence of the postrostral groove and the extent of the adrostral ridge as distinguishing features between *P. esculentus* and *P. semisulcatus*. The presence/absence of movable spines on the telson distinguished *M. endeavouri* from *M. ensis*. For a subset of the samples, these taxonomic features were confirmed at the SARDI laboratory by IS. Final identification of king prawns captured from latitudes south of Mackay to north of Maryborough was confirmed at the SARDI laboratory by author IS, using supplementary light and magnification. These central/southern Queensland waters encompass range overlaps for the Eastern King Prawn (*P. plebejus*) and the Western King Prawn, aka blue-leg prawn (*P. latisulcatus*). An important diagnostic feature that discriminates these two species is the gastro-frontal groove on the rostrum; the distinguishing characteristics on this particular anatomical feature are difficult to determine from photographs.

For identification of prawns in the genus *Penaeus*, we have adopted the recommendations of Ma *et al* and Flegel [244, 245]. These authors refute the reclassification of this genus proposed by Pérez-Farfante and Kensley [246], whereby subgenera were raised to the status of genus. This tradition has also been adopted for our listing of prawn species in Table 1, i.e. we maintain the original binomial nomenclature for the genus *Penaeus*.

4. RESULTS

4.1. PRAWN SAMPLING

Prawn samples were collected between April 2012 and August 2013 from the four surveyed fisheries. Sample collection from South Australia, Western Australia and Northern Prawn Fishery locations within the Gulf of Carpentaria surveyed by CSIRO was relatively straightforward, as we were able to access support from fishery staff that collected samples for this survey during the course of their routine stock assessment work. Sampling from the Queensland East Coast Prawn Fishery and NPF locations west of the Gulf of Carpentaria and within the Gulf but outside of CSIRO's survey sweep was more problematic, and was a significant factor influencing the delayed completion of this project. While most – but not all – industry operators we approached were apparently supportive of the aims of the project, general constraints relating to requirements to align with commercial fishing schedules, and logistical challenges pertaining to our requirements to be able to definitively locate each sample spatially and temporally hampered the progress of survey sampling from these two fisheries. We were not always able to identify trawlers or their catch at all locations that our random sampling program had identified, at times early in the survey period. However, with perseverance and late-stage discovery of particularly supportive industry contacts, we were able to secure a reasonable sampling program from across the fisheries. Table 3 presents the location and number of samples requested and received.

Table 3. Prawn samples requested and received for the 2012-13 survey

Fishery	Requested	Received
NPF	49	49
QLD	49	45
WA	24	29
SA	17	17
TOTAL	**139**	**140**

Discrepancies are seen with the Queensland East Coast Prawn Fishery, where we were not able to secure four samples from requested locations. However, the end result is a reasonable sweep across the fishery, with samples received from north of Cape Melville down the length of the coast to the Southport area, with sampling clusters particularly from the south-east corner of the state, as per the weighted instructions fed into our random sampling program.

We also over-sampled from the Western Australia prawn fisheries. This occurred inadvertently because some samples initially secured for us by WA Fisheries staff were intercepted by another Adelaide-based researcher not involved with this project. WA Fisheries subsequently collected further samples for us from requested sites; after we had received, processed and analysed these later samples we discovered that we had over-sampled.

All samples received were able to be identified to capture date and location from information supplied to us by sample collectors. A small number of samples received (some six to eight) could not be uniquely identified in space and time; in such cases these samples were either not analysed or were pooled with another sample sent by the same collector for which location and date/time information were provided. We are therefore confident that we avoided pseudoreplication; all samples accepted by the survey team were then processed and analysed on an "as received" basis.

Figure 11 shows the proportions of prawn species surveyed.

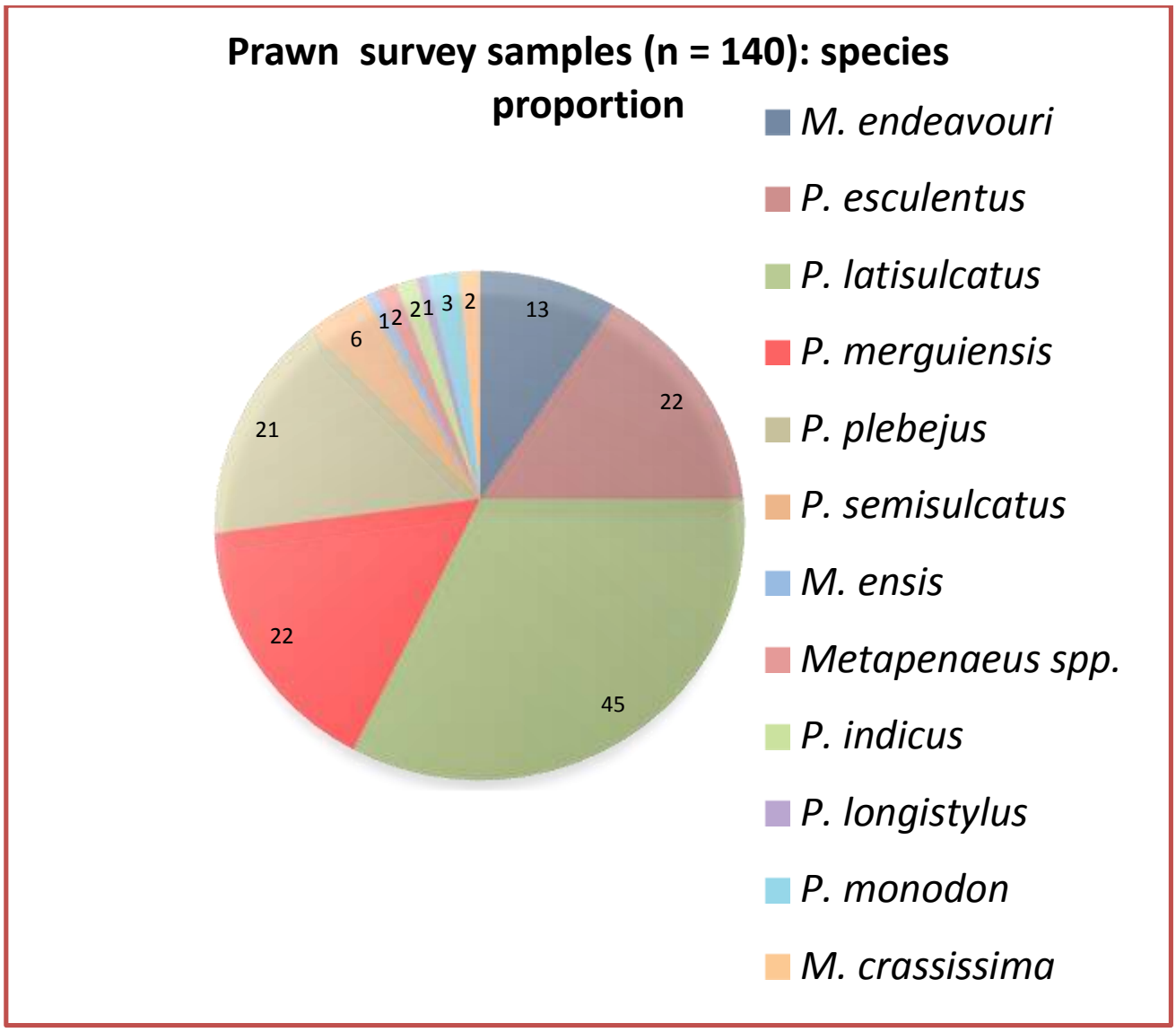


Figure 11. Prawn species surveyed

4.2. Summary statistics: cadmium and selenium analyses

Tables 4 and 5 present summary statistics for the survey, with results of cadmium and selenium analysed in de-veined prawn muscle tissues. Data are also partitioned by fishery and prawn species. Mean cadmium levels in analysed prawn species were seen in the sequence: *M. crassissima* > *M. endeavouri* > *P. indicus* > *P. longistylus* = *P. plebejus* > *P. esculentus* > *P. semisulcatus* > *P. merguiensis* = *P. latisulcatus* > *M. ensis* > *Metapenaeus* spp. > *P. monodon*. However, this sequence should be interpreted with caution for the following species, which were sampled and analysed in low numbers: *P. indicus* (*n* =2), *P. longistylus* (*n* =1), *M. ensis* (*n* =1), *Metapenaeus* spp. (*n* = 2) and *P. monodon* (*n* = 3). While *M. crassissima* was also subject to a small sample size in this survey (*n* = 2), the high cadmium levels detected in both samples deserve separate consideration, given the known capacity of this particular species to bioaccumulate excess cadmium [158, 163].

Mean selenium levels were found in the sequence: *M. crassissima* > *M. endeavouri* > *P. indicus* > *P. latisulcatus* > *P. merguiensis* > *Metapenaeus* spp. > *P. longistylus* > *P. semisulcatus* = *P. plebejus* = *M. ensis* > *P. esculentus* > *P. monodon*. As discussed above, the position in this sequence of species sampled and analysed in small numbers should be interpreted with caution.

For the four surveyed fisheries, mean cadmium concentration in prawn muscle was seen as Western Australia > Northern Prawn Fishery > Queensland East Coast fishery > Spencer Gulf fishery (SA). However, this sequence is somewhat biased because of skewed distributions, in particular the contribution of the two *M. crassissima* samples from Shark Bay in Western Australia, which returned very high cadmium levels. For mean selenium concentration, this sequence was seen as South Australia > Northern Prawn Fishery > Western Australia = Queensland.

Table 4. Summary statistics: cadmium and selenium in prawn muscle tissue, by fishery

		Fishery				
		Survey	**NPF**	**QLD**	**SA**	**WA**
	n	140	49	45	17	29
Cadmium mg kg^{-1} wet weight	Minimum	<LOQ	<LOQ	<LOQ	<LOQ	0.056
	Median	0.08	0.14	0.084	<LOQ	0.11
	Maximum	4.6	1.1	0.32	0.025	4.6
	Mean*	0.16	0.19	0.105	0.012	0.29
	>0.5mg/kg[#]	6	4	0	0	2
Selenium mg kg^{-1} wet weight	Minimum	0.38	0.47	0.38	0.58	0.41
	Median	0.64	0.65	0.61	0.80	0.58
	Maximum	1.0	1.0	0.97	0.95	1.0
	Mean	0.66	0.68	0.61	0.80	0.61

<LOQ: below reporting limit

*: samples reported at <LOQ allocated a cadmium concentration of 0.5*LOQ (= 0.01mg/kg)

[#]: 0.5mg/kg is the EC regulatory limit for cadmium in crustacean muscle

Table 5. Summary statistics: cadmium and selenium in prawn muscle tissue, by prawn species

		Prawn species												
		Survey	***M. endeavouri***	***P. merguiensis***	***M. ensis***	***P. longistylus***	***P. esculentus***	***P. plebejus***	***Metapenaeus***	***P. monodon***	***P. latisulcatus***	***P. semisulcatus***	***P. indicus***	***M. crassissima***
	n	140	13	22	1	1	22	21	2	3	45	6	2	2
Cadmium mg kg^{-1} wet weight	**Minimum**	<LOQ	0.17	<LOQ	0.057	0.16	<LOQ	0.03	0.02	<LOQ	<LOQ	0.046	0.20	0.70
	Median	0.08	0.22	0.03	0.057	0.16	0.125	0.15	0.0205	<LOQ	0.056	0.087	0.26	2.65
	Maximum	4.6	1.1	0.27	0.057	0.16	0.55	0.31	0.021	<LOQ	0.28	0.24	0.32	4.6
	Mean*	0.16	0.405	0.06	0.057	0.16	0.14	0.16	0.0205	<LOQ	0.06	0.12	0.26	2.65
	>0.5mg/kg[#]	6	3	0	0	0	1	0	0	0	0	0	0	2
Selenium mg kg^{-1} wet weight	**Minimum**	0.38	0.58	0.50	0.57	0.63	0.38	0.41	0.62	0.47	0.50	0.47	0.69	0.63
	Median	0.64	0.79	0.685	0.57	0.63	0.54	0.60	0.68	0.48	0.70	0.58	0.79	0.81
	Maximum	1.0	1.0	0.93	0.57	0.63	0.74	0.70	0.74	0.55	0.97	0.66	0.89	0.99
	Mean	0.66	0.79	0.70	0.57	0.63	0.53	0.57	0.68	0.50	0.71	0.57	0.79	0.81

<LOQ: below reporting limit

*: samples reported at <LOQ allocated a cadmium concentration of 0.5*LOQ (= 0.01mg/kg)

[#]: 0.5mg/kg is the EC regulatory limit for cadmium in crustacean muscle

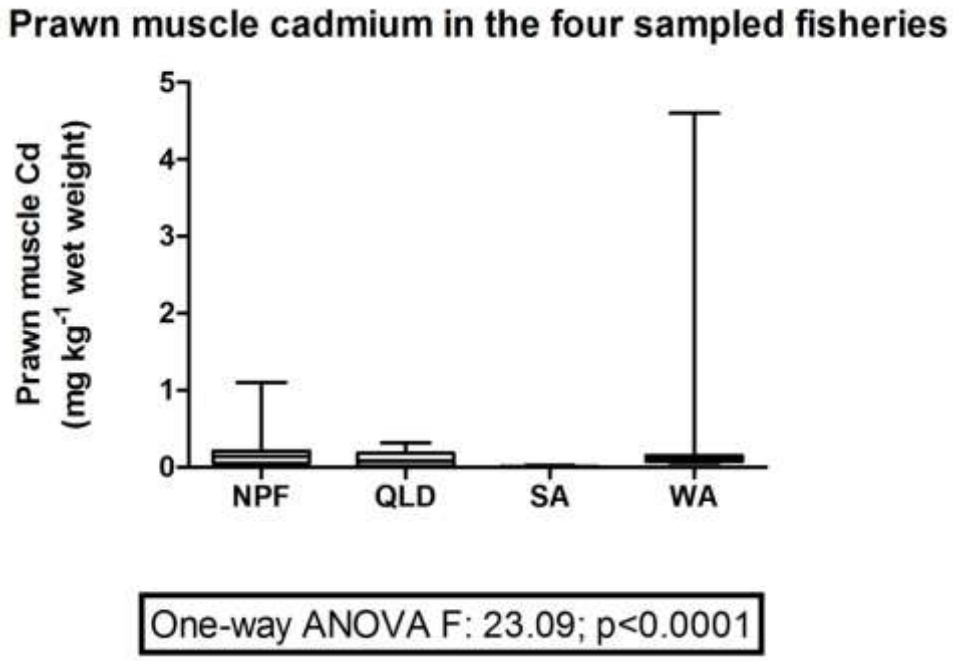
4.3. Cadmium: correlation coefficients and hypothesis tests

4.3.1. Residuals, logarithmic transformation of data

For each of the explanatory variables of fishery, genus, species, season and weight, a simple linear regression model was applied to assess the relationship between each of these explanatory variables and the prawn cadmium values after $\log_{10}$ transformation. From the diagnostic plots of each of these regressions (see Figures 22 & 30), the residuals were seen to be approximately normally distributed, satisfying the assumption of normality for ANOVA and subsequent hypothesis tests. Therefore all two-group, multiple-group and correlation co-efficient tests were undertaken with parametric methods: one way ANOVA, paired and unpaired t-tests and Tukey's comparison of multiple means and Pearson correlations.

4.3.2. Cadmium *vs.* prawn fishery

Figures 13 and 14 present prawn muscle cadmium levels, partitioned into the four surveyed fisheries. Figure 13 has cadmium concentration plotted on the *y*-axis; Figure 14 shows the same data with a $\log_{10}$ transformation of measured cadmium concentrations.



Tukey's Multiple Comparison Test *p*-values

	QLD	SA	WA
NPF	0.04	<0.0001	0.93
QLD		<0.0001	0.02
SA			<0.0001

Figure 13. Prawn muscle cadmium across surveyed fisheries

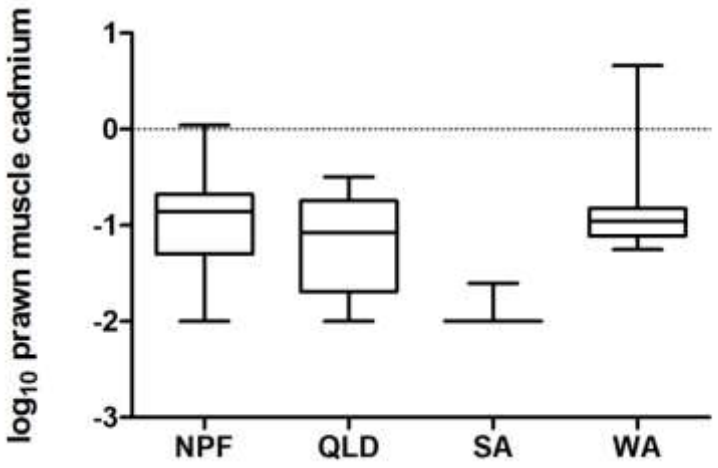


Figure 14. Prawn muscle cadmium across surveyed fisheries (log_{10} transform)

Cadmium levels were significantly different across the fisheries. Post-hoc Tukey's Multiple Comparison Tests showed that cadmium in prawns from the South Australian Spencer Gulf fishery was significantly lower than from each of the other fisheries.

4.3.3. Cadmium *vs.* genus and species

Apart from the two *Metapenaeopsis crassissima* samples from Western Australia, all sampled prawns belonged to either the genus *Penaeus* or *Metapenaeus*. Figure 15 presents a comparison between cadmium levels measured in these latter two genera.

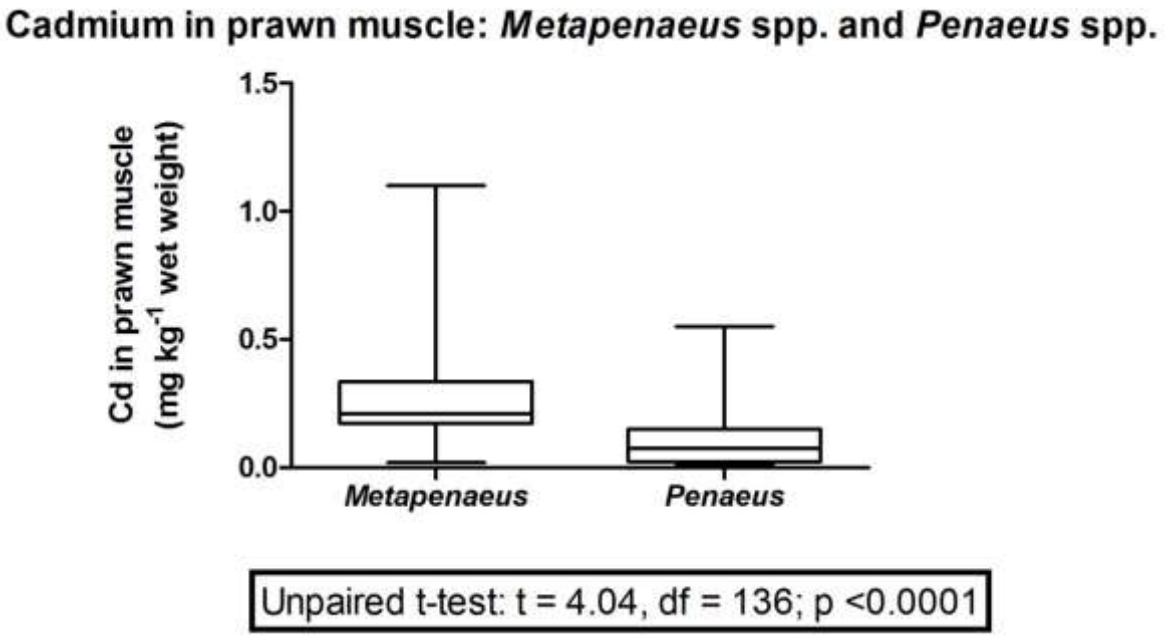
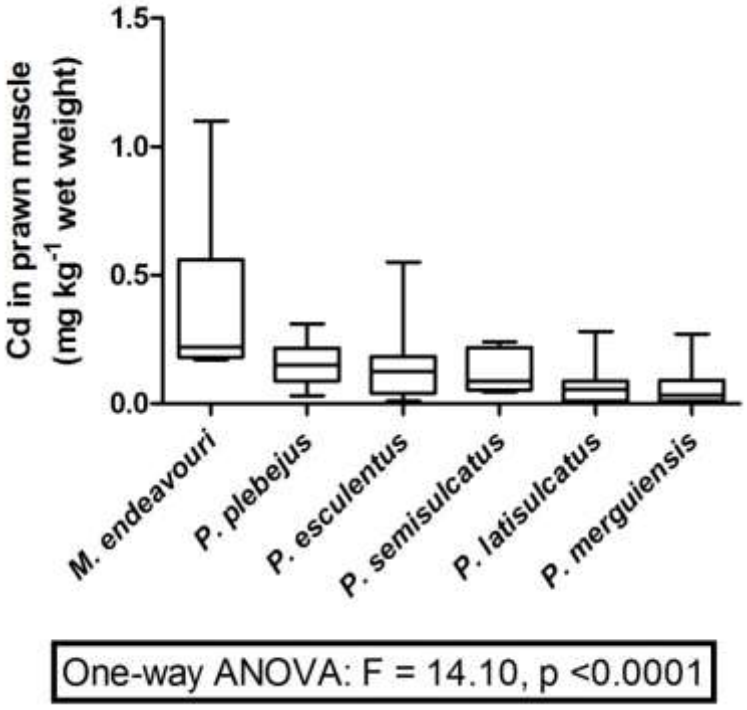


Figure 15. Cadmium in prawn muscle: *Penaeus* vs. *Metapenaeus*

Figure 15 shows that *Penaeus* spp. prawns had significantly lower cadmium levels than prawns belonging to the genus *Metapenaeus*. In order to investigate whether this relationship was sustained at the species level, a one-way ANOVA was conducted to compare means of all species that were sampled in numbers ≥6, i.e. we excluded from these analyses *M. crassissima*, *P. longistylus*, *P. indicus*, *M. ensis*, *Metapenaeus* spp. and *P. monodon* because these species were sampled only in low numbers. To identify where significant differences may lie between the various prawn species, post-hoc Tukey's Multiple Comparison Test compared cadmium levels across all species combinations.

Figure 16 presents the results of the ANOVA and post-hoc tests.



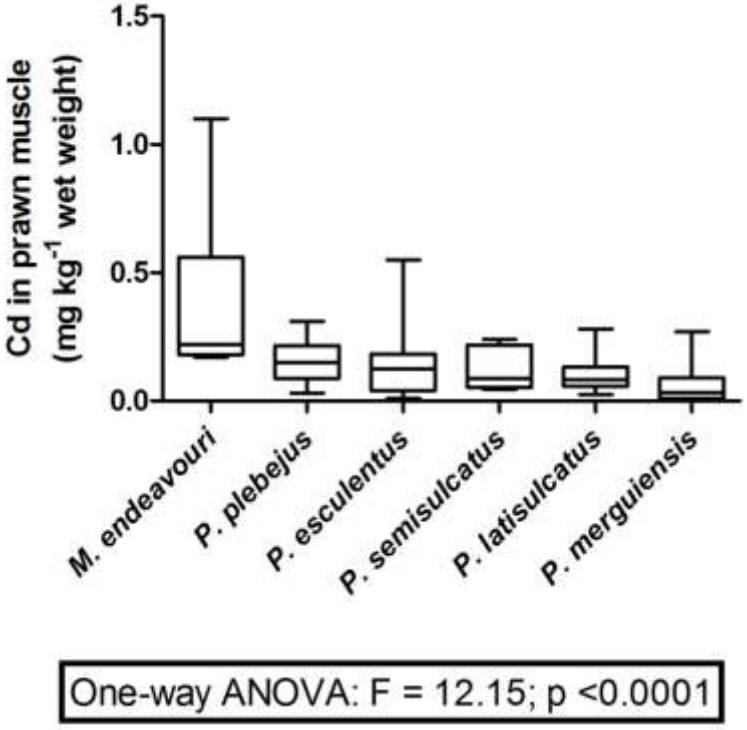
Tukey's Multiple Comparison Test *p*-values

	P. esculentus	*P. latisulcatus*	*P. merguiensis*	*P. plebejus*	*P. semisulcatus*
M. endeavouri	0.007	<0.0001	<0.0001	0.15	0.17
P. esculentus		0.008	0.02	0.81	>0.99
P. latisulcatus			>0.99	<0.0001	0.22
P. merguiensis				0.0003	0.22
P. plebejus					0.98

Figure 16. Cadmium in prawn muscle: analysis by prawn species

Figure 16 demonstrates that endeavour prawns (*M. endeavouri*) had significantly higher cadmium levels in muscle tissue than brown tiger prawns (*P. esculentus*), western king prawns (*P. latisulcatus*) and banana prawns (*P. merguiensis*). Banana prawns (*P. merguiensis*) were found to have lower cadmium levels than all species except western king prawns (*P. latisulcatus*) and green tigers. Western

king prawns were seen to have significantly lower cadmium levels than eastern king prawns (*P. plebejus*). However, with the knowledge that the single-species South Australian fishery returned prawns with very low cadmium levels (Figs. 13 & 14), these hypothesis tests were repeated to compare cadmium levels in western king prawns from the WA, NPF and Queensland fisheries (i.e. after removing SA prawns from the analysis). Figure 17 presents these results.



One-way ANOVA: F = 12.15; p <0.0001

Tukey's Multiple Comparison Test *p*-values

	P. esculentus	*P. latisulcatus*	*P. merguiensis*	*P. plebejus*	*P. semisulcatus*
M. endeavouri	0.001	<0.0001	<0.0001	0.06	0.08
P. esculentus		**0.99**	0.004	0.70	>0.99
P. latisulcatus			**0.02**	**0.25**	0.99
P. merguiensis				<0.0001	0.11
P. plebejus					0.97

NB: highlighted *p*-values indicate changes in statistical significance after removal of SA *P. latisulcatus* from analysis

Figure 17. Cadmium in prawn muscle after removal of South Australian *P. latisulcatus* from analysis

Those relationships were altered: western king prawns were seen to be significantly higher in cadmium than banana prawns, but cadmium levels were not significantly different between western king prawns, brown tiger prawns and eastern king prawns. We also examined cadmium levels in western king prawns, comparing samples from South Australia against those collected from the other three fisheries combined. Figure 18 shows the results of that test, revealing that geographic location may have an important bearing on bioaccumulated cadmium in prawns, as observed in *P. latisulcatus* alone.

