Assessment of heavy metals in tropical rock oysters (blacklip and milky) and implications for placement into the Australian seafood market and for Indigenous enterprise development in the NT

Principal Investigators:

Ann Fleming[1]

Karen Gibb[2]

Associate Investigators:

Dylan Campbell[2]

Niels Munksgaard[2]

Julia Fortune[3]

Andrea Birch[1]

Mirjam Kaestli[2]

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[1] Aquaculture Unit, Department of Primary Industry and Fisheries, Northern Territory Government, Darwin NT 0801

[2] Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin NT 0909

[3] Aquatic Health Unit, Water Resources Division, Department of Land Resource Management, Northern Territory Government, Darwin NT 0801

Warning for Aboriginal and Torres Strait Islanders

Please be aware that this report may contain the names or images of deceased people. NT Fisheries strives to treat Indigenous culture and beliefs with respect. We acknowledge that to some communities, it can be distressing and offensive to show images of people who are deceased.

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Researcher Contact Details

Name: Ann Fleming
Address: Aquaculture Unit
Department of Primary Industry and Fisheries
GPO Box 3000 Darwin NT 0801
Phone: 0428963681
Fax: 08 8924 4277
Email: AnnE.Fleming@nt.gov.au
Web: www.nt.gov.au/d/Fisheries/

FRDC Contact Details

Address: 25 Geils Court
Deakin ACT 2600
Phone: 02 6285 0400
Fax: 02 6285 0499
Email: frdc@frdc.com.au
Web: www.frdc.com.au

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Executive Summary

The study and the Need

This small, but extensive, sampling survey was conducted on South Goulburn Island, located off West Arnhem Land in the Northern Territory (NT) to assess the occurrence of heavy metals (both spatially and temporally) in tropical blacklip (*Saccostrea mytiloides*) and milky (*Saccostrea mordax*) oysters. Heavy metals tested where those identified by the Australian Shellfish Quality Assurance Program

Results were used to determine whether heavy metal levels exceeded the Maximum Residue Levels (MRLs - or MLs as the more commonly used terminology) set by Food Standards Australia New Zealand (FSANZ) within the Australia New Zealand Food Standards Code (ANZFSC). The range of metals tested were chosen based on previous national residue surveys in seafood across the NT (and our preliminary screening of the study site) that indicated likely contaminants. For example, in this study mercury was not tested as the preliminary screening test done on South Goulburn Island indicated mercury to be low (0.005-0.007 mg/kg; ML 0.5mg/kg) and previous extensive heavy metal testing done by various national surveys along the NT coastline over the last few decades reported consistently low levels of mercury in various seafood products.

This sampling survey was initiated in response to an unforeseen event that arose in the early development phase of the Indigenous oyster enterprise program of the NT Government's Aquaculture Unit. In December 2011 opportunistic samples of oyster flesh taken at two sites on Goulburn Island showed high levels of cadmium and arsenic, both at levels above the MLs for these elements. The implication of these results for Indigenous organisations planning to sell tropical oysters into Australian seafood markets was unknown at the time.

A more extensive assessment of the occurrence of heavy metals in potential growout areas was needed to assess the risk to human health and identify possible management strategies to ensure oyster product met the food safety standards set by the FSANZ. To assess the risk to human health from heavy metals in tropical oysters the following objectives were addressed:

1. Conduct a sampling survey of the spatial and temporal variability of heavy metals in tropical oysters (blacklip and milky) in the West Arnhem region.
2. Assess the implications of results on the development strategy of the oyster enterprise and the sale of tropical oysters into the Australian seafood market.
3. Employ Indigenous partners to conduct the shellfish monitoring outlined in this project to develop Indigenous capacity in fisheries sciences and an additional employment steam for Indigenous people.

The Aquaculture Unit of the Department of Primary Industry and Fisheries, the Goulburn Island Indigenous Aquaculture Team and Charles Darwin University (CDU) researchers collaborated to measure trace elements (metals) in blacklip and milky oysters collected from four sites around South Goulburn Island. Sampling (of oysters and seawater) was conducted during the dry season in September 2012, the wet season in February 2013, and again during the dry in September 2013. Samples were collected from the shore within a 24-hour period during extreme low daytime tides, flown to CDU's Environmental Chemistry and

Microbiology Unit (ECMU), where they were analysed for heavy metal content. A suite of heavy metals were analysed but of prime interest were arsenic (As) (note - FSANZ considers arsenic as a metal for the purposes of the Food Standards Code), cadmium (Cd) and lead (Pb) as MLs are set by FSANZ for these elements only. Oyster product must conform with MLs set for these metals to allow placement of product in the Australian seafood market.

The results

Ideally, oyster sampling would target market sized animals within a narrow size range (10-15 cm length), as the heavy metal content of these aniamls would be assumed to reflect heavy metal contect of harvestable animals from commercial operations. However this was not possible as the oyster sampling program conducted in this study was done on a remote island, at remote sites across the breadth of the island that were accessably only during dry weather conditions, and during a small window of opportunity when oyster beds were exposed during extreme low tides. As a result, the data is compromised due to the small sample size for some sampling sites and times. Every effort was made to meet the targeted sample size and number, but final oyster samples were limited to those that were available.. An initial collection trip failed to collect sufficient samples at most sites and so was not included in the dataset. Farmed blacklip oysters were deployed during the project to increase sample availability. Subsequent collections were sometimes done at night-time low tides to ensure all sites were sampled. It must be noted that accumulation of heavy metals may differ between oyster age classes (and size), most likely due to different exposure times. Thus the smaller size range of oysters collected in this study may be an underrepresenation of heavy metal content of marketable oysters.