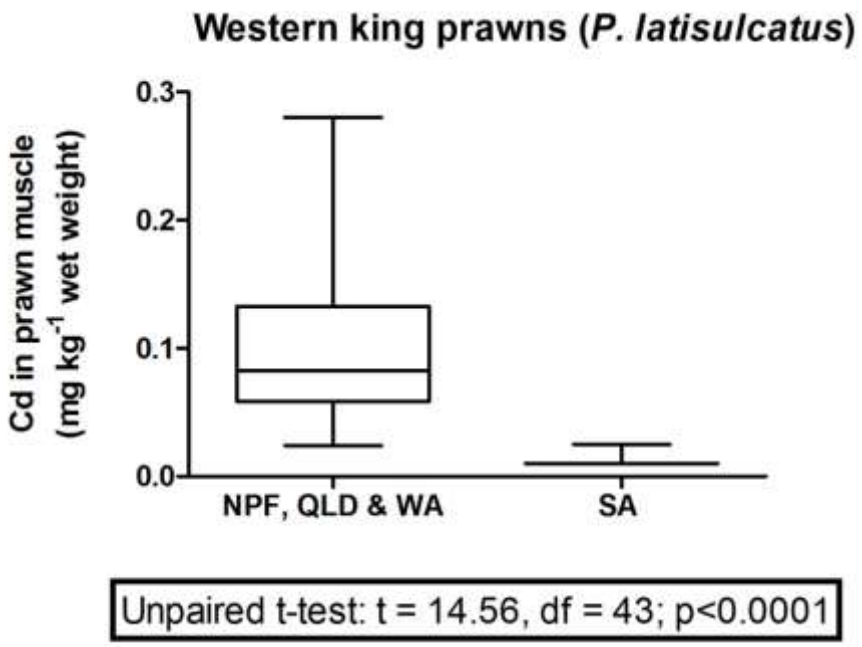


Figure 18. Cadmium in muscle tissues of western king prawns (*P. latisulcatus*)

4.3.4. Cadmium and other explanatory variables: prawn weight, season

Figure 19 shows a correlation plot for prawn muscle cadmium against mean prawn weight.

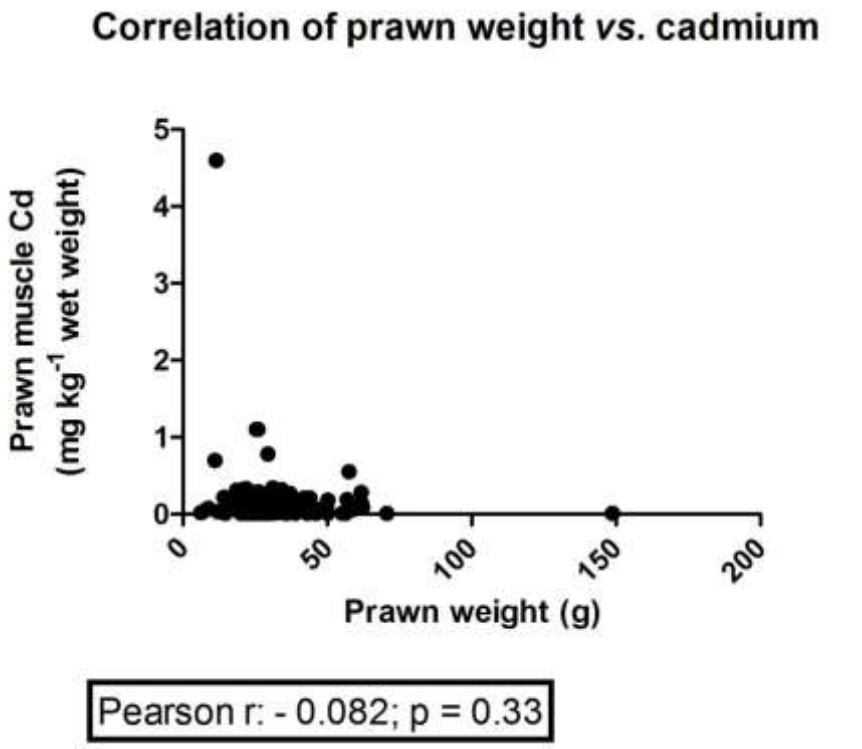


Figure 19. Average prawn weight and prawn muscle cadmium correlation plot

No significant correlation between prawn weight and cadmium in muscle tissues was seen. Two outliers exemplify the extreme ends of this particular finding:

- the prawn sample with the highest measured cadmium concentration (seen on the upper left quadrant of the Fig. 19 plot) was for *M. crassissima* from the WA Shark Bay fishery. These were quite small prawns, with an average weight of 11.5g
- the largest prawns sampled in this survey (plot point seen on the *x*-axis near to the 150g marker) were broodstock *P. monodon* captured from the Cairns area, mean weight 149g. Cadmium levels in this sample were below the detection limit.

In order to address the possibility of species-related variability in growth rates and accumulation efficiencies influencing the overall correlation of prawn size and cadmium concentration, we conducted separate correlation tests on muscle cadmium and weight for each of the six species that were sampled at $n \geq 6$. Only one statistically significant correlation was seen, for western king prawns (*P. latisulcatus*): Pearson r = -0.38; p = 0.01. However, the R^2 value was low, at 0.14. We conclude that this survey has not revealed good evidence for any cadmium and prawn weight relationships.

Figure 20 presents a comparison between cadmium levels in prawns from the Northern Prawn Fishery sampled in the dry season (May to September) and wet season (October to April). No statistically significant difference was seen. We applied this same hypothesis test to all prawns in the survey (i.e. from all fisheries). A significant difference was seen, where prawns sampled between May and September returned higher cadmium levels than those captured between October and April (Figure 21). Note the $\log_{10}$-transformed data plotted in Figure 21.

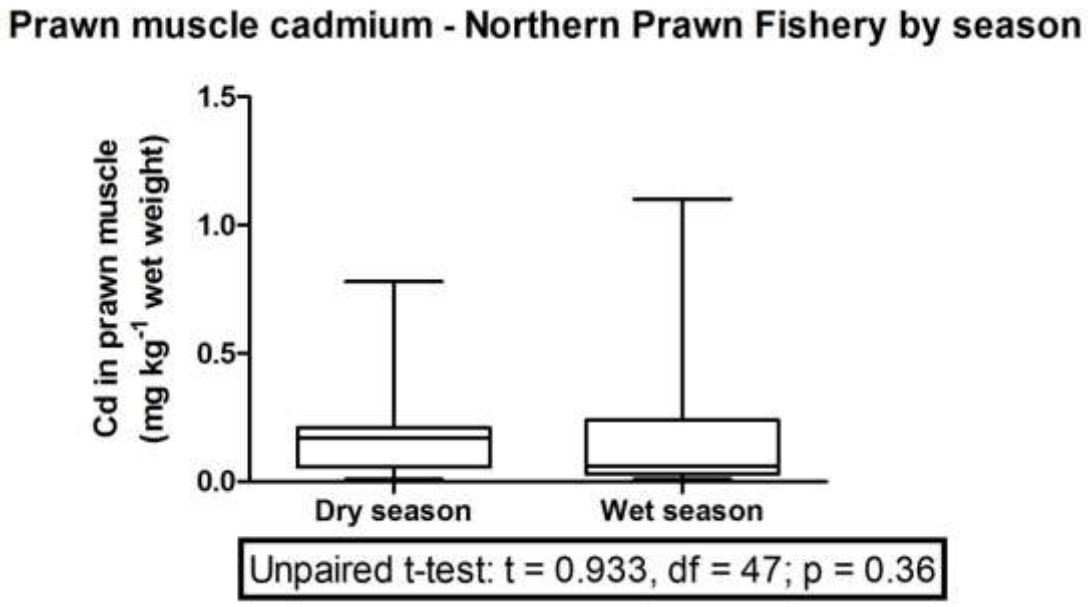


Figure 20. Prawn muscle cadmium in NPF samples captured in dry and wet seasons

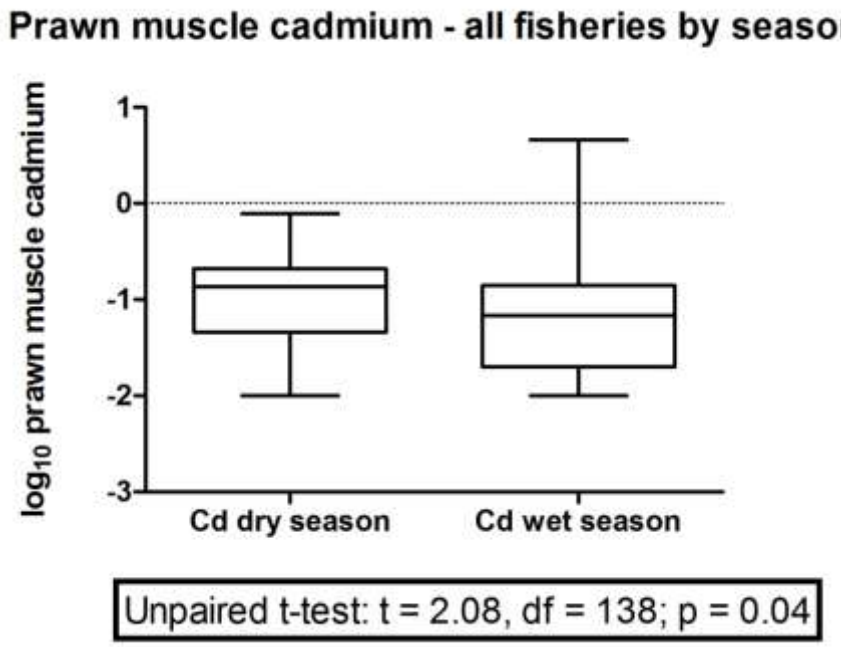


Figure 21. Prawn muscle cadmium in all samples captured in dry and wet seasons

4.3.5. Regression modelling: cadmium in prawn muscle tissue

To model the combined effect of the explanatory variables on $\log_{10}$-transformed prawn cadmium levels, the regression model

$\log_{10}$(Cadmium) ~ Fishery + Species + Season + Weight

was fitted (note that the variable "Genus" was not included in the regression model as it is completely correlated with species) and the most parsimonious model with the least number of predictor variables, minimising the measure of Akaike's Information Criterion, was achieved using stepAIC (contained in the MASS package in **R**). The final model identifies fishery and species to be the significant predictor variables for cadmium levels in prawn muscle; species was the more significant of the two explanatory variables. The multiple R^2 value for the final model was 0.728, so 73% of the variability in prawn muscle cadmium was explained by species and fishery.

Diagnostic plots for the model are given below in Figure 22.

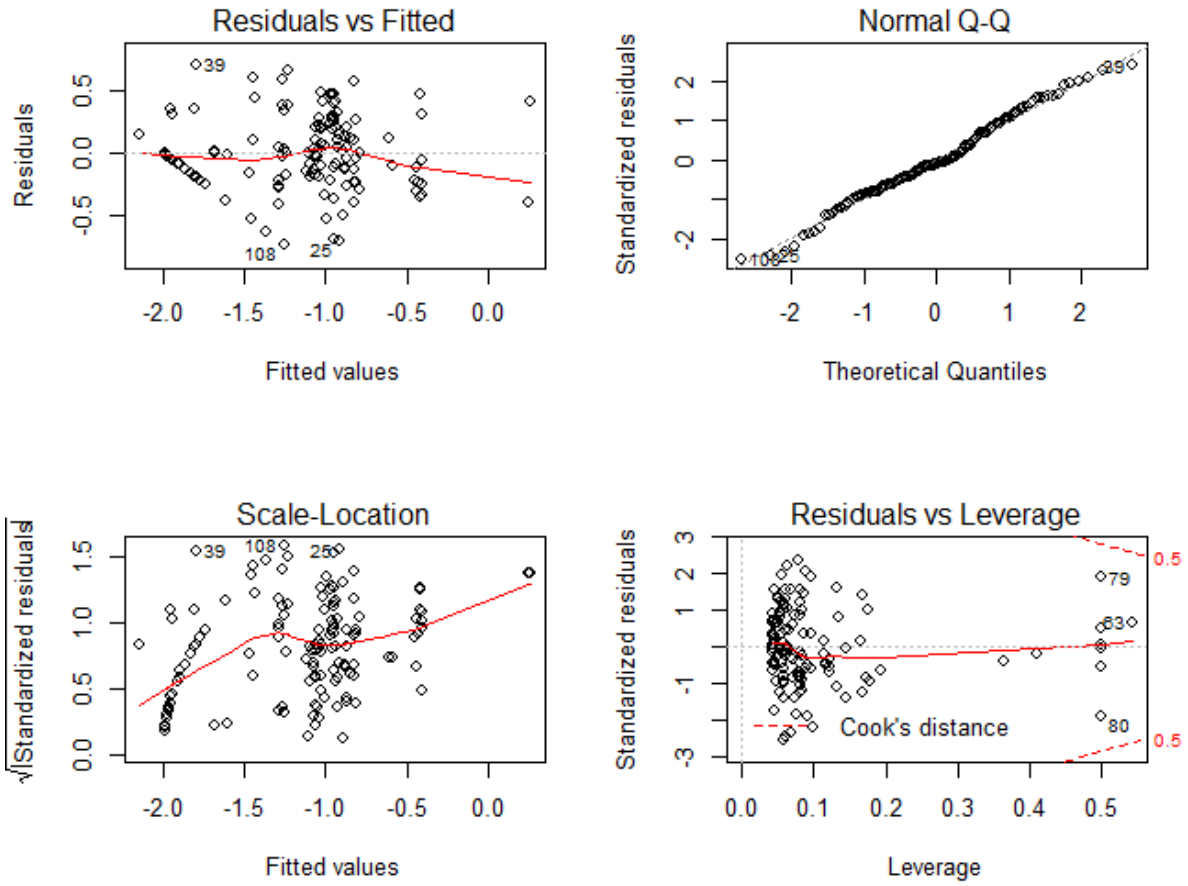


Figure 22. Diagnostic plots for the model $\log_{10}$(cadmium) ~ Fishery + Species.

4.4. SELENIUM: correlation coefficients and hypothesis tests

As was the case with the cadmium analyses, the diagnostic plots of models based on $\log_{10}$-transformed selenium data showed residuals which were adequately normally distributed (see Figure 30), thus satisfying the normality assumption for ANOVA and further parametric statistical tests.

4.4.1. Selenium *vs.* prawn fishery

Figure 23 presents prawn muscle selenium levels, partitioned into the four surveyed fisheries.

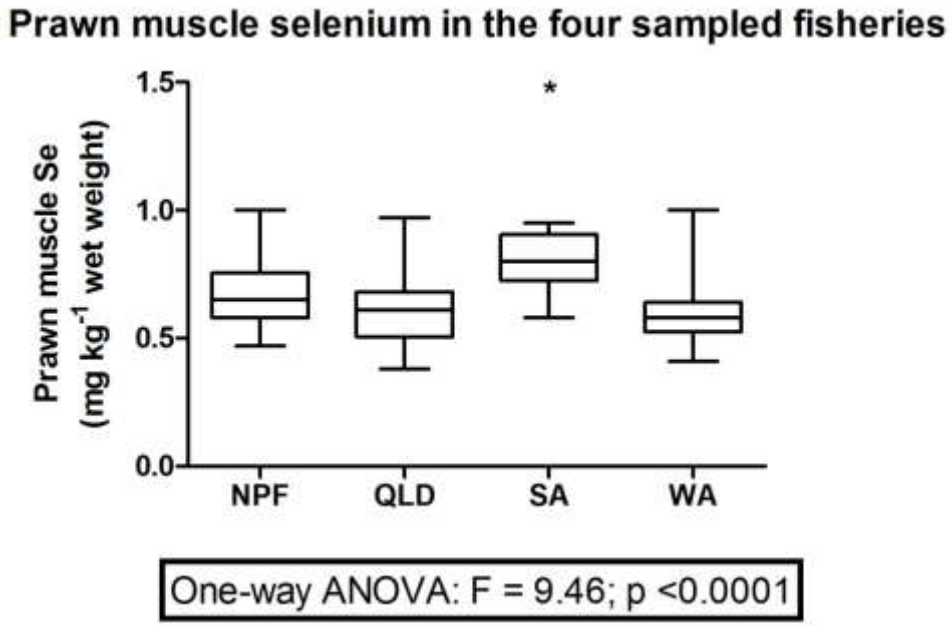


Figure 23. Prawn muscle selenium across surveyed fisheries

Selenium levels were significantly different across the fisheries. Post-hoc Tukey's Multiple Comparison Test showed that selenium in prawns from the South Australian Spencer Gulf fishery was significantly higher (p <0.02) than from each of the other fisheries.

4.4.2. Selenium *vs.* genus and species

Apart from the two *Metapenaeopsis crassissima* samples from Western Australia, all sampled prawns belonged to the genera *Penaeus* and *Metapenaeus*. Figure 24 presents a comparison between selenium levels measured in these latter two genera.

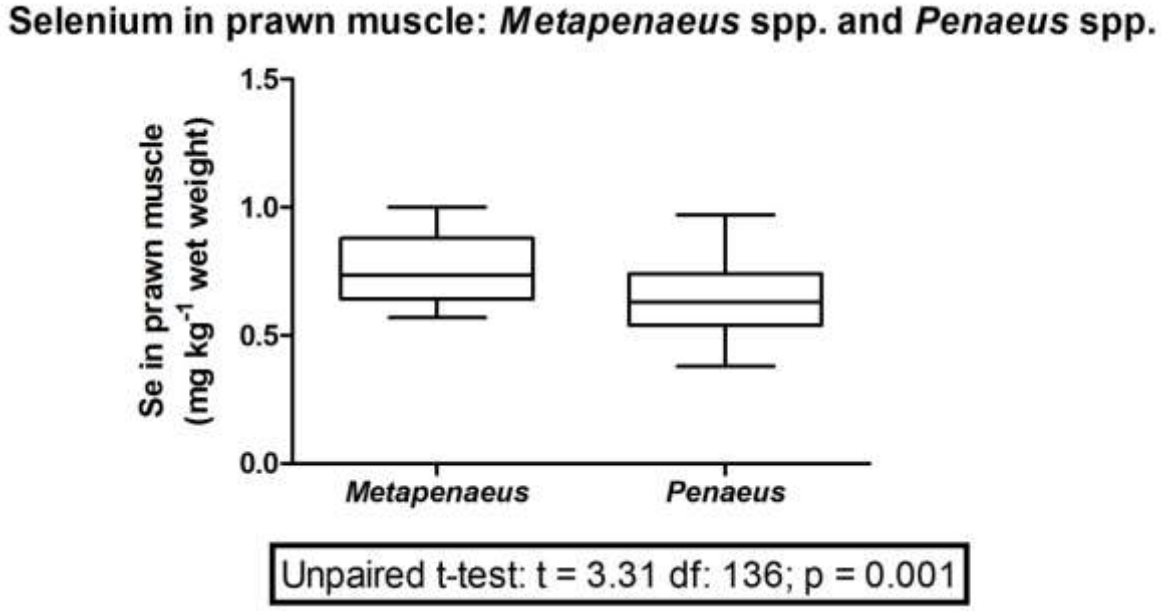
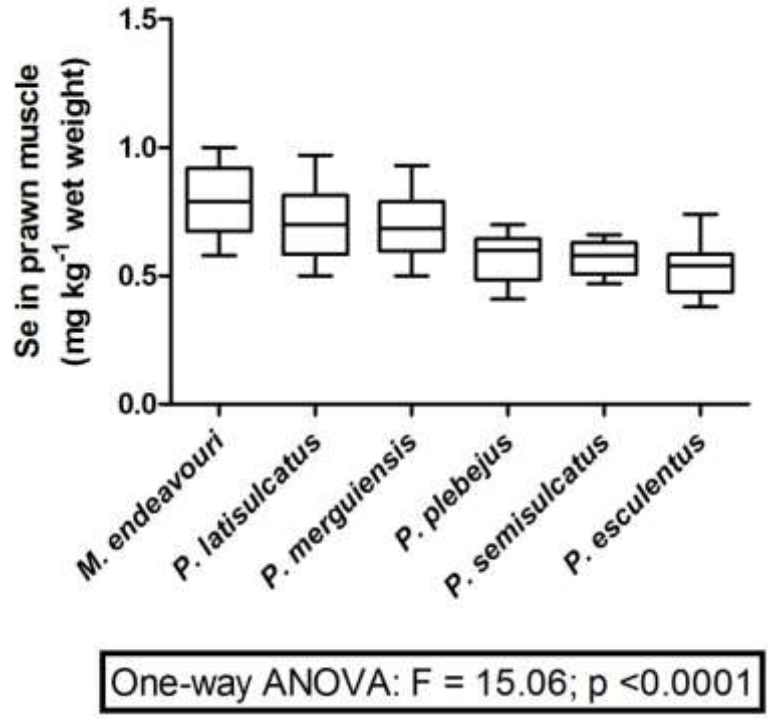


Figure 24. Selenium in prawn muscle: *Metapenaeus* vs. *Penaeus*

Figure 24 shows that *Metapenaeus* spp. prawns had significantly higher selenium levels than prawns belonging to the genus *Penaeus*. In order to investigate whether this relationship was sustained at the species level, a one way ANOVA and Tukey's post-hoc tests were run for selenium levels across all species combinations, after excluding those species that were sampled only in low numbers ($n \leq 3$).

Figure 25 presents results of the ANOVA and post-hoc tests.



Tukey's Multiple Comparison Test *p*-values

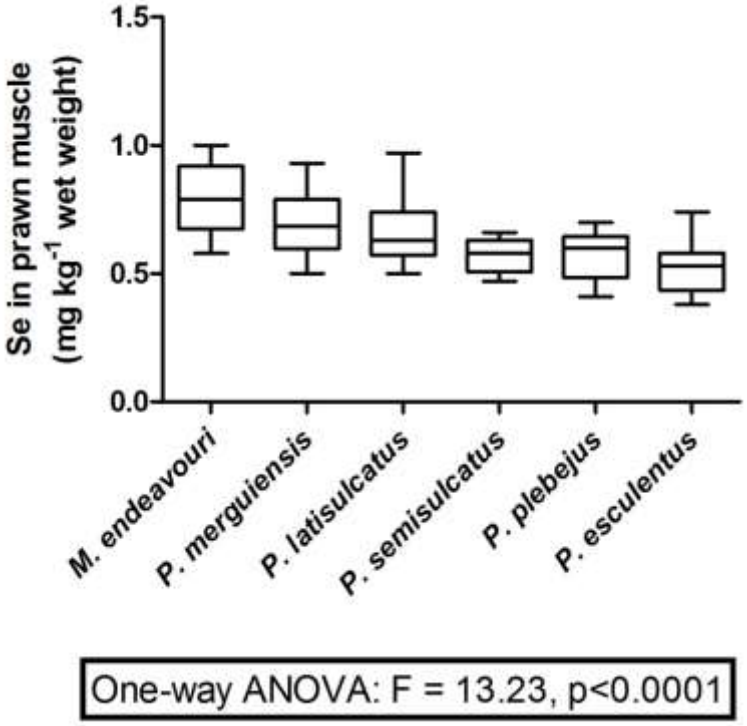
	P. esculentus	*P. latisulcatus*	*P. merguiensis*	*P. plebejus*	*P. semisulcatus*
M. endeavouri	<0.0001	0.33	0.29	<0.0001	0.005
P. esculentus		<0.0001	<0.0001	0.66	0.89
P. latisulcatus			>0.99	0.0002	0.08
P. merguiensis				0.005	0.19
P. plebejus					>0.99

Figure 25. Selenium in prawn muscle: analysis by prawn species

Figure 25 shows that endeavour prawns (*M. endeavouri*) had significantly higher selenium concentrations in muscle tissue all other examined species except western king prawns (*P. latisulcatus*) and banana prawns (*P. merguiensis*).

Brown tiger prawns (*P. esculentus*) had lower selenium levels than all species except green tiger prawns (*P. semisulcatus*) and eastern king prawns (*P. plebejus*). Both western king prawns and banana prawns had significantly higher selenium concentrations than in eastern kings (*P. plebejus*).

Because selenium levels in *P. latisulcatus* from the South Australian fishery were significantly higher than from all prawn samples from the other fisheries, we repeated this hypothesis test to examine the influence of selenium in *P. latisulcatus* after removing SA samples from the analysis. Those results are presented in Figure 26. Endeavour prawns were then seen to be significantly higher in selenium than western king prawns from NPF, QLD and WA. Selenium in western king prawns was not significantly higher than selenium in eastern king prawns. We also examined selenium levels in western king prawns, comparing samples from South Australia against those collected from the other three fisheries combined. Figure 27 shows the results of that test, revealing that western king prawns from South Australia had significantly higher selenium levels than western king prawns from the other fisheries. As seen for cadmium (Section 4.3.3. & Fig. 18), geographic location may have an important bearing on bioaccumulated selenium in prawns, as observed in *P. latisulcatus* alone.



Tukey's Multiple Comparison Test *p*-values

	P. esculentus	*P. latisulcatus*	*P. merguiensis*	*P. plebejus*	*P. semisulcatus*
M. endeavouri	<0.0001	**0.02**	0.26	<0.0001	0.004
P. esculentus		0.0002	<0.0001	0.63	0.87
P. latisulcatus			0.86	**0.06**	0.53
P. merguiensis				0.004	0.17
P. plebejus					>0.99

NB: highlighted *p*-values indicate changes in statistical significance after removal of SA *P. latisulcatus* from analysis

Figure 26. Selenium in prawn muscle after removal of South Australian *P. latisulcatus* from analysis

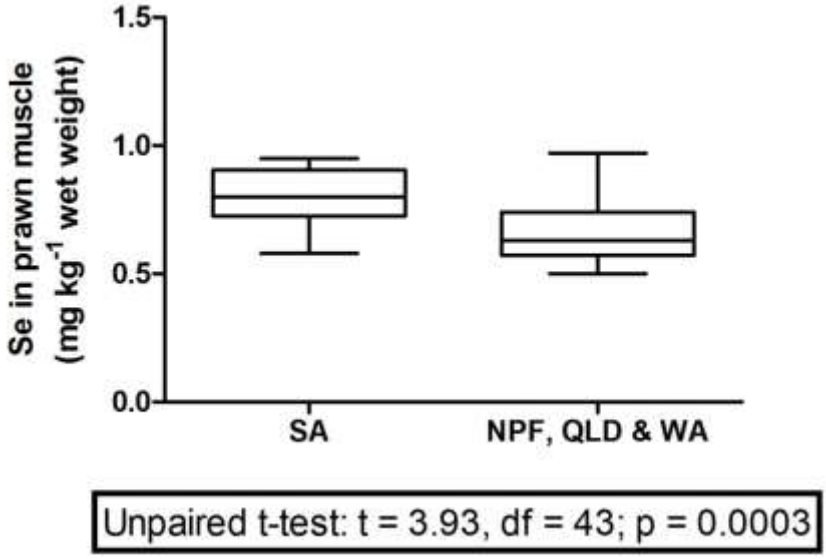


Figure 27. Selenium in muscle tissues of western king prawns (*P. latisulcatus*)

4.4.3. Selenium and other explanatory variables: prawn weight, season

Figure 28 shows a correlation plot for prawn muscle cadmium against mean prawn weight.

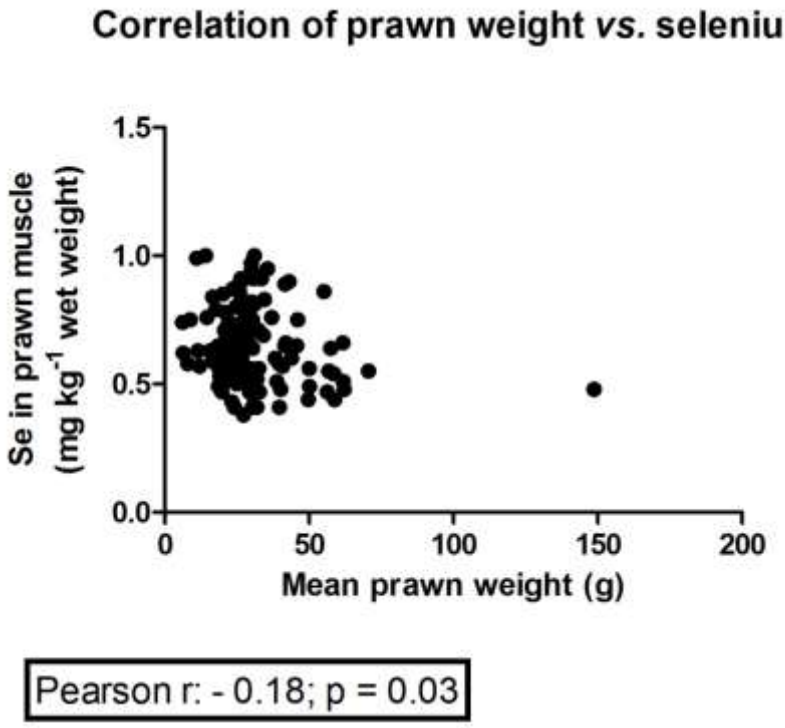


Figure 28. Average prawn weight and prawn muscle selenium correlation plot

A significant correlation between prawn weight and selenium in muscle tissues was seen (p = 0.03), but the R^2 value was low (0.033). The size outlier of 149g mean weight (a sample of *P. monodon* from the Cairns region) was found to have a relatively low selenium level.

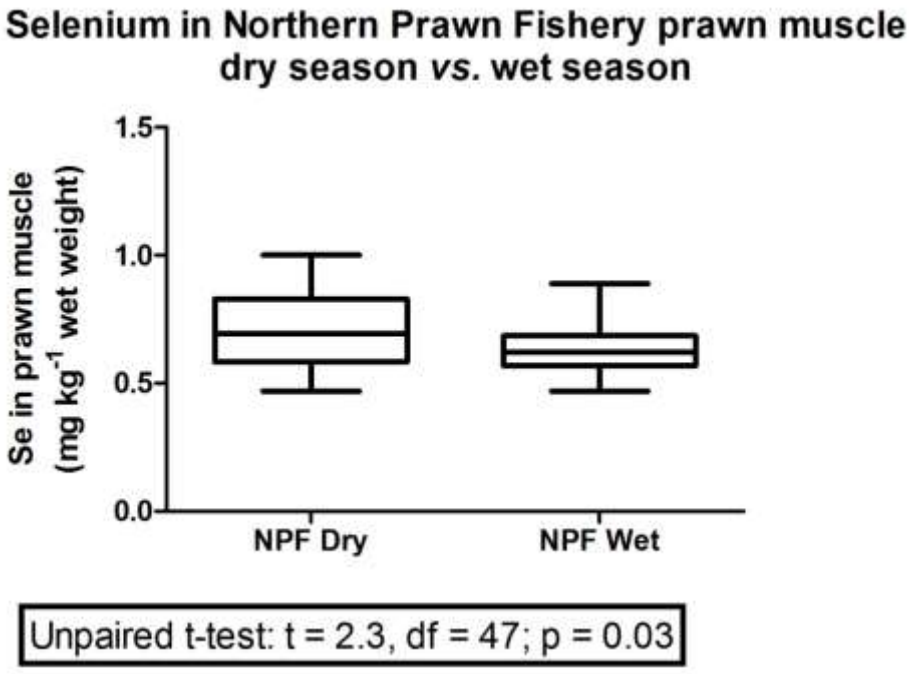


Figure 29. Prawn muscle selenium in NPF samples captured in dry and wet seasons

Figure 29 presents a comparison between selenium levels in prawns from the Northern Prawn Fishery sampled in the dry season (May to September) and wet season (October to April). Selenium concentrations were significantly higher in prawns sampled during the dry season. When prawns from the whole survey were considered, however, this relationship did not hold; no statistically significant seasonal differences in selenium levels were seen when prawns captured from all four fisheries were considered (p = 0.39).

4.4.4. Regression modelling: selenium in prawn muscle tissue

To model the combined effect of explanatory variables on $\log_{10}$-transformed prawn selenium levels, the regression model

$\log_{10}$(Selenium) ~ Fishery + Species + Season + Weight

was fitted (note that the variable of "Genus" was not included in the regression model as it is completely correlated with species) and the most parsimonious model with the least number of predictor variables, minimising the measure of Akaike's Information Criterion, was achieved using stepAIC. The final model identifies species, fishery and season – in decreasing order of relevance – to be significant predictor variables for selenium levels in prawn muscle. The multiple R^2 value for the final model was 0.546, so 55% of the variability in prawn muscle selenium was explained by species, fishery and season.

Diagnostic plots for the model are given below in Figure 30.

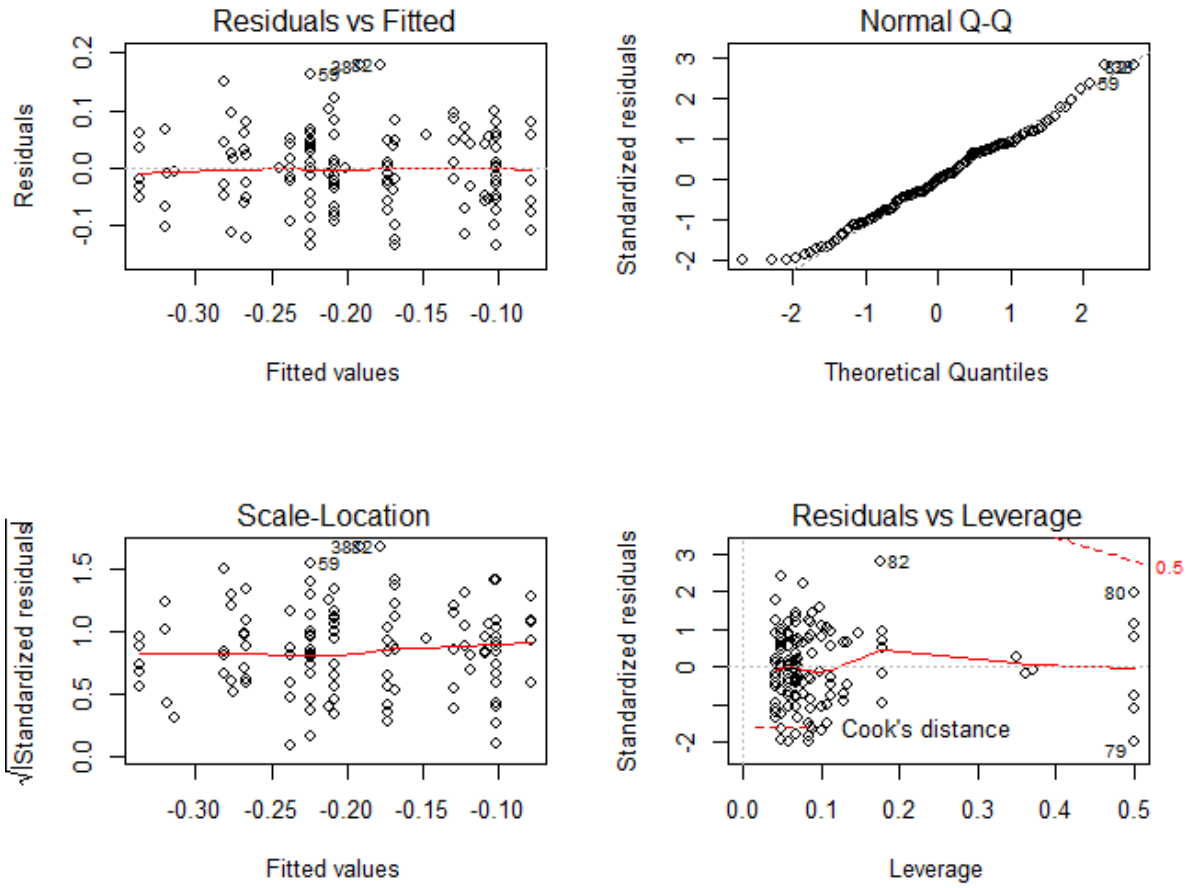


Figure 30. Diagnostic plots for the model $\log_{10}$(Selenium) ~ Fishery + Species + Season.

4.5. SUPPLEMENTARY TISSUES ANALYSIS AND ANALYTICAL QUALITY ASSURANCE

4.5.1. Routine laboratory QA

Table 6 presents a summary of routine QA procedures conducted by AAA, showing certified matrix reference concentrations, recoveries and spike recoveries.

Table 6. Advanced Analytical laboratory routine QA results

AGAL-3	*n*	Cadmium	Selenium
Certified (µg g^{-1})		0.14	2.7
Measured (µg g^{-1})	5	0.12 – 0.19	2.1 – 2.5
Recovery (%)		86 – 137	78 – 103
Matrix spike recovery (0.1µg mL^{-1} each analyte)	12	82 - 106	100 - 118

4.5.2. Cadmium supplementary tissues analysis

Table 7 presents cadmium data (replicate analyses), for each of the 21 samples subjected to re-analysis. Results of the original cadmium analyses for these samples – conducted over numerous analytical runs during 2012 and 2013 – are also tabulated. Results of analysis of hepatopancreas tissues are also listed.

Table 7. Supplementary tissues analysis for cadmium

Sample No.	Cadmium group	Muscle + alimentary tract replicates			Muscle-only replicates			Analytical laboratory (AAA) replicates			Original cadmium analyses	Hepatopancreas
ACPF-011	L	<LOQ	<LOQ	<LOQ	0.05	<LOQ					<LOQ	0.34
ACPF-126	L	0.03	<LOQ	<LOQ	<LOQ	<LOQ	0.03	<LOQ	0.04	0.05	<LOQ	2.9
ACPF-077	L	<LOQ	<LOQ	<LOQ	0.04	<LOQ	<LOQ				<LOQ	0.10
ACPF-064	L	0.04	0.09	<LOQ	0.02	<LOQ					0.05	0.90
ACPF-082	L	0.06	0.05	0.06	0.08						0.06	4.6
ACPF-083	L	0.10	0.08	0.08							0.08	4.8
ACPF-095	L	0.08	0.08	0.08							0.08	11.0
ACPF-043	L	0.11	0.10	0.11	0.10	0.09	0.09	0.09	0.09	0.09	0.10	18.5
ACPF-090	I	0.14	0.11	0.11	0.13	0.12	0.12	0.13	0.12	0.12	0.12	5.1
ACPF-152	I	0.14	0.16	0.17	0.17	0.17	0.14				0.13	4.6
ACPF-097	I	0.16	0.15	0.15	0.13	0.13	0.15				0.14	8.7
ACPF-021	I	0.17	0.19	0.22	0.15	0.17	0.16				0.18	4.3
ACPF-180	I							0.19	0.18	0.19	0.2	
ACPF-055	I	0.22	0.29	0.20	0.17	0.16	0.17				0.22	
ACPF-059	I	0.23	0.25	0.24	0.28	0.31	0.26				0.28	3.1
ACPF-017	I	0.39	0.39	0.39	0.32	0.37	0.37	0.35	0.33	0.34	0.34	
ACPF-019	H	0.49	0.66	0.53	0.71	0.52	0.74	0.64	0.53	0.38	0.55	41.7
ACPF-089	H	0.84	0.95	0.64							0.7	2.8
ACPF-161	H	1.00	1.01	0.99	1.0	0.95					1.1	22.0
ACPF-023	H	1.5	1.6	1.6	1.4	1.4	1.6	1.4	1.3	1.5	0.78	8.2
ACPF-088	H	4.34	4.34	4.06							4.6	4.6
RSD (%)				8.4					9.3			

<LOQ: Below detection limits

Empty cells: not analysed

Figure 31 presents data from Table 7 (except for hepatopancreas tissues) in graphical form, including results of five paired-sample t-tests.

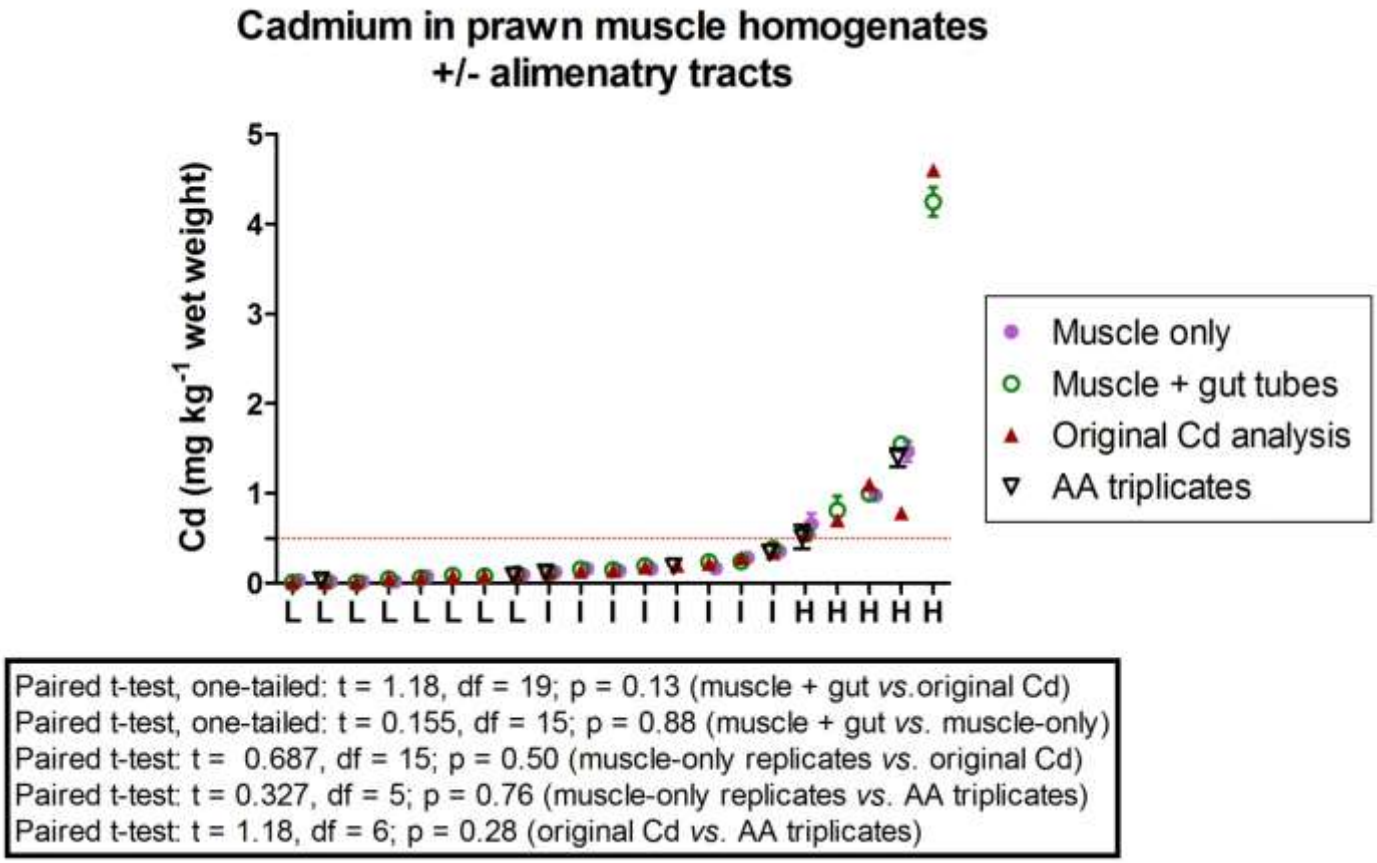


Figure 31. Replicate cadmium analyses. Data points are mean +/- SD

4.5.3. Selenium supplementary tissues analysis

Table 8 presents selenium data (replicate analyses), for each of the 21 samples subjected to re-analysis. Results of the original selenium analyses for these samples – conducted over numerous analytical runs during 2012 and 2013 – are also tabulated. Results of selenium analyses in hepatopancreas tissues are also listed.

Figure 32 presents data from Table 8 (except for hepatopancreas tissues) in graphical form, including results of five paired-sample t-tests.

Table 8. Supplementary tissues analysis for selenium

Sample No.	Muscle + alimentary tract replicates			Muscle-only replicates			Analytical laboratory (AAA) replicates			Original selenium analyses	Hepatopancreas
ACPF-077	0.55	0.56	0.50	0.42	0.40	0.40				0.41	0.68
ACPF-152	0.49	0.54	0.55	0.59	0.45	0.52				0.46	1.50
ACPF-126	0.58	0.48	0.46	0.55	0.53	0.43	0.75	0.35	0.53	0.47	1.49
ACPF-095	0.45	0.40	0.52							0.53	2.22
ACPF-090	0.53	0.59	0.54	0.45	0.54	0.49	0.75	0.78	0.71	0.53	1.66
ACPF-043	0.52	0.68	0.67	0.53	0.67	0.60	0.78	0.75	0.76	0.57	2.90
ACPF-082	0.63	0.69	0.74	0.82						0.57	2.32
ACPF-011	0.59	0.61	0.60	0.61	0.68					0.58	1.76
ACPF-083	0.70	0.76	0.61							0.58	1.91
ACPF-097	0.45	0.58	0.52	0.55	0.71	0.57				0.59	1.57
ACPF-055	0.66	0.62	0.62	0.57	0.61	0.55				0.61	
ACPF-088	0.66	0.84	0.78							0.63	1.24
ACPF-019	0.59	0.49	0.66	0.61	0.48	0.61	0.70	0.68	0.76	0.64	3.07
ACPF-059	0.72	0.55	0.70	0.69	0.68	0.73				0.64	1.57
ACPF-023	0.88	0.84	0.87	0.99	0.91	0.88	1.00	0.94	1.00	0.70	3.08
ACPF-064	0.77	0.79	0.83	0.81	0.65					0.87	1.76
ACPF-180							0.74	0.71	0.75	0.89	
ACPF-161	0.87	0.94	0.84	0.93	0.77					0.89	3.80
ACPF-021	1.01	0.93	0.87	0.83	0.82	1.00				0.95	3.41
ACPF-089	1.40	1.26	1.20							0.99	1.60
ACPF-017	0.91	0.93	1.01	0.97	0.87	0.87	1.00	1.00	1.10	1.00	
RSD (%)			7.9					11.9			

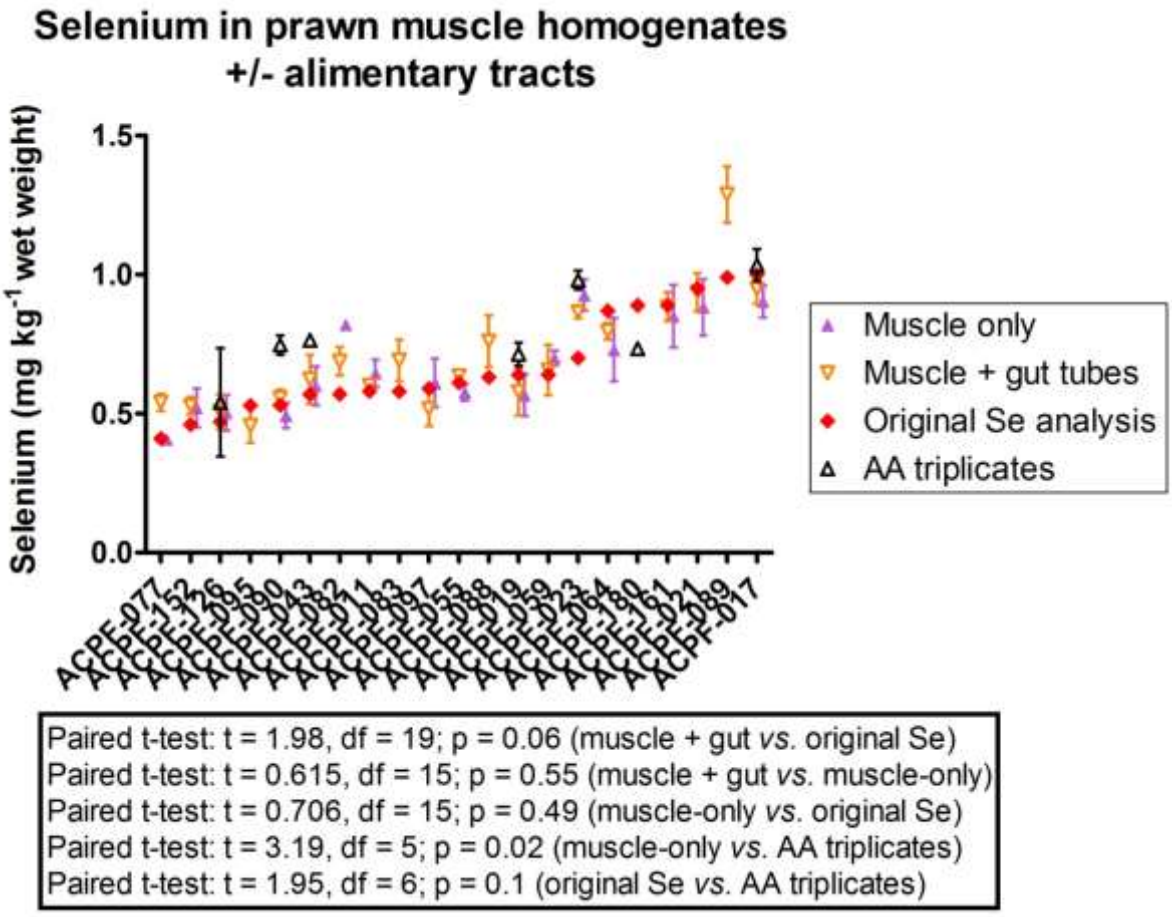


Figure 32. Replicate selenium analyses. Data points are mean +/- SD

4.6. ISOLATED PRAWN GUT TUBES AND HEPATOPANCREAS

4.6.1. Cadmium in prawn gut tube and hepatopancreas

Figure 33 presents a correlation plot for cadmium levels measured in isolated alimentary tracts, compared to muscle tissue cadmium levels in the same samples. Seven samples were randomly selected from the survey; the entire isolated gut tubes from these samples were oven-dried and subjected to cadmium analysis (i.e. these analyses were conducted on a different set of samples to those shown in Table 7). Cadmium dry-weight values reported for gut tube analyses have been converted back to wet weight for comparison in Figure 33, using a 75% moisture content, as determined in this study for prawn muscle tissues (see Section 4.7). Apart from the observation that cadmium levels were uniformly higher in isolated alimentary tracts than in muscle tissue, no obvious relationship was seen.

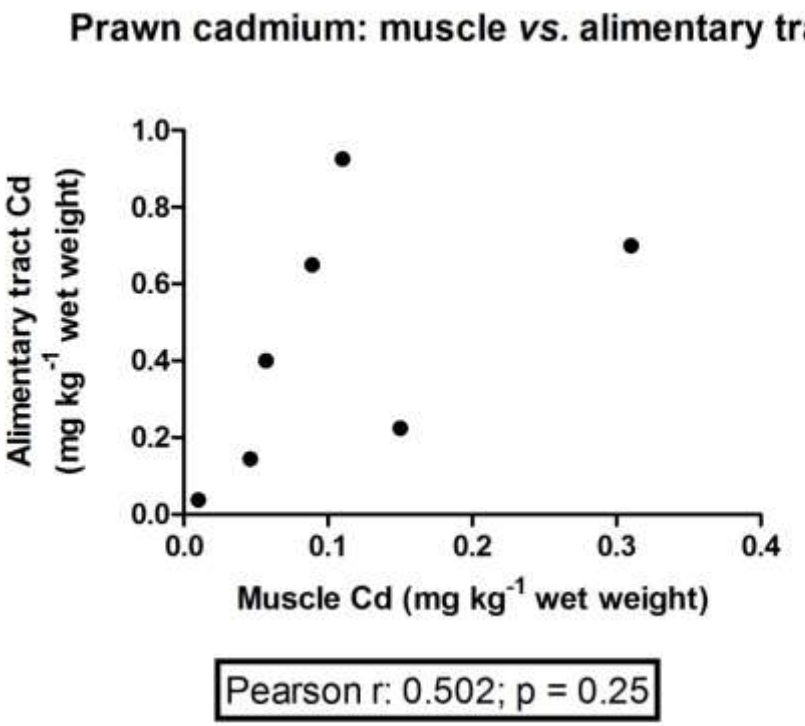


Figure 33. Correlation of cadmium in prawn muscle and prawn alimentary tract

Figure 34 shows a correlation plot for cadmium levels measured in hepatopancreas, compared to muscle tissue cadmium levels. Cadmium levels in HP tissues are also listed in Table 7. Apart from the prawn muscle cadmium outlier sample, this being the *M. crassissima* prawns from Shark Bay, where both muscle and hepatopancreas cadmium levels were identical at 4.6 mg kg^{-1} wet weight, all other samples showed that hepatopancreas cadmium levels were higher than seen in muscle tissues. In some samples, the hepatopancreas carried much higher cadmium concentrations (up to three orders of magnitude). A statistically significant correlation was seen between muscle and HP cadmium levels ($p = 0.007$); apart from the obvious outlier, some indications of a linear relationship can be seen from the plot, although the R^2 value was low at 0.37.

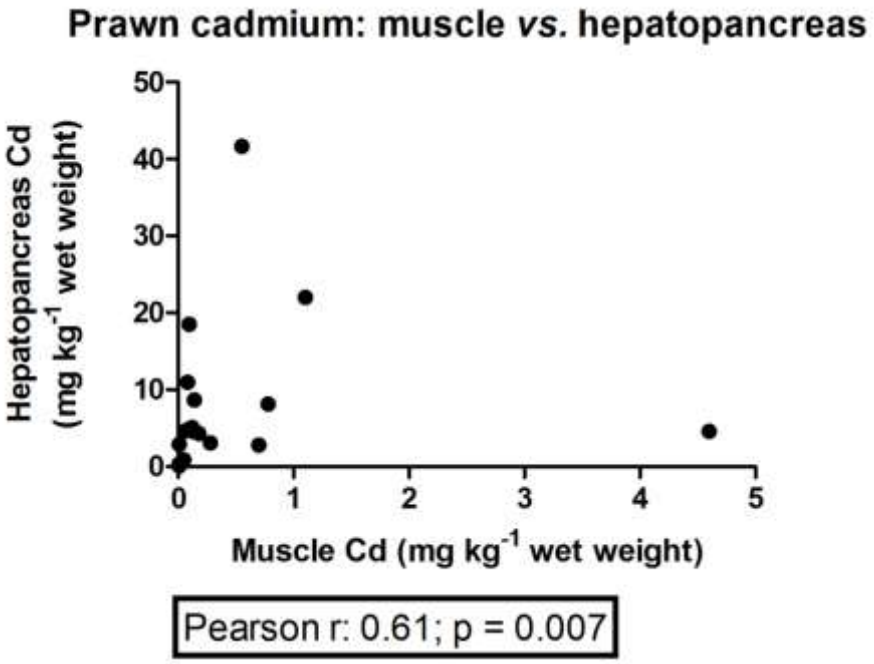


Figure 34. Correlation of cadmium in prawn muscle and prawn hepatopancreas

4.6.2. Selenium in prawn gut tube and hepatopancreas

Figure 35 presents a correlation plot for selenium levels measured in isolated alimentary tracts, compared to the muscle tissue selenium levels. Unlike cadmium, selenium levels in six of the seven isolated alimentary tracts were lower than those found in muscle tissues. No obvious linear relationship between gut tube and muscle selenium was seen.

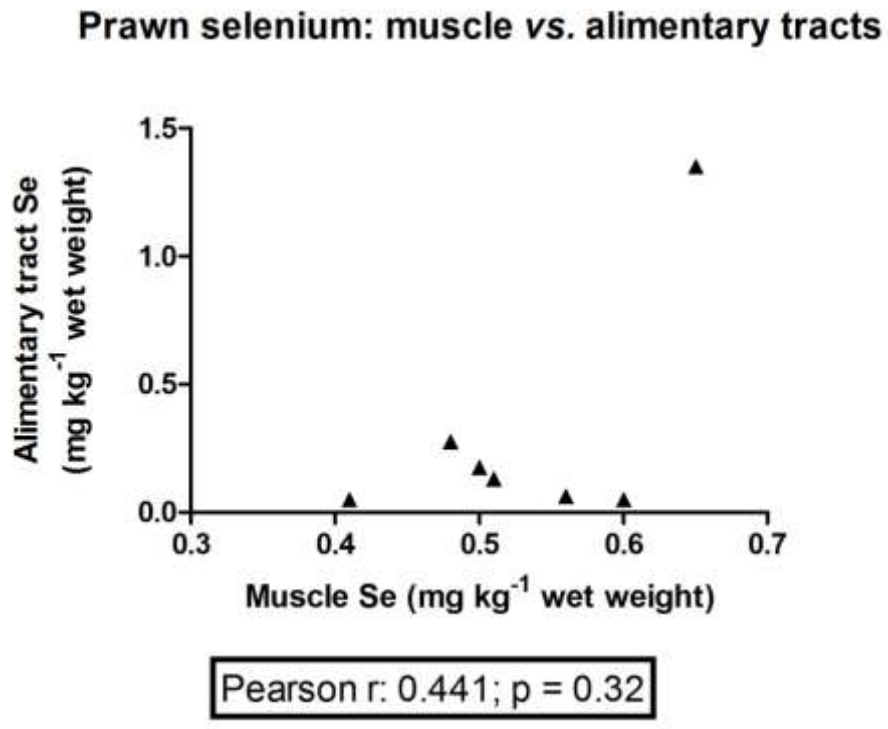


Figure 35. Correlation of selenium in prawn muscle and prawn alimentary tract

Figure 36 shows a correlation plot for selenium levels measured in hepatopancreas, compared to muscle tissue selenium levels. Selenium levels in HP tissues are also listed in Table 8. A weak linear relationship (R^2 = 0.29) was seen.

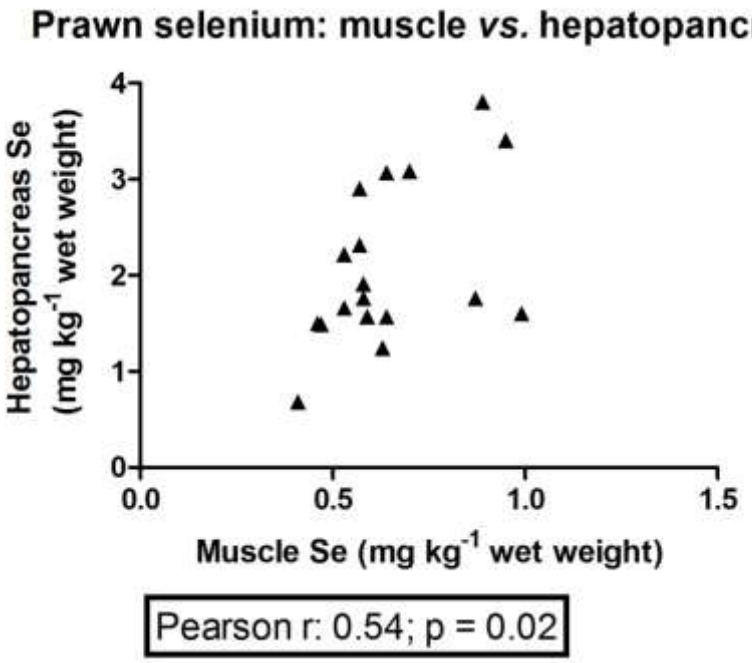


Figure 36. Correlation of selenium in prawn muscle and prawn hepatopancreas

4.7 PRAWN TISSUE WATER CONTENT

Table 9 presents results of the lyophilisation process conducted (in triplicate) on muscle tissues of three prawn species. This method has excellent reproducibility; each of these three *Penaeus* species had identical moisture content in muscle tissues.

Table 10 shows water content determined in prawn hepatopancreas by oven-drying. Note that cadmium and selenium were determined in *n* = 18 samples (Tables 7 & 8). Water content determinations were made on *n* = 13 of these samples, as seen in Table 10. For the outstanding *n* = 5 samples in which hepatopancreas tissues were dried, tare weight of the watch-glasses was not recorded. For those samples, mean prawn HP water contents, as shown in Table 10, were adopted to calculate dry weight to wet weight conversions.

Table 9. Prawn muscle water content determined by lyophilisation

		Sample No.	Tare weight (g)	Gross weight wet (g)	Gross weight dry (g)	Net weight dry (g)	Water content (g)	Water proportion (%)	Median water (%)	Mean water (%)	SD	RSD
PRAWN SPECIES	***Penaeus indicus***	180A	13.497	27.878	17.108	3.611	10.77	74.9	74.9	74.9	0.072	0.1%
		180B	13.381	27.028	16.792	3.41	10.236	75				
		180C	13.597	25.905	16.689	3.092	9.216	74.9				
	Penaeus latisulcatus	001A	13.563	34.356	18.825	5.262	15.531	74.7	74.6	74.6	0.039	0.05%
		001B	13.347	34.079	18.608	4.964	15.471	74.6				
		001C	13.73	33.29	18.694	3.407	14.596	74.6				
	Penaeus esculentus	086A	13.227	26.779	16.634	3.407	10.145	74.9	74.9	74.8	0.029	0.04%
		086B	13.544	26.652	16.846	3.302	9.805	74.8				
		086C	13.403	26.131	16.603	3.2	9.528	74.9				

Table 10. Prawn hepatopancreas water content determined by oven-drying

Sample No.	Tare weight (g)	Gross weight wet (g)	Gross weight dry (g)	Net weight dry (g)	Water content (g)	Water proportion (%)	Median water proportion (%)	Mean water proportion (%)	SD
ACPF-019	127.5	169	136.6	9.1	32.4	78.1	80.6	80.0	2.57
ACPF-021	138	156.5	142.2	4.2	14.3	77.3			
ACPF-023	144.8	174.3	151.3	6.5	23	78			
ACPF-043	144.7	168	149.9	5.2	18.1	77.7			
ACPF-059	132	156.8	136.8	4.8	20	80.1			
ACPF-077	140.1	149.9	141.7	1.6	8.2	83.7			
ACPF-082	131.1	148.2	134.4	3.3	13.8	80.7			
ACPF-083	130.7	148.5	134.1	3.4	14.4	80.9			
ACPF-088	130.7	142.3	132.6	1.9	9.7	83.6			
ACPF-090	145.4	159.7	148.1	2.7	11.6	81.1			
ACPF-095	127.5	138.2	130	2.5	8.2	76.6			
ACPF-097	129.3	147	132.2	2.9	14.8	83.6			
ACPF-126	130.3	149.2	134.5	4.2	14.7	77.8			

4.8. PRAWN SPECIES IDENTIFICATION

Of the *n* = 140 prawn samples received from various sample collectors, we identified only a single case of species misidentification. This sample was sent to us by an industry operator in Queensland; it was presented to us as "red spot" prawn (i.e. *P. longistylus*). Close examination of both the photographs and re-examination of unprocessed prawns retained in cold storage from that particular sample revealed those prawns to be *P. latisulcatus*. No diagnostic red spot could be seen on the 3rd abdominal segment of any of the remaining prawns, and the blue legs and yellow pleopods characteristic of *P. latisulcatus* were noted. The extent of the postrostral groove on the carapace was also diagnostic. Figure 37 is an image of this sample.

Figure 37. *P. latisulcatus* Sample collected from the Queensland East Coast Prawn Fishery.

Apart from this single incidence of misidentification, all other samples were found to correspond with the information initially provided by sample collectors. Eight samples were sent to us without presumptive species identification; those samples were identified by the methods described here. Tiger prawns sent to us by industry operators from the Queensland fisheries and the Northern Prawn Fishery, as well as tiger prawns from WA, were presented to us as such, i.e. "tiger prawns." Our blinded assessment using photographs and keys allowed us to formally categorise these samples as either *P. esculentus* (brown tiger prawn) or *P. semisulcatus* (green tiger prawn). Three *P. monodon* samples received in the survey were identified by sample providers as either "*P. monodon*" or "leader prawns."

5. DISCUSSION

5.1. Results of the survey

5.1.1. CADMIUM

5.1.1.1. Prawns that failed the EC regulatory guideline level of 0.5mg/kg cadmium

One hundred and thirty-four samples (95.7%) were found to have cadmium concentrations in de-veined muscle tissue that was below the EC food safety regulatory limit of 0.5 mg/kg for cadmium in prawns [247]. Six of the 140 prawn samples (i.e. 4.3%) were found to have muscle cadmium levels in excess of 0.5mg/kg. Two of these latter samples were *Metapenaeopsis crassissima* captured from Shark Bay in WA. The other four samples came from the Gulf of Carpentaria, in the Commonwealth-managed Northern Prawn Fishery. Specific details of sampling locations and species for these four samples were as follows:

- *P. esculentus*, cadmium measured at 0.55mg/kg, captured in July 2012 from Queensland waters west of Mornington Island, map cell 2444 or 2555 (actual capture location was from waters bordering these two cells – see Fig. 3 for map cell locations).
- *M. endeavouri*, cadmium 0.78mg/kg, July 2012, map cell 2592 (NT waters south of Groote Eylandt)
- *M. endeavouri*, cadmium 1.1mg/kg, February 2013, cell 2667 (NT waters between Cape Grey and Groote Eylandt)
- *M. endeavouri*, cadmium 1.1mg/kg, February 2013, cell 2747 (Qld waters off Weipa)

The two *M. crassissima* samples from Shark Bay had cadmium concentrations measured in muscle tissue at 0.7mg/kg and 4.6mg/kg. These two samples were the sole representatives of this particular prawn species in the survey, because our random sampling program, weighted for prior species-specific production from the WA prawn fisheries, had only requested two *M. crassissima* samples. The Shark Bay coral prawn fishery is a small by-catch fishery, of relatively low economic value. Between 1989 and 2000, landings of coral prawns averaged 160 tonnes, ranging between 80 to 280 tonnes annually [248]. This species would appear to be somewhat unusual because of its known propensity to bioaccumulate excessive concentrations of cadmium [158, 163]. Our findings reinforce the earlier observations of Francesconi and colleagues. Because of this small number of *M. crassissima* samples in the survey, we did not subject these samples to statistical consideration of species-specific relationships to prawn cadmium levels (see Section 4.3.3). However, the fact that both of our *M. crassissima* samples (i.e. 100%) had high cadmium levels is nonetheless an important outcome of this investigation.

As for the NPF prawns with muscle cadmium >0.5mg/kg, the observation that three of the four samples were endeavour prawns is noteworthy. In her investigation of metals in Torres Strait prawns, Evans-Illidge found that endeavour prawns carried the highest cadmium loads of the three species studied (*M. endeavouri*, *P. esculentus*, *P. longistylus*)[117]. Further insights regarding cadmium in endeavour prawns were revealed as a result of statistical testing, discussed in the following section.

5.1.1.2. Inferential statistical tests of cadmium in prawns

Of the four explanatory variables investigated in this survey (prawn genus & species, fishery, prawn size, season) the most important predictor of variability in cadmium levels was the species. Apart from the observation of high cadmium levels in *M. crassissima*, as outlined in the preceding section, analysis of cadmium levels as a continuous variable has shown that endeavour prawns are significantly more likely to carry higher cadmium levels than brown tiger prawns, western king prawns and banana prawns. This finding further reinforces the observations presented in the previous section, that three of the four high-cadmium samples from the Gulf of Carpentaria were endeavour prawns, as well as the earlier findings of higher cadmium levels in endeavours from the Torres Strait prawn fishery [117].

Some other species-specific relationships to prawn muscle cadmium concentration were revealed by our statistical testing, as presented in Section 4.3.3. Of the six species subjected to inferential statistical testing, banana prawns (*P. merguiensis*) and western king prawns (*P. latisulcatus*) returned the lowest cadmium concentrations.

A fishery-specific relationship to cadmium levels was also revealed. Prawns from the South Australian Spencer Gulf fishery were found to have remarkably low cadmium concentrations (see Figs. 13, 14 & 18).

Prawns sampled between May and September were found to have higher cadmium levels across all fisheries than those sampled between October and April (Fig. 21). Some overseas reports have also noted seasonal variation in prawn cadmium concentration, e.g. [134, 140]. When considering only samples from the Northern Prawn Fishery, mean cadmium levels were higher in the dry season, but the differences did not reach statistical significance (Fig. 20).

No relationship between prawn size and cadmium concentration in muscle tissue was seen. As noted in Section 4.3.4, some large *P. monodon* had undetectable cadmium levels, while the small *M. crassissima* samples had high levels.

Regression modelling of cadmium in prawn muscle reinforces the findings of the two-group and multiple-group hypothesis tests. Prawn species and, to a lesser extent fishery, were seen to be the most significant predictors of variability in cadmium concentration.

5.1.1.3. Supplementary tissues analysis: cadmium

The hypothesis that eating prawn muscle with alimentary tracts *in situ* will lead to higher cadmium exposure in consumers could not be supported by this investigation. Considering the null hypothesis that prawn muscle homogenates with and without the gut tubes would not differ statistically, the findings presented in Fig. 31 indicate that the null hypothesis could not be rejected. Fig. 33 shows that cadmium concentrations in the alimentary tract were always higher than the corresponding levels seen in muscle tissues. Therefore we conducted one-tailed t-tests to examine cadmium concentrations in homogenates with and without the proportional addition of gut tube tissues, comparing both the original cadmium analyses for those samples conducted over several analytical

runs throughout the previous year, as well as repeat replicate analyses conducted within the same analytical run. Those results were non-significant, at $p = 0.13$ and $p = 0.88$ respectively. The most likely explanation here is that of a dilution effect. A typical sample of eight prawns having full alimentary tracts, whole weight 310g, had 155g muscle tissue recovered after disassembly, and gut tubes weighing 1.2g. So the alimentary tracts constituted only 0.8% of the weight of muscle tissue. European Standard EN 13804:2002 stipulates that "In crustaceans the visible digestive tract shall be removed prior to analysis." This requirement presumably reinforces the approach in ES 13804:2002 that "In trace element analysis only the part intended for eating should be investigated…"

In Australia, "Food Standard Code 1.4.1. – contaminants and natural toxicants" states that: "The maximum level must be calculated for the edible content of the food that is ordinarily consumed." The wording of this Australian code would appear to leave more room for interpretation than is the case with the European standard. During informal discussions with some Australian analytical laboratories, we asked what tissues they would test in the event that a client presented prawns for trace element analysis without specifying which tissues should be analysed. Responses varied between "Muscle tissue" and "whole prawns." The laboratory that offered the latter response interpreted Code 1.4.1 such that whole prawns should be analysed because whole prawns may be prepared in dishes such as tapas.

Our analysis of cadmium in hepatopancreas tissues (see Table 7) demonstrates that, with one possible exception, cadmium in HP was always higher than found in muscle tissues, sometimes by several orders of magnitude. The exception, interestingly, was that of one of the *M. crassissima* samples, which had essentially equivalent concentrations of cadmium in muscle and hepatopancreas tissues (sample listed in Table 7 with 4.6mg/kg cadmium in hepatopancreas). That finding, however, did not hold for the other *M. crassissima* sample collected from Shark Bay, where the HP cadmium level of 8.2mg/kg was some five times higher than that measured in muscle tissue. These findings of higher cadmium levels in hepatopancreas tissue reinforce the findings from other studies worldwide, listed in Table 1, which show that the hepatopancreas is the principal storage organ for cadmium in decapod prawns.

One recommendation arising from these findings is that seafood industry operators having their prawns sampled and tested for cadmium may wish to consider specifying which tissues are to be analysed, depending on the principal reason for having the analyses conducted. If samples are being tested within Australia in order to gauge likely compliance for export markets (particularly into the EU) then we recommend testing of de-veined muscle tissue, in line with the European Standard EN 13804:2002 (despite our analyses of gut veins indicating that the contribution of cadmium in alimentary tracts to the overall prawn muscle cadmium concentration is undetectable).

Replicate testing of cadmium in prawn muscle homogenates showed that, as a further quality control measure, the analysis was reproducible and repeatable. Combined relative standard deviations (RSDs) of blinded analysis of muscle + gut tube triplicate homogenates and muscle-only replicates, at 8.4%, are essentially equivalent to the replicate analyses conducted unblinded by AAA (RSD 9.3%). The plots in Fig. 31 demonstrate that repeat analysis of prawn samples was repeatable and reproducible. One apparent failure is seen in the penultimate sample listed in Table 7 and plotted in Fig. 31. That sample had cadmium measured at 0.78mg/kg in the initial analysis, but replicate analyses conducted for the supplementary tissues analysis show all three subsequent analyses (each in triplicate) clustering

around the 1.3 mg/kg to 1.6 mg/kg mark. This would suggest that the initial 0.78mg/kg cadmium result may have been lower than the actual value. Otherwise, the rest of the samples show excellent agreement with each other and with the original analyses. The series of paired t-tests comparing sample cadmium levels tested and re-tested were all above statistical significance, indicating that the null hypotheses that mean cadmium levels measured by each of these methods were not different from one another could not be rejected.

5.1.2. SELENIUM RESULTS

5.1.2.1. Inferential statistical tests of selenium in prawns

As was seen for cadmium in prawn muscle tissue, there was a statistically significant influence of fishery on the overall selenium levels in this survey, as shown in Fig. 23. Post-hoc tests revealed that only samples from the South Australian fishery were different to those from each of the other three fisheries. So the combined picture for both these elements is that SA prawns had lower cadmium and higher selenium than prawns from the other fisheries.

Analysis of prawn by genus (without considering n = 2 *M. crassissima*) demonstrated that *Metapenaeus* spp. carried higher selenium levels than *Penaeus* spp. (Fig. 24). Further investigation of the n = 6 species surveyed in quantities greater than 5 samples showed a similar but not identical picture to that seen for cadmium. *M. endeavouri* were seen to have significantly higher selenium levels than other species, with the exception of *P. merguiensis*, where differences were non-significant (Figs. 25 & 26).

Unlike cadmium, selenium and prawn weight were correlated but the relationship would appear to be weak (R^2 = 0.33) (Fig. 28). Prawn weight was not identified as a significant explanatory variable in the regression model.

A seasonal relationship to selenium concentration was seen, but unlike that seen for cadmium, only prawns from the Northern Prawn Fishery were higher in selenium during the dry season (Fig. 29). That relationship did not hold when considering prawn samples from across the survey as a whole.

Regression modelling of selenium in prawn muscle essentially reinforces the findings of the two-group and multiple-group hypothesis tests. Prawn species was seen to be the most significant predictor of variability in selenium concentration.

5.1.2.2. Supplementary tissues analysis: selenium

Replicate analysis of prawn tissue homogenates for selenium was not as successful as for cadmium, but was mostly within acceptable limits. The initial results of the blinded, randomly-coded analyses were unacceptable, which led to us breaking the randomisation code with AAA in order to demonstrate that the repeat analyses had failed. AAA then identified a quality-control issue pertaining to the selenium re-analyses, and the muscle + gut tube homogenates and muscle-only homogenates prepared by SARDI were then re-analysed, in the same sequence (i.e. unblinded but with replicates randomly distributed throughout the analytical run). The re-analysed replicates are presented in Table 8 and plotted in Figure 32. Reproducibility was then found to be excellent, with combined RSD of 7.9%.

Reproducibility of the *n* = 7 AAA triplicates was somewhat weaker, with an RSD of 11.9%. Sample ACPF-126 would seem to be the main contributor to that uncertainty, with replicate selenium results of 0.75, 0.35 and 0.53 mg/kg. The AAA replicate analyses were not subjected to re-analysis after the QA failure was recognised. Note that the error bars and mean data point spread seen in Fig. 32 should not be compared directly with the cadmium plots in Fig. 31, because of the differing *y*-axis ranges of these graphs. The range of selenium concentrations seen in this survey was narrower than for cadmium. Therefore the spread of data points and error bars would appear to be worse than for cadmium from a cursory viewing of these two plots. In fact the reproducibility of the muscle + gut tube homogenates and the muscle-only replicates – as represented by combined RSD values in Tables 7 & 8, and the error bars in Figs. 31 and 32 – was essentially equivalent. The weaker reproducibility of the AAA triplicate selenium analyses possibly influenced the failure of that particular statistical test to demonstrate that mean selenium concentrations were not different (p= 0.02 for muscle-only replicates *vs.* AAA triplicates).

Selenium concentrations in isolated alimentary tracts, unlike cadmium, were – with one exception – lower than in muscle homogenates. But as was the case for cadmium, addition of gut tube contents in proportion to their original weight to prawn muscle homogenates did not result in any statistically-significant change in mean selenium concentration. Selenium was also found to be higher in hepatopancreas tissues than in muscle, although differentials were not greater than one order of magnitude (see Table 8). Selenium in prawn muscle was found to correlate with hepatopancreas at α = 5% level (Fig. 36), but the relationship appears weak (R^2 = 0.29).

5.2. Prawn species identification

The single-blind procedure we developed to identify prawn samples from photographs was successful. Some samples required re-photographing in order to capture better images of particular anatomical features – particularly the rostrum – but all samples were able to be identified to species level. The only exception was in the case of two samples identified only to genus level (*Metapenaeus* spp.) that were presented to us by sample providers as “bay prawns.” Direct observation under enhanced lighting and magnification was required in order to discriminate western king prawns (*P. latisulcatus*) and eastern king prawns (*P. plebejus*); the particular differentiating diagnostic feature in this case is the gastro-frontal groove on the ventral aspect of the rostrum.

The ability to formally identify prawn species tested in this survey has been of paramount importance for the design and implementation of this survey. The species-related variability in both cadmium and selenium would likely not have been revealed – or would at least have been incorrect – if our statistical inference testing had been informed by use of common names (and a mis-identified sample).

5.3. Cadmium in prawns in the peer-reviewed literature

The extensive list of papers and reports presented in Table 1 indicates that the topic of cadmium in prawns is of global interest, and has been so for several decades, presumably since the availability of accurate and reproducible analytical methods in the 1970s. The reports listed in Table 1 would seem to be the first published review of this subject to be conducted systematically. We have not attempted to critically appraise these reports and rank them accordingly; results presented in Table 1 are “as

published." In a compendium of this size, we may expect the quality of the research to vary. We were intrigued, for example, by the two reports from a group in Tamil Nadu, India, published in different journals three years apart, in which different treatment group numbers of freshwater prawns were dosed with an identical cadmium regime. Cadmium concentrations in three tissue compartments were identical across both studies, which is a remarkable result [159, 160]. Note also that there are likely to be implications for interpreting some of the reports published in Table 1 that pertain to advances in analytical capability since the 1970s. For example, a report from work conducted in the late 1970s notes several cadmium non-detects in prawn samples where the AAS detection limit was 0.7mg/kg [139]. The sensitivity of AAS techniques and the more recently-adopted ICP-MS methods have improved substantially across ensuing decades.

Many, indeed probably the majority, of the observational studies listed in Table 1 have as the main focus of their investigation a broader picture than cadmium in prawns. A large proportion of Table 1 reports are concerned with the investigation of other metals and/or other biota. We have extracted and tabulated information only on cadmium in edible decapod prawns from those studies.

No obvious temporal or spatial trends for changes in prawn cadmium concentration are discernible from the published literature tabled in Table 1 (table columns "Year" and "Location"). Both low and high levels of cadmium in Australian prawns are reported, as was the case for the broader Western Europe (including the U.K.) and northeast Atlantic region. Observational studies on prawns from the Americas tended to cluster at lower reported cadmium concentrations in muscle tissues, although high levels in hepatopancreas were noted. Prawns sampled from aquaculture facilities had low cadmium loads (table column "Species"). The range of species investigated is broad, which essentially precludes any substantial interrogation of the data presented in Table 1 for species-specific cadmium levels. Most of the more frequently-sampled species, e.g. *P. monodon*, *P. merguiensis*, *C. crangon*, are seen ranging across the spectrum of reported cadmium levels. However, observational studies on *P. vannamei* all reported cadmium concentrations below 0.3mg/kg.

Reported cadmium concentrations are tabulated as reported in each study, as either in whole prawns, in muscle tissues only, in muscle and other tissues, or in different tissues as well as whole prawns. These various studies, both observational and experimental, from around the world, and conducted across several decades, clearly show that prawns have higher concentrations of cadmium in hepatopancreas and gill tissues than are found in muscle tissues.

We have included in Table 1 reports of experimental studies where cadmium was dosed and/or quantified in tissues of edible decapod prawns. Studies in which uptake occurs from the gills via aqueous concentrations of cadmium that would be unlikely to be encountered in natural waters therefore do not invite direct consideration of food safety risks, but such studies serve to illustrate the capacity of decapod prawns to bioaccumulate cadmium over short time-scales, and to sequester very high concentrations in the hepatopancreas.

5.4. Sample size estimates, random sampling program and sample collection

To inform our sample size calculation, we adopted a proportion of 10.1% of prawn samples returning cadmium levels >0.5mg/kg. This figure was taken from a historical dataset of >2,000 laboratory

analyses conducted over two decades. We applied a 5% minimum precision level for our calculations; the sample size of *n* = 139 so calculated would therefore have predicted that between 5% and 15% of the population of prawns sampled would have cadmium levels above 0.5mg/kg. In fact, this study showed that cadmium levels >0.5mg/kg were just outside of these predictions, at six of 140 samples, = 4.3%. However, the reason for this discrepancy would appear to lie with the observation that the historical dataset proportion of 10.1% included analyses of cadmium in prawn muscle, whole prawns and prawn heads. Our study was principally concerned with analysis of cadmium in de-veined prawn muscle; measurement of cadmium in whole prawns and in particular in prawn heads will raise the proportion of cadmium detects >0.5mg/kg because of the known capacity of prawns to concentrate this metal in hepatopancreas tissues.

After conducting our sample size calculations and initiating the survey with the aim of securing *n* = 139 samples, we subsequently re-interrogated the historical dataset and discovered that information was available to identify the prawn tissues analysed for each of the samples (information that had escaped our attention at the time of developing sample size estimates and calculations). 136 prawn samples analysed for cadmium in muscle tissue only were above the 0.5mg/kg threshold, i.e. 6.65% of the historical dataset samples. Using that figure to populate our sample size estimates would have recommended a sample size of *n* = 95, which would have predicted a range of 1.7% to 11.7% of muscle-only cadmium >0.5mg/kg in the population to be sampled. However, we are confident that our actual sampling program, which arguably represents over-sampling given the considerations discussed here, has resulted in a study that has facilitated more robust statistical conclusions than might otherwise have been possible with a smaller sampling program.

The random sampling strategies we adopted were successful in the main, and allowed us to present in large part a sample of prawns from Australia's main fisheries that reflects recent productivity and is essentially free of concerns regarding the potential for bias. The small proportion of samples that we were either unable to secure or were replaced by samples from outside of discrete locations identified by the random sampling program was largely influenced by the vagaries and challenges of having small batches of prawns collected, labelled, packed, stored and air-freighted to our laboratory by busy seafood-industry workers. One of the specific challenges identified by a seafood industry representative concerned the number of hands that one of our requested samples needed to pass through in order to get to us. After relaying our request to a trawler skipper operating in a location that our random sampling program had selected, the crew of that vessel may have collected a sample, bagged, labelled and frozen it, but then handed it to another crew (say on a mothership) who would then hand it to yet another staffer at the company's onshore distribution centre, who would then need to arrange air-freight to us. So this presented something of a "what's in it for us?" dilemma: having our samples pass through a chain of workers, not all of whom may have been fully and comprehensively apprised of the aims of our study. This despite the obvious goodwill and enthusiasm we encountered from many, indeed most, of our seafood industry contacts.

So while we eventually succeeded in the main with our sampling program, we must emphasise for the sake of planning future investigations of this kind that the collection and transport of fresh prawn samples is not an inconsequential consideration. The difficulties we experienced, particularly with securing samples from the Queensland east coast prawn fishery, were the principal cause of the delays in conducting and completing the work presented here. For consideration of scoping, funding and planning of future investigations like this one, we recommend careful and detailed attention to the

question of sampling logistics. In our experience this is not a trivial matter, and significant efficiency gains should be realisable in future if the sampling strategy is given a more strategic focus.

5.5. SOURCES OF CADMIUM IN PRAWNS

The results of this survey of Australia's principal wild-capture prawn fisheries have shown that cadmium levels in prawns which exceeded the EC's regulatory alert concentration of 0.5mg/kg in muscle tissue were restricted to two geographical locations: Shark Bay in Western Australia, and the Gulf of Carpentaria in the Northern Prawn fishery. While our data should not be interpreted in such a way as to draw any conclusions regarding the sources of cadmium in prawns – the survey was not designed to attempt any such interrogation – some potential and theoretical issues arising from this work arguably deserve some consideration in this regard.

Firstly to Shark Bay in Western Australia. Other marine biota from this site, mainly bivalve molluscs, are known to be capable of carrying high cadmium loads [249, 250]. McConchie *et al* [250] note that Shark Bay is sufficiently spatially removed from intensive industrial activities to reject attribution of these excessive cadmium bioaccumulation findings to anthropogenic cadmium contamination. These authors, however, also discuss the geomorphology of the Bay, observing that "No rocks with high heavy metal contents have yet been recognised along the eastern margins of Shark Bay or in any other nearby strata." McConchie *et al* do not make strong conclusions regarding the sources of cadmium in Shark Bay biota, suggesting a range of potential mechanisms that invite further investigation. Francesconi *et al* [249], in their study of cadmium in Shark Bay scallops, take the default position that high levels of cadmium found in these molluscs "…must be of natural origin because [this area is] far removed from any possible anthropogenic source of cadmium." Subsequent studies by Francesconi *et al* [158, 163] have shown that coral prawns (*M. crassissima*) captured from both Shark Bay and Exmouth Gulf can bioaccumulate high levels of cadmium.

Next, the issue of cadmium in Spencer Gulf biota. In their 1986 report, Ward *et al* [145]reported a survey of a range of fish, molluscs, crustaceans (including edible prawns), an ascidian and seagrasses from Spencer Gulf, investigating the impact of the Port Pirie lead smelter on cadmium, zinc and lead contamination in the Gulf. The Port Pirie facility became the world's largest lead smelter in 1934, and is still one of the world's biggest lead refining operations. Ward *et al* conducted a detailed mapping and modelling investigation of metal contamination, showing decreasing concentration gradients in marine biota with distance from the smelter, and discussing other interacting variables, particularly retention and mobilisation of toxic metals from sediments. As listed in Table 1, Ward *et al* measured cadmium in *P. latisulcatus* at up to 10mg/kg dry weight (approx 2.5mg/kg wet weight) in whole Spencer Gulf prawns; Ward *et al* demonstrated a gradient for cadmium in *P. latisulcatus*, with higher levels seen in samples collected closest to the smelter. More recent work by Edwards *et al* [251] found high metal concentrations in Spencer Gulf seston and fish. Edwards *et al* cited investigations from the 1970s that found extensive contamination of upper Spencer Gulf by toxic metals, noting that: "The geographic pattern of cadmium, lead and zinc in the sediments suggested that metal-rich particulates from smelter stack emissions, from ore spillage in handling and shipping operations, and from fugitive dusts were the main general sources of contamination of the sediments." [251].

The findings from this survey, with prawns sampled during 2012 and 2013, are that *P. latisulcatus* from Spencer Gulf had remarkably low levels of cadmium. Samples were collected from across the entire fishery, including two samples from the upper Gulf (See Figure 5, map grids 14 and 15) both of which had undetectable levels of cadmium. So this is a noteworthy departure from the earlier published reports of high cadmium contamination in Spencer Gulf prawns. Such comparisons should be viewed with caution, again because this survey was not designed to address complex and multivariable issues influencing the transfer of point-source anthropogenic cadmium contamination into marine biota. However, the detailed environmental mapping and modelling studies into metals contamination in Spencer Gulf that are published in the peer-reviewed literature are now several decades old. Our current survey, which has not identified any cadmium contamination in western king prawns from Spencer Gulf, hints at the possibility of remediation if environmental licensing and pollutant discharges from the Port Pirie smelter have incrementally improved over ensuing decades (which we assume has occurred, though we have not attempted to ascertain detailed changes in the environmental management program of the smelter operation). These improvements may have occurred over more recent time-scales, however. The Australian Government's National Pollutant Inventory website shows that reported airborne emissions of cadmium from the smelter were greater than 3 tonnes for each year between 2000/01 and 2007/08, whereas the reported airborne emissions were 0.47 tonnes for 2008/09, 0.45 tonnes for 2009/10, 1.2 tonnes (2010/11) and 0.36 tonnes (2011/12). Aqueous discharges of cadmium across this period were less variable, ranging from 0.89 tonnes in 2000/01 to 0.31 tonnes for 2009/10 http://www.npi.gov.au/npidata/action/load/emission-by-individual-facility-result/criteria/state/SA/year/2012/jurisdiction-facility/SA0018

The historical dataset, comprising industry and National Residue Survey monitoring of cadmium in prawns, lists results for n = 264 analyses of South Australian king prawns conducted between 1991 and 2007. The majority of these tests were presumably made on product from Spencer Gulf, this being the state's principal prawn fishery. No samples returned cadmium levels above the EC 0.5 mg/kg level; the highest concentration recorded in muscle tissue was 0.14 mg/kg, and the highest head-only concentration was 0.36 mg/kg.

The results of this survey would appear to invite a more comprehensive, current investigation of cadmium and other toxic metals in Spencer Gulf sediments and marine biota – including prawns and other seafood – with the aim of addressing the question of whether or not improvements in environmental metals contamination attributable to the operations at the Port Pirie facility have occurred over time.

Finally, to the situation in the Gulf of Carpentaria. It is likely that cadmium in Gulf waters occurs through both natural and anthropogenic inputs. Studies of the geological profile of the Gulf show that the upper layers are sedimentary in origin [252]; sedimentary rocks carry higher cadmium concentrations than igneous and metamorphic forms [171, 173]. Cadmium is reported to occur naturally in Torres Strait marine sediments [253]. However, some significant metallurgical mining and processing operations are sited in this region – most notably the large-scale bauxite mining and alumina processing industries at both peninsular entrances to the Gulf, at Weipa on the eastern entrance and Gove on the western entrance. The McArthur River zinc and lead mine is also a very large operation, as is the Groote Eylandt manganese plant. As discussed in Section 2.2, bauxite and its alumina waste product, red mud, have a particularly important *potential* consideration with regard to environmental cadmium contamination, regardless of the proportional source contributions (natural

and/or anthropogenic) of cadmium. Bauxite and red mud have a particular and potent affinity for metals, including cadmium; this characteristic affinity is exemplified by the multifarious applications being researched and commercialised for red mud and bauxite to function as cadmium sequestrants and metals pollution remediation agents. If cadmium is present in marine systems, whether from natural and/or industrial sources, and potent chemical adsorbents like bauxite and red mud are also in these same systems, it can be argued that the physical, chemical and biological dynamics of such interactions are worthy of research investigation with the aim of understanding how and to what degree such interactions may influence both food web and dissolved (i.e. gill-mediated) uptake of metals into marine biota. Complementary investigations, perhaps involving the discipline of isotope geochemistry, may assist in determining and differentiating the various sources of contaminant cadmium in sediments, waters and biota.

In the Gulf of Carpentaria, cadmium is clearly present in the marine ecosystem such that at some times and in some locations, it can be transferred to seafood products in concentrations sufficient to trigger public health concerns in some jurisdictions and attendant economic disruption to the seafood industry. While the overall intensity and scale of industrial activities that emit pollutant metals into the environment in the southern hemisphere has always been much lower than is the case in the northern hemisphere, it is not the case that all of Australia's coastal waters can continue to be viewed as pristine by comparison. Some very large-scale metalliferous mining and processing operations have the potential to influence marine metal pollution dynamics via both land-based runoff and emission of airborne dusts. Industries that have the potential to disseminate toxic metals into marine ecosystems are required to adhere to relevant environmental management programs and regulations, and their activities are subject to oversight by agencies such as state government environmental protection authorities. Yet the potential exists for unusual and difficult-to-predict events to disrupt routine operations. An example from recent years that illustrates the potential for this kind of acute economic disruption is that of the abandonment of the ore carrier *Wunma* in the Gulf of Carpentaria during a cyclone in 2007 [254]. The ship was loaded with zinc concentrate; some 200 tonnes was lost overboard. Expert opinion on the impacts of that event that pertain to the risks of toxic metals contamination and uptake into marine biota focused on the chemical properties of the discharged concentrate. Because metals were in the form of sulfides, which are weakly soluble in water, expert opinion was that "no significant bioavailability of the metals, and … no significant chemical toxicity or bioaccumulation of metals such as lead, zinc or cadmium [would be anticipated]" [254]. So while that particular event may, in retrospect (and in the short-term), be viewed as something of a "close call" for the local seafood industry, some other conclusions of the Marine Board of Inquiry report into this incident are particularly pertinent to the discussion here. The panel noted that:

> "*The conclusion that the spillage of zinc concentrate at around the time of the incident has not been shown to have produced any significant impact on the marine environment does not diminish the concerns of local communities, persons involved in the fishing industry and members of the general public about the spillage, and the need to avoid a repetition of it. The waters of the Gulf are part of a unique ecosystem. … The fishing industry and those who rely upon it for their livelihoods depend upon the protection of the marine environment, and, to some extent, upon the Gulf's reputation as a relatively pristine body of water.*
>
> *"The preservation of the Gulf as a unique and relatively pristine body of water serves a variety of private interests and the public interest. The public interest in*

preventing the spillage of cargo into the marine environment is reflected in both international conventions and domestic law. ….The importance of that objective is not diminished by the fact that the spillage in February 2007 has not been shown to have produced any significant impact on the marine environment." [254].

While the potential impact of such near-catastrophic incidents on the seafood industry is obvious and dramatic, we suggest that there is still much to learn about incremental and inconspicuous interactions between anthropogenic activities and natural geochemistry that may influence the presence and dissemination of toxic metals into marine environmental compartments and food webs. Once again, we emphasise that this study of cadmium and selenium in Australia's wild-caught prawns cannot draw conclusions about the sources – whether natural and/or anthropogenic – of cadmium in Gulf of Carpentaria prawns, nor indeed about the reasons for the essential absence of cadmium in Spencer Gulf prawns. The sources – and sinks – for cadmium are likely to be complex, dynamic, and subject to interactions from various physical, chemical and biological factors. These sources, sinks and interactions are poorly understood, but are amenable to incremental improvement in understanding by the application of well-designed observational and experimental research investigations.

6. Conclusions and Recommendations

This large survey of cadmium and selenium in prawns from Australia's main commercial prawn fisheries has found that a low proportion (4.3%) of prawns overall had cadmium concentrations in muscle tissue that exceeded the EC regulatory level of 0.5 mg/kg. Within the survey, particular prawn species and locations were identified that may represent avenues for future seafood industry-wide interventions that may help to address market access problems and the broader reputation of Australian wild-caught prawns as a high-quality product. Because coral prawn landings represent only a small component of Western Australia's wild-catch prawn fisheries, we analysed only two samples of *M. crassissima*, and for that reason we did not include that species in our statistical testing to examine the relative contribution of prawn species to explaining variability in cadmium levels. However, because both of the *M. crassissima* prawn samples in the survey were found to be in excess of the 0.5mg/kg cadmium level, this finding probably represents an important qualitative consideration. One of those samples had the highest cadmium concentration measured in the whole survey, at 4.6mg/kg. *M. crassissima*, marketed as coral prawns, are a small component of the WA prawn fishery, and a relatively low-value product. *M. crassissima* has been known to have an unusual capacity to bioaccumulate cadmium since reports were published on this matter in the peer-reviewed scientific literature in the 1990s [158, 163].

The other area of interest identified in this survey was the Gulf of Carpentaria. Four prawn samples, three of which were endeavour prawns, and one brown tiger prawn sample, were found to have excess cadmium concentrations. However, this still represents a reasonably small proportion of prawns sampled from the intensively-fished Gulf: 43 samples overall were collected for the survey from within the Gulf of Carpentaria, so only 9.3% of prawns from this area could be viewed as having a cadmium problem (again, by the EC maximum level). All samples from the Northern Prawn Fishery captured from waters west of the Gulf were below the ML, as were all prawns from South Australia and the Queensland east coast fishery. Prawns from South Australia's Spencer Gulf fishery all had

remarkably low levels of cadmium, and also the highest selenium levels, which may mark that particular product as having excellent potential for future marketing campaigns as a low-risk, high micronutrient item (at least for the two trace elements surveyed, cadmium and selenium).

Endeavour prawns were found to accumulate higher cadmium levels overall than other prawn species (except for *M. crassissima*, as noted above). Banana prawns and western king prawns had the lowest cadmium levels across surveyed species.

A weak relationship to season was seen, whereby prawns sampled between May and September across all fisheries had higher mean cadmium levels than prawns captured between October and April. That relationship did not hold at a statistically significant level for prawns fished from the Northern Prawn Fishery, where those dry / wet season periods apply.

M. crassissima and endeavour prawns were also found to have higher levels of selenium than the other species. Australian wild-caught prawns can be viewed as good sources of dietary selenium. The criteria for formally describing prawns as a "good source" of selenium have been met when considering all samples collected for this representative survey of Australia's wild-capture prawn fisheries: Food Standard 1.2.7. requires that a food item making a claim of "good source" for vitamins and minerals must contain at least 25% of the Recommended Daily Intake for the specified item [255]. The RDI for selenium is 70µg for adults, and 25µg for children aged 1-3 [256]. The lowest level of selenium measured in prawns for this survey was 0.38µg/kg, so a 0.25x adult RDI of 17.5µg selenium would be exceeded by a 50g portion of any of the prawns we surveyed, and a 0.25x child RDI of 6.25µg selenium would be met by a 20g portion of prawns.

Selenium concentrations were analysed in five samples of wild and farmed prawn species for the recent ASCRC study on seafood nutrient compositional profiles [257]. Measured selenium concentrations were all greater than 270 µg/kg, which, by Food Standard 1.2.7. criteria, qualifies those samples for description as selenium "good sources" with portion sizes of 100g [255]. The sampling strategy for this 2012-13 survey of wild-caught prawns means that this work is a more representative study of Australia's wild-capture prawn fisheries. Therefore both this work and the compositional profiles study complement the conclusion that Australian prawns can be confidently marketed as "good sources" of selenium.

Prawn hepatopancreas was found to contain higher levels of cadmium than muscle tissue. This finding reinforces studies conducted elsewhere in the world, and mirrors the picture seen in other decapod food items, i.e. lobster and crab. The understanding that prawn hepatopancreas, like lobster and crab tomalley, can concentrate toxic metals and other contaminants may have implications for future product development and marketing strategies that focus on whole prawns or, in particular, prawn "mustard" or tomalley. Cadmium contamination in crab hepatopancreas (aka "brown meat") was recently investigated by the UK's Food Standards Agency, which noted that "… eating brown crabmeat products can potentially make a large contribution to dietary exposure to cadmium for adults and toddlers." [258].

The following recommendations arise from the results and interpretation of this study:

1. Targeting prawn species as a potential cadmium risk reduction strategy

Our findings indicate that the greatest gains with regard to addressing market access problems for export prawns may be realised by strategic marketing of particular species. Banana prawns and western king prawns would appear to be excellent, low-cadmium-risk species.

2. Targeting prawn harvest location as a potential cadmium risk reduction strategy

Queensland's east coast, South Australia, Western Australia (with the exception of coral prawns, as previously discussed) and the Northern Prawn Fishery west of the Gulf of Carpentaria would all appear to represent low-cadmium-risk prawn fishing locations. While we identified 9.3% of samples captured from within the Gulf of Carpentaria as representing potential problems with regard to cadmium non-compliance into the European export market, we recognise that prawn productivity from the Gulf is one of the most important in the country. Addressing this particular aspect of the overall challenge represented by cadmium will be difficult and not readily dealt with in the short term. A better understanding of the sources of cadmium in Gulf of Carpentaria waters and prawns, and the degree to which industrial activities may or may not influence cadmium dynamics in that system could inform and direct strategies and interventions that may help manage the problem in future.

3. Assess the potential for continued export of high-cadmium-risk *M. crassissima* to negatively impact the reputation of other Australian prawn species

This survey has reinforced the discovery made twenty years ago that there may be a particular problem with *M. crassissima* in regard to cadmium. Western Australia's by-catch coral prawn fishery represents six percent of WA prawn fishery production. Western Australia contributes 15 percent of Australia's wild-capture prawn production, so WA coral prawns comprise only 0.9% of Australia's wild prawns (ABARES and WA Fisheries data, 2000-2010). While it is important to not overstate the significance of analyses of $n = 2$ *M. crassissima* samples conducted for this survey to the overall conclusions, this project reinforces previously published studies that indicate *M. crassissima* may have an unusual capacity to bioaccumulate cadmium [158, 163].

4. Australian wild-caught prawns can be marketed as a good source of selenium

This survey has found that Australian prawns may be considered good sources of dietary selenium. The terminology of "good source" satisfies the formal requirements established by FSANZ for nutritional claims relating to vitamins and minerals, so this aspect of the work may serve as a positive marketing message for the prawn industry.

4. Commercial laboratory analysis of prawns for industry clients: specify tissues for testing

In any batch of prawns, testing for cadmium in whole prawns will likely return higher levels than if the muscle tissues only are tested. This is because of the contribution of higher hepatopancreas cadmium concentrations to the whole prawn tissue matrix. Arising from this understanding should be the response that the client (i.e. the seafood industry operator in each case) should specify which tissues should be analysed, depending on the reasons for commissioning the analysis. Interpretation and discretion as to which tissues should be analysed should not be left to the analytical laboratory. By specifying which tissues are to be analysed, industry operators can make more informed comparisons regarding trends in prawn cadmium levels over time and across various locations. If the purpose of monitoring cadmium levels in particular fisheries is to assess compliance for export, the tissues specified for analysis should align with requirements for food safety regulations in the specific export destination. In the case of Europe, the current analytical standard for prawns is to measure cadmium in de-veined muscle only. If, for example, future product development involving whole prawns or prawn tomalley (mustard) is being scoped, then the appropriate tissues to analyse would be whole prawns and prawn hepatopancreas respectively. Therefore, we recommend that the Australian Council of Prawn Fisheries ensures that its constituent members understand that different prawn tissues will return different levels of cadmium when analysed, and where possible promotes a consistent analysis approach (e.g. de-veined muscle) so that data generated from the current time into the future can meaningfully be analysed for trends to identify any new and emerging threats.

5. Industry database of cadmium and other contaminants in prawns

A potential follow-on from Recommendation 4 above is that the prawn industry establishes a database of cadmium and other contaminants in prawn tissues that all industry partners contribute to. This database could be maintained by a service provider on behalf of the industry; the prawn industry would own the data. A simple set of guidelines for sample preparation and analytical methods could be developed and sent to industry partners so that monitoring tests could be standardised across the industry. This approach would be of benefit for future studies of the kind presented in this report, as data currently held by individual companies could be captured, collated and summarised. This information, currently held by individual prawn industry companies, can only be accessed by researchers after a call for data; piecemeal and incomplete datasets limit the usefulness of this information. A comprehensive database holding results of metals testing across time and location could be of considerable long-term benefit to the industry. Access to a comprehensive and unbiased database by researchers may considerably lower the cost to industry for commissioning future surveys of trace metals and other contaminants, or beneficial trace elements and nutrients.

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8. REFERENCES

1. Khan, A.T., J.S. Weis, and L. D'Andrea, *Studies of cadmium tolerance in two populations of grass shrimp, Palaemonetes pugio.* Bull Environ Contam Toxicol, 1988. **40**(1): p. 30-4.
2. Khan, A.T., J.S. Weis, and L. D'Andrea, *Bioaccumulation of four heavy metals in two populations of grass shrimp, Palaemonetes pugio.* Bull Environ Contam Toxicol, 1989. **42**(3): p. 339-43.
3. Frenet, M. and A. Alliot, *Comparative bioaccumulation of metals in Palaemonetes varians in polluted and non-polluted environments.* Mar Environ Res, 1985. **17**(1): p. 19-44.
4. Nimmo, D.W.R., D.V. Lightner, and L.H. Bahner, *Effects of cadmium on the shrimps, Penaeus duorarum, Palaemonetes pugio and Palaemonetes vulgaris*, in *Physiological responses of marine biota to pollutants*, F.J. Vernberg, et al., Editors. 1977, Academic Press: New York. p. 131-83.
5. Ahsanullah, M., D.S. Negilski, and F. Tawfik, *Heavy metal content of Callianassa spp. in Western Port.* Aust J Mar Freshw Res, 1980. **31**(6): p. 847-850.
6. Ahsanullah, M., M.C. Mobley, and D.S. Negilski, *Accumulation of cadmium from contaminated water and sediment by the shrimp Callianassa australiensis.* Mar Biol, 1984. **82**(2): p. 191-197.
7. Blasco, J., A.M. Arias, and V. Sáenz, *Heavy metal concentrations in Squilla mantis (L.) (Crustacea, Stomatopoda) from the Gulf of Cádiz: evaluation of the impact of the Aznalcollar mining spill.* Environ Int, 2002. **28**(1-2): p. 111-6.
8. Demina, L.L. and S.V. Galkin, *On the role of abiogenic factors in the bioaccumulation of heavy metals by the hydrothermal fauna of the Mid-Atlantic Ridge.* Oceanology, 2008. **48**(6): p. 784-797.
9. Tam, S.Y. and C.S. Mok, *Metallic contamination in oyster and other seafood in Hong Kong.* Food Addit Contam, 1991. **8**(3): p. 333-42.
10. Ip, C.C., et al., *Heavy metal and Pb isotopic compositions of aquatic organisms in the Pearl River Estuary, South China.* Environ Pollut, 2005. **138**(3): p. 494-504.
11. Goncalves, M.L., et al., *Effect of speciation on uptake and toxicity of cadmium to shrimp Crangon Crangon (L.).* Bull Environ Contam Toxicol, 1989. **43**(2): p. 287-94.
12. Shuhaimi-Othman, M., et al., *Sensitivity of the freshwater prawn, Macrobrachium lanchesteri (Crustacea: Decapoda), to heavy metals.* Toxicol Ind Health, 2011. **27**(6): p. 523-30.

13. Jung, K. and G.P. Zauke, *Bioaccumulation of trace metals in the brown shrimp Crangon crangon (Linnaeus, 1758) from the German Wadden Sea.* Aquat Toxicol, 2008. **88**(4): p. 243-249.
14. Carvalho, R.A., M.C. Benfield, and P.H. Santschi, *Comparative bioaccumulation studies of colloidally complexed and free-ionic heavy metals in juvenile brown shrimp Penaeus aztecus (Crustacea : Decapoda : Penaeidae).* Limnol Oceanogr, 1999. **44**(2): p. 403-414.
15. Munshi, A.B., A.S. Hina, and T.H. Usmani, *The effect of water salinity on the uptake of metals related to survival of penaeid shrimp.* J Chem Soc Pak, 2006. **28**(3): p. 288-294.
16. Papathanassiou, E., *Cadmium accumulation and ultrastructural alterations in oogenesis of the prawn Palaemon serratus (Pennant).* Bull Environ Contam Toxicol, 1986. **36**(2): p. 192-8.
17. Wu, J.P. and H.C. Chen, *Effects of cadmium and zinc on oxygen consumption, ammonium excretion, and osmoregulation of white shrimp (Litopenaeus vannamei).* Chemosphere, 2004. **57**(11): p. 1591-1598.
18. Wu, J.P., H.C. Chen, and D.J. Huang, *Histopathological and biochemical evidence of hepatopancreatic toxicity caused by cadmium and zinc in the white shrimp, Litopenaeus vannamei.* Chemosphere, 2008. **73**(7): p. 1019-26.
19. Nunez-Nogueira, G., et al., *Subcellular distribution of zinc and cadmium in the hepatopancreas and gills of the decapod crustacean Penaeus indicus.* Mar Biol, 2006. **150**(2): p. 197-211.
20. Sharif, R., et al., *Toxicological evaluation of some Malaysian locally processed raw food products.* Food Chem Toxicol, 2008. **46**(1): p. 368-74.
21. Custer, T.W. and C.A. Mitchell, *Trace elements and organochlorines in the sholagrass community of the lower Laguna Madre, Texas.* Environ Monit Assess, 1993. **25**(3): p. 235-246.
22. Mansour, S.A. and M.M. Sidky, *Ecotoxicological studies. 3. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt.* Food Chem, 2002. **78**(1): p. 15-22.
23. Sriket, P., et al., *Comparative studies on chemical composition and thermal properties of black tiger shrimp (Penaeus monodon) and white shrimp (Penaeus vannamei) meats.* Food Chem, 2007. **103**(4): p. 1199-1207.
24. Biney, C.A. and E. Ameyibor, *Trace metal concentrations in the pibnk shrimp Penaeus notialis from the coast of Ghana.* Water Air Soil Pollut, 1992. **63**(3-4): p. 273-279.
25. Fraser, M., C. Surette, and C. Vaillancourt, *Fish and seafood availability in markets in the Baie des Chaleurs region, New Brunswick, Canada: a heavy metal contamination baseline study.* Environ Sci Pollut Res, 2013. **20**(2): p. 761-770.
26. dos Santos, É.J., et al., *Simultaneous determination of Pb and Cd in seafood by ICP OES with on-line pre-concentration by solid phase extraction with Amberlite XAD-4 after complex formation with DDTP.* Braz Arch Biol Technol, 2013. **56**(1): p. 127-134.
27. Páez-Osuna, F. and L. Tron-Mayen, *Concentration and distribution of heavy metals in tissues of wild and farmed shrimp Penaeus vannamei from the northwest coast of Mexico.* Environ Int, 1996. **22**(4): p. 443-450.
28. Wu, X.Y. and Y.F. Yang, *Heavy metal (Pb, Co, Cd, Cr, Cu, Fe, Mn and Zn) concentrations in harvest-size white shrimp Litopenaeus vannamei tissues from aquaculture and wild source.* J Food Comp Anal, 2011. **24**(1): p. 62-65.
29. Darmono, D. and G.R. Denton, *Heavy metal concentrations in the banana prawn, Penaeus merguiensis, and leader prawn, P. monodon, in the Townsville region of Australia.* Bull Environ Contam Toxicol, 1990. **44**(3): p. 479-86.
30. Peerzada, N., M. Nojok, and C. Lee, *Distribution of heavy metals in prawns from Northern Territory, Australia.* Mar Pollut Bull, 1992. **24**(8): p. 416-418.
31. Páez-Osuna, F. and L. Tron-Mayen, *Distribution of heavy metals in tissues of the shrimp Penaeus californiensis from the northwest coast of Mexico.* Bull Environ Contam Toxicol, 1995. **55**(2): p. 209-15.

32. Jeckel, W.H., R.R. Roth, and L. Ricci, *Patterns of trace-metal distribution in tissues of Pleoticus muelleri (Crustacea: Decapoda: Solenoceridae).* Mar Biol, 1996. **125**(2): p. 297-306.
33. Soegianto, A., B. Irawan, and Hamami, *Bioaccumulation of heavy metals in aquatic animals collected from coastal waters of Gresik, Indonesia.* Asian J Water Environ Pollut, 2008. **6**(2): p. 95-100.
34. Burger, J. and M. Gochfeld, *Heavy metals in commercial fish in New Jersey.* Environ Res, 2005. **99**(3): p. 403-12.
35. Pigeot, J., et al., *Cadmium pathways in an exploited intertidal ecosystem with chronic cadmium inputs (Marennes-Oléron, Atlantic coast, France).* Mar Ecol Prog Ser, 2006. **307**: p. 101-114.
36. Xie, B., W. Jiang, and H. Yang, *Growth performance and nutrient quality of Chinese shrimp Penaeus chinensis in organic polyculture with razor clam Sinonovacula constricta or hard clam Meretrix meretrix.* Bulg J Agric Sci, 2011. **17**(6): p. 851-858.
37. Cheung, K.C. and M.H. Wong, *Risk assessment of heavy metal contamination in shrimp farming in Mai Po Nature Reserve, Hong Kong.* Environ Geochem Health, 2006. **28**(1-2): p. 27-36.
38. Sheppard, B.S., D.T. Heitkemper, and C.M. Gaston, *Microwave digestion for the determination of arsenic, cadmium and lead in seafood products by inductively coupled plasma atomic emission and mass spectrometry.* Analyst, 1994. **119**(8): p. 1683-1686.
39. Kikuchi, Y., et al., *Cadmium concentration in current Japanese foods and beverages.* J Occup Health, 2002. **44**(4): p. 240-247.
40. Ruelas-Inzunza, J., et al., *Biomonitoring of Cd, Cr, Hg and Pb in the Baluarte River basin associated to a mining area (NW Mexico).* Sci Total Environ, 2011. **409**(18): p. 3527-3536.
41. Lei, B., et al., *Trace elements in animal-based food from Shanghai markets and associated human daily intake and uptake estimation considering bioaccessibility.* Ecotoxicol Environ Saf, 2013. **96**: p. 160-7.
42. Tu, N.P.C., et al., *Regional variations in trace element concentrations in tissues of black tiger shrimp Penaeus monodon (Decapoda : Penaeidae) from South Vietnam.* Mar Pollut Bull, 2008. **57**(6-12): p. 858-866.
43. Park, J. and B.J. Presley, *Trace metal contamination of sediments and organisms from the Swan Lake area of Galveston Bay.* Environ Pollut, 1997. **98**(2): p. 209-221.
44. Pastorelli, A.A., et al., *Human exposure to lead, cadmium and mercury through fish and seafood product consumption in Italy: a pilot evaluation.* Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 2012. **29**(12): p. 1913-21.
45. Kaoud, H.A. and A. Rezk, *Effect of exposure to cadmium on the tropical freshwater prawn Macrobrachium rosenbergii.* Afr J Aquat Sci, 2011. **36**(3): p. 253-260.
46. Tu, N.P.C., et al., *Biomagnification profiles of trace elements through the food web of an integrated shrimp mangrove farm in Ba Ria Vung Tau, South Vietnam.* Am J Environ Sci, 2012. **8**(2): p. 117-129.
47. Deng, L., S. Lin, and H. Zhang, *Heavy metals in marine mollusks, crustaceans and fish from the Shenzhen coastal region*. Progress in Environmental Science and Technology, Vol II, Pts A and B, ed. S.C. Li, et al. 2009. 142-146.
48. Pierron, F., et al., *Effects of salinity and hypoxia on cadmium bioaccumulation in the shrimp Palaemon longirostris.* Environ Toxicol Chem, 2007. **26**(5): p. 1010-1017.
49. Opuene, K. and I.E. Agbozu, *Relationships between heavy metals in shrimp (Macrobrachium felicinum) and metal levels in the water column and sediments of Taylor Creek.* Int J Environ Res, 2008. **2**(4): p. 343-8.
50. Carbonell, G., C. Ramos, and J.V. Tarazona, *Heavy metals in shrimp culture areas from the Gulf of Fonseca, Central America. II. Cultured shrimps.* Bull Environ Contam Toxicol, 1998. **60**(2): p. 260-5.

51. Falcó, G., et al., *Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species.* J Agric Food Chem, 2006. **54**(16): p. 6106-12.
52. Santos, L.F.P., et al., *Assessment of cadmium and lead in commercially important seafood from São Francisco do Conde, Bahia, Brazil.* Food Control, 2013. **33**(1): p. 193-199.
53. Olmedo, P., et al., *Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers.* Environ Int, 2013. **59**: p. 63-72.
54. Maher, W.A., *Trace metal concentrations in marine organisms from St. Vincent Gulf, South Australia.* Water Air Soil Pollut, 1986. **29**(1): p. 77-84.
55. Pourang, N. and G. Amini, *Distribution of trace elements in tissues of two shrimp species from Persian Gulf and effects of storage temperature on elements transportation.* Water Air Soil Pollut, 2001. **129**(1-4): p. 229-243.
56. Méndez, L., et al., *Effect of stocking densities on trace metal concentration in three tissues of the brown shrimp Penaeus californiensis.* Aquaculture, 1997. **156**(1-2): p. 21-34.
57. Phuong, N.T.K. and N.C. Khoa, *Evaluation of heavy metals in tissue of shellfish from Can Gio coastline in Ho Chi Minh City, Vietnam.* Asian J Chem, 2013. **25**(15): p. 8552-6.
58. Medina, J., et al., *Determination of mercury, cadmium, chromium and lead in marine organisms by flameless atomic absorption spectrophotometry.* Mar Pollut Bull, 1986. **17**(1): p. 41-44.
59. Ahdy, H.H.H., A.M.A. Abdallah, and F.T. Tayel, *Assessment of heavy metals and nonessential content of some edible and soft tissues.* Egypt J Aquat Res, 2007. **33**(1): p. 85-97.
60. Storelli, M.M., *Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs).* Food Chem Toxicol, 2008. **46**(8): p. 2782-8.
61. Alam, L., C.A. Mohamed, and M. Bin Mokhtar, *Accumulation pattern of heavy metals in marine organisms collected from a coal burning power plant area of Malacca Strait.* ScienceAsia, 2012. **38**(4): p. 331-339.
62. Jensen, S., et al., *Undesirable substances in seafood products - results from the Icelandic marine monitoring activities in the year 2012*. 2013, Matís: Reykjavík. 34 pp.
63. Madany, I.M., A.A.A. Wahab, and Z. Al-Alawi, *Trace metals concentrations in marine organisms from the coastal areas of Bahrain, Arabian Gulf.* Water Air Soil Pollut, 1996. **91**(3-4): p. 233-248.
64. Miramand, P., et al., *Contamination of the biological compartment in the Seine estuary by Cd, Cu, Pb, and Zn.* Estuaries, 2001. **24**(6B): p. 1056-1065.
65. Turkmen, G., *Seasonal variation of heavy metals in shrimp Penaeus kerathurus (Forskal, 1775) from Izmir Bay, Turkey.* J Anim Vet Adv, 2012. **11**(15): p. 2839-2844.
66. D'Adamo, R., et al., *Migratory crustaceans as biomonitors of metal pollution in their nursery areas. The Lesina lagoon (SE Italy) as a case study.* Environ Monit Assess, 2008. **143**(1-3): p. 15-24.
67. Krelowska-Kulas, M., *Content of some metals in mean tissue of salt-water and fresh-water fish and in their products.* Nahrung, 1995. **39**(2): p. 166-72.
68. Everaarts, J.M., R. Heesters, and C.V. Fischer, *Heavy metals (Cu, Zn, Pb, Cd) in sediment, zooplankton and epibenthic invertebrates from the area of the continental slope of the Banc d'Arguin (Mauritania).* Hydrobiologia, 1993. **258**: p. 41-58.
69. Olowu, R.A., et al., *Determination of heavy metals in crab and prawn in Ojo Rivers Lagos, Nigeria.* J Chem, 2010. **7**(2): p. 526-530.
70. Balkas, T.I., S. Tuğrul, and I. Salihoğlu, *Trace metal levels in fish and crustacea from northeastern Mediterranean coastal waters.* Mar Environ Res, 1982. **6**(4): p. 281-289.
71. Tyrrell, L., et al., *Trace metal concentrations in various fish species landed at selected Irish ports, 2003*, in *Marine Environment and Health Series, No. 20, 2005*. 2005, Marine Institute, Marine Environment and Food Safety Services: Dublin. 19pp.

72. Ásmundsdóttir, Á.M., et al., *Undesirable substances in seafood products - results from monitoring activities in 2006*. 2008, Matís: Reykjavík. p. i-iv; 1-39.
73. Hossain, M.S. and Y.S.A. Khan, *Trace metals in Penaeid shrimp and spiny lobster from the Bay of Bengal.* ScienceAsia, 2001. **27**(3): p. 165-8.
74. Tu, N.P.C., et al., *Bioaccumulation and distribution of trace elements in tissues of giant river prawn Macrobrachium rosenbergii (Decapoda : Palaemonidae) from South Vietnam.* Fish Sci, 2008. **74**(1): p. 109-119.
75. Panutrakul, S., et al., *Heavy metals in wild banana prawn (Fenneropenaeus merguiensis de Man, 1888) from Chantaburi and Trat provinces, Thailand.* J Shellfish Res, 2007. **26**(4): p. 1193-1202.
76. Abdolahpur Monikh, F., et al., *Heavy metals' concentration in sediment, shrimp and two fish species from the northwest Persian Gulf.* Toxicol Ind Health, 2013.
77. Lobato, R.O., et al., *The role of lipoic acid in the protection against of metallic pollutant effects in the shrimp Litopenaeus vannamei (Crustacea, Decapoda).* Comp Biochem Physiol A Mol Integr Physiol, 2013. **165**(4): p. 491-7.
78. Guns, M., et al., *Trace metals in selected benthic invertebrates from Belgian coastal waters (1981-1996).* Mar Pollut Bull, 1999. **38**(12): p. 1184-1193.
79. Mdegela, R.H., et al., *Heavy metals and organochlorine residues in water, sediments, and fish in aquatic ecosystems in urban and peri-urban areas in Tanzania.* Water Air Soil Pollut, 2009. **203**(1-4): p. 369-379.
80. Brunner, B., H. Marx, and A. Stolle, *Lead, mercury and cadmium burden of the German mud flats (Wattenmeer) does not hamper quality of common shrimp.* Rev Med Vet (Toulouse), 2002. **153**(11): p. 747-750.
81. Marx, H. and B. Brunner, *Heavy metal contamination of North Sea shrimp (Crangon crangon L.).* Z Lebensm Unters Forsch A, 1998. **207**(4): p. 273-275.
82. Sadiq, M., et al., *Heavy metal concentrations in shrimp, crab, and sediment obtained from AD-Dammam sewage outfall area.* Bull Environ Contam Toxicol, 1982. **29**(3): p. 313-9.
83. White, S.L. and P.S. Rainbow, *Accumulation of cadmium by Palaemon elegans (Crustacea: Decapoda).* Mar Ecol Prog Ser, 1986. **32**(1): p. 17-25.
84. White, S.L. and P.S. Rainbow, *A preliminary study of Cu-, Cd- and Zn-binding components in the hepatopancreas of Palaemon elegans (Crustacea: Decapoda).* Comp Biochem Physiol C, 1986. **83**(1): p. 111-6.
85. Horowitz, A. and B.J. Presley, *Trace metal concentrations and partitioning in zooplankton, neuston, and benthos from the south Texas outer continental shelf.* Arch Environ Contam Toxicol, 1977. **5**(2): p. 241-55.
86. Sivaperumal, P., T.V. Sankar, and P.G. Viswanathan Nair, *Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards.* Food Chem, 2007. **102**(3): p. 612-620.
87. Vos, G., J.P. Hovens, and P. Hagel, *Chromium, nickel, copper, zinc, arsenic, selenium, cadmium, mercury and lead in Dutch fishery products 1977-1984.* Sci Total Environ, 1986. **52**(1-2): p. 25-40.
88. Pourang, N., J.H. Dennis, and H. Ghourchian, *Distribution of heavy metals in Penaeus semisulcatus from Persian Gulf and possible role of metallothionein in their redistribution during storage.* Environ Monit Assess, 2005. **100**(1-3): p. 71-88.
89. Harding, L. and D. Goyette, *Metals in northeast Pacific coastal sediments and fish, shrimp, and prawn tissues.* Mar Pollut Bull, 1989. **20**(4): p. 187-189.
90. Martorell, I., et al., *Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend.* Biol Trace Elem Res, 2011. **142**(3): p. 309-22.
91. Storelli, M.M., et al., *Toxic metals (Hg, Cd, and Pb) in fishery products imported into Italy: suitability for human consumption.* J Food Prot, 2012. **75**(1): p. 189-94.

92. Páez-Osuna, F. and C. Ruiz-Fernández, *Comparative bioaccumulation of trace metals in Penaeus stylirostris in estuarine and coastal environments.* Estuar Coast Shelf Sci, 1995. **40**(1): p. 35-44.
93. Drava, G., et al., *Trace elements in the muscle of red shrimp Aristeus antennatus (Risso, 1816) (Crustacea, Decapoda) from Ligurian sea (NW Mediterranean): variations related to the reproductive cycle.* Sci Total Environ, 2004. **321**(1-3): p. 87-92.
94. Pastor, A., et al., *Levels of heavy metals in some marine organisms from the western Mediterranean area (Spain).* Mar Pollut Bull, 1994. **28**(1): p. 50-53.
95. Everaarts, J.M. and C. Swennen, *Heavy metals (Zn, Cu, Cd, Pb) in some benthic invertebrate species and in sediment from three coastal areas in Thailand and Malaysia.* J Sci Soc Thailand, 1987. **13**(4): p. 189-203.
96. Prabhu Dass Batvari, B., et al., *Heavy metals accumulation in crab and shrimps from Pulicat lake, north Chennai coastal region, southeast coast of India.* Toxicol Ind Health, 2013.
97. Whyte, J.N.C. and J.A. Boutillier, *Concentrations of inorganic elements and fatty acids in geographic populations of the spot prawn Pandalus platyceros* Can J Fish Aquat Sci, 1991. **48**(3): p. 382-390.
98. Deshpande, A., et al., *Analysis of heavy metals in marine fish from Mumbai Docks.* Environ Monit Assess, 2009. **159**(1-4): p. 493-500.
99. Capelli, R., et al., *A four-year study on the distribution of some heavy metals in five marine organisms of the Ligurian Sea.* Mar Chem, 1983. **12**(4): p. 281-293.
100. Ray, S., et al., *The disposition of cadmium and zinc in Pandalus montagui* Arch Environ Contam Toxicol, 1980. **9**(6): p. 675-681.
101. Hendrickx, M.E., F. Páez-Osuna, and H.M. Zazuet-Padilla, *Biology and biochemical composition of the deep-water shrimp Heterocarpus vicarius Faxon (Crustacea : Decapoda : Caridea : Pandalidae) from the southeastern Gulf of California, Mexico.* Bull Mar Sci, 1998. **63**(2): p. 265-275.
102. Skwarzec, B., et al., *Influence of accumulation of cadmium on the content of other microelements of two species of Black Sea decapods.* Bull Environ Contam Toxicol, 1984. **32**(1): p. 93-101.
103. Hashmi, M.I., S. Mustafa, and S.A. Tariq, *Heavy metal concentrations in water and tiger prawn (Penaeus monodon) from grow-out farms in Sabah, North Borneo.* Food Chem, 2002. **79**(2): p. 151-156.
104. Canli, M., M. Kalay, and Ö. Ay, *Metal (Cd, Pb, Cu, Zn, Fe, Cr, Ni) concentrations in tissues of a fish Sardina pilchardus and a prawn Peaenus japonicus from three stations on the Mediterranean Sea.* Bull Environ Contam Toxicol, 2001. **67**(1): p. 75-82.
105. Bayen, S., et al., *Persistent organic pollutants and heavy metals in typical seafoods consumed in Singapore.* J Toxicol Environ Health A, 2005. **68**(3): p. 151-166.
106. Kumar, K.A. and H. Achyuthan, *Heavy metal accumulation in certain marine animals along the East Coast of Chennai, Tamil Nadu, India.* J Environ Biol, 2007. **28**(3): p. 637-643.
107. Petersen, A. and G.K. Mortensen, *Trace elements in shellfish on the Danish market.* Food Addit Contam, 1994. **11**(3): p. 365-373.
108. Frías-Espericueta, M.G., et al., *Metal content of the gulf of California blue shrimp Litopenaeus stylirostris (Stimpson).* Bull Environ Contam Toxicol, 2007. **79**(2): p. 214-7.
109. Amaraneni, S.R., *Distribution of pesticides, PAHs and heavy metals in prawn ponds near Kolleru Lake wetland, India.* Environ Int, 2006. **32**(3): p. 294-302.
110. Fowler, S.W., *Trace metal monitoring of pelagic organisms from the open Mediterranean Sea.* Environ Monit Assess, 1986. **7**(1): p. 59-78.
111. Kurun, A., H. Balkis, and N. Balkis, *Accumulations of total metal in dominant shrimp species (Palaemon adspersus, Palaemon serratus, Parapenaeus longirostris) and bottom surface sediments obtained from the Northern Inner Shelf of the Sea of Marmara.* Environ Monit Assess, 2007. **135**(1-3): p. 353-67.

112. Nuñez-Nogueira, G. and P.S. Rainbow, *Cadmium uptake and accumulation by the decapod crustacean Penaeus indicus.* Mar Environ Res, 2005. **60**(3): p. 339-354.
113. Páez-Osuna, F. and C. Ruiz-Fernández, *Trace metals in the Mexican shrimp Penaeus vannamei from estuarine and marine environments.* Environ Pollut, 1995. **87**(2): p. 243-7.
114. Marcovecchio, J.E., *Trace metal residues in tissues of two crustacean species from the Bahía Blanca estuary, Argentina.* Environ Monit Assess, 1994. **29**(1): p. 65-73.
115. Tungare, S.M. and A.D. Sawant, *Lead and cadmium in selected species of shrimp around the Mumbai coast, India.* Bull Environ Contam Toxicol, 2002. **68**(3): p. 455-62.
116. Barwick, M. and W. Maher, *Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia.* Mar Environ Res, 2003. **56**(4): p. 471-502.
117. Evans-Illidge, E., *Heavy metals in commercial prawn and crayfish species in Torres Strait*, in *Report Series*. 1997, Great Barrier Reef Marine Park Authority: Townsville. p. i-xvii; 1-111.
118. Lakshmanan, P.T., *Levels of cadmium in seafood products.* Fish Technol, 1988. **25**(2): p. 142-6.
119. Joseph, K.O. and J.P. Srivastava, *Heavy metal load in prawn, Penaeus indicus (H. Milne Edwards) inhabiting Ennore estuary in Madras.* J Inland Fish Soc India, 1992. **24**(1): p. 30-3.
120. Tariq, J., et al., *Heavy metal concentrations in fish, shrimp, seaweed, sediment, and water from the Arabian Sea, Pakistan.* Mar Pollut Bull, 1993. **26**(11): p. 644-647.
121. Kargin, F., A. Dönmez, and H.Y. Çoğun, *Distribution of heavy metals in different tissues of the shrimp Penaeus semiculatus and Metapenaeus monocerus from the Iskenderun Gulf, Turkey: seasonal variations.* Bull Environ Contam Toxicol, 2001. **66**(1): p. 102-9.
122. Scrudato, R.J., B.F. Henningsen, and E.L. Estes, *Trace metal concentrations of selected macro-fauna from a southeast Texas estuary.* Texas J Sci, 1976. **27**(4): p. 419-26.
123. Kalogeropoulos, N., et al., *Heavy metals in raw, fried and grilled Mediterranean finfish and shellfish.* Food Chem Toxicol, 2012. **50**(10): p. 3702-3708.
124. Wharfe, J.R. and W.L.F. van den Broek, *Heavy metals in macroinvertebrates and fish from the Lower Medway Estuary, Kent.* Mar Pollut Bull, 1977. **8**(2): p. 31-4.
125. Guhathakurta, H. and A. Kaviraj, *Heavy metal concentration in water, sediment, shrimp (Penaeus monodon) and Mullet (Liza parsia) in some brackish water ponds of Sunderban, India.* Mar Pollut Bull, 2000. **40**(11): p. 914-920.
126. Krishnakumar, P.K., V.K. Pillai, and K.K. Valsala, *Bioaccumulation of trace metals by marine flora and fauna near a caustic soda plant (Karwar, India).* Indian J Fish, 1990. **37**(2): p. 129-37.
127. Wu, J.P. and H.C. Chen, *Metallothionein induction and heavy metal accumulation in white shrimp Litopenaeus vannamei exposed to cadmium and zinc.* Comp Biochem Physiol C Toxicol Pharmacol, 2005. **140**(3-4): p. 383-94.
128. Munshi, A.B., H.A. Siddiqi, and F.A. Ansari, *The effect of temperature on the uptake of copper and cadmium by penaeid shrimp.* J Chem Soc Pak, 2008. **30**(6): p. 840-844.
129. Everaarts, J.M., E. Otter, and C.V. Fischer, *Cadmium and polychlorinated biphenyls: different distribution pattern in North Sea benthic biota.* Neth J Sea Res, 1990. **26**(1): p. 75-82.
130. Rahimi, E., et al., *Cadmium concentrations in shrimp (Penaeus semisulcatus and Penaeus monodon) caught from the coastal areas in Southern Iran.* Toxicol Ind Health, 2013. **29**(3): p. 272-5.
131. Mantelatto, F.L.M., et al., *Heavy metals in the shrimp Xiphopenaeus kroyeri (Heller, 1862) (Crustacea, Penaeidae) from Ubatuba Bay, Sao Paulo, Brazil.* Bull Environ Contam Toxicol, 1999. **62**(2): p. 152-159.
132. Pourang, N. and J.H. Dennis, *Distribution of trace elements in tissues of two shrimp species from the Persian Gulf and roles of metallothionein in their redistribution.* Environ Int, 2005. **31**(3): p. 325-341.

133. Kureishy, T.W., *Concentration of heavy metals in marine organisms around Qatar before and after the gulf war oil spill.* Mar Pollut Bull, 1993. **27**: p. 183-186.
134. Çoğun, H., et al., *Seasonal variation and tissue distribution of heavy metals in shrimp and fish species from the Yumurtalik coast of Iskenderun Gulf, Mediterranean.* Bull Environ Contam Toxicol, 2005. **75**(4): p. 707-15.
135. Vazquez, F.G., et al., *Metals in fish and shrimp of the Campeche Sound, Gulf of Mexico.* Bull Environ Contam Toxicol, 2001. **67**(5): p. 756-62.
136. Ridout, P.S., et al., *Concentrations of V, Cr, Mn, Fe, Ni, Co, Cu, Zn, As and Cd in mesopelagic crustaceans from the North East Atlantic Ocean.* Mar Biol, 1989. **100**(4): p. 465-471.
137. Mitra, A., K. Banerjee, and S. Sinha, *Shrimp tissue quality in the lower Gangetic delta at the apex of Bay of Bengal.* Toxicol Environ Chem, 2011. **93**(3): p. 565-574.
138. Abdennour, C., et al., *Trace metals in marine, brackish and freshwater prawns (Crustacea, Decapoda) from northeast Algeria.* Hydrobiologia, 2000. **432**(1-3): p. 217-227.
139. Shiber, J.G., *Heavy metals in Ras Beirut prawns and sea urchin eggs.* J Environ Sci Health B, 1979. **14**(1): p. 73-95.
140. Everaarts, J.M., et al., *Copper, zinc and cadmium in benthic organisms from the Java Sea and estuarine and coastal areas around East Java.* Neth J Sea Res, 1989. **23**(4): p. 415-426.
141. Everaarts, J.M. and J. Nieuwenhuize, *Heavy metals in surface sediment and epibenthic macroinvertebrates from the coastal zone and continental slope of Kenya.* Mar Pollut Bull, 1995. **31**(4–12): p. 281-289.
142. Mitra, A., et al., *Analysis of trace metals in commercially important crustaceans collected from UNESCO protected World Heritage Site of Indian Sundarbans.* Turkish J Fish Aquat Sci, 2012. **12**(1): p. 49-61.
143. Soegianto, A., et al., *Bioaccumulation, elimination, and toxic effect of cadmium on structure of gills and hepatopancreas of freshwater prawn Macrobrachium sintangese (De Man, 1898).* Water Air Soil Pollut, 2013. **224**(5).
144. Gokoglu, N., P. Yerlikaya, and M. Gokoglu, *Trace elements in edible tissues of three shrimp species (Penaeus semisulcatus, Parapenaeus longirostris and Paleomon serratus).* J Sci Food Agric, 2008. **88**(2): p. 175-178.
145. Ward, T.J., R.L. Correll, and R.B. Anderson, *Distribution of cadmium, lead and zinc amongst the marine sediments, seagrasses and fauna, and the selection of sentinel accumulators, near a lead smelter in South Australia.* Aust J Mar Freshw Res, 1986. **37**(5): p. 567-585.
146. Leatherland, T.M., et al., *Concentrations of some trace metals in pelagic organisms and of mercury in Northeast Atlantic Ocean water.* Deep Sea Res, 1973. **20**(8): p. 679-85.
147. Wright, D.A., *Heavy metals in animals from the north east coast.* Mar Pollut Bull, 1976. **7**(2): p. 36-8.
148. Culshaw, C., et al., *Concentrations of Cd, Zn and Cu in sediments and brown shrimp (Crangon crangon L.) from the Severn Estuary and Bristol Channel, UK.* Mar Environ Res, 2002. **54**(3-5): p. 331-4.
149. Peden, J.D., et al., *Heavy metals in Somerset marine organisms.* Mar Pollut Bull, 1973. **4**(1): p. 7-9.
150. White, S.L. and P.S. Rainbow, *Heavy metal concentrations and size effects in the mesopelagic decapod crustacean Systellaspis debilis.* Mar Ecol Prog Ser, 1987. **37**(2-3): p. 147-151.
151. Soegianto, A., et al., *Impact of cadmium on the structure of gills and epipodites of the shrimp Penaeus japonicus (Crustacea : Decapoda).* Aquat Living Resour, 1999. **12**(1): p. 57-70.
152. Dietz, R., F. Riget, and P. Johansen, *Lead, cadmium, mercury and selenium in Greenland marine animals.* Sci Total Environ, 1996. **186**(1-2): p. 67-93.
153. Darmono, D., *Uptake of cadmium and nickel in banana prawn (Penaeus merguiensis de Man).* Bull Environ Contam Toxicol, 1990. **45**(3): p. 320-8.

154. Núñez-Nogueira, G., et al., *Accumulation and regulation effects from the metal mixture of Zn, Pb, and Cd in the tropical shrimp Penaeus vannamei.* Biol Trace Elem Res, 2012. **150**(1-3): p. 208-13.
155. Albrecht, W.N., C.A. Woodhouse, and J.N. Miller, *Nearshore dredge-spoil dumping and cadmium, copper, and zinc levels in a dermestid shrimp.* Bull Environ Contam Toxicol, 1981. **26**(2): p. 219-23.
156. Firat, O., et al., *Concentrations of Cr, Cd, Cu, Zn and Fe in crab Charybdis longicollis and shrimp Penaeus semisulcatus from the Iskenderun Bay, Turkey.* Environ Monit Assess, 2008. **147**(1-3): p. 117-23.
157. Ridout, P.S., et al., *Concentrations of Mn, Fe, Cu, Zn and Cd in the mesopelagic decapod Systellaspis debilis from the East Atlantic Ocean.* Mar Biol, 1985. **87**(3): p. 285-288.
158. Francesconi, K.A., K.L. Pedersen, and P. Højrup, *Sex-specific accumulation of Cd-metallothionein in the abdominal muscle of the coral prawn Metapenaeopsis crassissima from a natural population.* Mar Environ Res, 1998. **46**(1-5): p. 541-544.
159. Vijayram, K. and P. Geraldine, *Are the heavy metals cadmium and zinc regulated in freshwater prawns?* Ecotoxicol Environ Saf, 1996. **34**(2): p. 180-3.
160. Vijayaraman, K., et al., *Uptake and loss of heavy metals by the freshwater prawn, Macrobrachium malcolmsonii.* J Environ Biol, 1999. **20**(3): p. 217-222.
161. White, S.L. and P.S. Rainbow, *Regulation and accumulation of copper, zinc and cadmium by the shrimp Palaemon elegans* Mar Ecol Prog Ser, 1982. **8**(1): p. 95-101.
162. Keating, J., et al., *Histological findings, cadmium bioaccumulation, and isolation of expressed sequence tags (ESTs) in cadmium-exposed, specific pathogen-free shrimp, Litopenaeus vannamei postlarvae.* J Shellfish Res, 2007. **26**(4): p. 1225-1237.
163. Francesconi, K., C. Soames, and M. Jackson, *Remarkable sex related differences in the cadmium concentrations of the coral prawn Metapenaeopsis crassissima.* Bull Mar Sci, 1995. **56**(1): p. 337-339.
164. Hardisty, M.W., et al., *Ecological implications of heavy metal in fish from the Severn estuary.* Mar Pollut Bull, 1974. **5**(1): p. 12-15.
165. Pan, J., et al., *Cadmium levels in Europe: implications for human health.* Environ Geochem Health, 2010. **32**(1): p. 1-12.
166. Costa, M., *Trace elements: aluminium, arsenic, cadmium, and nickel*, in *Environmental toxicants: human exposures and their health effects*, M. Lippmann, Editor. 2000, John Wiley & Sons: New York. p. 811-50.
167. Kabir, E., et al., *Current status of trace metal pollution in soils affected by industrial activities.* ScientificWorldJournal, 2012. **2012**: p. 916705.
168. Thornton, I., *Metal content of soils and dusts.* Sci Total Environ, 1988. **75**(1): p. 21-39.
169. Johnson, A.H.M., et al., *Heavy metals in Jamaican surface soils.* Environ Geochem Health, 1996. **18**(3): p. 113-121.
170. Han, F.X., *Global perspectives of anthropogenic interferences in the natural trace element distribution*, in *Biogeochemistry of trace elements in arid environments*. 2007, Springer: Dordrecht. p. 303-327.
171. Hutton, M., *The release and pathways of environmental cadmium*, in *Sources of cadmium in the environment*. 1996, OECD: Paris. p. 19-48.
172. Hutton, M., et al., *Group report: cadmium*, in *Lead, mercury, cadmium and arsenic in the environment*, T.C. Hutchinson and K.M. Meema, Editors. 1987, John Wiley & Sons: New York. p. 35-41.
173. Han, F.X., *Trace element distribution in arid zone soils*, in *Biogeochemistry of trace elements in arid environments*. 2007, Springer: Dordrecht. p. 47-68.
174. Williams, C.H., *Heavy metals and other elements in fertilizers: environmental considerations*, in *Fertilizers and the environment*, D.R. Leece, Editor. 1974, Australian Institute of Agricultural Science (NSW Branch): Sydney. p. 123-130.

175. Lalor, G.C., et al., *Heavy metals in Jamaica. Part 3: the distribution of cadmium in Jamaican soils.* Rev Int Contam Ambient, 1988. **14**(1): p. 7-12.
176. Linak, W.P., R.K. Srivastava, and J.O.L. Wendt, *Sorbent capture of nickel, lead, and cadmium in a laboratory swirl flame incinerator.* Combust Flame, 1995. **100**(1-2): p. 241-250.
177. Uberoi, M. and F. Shadman, *High-temperature removal of cadmium compounds using solid sorbents.* Environ Sci Technol, 1991. **25**(7): p. 1285-9.
178. Liang, Z., X. Peng, and Z. Luan, *Immobilization of Cd, Zn and Pb in sewage sludge using red mud.* Environ Earth Sci, 2012. **66**(5): p. 1321-1328.
179. Apak, R., K. Güçlü, and M.H. Turgut, *Modeling of copper(II), cadmium(II), and lead(II) adsorption on red mud.* J Colloid Interface Sci, 1998. **203**(1): p. 122-130.
180. Gupta, V.K. and S. Sharma, *Removal of cadmium and zinc from aqueous solutions using red mud.* Environ Sci Technol, 2002. **36**(16): p. 3612-7.
181. Hizal, J., et al., *Heavy metal removal from water by red mud and coal fly ash: an integrated adsorption-solidification / stabilization process.* Desalin Water Treat, 2013. **51**(37-39): p. 7181-93.
182. Zhu, C.L., et al., *Removal of cadmium from aqueous solutions by adsorption on granular red mud (GRM).* Sep Purif Technol, 2007. **57**(1): p. 161-169.
183. Barrow, N.J., *Possibility of using caustic residue from bauxite for improving the chemical and physical properties of sandy soils.* Aust J Agric Res, 1982. **33**(2): p. 275-285.
184. Lombi, E., et al., *In situ fixation of metals in soils using bauxite residue: chemical assessment.* Environ Pollut, 2002. **118**(3): p. 435-43.
185. Tse, P.K., *The mineral industry of Australia*, in *U.S. Geological Survey Minerals Yearbook - 2011*. 2012, U.S. Gelogical Survey: Reston, VA. p. 3.1 - 3.27.
186. Jones, B.E.H. and R.J. Haynes, *Bauxite processing residue: a critical review of its formation, properties, storage, and revegetation.* Crit Rev Environ Sci Technol, 2011. **41**(3): p. 271-315.
187. Alvarado, J. and A. Petrola, *Determination of cadmium, chromium, lead, silver and gold in Venezuelan red mud by atomic absorption spectrometry.* J Anal At Spectrom, 1989. **4**(5): p. 411-414.
188. Summers, R.N., et al., *Bauxite residue (red mud) improves pasture growth on sandy soils in Western Australia.* Aust J Soil Res, 1996. **34**(4): p. 569-581.
189. Wagh, A.S. and B. Thompson, *A study of inter-particle bonds in dry bauxite waste resulting in atmospheric aerosols.* Phys Scr, 1988. **37**(2): p. 305-309.
190. Csavina, J., et al., *A review on the importance of metals and metalloids in atmospheric dust and aerosol from mining operations.* Sci Total Environ, 2012. **433**: p. 58-73.
191. International Programme on Chemical Safety (IPCS), *Cadmium*, in *Environmental Health Criteria*. 1992, World Health Organization: Geneva.
192. Nriagu, J.O. and J.M. Pacyna, *Quantitative assessment of worldwide contamination of air, water and soils by trace metals.* Nature, 1988. **333**(6169): p. 134-9.
193. Nriagu, J.O., *A global assessment of natural sources of atmospheric trace metals.* Nature, 1989. **338**(6210): p. 47-49.
194. Rothlisberg, P.C., *Aspects of penaeid biology and ecology of relevance to aquaculture: a review.* Aquaculture, 1998. **164**(1–4): p. 49-65.
195. International Programme on Chemical Safety (IPCS), *Cadmium - environmental aspects*, in *Environmental Health Criteria*. 1992, World Health Organization: Geneva.
196. Wu, X., Y. Jia, and H. Zhu, *Bioaccumulation of cadmium bound to ferric hydroxide and particulate organic matter by the bivalve M. meretrix.* Environ Pollut, 2012. **165**: p. 133-9.
197. Wu, X., et al., *Effects of sediment composition on cadmium bioaccumulation in the clam Meretrix meretrix Linnaeus.* Environ Toxicol Chem, 2013. **32**(4): p. 841-7.
198. GESAMP: IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution, *Cadmium, lead and tin in the marine environment*, in

UNEP Regional Seas Reports and Studies No. 56; GESAMP Reports and Studies No. 22. 1985, UNEP: Nairobi. 90pp.
199. Mart, L. and H.W. Nürnberg, *The distribution of cadmium in the sea*, in *Cadmium in the environment*, H. Mislin and O. Ravera, Editors. 1986, Birkhäuser: Basel. p. 28-40.
200. Rainbow, P.S., *Kenneth Mellanby Review Award. Trace metal concentrations in aquatic invertebrates: why and so what?* Environ Pollut, 2002. **120**(3): p. 497-507.
201. Rainbow, P.S., *Trace metal bioaccumulation: models, metabolic availability and toxicity.* Environ Int, 2007. **33**(4): p. 576-82.
202. Nunez-Nogueira, G., P.S. Rainbow, and B.D. Smith, *Assimilation efficiency of zinc and cadmium in the decapod crustacean Penaeus indicus.* J Exp Mar Biol Ecol, 2006. **332**(1): p. 75-83.
203. Fraga, C.G., *Relevance, essentiality and toxicity of trace elements in human health.* Mol Aspects Med, 2005. **26**(4-5): p. 235-44.
204. Cotton, P.A., et al., *Dietary sources of nutrients among US adults, 1994 to 1996.* J Am Diet Assoc, 2004. **104**(6): p. 921-30.
205. Mahabir, S., et al., *Dietary zinc, copper and selenium, and risk of lung cancer.* Int J Cancer, 2007. **120**(5): p. 1108-15.
206. Finley, J.W., *Bioavailability of selenium from foods.* Nutr Rev, 2006. **64**(3): p. 146-51.
207. Fairweather-Tait, S.J., R. Collings, and R. Hurst, *Selenium bioavailability: current knowledge and future research requirements.* Am J Clin Nutr, 2010. **91**(5): p. 1484s-1491s.
208. Thomson, C.D. and M.F. Robinson, *Urinary and fecal excretions and absorption of a large supplement of selenium: superiority of selenate over selenite.* Am J Clin Nutr, 1986. **44**(5): p. 659-63.
209. Yoshida, S., et al., *Selenium in seafood materials.* J Health Sci, 2011. **57**(3): p. 215-224.
210. Yamashita, Y., T. Yabu, and M. Yamashita, *Discovery of the strong antioxidant selenoneine in tuna and selenium redox metabolism.* World J Biol Chem, 2010. **1**(5): p. 144-50.
211. Yamashita, Y. and M. Yamashita, *Identification of a novel selenium-containing compound, selenoneine, as the predominant chemical form of organic selenium in the blood of bluefin tuna.* J Biol Chem, 2010. **285**(24): p. 18134-8.
212. Barclay, M.N.I., A. MacPherson, and J. Dixon, *Selenium content of a range of UK foods.* J Food Compost Anal, 1995. **8**(4): p. 307-318.
213. McNaughton, S.A. and G.C. Marks, *Selenium content of Australian foods: a review of literature values.* J Food Compost Anal, 2002. **15**(2): p. 169-182.
214. Rayman, M.P., *Selenium and human health.* Lancet, 2012. **379**(9822): p. 1256-68.
215. Brenneisen, P., H. Steinbrenner, and H. Sies, *Selenium, oxidative stress, and health aspects.* Mol Aspects Med, 2005. **26**(4-5): p. 256-67.
216. Klein, E.A., et al., *Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT).* JAMA, 2011. **306**(14): p. 1549-56.
217. Lippman, S.M., et al., *Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT).* JAMA, 2009. **301**(1): p. 39-51.
218. Hurst, R., et al., *Selenium and prostate cancer: systematic review and meta-analysis.* Am J Clin Nutr, 2012. **96**(1): p. 111-22.
219. Richman, E.L. and J.M. Chan, *Selenium and prostate cancer: the puzzle isn't finished yet.* Am J Clin Nutr, 2012. **96**(1): p. 1-2.
220. Loef, M., G.N. Schrauzer, and H. Walach, *Selenium and Alzheimer's disease: a systematic review.* J Alzheimers Dis, 2011. **26**(1): p. 81-104.
221. Schrauzer, G.N., *Selenium and selenium-antagonistic elements in nutritional cancer prevention.* Crit Rev Biotechnol, 2009. **29**(1): p. 10-7.
222. Schrauzer, G.N., *Interactive effects of selenium and cadmium on mammary tumor development and growth in MMTV-infected female mice. A model study on the roles of*

cadmium and selenium in human breast cancer. Biol Trace Elem Res, 2008. **123**(1-3): p. 27-34.
223. Li, S., T. Xiao, and B. Zheng, *Medical geology of arsenic, selenium and thallium in China.* Sci Total Environ, 2012. **421-422**: p. 31-40.
224. Aldosary, B.M., et al., *Case series of selenium toxicity from a nutritional supplement.* Clin Toxicol (Phila), 2012. **50**(1): p. 57-64.
225. See, K.A., et al., *Accidental death from acute selenium poisoning.* Med J Aust, 2006. **185**(7): p. 388-9.
226. Al-Matary, A., M. Hussain, and J. Ali, *Selenium: a brief review and a case report of selenium responsive cardiomyopathy.* BMC Pediatr, 2013. **13**: p. 39.
227. Bodnar, M., P. Konieczka, and J. Namiesnik, *The properties, functions, and use of selenium compounds in living organisms.* J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 2012. **30**(3): p. 225-52.
228. Combs, G.F., Jr., *Selenium in global food systems.* Br J Nutr, 2001. **85**(5): p. 517-47.
229. Drutel, A., F. Archambeaud, and P. Caron, *Selenium and the thyroid gland: more good news for clinicians.* Clin Endocrinol (Oxf), 2013. **78**(2): p. 155-64.
230. Torres-Vega, A., et al., *Limbic system pathologies associated with deficiencies and excesses of the trace elements iron, zinc, copper, and selenium.* Nutr Rev, 2012. **70**(12): p. 679-92.
231. Dalton, C. and P. Bird, *Risk assessment for the consumption of fish with elevated selenium levels.* N S W Public Health Bull, 2003. **14**(8): p. 174-6.
232. Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. 2000: Washington, D.C. p. i-xxii; 1-506.
233. National Health and Medical Research Council, *Nutrient reference values for Australia and New Zealand, including recommended dietary intakes*. 2006, NHMRC: Canberra. p. i-viii; 1-333.
234. Fialkowski, M.K., et al., *Estimated nutrient intakes from food generally do not meet dietary reference intakes among adult members of Pacific Northwest tribal nations.* J Nutr, 2010. **140**(5): p. 992-8.
235. Szlinder-Richert, J., et al., *Marine and farmed fish on the Polish market: comparison of the nutritive value and human exposure to PCDD/Fs and other contaminants.* Chemosphere, 2011. **85**(11): p. 1725-33.
236. Perugini, M., et al., *Contamination of different portions of raw and boiled specimens of Norway lobster by mercury and selenium.* Environ Sci Pollut Res Int, 2013.
237. Moreno, P., et al., *Fractionation studies of selenium compounds from oysters, and their determination by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry.* J Anal At Spectrom, 2001. **16**(9): p. 1044-1050.
238. Cochran, W.G., *Sampling techniques*. 3rd ed. 1977, New York: John Wiley & Sons. i-xvi; 1-428.
239. Sumner, J., *ISO Best Practice Manual: catching and processing of wild-caught prawns*. 1997, Seafood Services Australia: Ascot. 93 pp.
240. European Committee for Standardization, *Foodstuffs - determination of trace elements - performance criteria, general considerations and sample preparation*. 2002, CEN: Brussels. 14 pp.
241. Creed, J.T., C.A. Brockhoff, and T.D. Martin, *Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry - Method 200.8 Revision 5.4*. 1994, U.S. Environnmetal Protection Agency: Cincinnati. 57 pp.
242. Lubin, J.H., et al., *Epidemiologic evaluation of measurement data in the presence of detection limits.* Environ Health Perspect, 2004. **112**(17): p. 1691-6.

243. Grey, D.L., W. Dall, and A. Baker, *A guide to the Australian penaeid prawns*. 1983, Darwin: Department of Primary Production of the Northern Territory. 140pp.
244. Flegel, T.W., *The right to refuse revision in the genus Penaeus.* Aquaculture, 2007. **264**(1-4): p. 2-8.
245. Ma, K.Y., T.Y. Chan, and K.H. Chu, *Refuting the six-genus classification of Penaeus s.l. (Dendrobranchiata, Penaeidae): a combined analysis of mitochondrial and nuclear genes.* Zool Scr, 2011. **40**(5): p. 498-508.
246. Pérez-Farfante, I. and B. Kensley, *Penaeoid and sergestoid shrimps and prawns of the world. Keys and diagnoses for the families and genera*. 1997, Muséum National d'Histoire Naturelle: Paris. 233pp.
247. The Commission of the European Communities, *Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance)*, in *2006R 1881 - EN - 01.07.2010 - 004.001*, 2006, European Union: Brussels.
248. Kangas, M., et al., *Shark Bay prawn fishery*, in *ESD Report Series No. 3*. 2006, Department of Fisheries, Western Australia: Perth. 128pp.
249. Francesconi, K.A., E.J. Moore, and L.M. Joll, *Cadmium in the saucer scallop, Amusium balloti, from Western Australian waters: concentrations in adductor muscle and redistribution following frozen storage.* Aust J Mar Freshw Res, 1993. **44**(6): p. 787-797.
250. McConchie, D.M., et al., *Heavy metals in marine biota, sediments and waters from the Shark Bay area, Western Australia.* J Coast Res, 1988. **4**(1): p. 37-58.
251. Edwards, J.W., et al., *Metal levels in seston and marine fish flesh near industrial and metropolitan centres in South Australia.* Mar Pollut Bull, 2001. **42**(5): p. 389-96.
252. Torgersen, T., et al., *Late Quaternary hydrological changes in the Gulf of Carpentaria.* Nature, 1985. **313**(6005): p. 785-787.
253. Gladstone, W., *Trace metals in sediments, indicator organisms and traditional seafoods of the Torres Strait*. 1996, Great Barrier Reef Marine Park Authority: Townsville. p. i-vii; 1-184.
254. Maritime Safety Queensland, *Report of the Board of Inquiry into the marine incident involving the ship Wunma in the waters of the Gulf of Carpentaria on 6 and 7 February 2007*. 2008, State of Queensland: Brisbane. 518pp.
255. Food Standards Australia New Zealand, *Standard 1.2.7. Nutrition, health and related claims*. 2013, FSANZ: Canberra. 48pp.
256. Food Standards Australia New Zealand, *Standard 1.1.1. Preliminary provisions - application, interpretation and general provisions*. 2011, FSANZ: Canberra. 14pp.
257. Padula, D., et al., *Australian seafood compositional profiles*. 2013, Australian Seafood Cooperative Research Centre: Adelaide. 57pp.
258. Food Standards Agency, *Cadmium in brown meat from crabs and products made with the brown meat from crabs*, in *Food Survey Information Sheet No. 01/13*. 2013, Food Standards Agency: London.
259. Olsen, A.M., *Heavy metal concentrations of fish, other aquatic biota, River Murray and South Australian aquatic environments.* Fisheries Research Paper No. 10. 1983, Department of Fisheries South Australia. 116pp.

APPENDIX 1

Table 1. Cadmium concentrations (mg kg^{-1}) measured in decapod prawn species that comprise human food items

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
NS (2008)	Malaysia	NS: "dried shrimp" and "shrimp paste"	ND				NS	O	ICP-OES	[20]
1986 – 1987	Laguna Madre, Texas, USA	*Penaeus aztecus*	ND				dry	O	AAS	[21]
1997 – 1999	Lake Qarun, Egypt	*Penaeus* sp.		ND	ND		NS	O	AAS	[22]
NS (2007)	Prawn farms, Thailand	*P. monodon* *P. vannamei* (farmed)		ND[#]			wet	O	ICP-OES	[23]
1987 – 1988	Korle Lagoon, Accra, Ghana	*P. notialis*		ND			wet	O	AAS	[24]
2008	Retail outlets, New Brunswick, Canada	NS ("shrimp")		ND			wet	O	ICP-MS	[25]
NS (2013)	Retail market, Paranaguá, Brazil	*P. vannamei*		ND			wet	O	ICP-OES	[26]
1992	Pacific coast, Mexico	*P. vannamei* (wild and farmed)		ND	0.02[•] (farmed) – 0.74[•] (wild)	ND (farmed) – 0.7 (wild)	dry	O	AAS	[27]
NS (2011)	Guangdong Province, China	*P. vannamei* (wild and farmed)		ND	[0.34 – 1.05][•]		dry	O	ICP-MS	[28]
NS (1990)	Townsville, Queensland, Australia	*P. monodon* (farmed)		ND	1.4	ND	wet	O	AAS	[29]
1986	Townsville, Queensland, Australia	*P. merguiensis*		ND	[0.7 – 1.6]	ND	wet	O	AAS	[29]
1990	Adelaide River estuary, NT, Australia	*Macrobrachium rosenbergii* *M. novaehollandiae*		ND	0.8 1.5 – 2.0	ND	wet	O	AAS	[30]
1992	Baja California, Mexico	*P. californiensis*		ND	2.6[•], 4.0[•]	2.2, 2.5	dry	O	AAS	[31]
1992	San Jorge Gulf, Argentina	*Pleoticus muelleri*		ND	3.0 – 11.8		wet	O	AAS	[32]
2005	Gresik, Indonesia	*P. merguiensis*		0.00005[•]			dry	O	ICP-MS	[33]
NS (2005)	Retail outlets, New Jersey, USA	NS ("shrimp")		0.0001 – 0.004			wet	O	AAS	[34]
1997	Marennes-Oléron Bay, France	*Palaemon elegans*	0.005[•]				dry	O	AAS	[35]
2002	Prawn farm, Shandong Province, China PR	*P. chinensis* (farmed)		0.002 – 0.005[Δ]			dry	E – aquaculture experiment comparing different polyculture	AAS	[36]

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
								methods,, i.e. no added cadmium		
NS (2006)	Mai Po wetland, Hong Kong	*Metapenaeus ensis* (farmed) *M. nipponensis* (farmed)	0.005 – 0.008*• ND – 0.01*•				dry	O	AAS	[37]
NS (1994)	Presumed retail outlet, Ohio USA	NS ("frozen shrimp")	0.01				NS – presume wet weight	O	ICP-MS	[38]
NS (2002)	Japan	"giant tiger" (farmed)		[ND – 0.01]			wet	O	AAS	[39]
2005 – 2006	Baluarte River, Mexico	*M. digueti* *Atya* sp. *M. americanum*		ND – 0.008*• ND – 0.088*• ND – 0.01*•			dry	O	AAS	[40]
2008 – 2009	Shanghai❁	*P. vannamei*	[0.002 – 0.01]				wet	O	AAS	[41]
2003 – 2005	Mekong River delta and South Key Economic Zone, Vietnam	*P. monodon* (wild & farmed)		0.00075 – 0.011•	0.11 – 1.6•		dry	O	ICP-MS	[42]
1994	Galveston Bay, Texas, USA	*Penaeus* sp.	[0.005 – 0.013]•				dry	O	AAS	[43]
NS (2012)	Retail outlet, Italy	*Parapenaeus longirostris*		0.014			wet	O	ICP-MS	[44]
NS (2011)	Prawn farms, Egypt	*M. rosenbergii*		0.016•	0.4•	1.1	dry	E – exposed to 0.1 mg L^{-1} for 96 hours	AAS	[45]
2007	Ba Ria Vung Tau, Vietnam	*P. monodon* *P. merguiensis*		[0.008 – 0.018]Δ 0.003Δ			dry	O	ICP-MS	[46]
2006	Shenzhen coast, China	*Palaemon carincauda* *P. penicillatus* *Metapenaeus affinis*		0.01 – 0.02 ND – 0.02 ND			wet	O	ICP-MS	[47]
NS (2007)	Arcachon, France	*Palaemon longirostris*		0.01 – 0.02*•	0.8 – 1.9*•	4.4 – 21*	dry	E – exposed to 2µg/L Cadmium for 7 days at different salinities	AAS	[48]
1973	Spencer Gulf, South Australia	*P. latisulcatus*		0.02			wet	O	Colorimetric	[259]
2006	Taylor Creek, Nigeria	*M. felicinum*		0.02•			dry	O	AAS	[49]
NS (1998)	Gulf of Fonseca, Nicaragua & Honduras	NS (farmed prawns)		0.002 – 0.03			wet	O	AAS	[50]
2005	Spain	*P. setiferus*		[0.01 – 0.03]			wet	O	ICP-MS	[51]
2010 – 2011	Bahia, Brazil	*P. brasiliensis*		[0.02 – 0.03]•			dry	O	AAS	[52]
2009 – 2010	Spain (fresh) Tunisia (frozen)	*Parapenaeus longirostris*		0.03 (median) 0.01 (median)			wet	O	AAS	[53]

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
1981-3	Gulf St. Vincent, South Australia	*P. latisulcatus*		0.03•	0.02• ("viscera")		dry	O	AAS	[54]
1998	Qeshm Is, Iran	*P. merguiensis* *M. affinis*		0.02• 0.03•	0.3• 0.15• (analyses of viscera incl. hepatopancreas, gonads & heart)		dry	O	ICP-OES	[55]
1994	Ensenada de La Paz, Mexico	*P. californiensis* (farmed)		0.025*• – 0.03*•	0.1*• – 0.2*•		dry	E – comparing different stocking densities (i.e. no Cadmium dosing)	AAS	[56]
2012	Local markets, Ho Chi Minh City, Vietnam	*P. vannamei*		0.036			wet	O	AAS	[57]
1983	Mediterranean coast, Spain	*Parapenaeus longirostris*	0.02 – 0.04				wet	O	AAS	[58]
2005	Alexandria, Egypt	*Penaeus* spp.		0.01 – 0.04•			dry	O	AAS	[59]
2006	Adriatic Sea, Italy	*Aristeus antennatus* *Parapenaeus longirostris* *Plesionika martia*		0.01 – 0.04			wet	O	AAS	[60]
2008 – 2009	Fish market, Tok Muda, Malaysia	*P. merguiensis*		0.045•			dry	O	ICP-MS	[61]
1974	Gulf St. Vincent, South Australia	*P. latisulcatus*		0.05			wet	O	AAS	[259]
2012	Iceland	*Pandalus borealis*		[0.02 – 0.05]			wet	O	ICP-MS	[62]
1993	Arabian Gulf, Bahrain	*P. semisulcatus*	[0.001 – 0.06]				wet	O	AAS	[63]
1996	Seine Estuary, France	*Crangon crangon* *Palaemon longirostris*	[0.008 – 0.04]• [0.008 – 0.06]•				dry	O	AAS	[64]
2010 – 2011	Izmir Bay, Turkey	*P. kerathurus*		0.005 – 0.06•			dry	O	AAS	[65]
2004	Lesina Lagoon, Italy	*P. kerathurus*		0.03 – 0.06•			dry	O	AAS	[66]
1992	Atlantic Ocean (purchased in Poland)	*Parapenaeus* sp. (canned product)		[0.04 – 0.06]			wet	O	AAS	[67]
1988	Banc d'Arguin, Mauritania	*Processa elegantula*	[0.01 – 0.07] •				dry	O	AAS	[68]
NS (2010)	Ojo River, Nigeria	NS ("prawn")		[0.003 – 0.07] •			dry	O	AAS	[69]
1977 – 1980	Mediterranean coast, Turkey	*P. kerathurus*		[0.01 – 0.07]			wet	O	AAS	[70]
2003	Rossaveal, Ireland	*Nephrops norvegicus*		0.07			Wet	O	AAS	[71]
2006	Iceland	*P. borealis*		[0.015 – 0.075]			wet	O	ICP-MS	[72]

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
NS (2001)	Bay of Bengal, Bangladesh	*P. monodon*		0.05 – 0.075•	0.06 – 0.11•		dry	O	AAS	[73]
2003 – 2005	Ho Chi Minh City & surrounds, Vietnam	*M. rosenbergii*		[0.0002 – 0.076] •	[0.36 – 12.9] •		dry	O	ICP-MS	[74]
2004	Chantaburi & Trat provinces, Thailand	*P. merguiensis*		[0.01 – 0.08]	[0.02 – 2.3] ("cephalothorax")		wet	O	AAS	[75]
2010	Persian Gulf estuaries, Iran	*M. affinis*		0.04 – 0.08•	0.5 – 1.1•		dry	O	AAS	[76]
NS (2013)	Brazil	*P. vannamei*		0.08•	12.8•		dry	E – exposed to 1mg/kg Cadmium for two days	AAS	[77]
1994 – 1996	Coastal shelf, Belgium	*C. crangon*	[0.008 – 0.083]				wet	O	AAS	[78]
NS (2009)	Mindu Dam, Tanzania	*M. rude*	0.09				wet	O	AAS	[79]
1995	Weser estuary, Germany	*C. crangon*		[0.014 – 0.091]			wet	O	AAS	[80, 81]
1981	Ad Dammam, Saudi Arabia	*P. semisulcatus*		[0.07 – 0.1]			wet	O	AAS	[82]
NS (1986)	Firth of Clyde, Scotland	*P. elegans*	0.23•	0.1•	0.3•	4.7	dry	O (pre-dose concentrations in prawn batch used for subsequent experiments)	AAS	[83, 84]
NS (1977)	Texas coast, USA	"brown shrimp" "rock shrimp"		[0.01 – 0.08] • [0.06 – 0.1] •	[0.3 – 0.7] • ("viscera")		dry	O	AAS	[85]
2003 – 2005	Fish markets, Cochin, India	*P. indicus* *P. monodon* *Parapenaeopsis stylifera* Frozen, peeled shrimp		ND ND ND 0.07 – 0.12			wet	O	AAS	[86]
1977 – 1984	Wadden Sea, The Netherlands	*C. crangon*	0.003 – 0.12				wet	O	DPASV	[87]
2002	Persian Gulf coast, Iran	*P. semisulcatus*		[0.08 – 0.13]	[0.3 – 1.5]		wet	O	ICP-OES	[88]
NS (1989)	British Columbia, Canada	*Pandalopsis dispar* *P. borealis* *Pandalus platyceros*		0.025 – 0.06• 0.03 – 0.13• 0.03 – 0.04•			dry	O	ICP-OES & AAS	[89]
2008	Catalonia, Spain	NS		0.13			wet	O	ICP-MS	[90]
NS (2012)	SE Pacific SE Pacific SW Atlantic	*P. vannamei* *P. stylirostris* *P. schmitti*		0.05 0.15 0.08			wet	O	AAS	[60, 91]
1991	Pacific coast, Mexico	*P. stylirostris*		0.11•, 0.15•			dry	O	AAS	[92]

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
1996-1999	Gulf of Genoa, Italy	*A. antennatus*		[0.005 – 0.17]#•			dry	O	AAS	[93]
1998	Mediterranean coast, Spain	*Aristeomorpha* spp. *N. norvegicus* *P. kerathurus*		[0.02 – 0.18] ND [0.03 – 0.05]			wet	O	AAS	[94]
1985	Malay Peninsula, Malaysia & Thailand	*Exopalaemon styliferus* *Metapenaeus lysianassa* *Palaemon semmelinkii*		0.18•			dry	O	AAS	[95]
NS (2012)	Bay of Bengal estuarine system, India	*P. semisulcatus* *P. indicus* *P. monodon*		0.04 – 0.15 0.04 – 0.17 0.03 – 0.18	0.12 – 0.2 0.1 – 0.2 0.1 – 0.2		wet	O	AAS	[96]
1988	British Columbia, Canada	*P. platyceros*	0.3•	0.003 – 0.18•	2.8•		dry	O	ICP-MS	[97]
2007	Mumbai, India	*Metapenaeus monoceros*		[ND – 0.2]			wet	O	DPASV	[98]
1977-80	Ligurian Sea, Italy	*N. norvegicus*		0.14 [0.09 – 0.2] (?muscle)			wet	O	AAS	[99]
NS (1980)	St Andrews, New Brunswick, Canada	*Pandalus montagui*	1.5•	0.2•	4.9•		dry	E – exposed to 37µg/L Cadmium for 14 days	AAS	[100]
1991	Gulf of California, Mexico	*Heterocarpus vicarius*		0.19•, 0.22•			dry	O	AAS	[101]
NS (1984)	Sozopol, Bulgaria	*P. elegans*	5.4Δ	0.22Δ	18.1Δ	25.7Δ	dry	E – exposed to 1mg/L Cadmium for 3 days	AAS	[102]
NS (2002)	Sabah, Malaysia	*P. monodon* (farmed)		0.11* 0.23*			wet	O	AAS	[103]
NS (2001)	NE Mediterranean (Turkish coast)	*P. japonicus*		0.16 – 0.23•	0.86 – 3.2•	7.7 – 17.9	dry	O	AAS	[104]
2002 – 2003	Retail outlets, Singapore	NS (“gray prawn”) *P. monodon*		0.24 NS			wet	O	ICP-MS	[105]
NS (2007)	Chennai, India	*Solenocera crassicornis*		0.25*•		ND	dry	O	AAS	[106]
NS (1994)	Imported + local, Denmark	NS (“shrimp”)	[0.003 – 0.26]				wet	O	AAS	[107]
2004 – 2005	Gulf of California, Mexico	*P. stylirostris*		[0.1 – 0.26]			dry	O	AAS	[108]
NS (2006)	Kolleru Lake wetland, India	NS – farmed (? *P. monodon*)	0.05*• – 0.27•				dry	O	AAS	[109]
1974 – 1975	Mediterranean Sea	*Sergestes* spp. *Gennadas elegans*	0.1 – 0.28• 0.18 – 0.28•				dry	O	AAS	[110]
2003	Sea of Marmara, Turkey	*Palaemon adspersus* *Palaemon serratus* *Parapenaeus longirostris*		0.02 – 0.26Δ 0.18 – 0.26Δ 0.13 – 0.34Δ			dry	O	AAS	[111]

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
NS (2005)	London, England	*P. indicus*		0.3•	23•	80	dry	E – exposed to 10µg/L Cadmium for 20 days	AAS	[112]
NS (1995)	Pacific coast, Mexico	*P. vannamei*		[0.01 – 0.33] •			dry	O	AAS	[113]
1986 – 1987	Bahía Blanca estuary, Argentina	*P. muelleri* *Artemesia longinaris*		[0.09 – 0.34] [0.06 – 0.23]	[0.15 – 0.34]		wet	O	AAS	[114]
NS (2002)	Mumbai coast, India	*P. stylifera* *S. crassicornis* *M. monoceros*	0.16* - 0.34 0.06* - 0.33 0.14* - 0.36				wet	O	AAS	[115]
1999	Lake Macquarie, NSW, Australia	*Metapenaeus bennettae*		0.4•			dry	O	AAS	[116]
1992	Torres Strait, Queensland, Australia	*P. esculentus* *P. longistylus* *Metapenaeus endeavouri*		0.16# 0.08# 0.4#	5.8		wet	O	ICP-MS	[117]
1983 - 1987	Seafood processors & retail outlets, Kerala, India	*M. affinis* *Metapenaeus dobsoni* *M. monoceros* *P. stylifera* *P. indicus* Canned prawns		[ND – 0.12] [0.04 – 0.23] [ND – 0.09] [0.05 – 0.37] [ND – 0.22] [ND – 0.43]			wet	O	AAS	[118]
1984	Ennore estuary, Madras, India	*P. indicus*		[0.06 – 0.45] •			dry	O	AAS	[119]
1987 – 1988	Arabian Sea coast, Pakistan	*M. monoceros* *P. japonicus*		0.47 0.47			wet	O	AAS	[120]
1999	Iskenderun Gulf, Turkey	*P. semisulcatus* *M. monoceros*		0.25 – 0.48•	1.5 – 2.4•	2.4 – 4.5	dry	O	AAS	[121]
1971-1972	Sabine Lake, Texas, USA	*P. setiferus*	[0.43 – 0.50]				wet	O	AAS	[122]
2011	Saronikos Gulf, Greece	*P. longirostris*		0.24 (raw) 0.52 (pan-fried)			wet	O	AAS	[123]
1973-6	Lower Medway Estuary, Kent, U.K.	*C. vulgaris*	0.59 [0.53 – 0.65]				wet	O	AAS	[124]
1998	Sunderban, India	*P. monodon*		[0.03 – 0.8] •			dry	O	AAS	[125]
NS (1990)	Karwar, West India	*P. merguiensis* *P. stylifera*		0.38 0.83			wet	O	AAS	[126]
NS (2005)	Taiwan	*P. vannamei*		0.85•	97*•	67*•	dry	E – exposed to 0.2 mg/kg Cadmium for 84 days	AAS	[127]

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
NS (2008)	Prawn hatchery, Pakistan	*P. penicillatus* *P. monodon*	0.0007 – 0.30• 0.03 – 0.93•				dry	E – exposed to Cadmium at 10 – 100 µg L^{-1} for unspecified periods	AAS	[128]
1986	North Sea & Wadden Sea	*C. crangon* *C. allmanni*	0.04 – 0.1• 0.25 – 0.95•				dry	O	AAS	[129]
2009 – 2010	Coast of southern Iran	*P. monodon* *P. semisulcatus*		[0.01 – 0.96] [0.02 – 0.59]			wet	O	AAS	[130]
1995	Ubatuba Bay, Brazil	*Xiphopenaeus kroyeri*	0.27 – 1.1				dry	O	AAS	[131]
2001 – 2002	Persian Gulf, Iran	*P. merguiensis* *P. semisulcatus*		[0.01 – 0.18] [0.001 – 1.2]	[0.14 – 2.8] [0.1 – 2.8]		wet	O	ICP-OES & ICP-MS	[132]
1991	Arabian Gulf, Qatar	*P. semisulcatus*		[0.5 – 1.2]			wet	O	AAS	[133]
2002	Iskenderun Gulf, Turkey	*P. semisulcatus*		0.68 – 1.3•	3.8 – 7.1•	13.3 – 24.8	dry	O	AAS	[134]
NS (1984)	Sozopol, Bulgaria	*P. elegans*	0.35Δ – 1.3Δ				dry	O	AAS	[102]
1997	Campeche Sound, Mexico	*P. setiferus*		[0.3 – 1.5]•	[2.5 – 3.0]•		dry	O	AAS	[135]
1985	NE Atlantic	Various penaeid and caridean spp	0.45 – 1.7•				dry	O	AAS & ICP-MS	[136]
2010	Bay of Bengal, India	*P. monodon* *P. indicus* *P. semisulcatus* *P. merguiensis* *Metapenaeus brevicornis*		ND – 1.8•			dry	O	ICP-MS	[137]
1997	NE Algeria	*Palaemonetes varians* *Parapenaeus longirostris* *A. antennatus*	0.8 – 1.8• 0.12 – 0.22• 0.09 – 0.21•				dry	O	AAS	[138]
1977 – 1978	Mediterranean coast, Lebanon	*P. elegans*	[ND – 1.9]•				dry	O	AAS	[139]
1984	East Java, Indonesia	NS "Penaeidae"	0.3 – 2.0•				dry	O	AAS	[140]
1992	Coast & Indian Ocean off Kenya	NS "Penaeidae"	0.3 – 2.1•				dry	O	AAS	[141]
2011	Bay of Bengal, India	*P. monodon* *P. indicus* *P. semisulcatus* *P. merguiensis* *M. brevicornis*		[ND – 2.1]•			dry	O	ICP-MS	[142]
NS (2013)	East Java, Indonesia	*Macrobrachium sintangese*		2.1•	12.8•	187.5	dry	E – exposed to 0.03 mg L^{-1} Cadmium for 7 days	AAS	[143]

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
NS (2008)	Gulf of Antalya, Turkey	*P. semisulcatus* *Parapenaeus longirostris* *P. serratus*		2.4 0.23 0.88			NS (assumed wet weight)	O	AAS	[144]
1979-80	Spencer Gulf, South Australia	*P. latisulcatus*	[0.08 – 2.5] *•				dry	O	AAS	[145]
1985	NE Atlantic	*Systellaspis debilis*	2.2 – 3.3•				dry	O	ICP-MS	[136]
NS (1973)	NE Atlantic – off NW Africa & Azores	*S. debilis* *Oplophorus* sp. *Acanthephyra eximia*	[0.8 – 3.3] •				dry	O	Anion exchange & "neutron activation analysis"	[146]
1972-3	Northumberland coast, U.K.	*C. vulgaris* *P. serratus*	3.5 2.8				wet	O	AAS	[147]
1999	Bristol Channel & Severn Estuary, UK	*C. crangon*	0.4 – 3.8•				dry	O	AAS	[148]
NS (1973)	Bristol Channel, UK	NS (cooked & raw shrimps)	2.8 – 4.4				wet	O	AAS	[149]
1984	NE Atlantic, east of Azores	*S. debilis*	[1.4 – 4.5] •				dry	O	AAS	[150]
2012 – 2013	Major coastal prawn fisheries, Australia	*P. latisulcatus* *P. plebejus* *P. merguiensis* *P. esculentus* *M. endeavouri* + others		[ND – 0.28] [0.03 – 0.31] [ND – 0.27] [ND – 0.55] [0.17 – 1.1] [ND – 4.6]			wet	O	ICP-MS	This study
NS (1977)	Florida, USA	*P. duorarum*		0.6 – 4.7	50 – 166		wet	E – 96-hr exposures ranging from 1.25 to 5.0 mg/kg Cadmium	AAS	[4]
NS (1999)	Prawn farm, France	*P. japonicus*		0.28 – 5.2•	0.7 – 62•		dry	E – 4-day exposures ranging from 0 to 4mg L^{-1} Cadmium	AAS	[151]
1985 & 1987	Greenland	*P. borealis*	1.4 – 5.2 (geometric means)				wet	O	AAS	[152]
NS (1990)	Townsville, Queensland, Australia	*P. merguiensis*		5.5*	240*	40*	wet	E – exposed to 0.5mg/L Cadmium for 15 days	AAS	[153]
NS (2012)	Mexico	*P. vannamei*	5.7•				dry	E – exposed to 0.09mg/kg Cadmium for 10 days	ICP-MS	[154]
1978 – 1979	Oahu, Hawaii, USA	*H. ensifer*	1.8 – 5.8•				dry	O	AAS	[155]
2006	Iskenderun Bay, Turkey	*P. semisulcatus*		[2.5 – 6.5]•	[13.4 – 18.3] •	[59 – 86]	dry	O	AAS	[156]

Year sampled (Year of publication)	Source of prawns	Species	Cadmium in whole prawns; mean [range]	Cadmium in prawn muscle; mean [range]	Cadmium in prawn hepatopancreas; mean [range]	Cadmium in prawn gills; mean [range]	Wet or dry weight reported	Observational or experimental	Analytical method	Reference
1970-1984	East Atlantic, off W. Africa	*S. debilis*	[1.4 – 11]•				dry	O	AAS	[157]
1996	Shark Bay, Western Australia	*Metapenaeopsis crassissima*		[0.1 – 11.3]#	[1.0 – 22.7] (midgut)		wet	O	AAS	[158]
NS (1996)	Tamil Nadu, India	*Macrobrachium malcolmsonii*		11.5	39.7	26.5	wet	E – exposed to 0.16 mg/kg Cadmium for 22 days	AAS	[159]
Ns (1999)	Tamil Nadu, India	*M. malcolmsonii*		11.5	39.7	26.5	wet	E – exposed to 0.16 mg/kg Cadmium for 22 days	AAS	[160]
NS (1982)	Firth of Clyde, Scotland	*P. elegans*	12.5*•				dry	E – exposed to 1mg/L Cadmium for 21 days	AAS	[161]
NS (2008)	Hydrothermal vents, mid-Atlantic ridge	*Rimicaris exoculata* *Mirocaris fortunata*		12.8• 0.2•			dry	O	AAS	[8]
NS (2007)	SPF breeding line, Hawaii, USA	*P. vannamei*	0.17 (controls) 15.8 (experimental)				wet	E – exposed to 1mg/kg Cadmium for 48 hours	ICP-MS	[162]
1993	Shark Bay & Exmouth Gulf, Western Australia	*M. crassissima*		[0.05 – 23]#			wet	O	AAS	[163]
1972-3	Severn estuary, England & Milford Haven, Wales	*C. vulgaris*	1.2• (Milford Haven) 31• (Severn estuary)				dry	O	AAS	[164]
NS (1977)	Florida, USA	*P. duorarum*		3.8 – 31			wet	E – 30-day exposure studies, ranging from 0.08 mg/kg to 1.3 mg/kg Cadmium	AAS	[4]

*: values interpolated from published line graphs and histograms, therefore tabulated values are approximations

#: muscle tissues with alimentary tracts reportedly removed

Δ: tissue dry weight Cadmium concentrations converted to wet weight concentrations using cited authors' reported tissue moisture content

•: tissue dry weight Cadmium concentrations converted to wet weight using moisture content of 75% (muscle or whole prawns) and/or 80% (hepatopancreas), as determined in this study for muscle and hepatopancreas (see Tables 9 & 10).

❁: purchased product, therefore original source of prawns not specified

NS: not stated

ND: not detected