Our analysis of trace elements in milky and blacklip oysters in the West Arnhem region showed that the heavy metal content of oysters differed between sites and sampling times and that the two species accumulated heavy metals differently. Farmed blacklip oysters showed different heavy metal accumulations than wild caught blacklips at some sites.

Wild harvest blacklip oysters accumulated Cd levels that exceeded the food safety standards at all sites and on each of the three sampling events (two during the wet season and one during the dry) over the 12-month survey period.

Farmed blacklip deployed for up to 12 months repeatedly exceeded Cd at only one site (site 2) for the three sampling event. There were no other exceedences of Cd by farmed blacklip at any other sites or sampling events.

Wild harvest milky oysters also exceeded Cd levels at site 2 for each of the three sampling events. They also exceeded Cd at one site (site 1b) on the first sampling event.

We also tested total arsenic in the two oyster species. Levels of total As recorded in this study suggests that the inorganic component to which the guidelines relate are not likely to have been exceeded. Further As speciation analysis would be needed to confirm this.

The lead content of oysters was below MLs for all sites and at all sampling events.

Implications for stakeholders and recommendations

The implications of these results for the development of an Indigenous oyster enterprise and the sale of tropical oysters into the Australian seafood market are to limit harvesting to particular sites and species, and possibly to avoid wild harvest blacklip and consider using only farmed blacklip.

Our work has demonstrated that avoiding some sites and relying on farmed blacklip will significantly reduce the risk of exceedence. While this gives a cautious green light to this Indigenous enterprise, it should be noted however that there is still an element of risk for Cd exceedence. While some sites seem better than others, they are still geographically close and site characteristics could change. Ultimately a commercial industry would be coupled to an appropriate Quality Assurance (QA) Program ensuring that product placed in the Australian seafood market met the FSANZ guidelines. Currently a follow up project is underway to establish a database (and associated shoreline sanitary survey) to inform the future QA program.

Indigenous scientific investigation partnership

The project was conducted in partnership with the local Indigenous aquaculture team on Goulburn Island. This team consists of about 10 men who are working towards gaining a vocational training certificate in aquaculture (VETII), through the CDU's Vocational Education and Training School. The indigenous team supported Fisheries and CDU staff in conducting the sampling program in the field. The technical work was integrated into the training program so that students understood the reason for the survey, its practical application in the future oyster enterprise and to reinforced skill development in survey methods, and handling and processing samples.

The engagement of the aquaculture team during the project improved over time. This work was carried out during a period when a number of tensions existed within the community and, for a period, there was a certain level of mistrust between the local aquaculture team and staff. Negotiation and formalisation of payment for hours worked improved trust and participation. Lessons learnt during this project have improved Fisheries staff's processes for effectively engaging local support teams, building trust and communication.

The work done subsequent to this project (investigating broader quality assurance needs) has provided further insight and detail into the support processes needed to build Indigenous science capacity. Support and mentoring needs to gradually build understanding, familiarity, confidence, trust and skills in local support teams.

This transition to independent research activities without external support is a key step in achieving the community's aspirations to be in control of their own affairs and to take pride in supporting external scientists. In this way the community is a full participant in co-developing fisheries opportunities that allow communities to operate and work in local fisheries-based businesses.

Keywords

oysters, shellfish, aquaculture, Indigenous, trace elements, heavy metals, contaminants, quality assurance, food safety

Introduction

The Need - The Indigenous aquaculture program of the Northern Territory Government

For the past five years the Aquaculture Unit of the Northern Territory Fisheries Division has worked in partnership with Indigenous communities, commercial partners and Indigenous agencies to develop low technology sea-based aquaculture enterprises suitable for remote Indigenous coastal communities. Trials continue on Goulburn Island, Tiwi Islands and Groote Eylandt, including sandfish (*Holothuria scabra*) ranching, blacklip oyster (*Saccostrea mytiloides*) farming and wild harvest, and giant clam (*Tridacna squamosa*) farming.

Over the same period, social research conducted by the Aquaculture Unit in partnership with various university researchers investigated Indigenous people's preferred development pathways and employment aspirations. Results showed that Indigenous people see sea-based aquaculture as a culturally integrated form of work that aligns naturally with their customary practices on sea country (Fleming *et al*., 2015). Indigenous people expressed a desire for aquaculture development to provide benefit across their cultural priorities (harvesting and visiting sea country and the associated deeply spiritual wellbeing that comes from this), as well as social (healthy food enterprises), economic (jobs and local businesses) and environmental (sustainable use of marine assets) priorities. People aspire to have autonomy over their lives and want to run their own local seafood businesses, but recognise the need ongoing support through business management training and community governance capacity development. Some people were keen to begin engaging in aquaculture activities by growing and harvesting seafood for local food supply enterprises within the community, and, as people gradually develop capacity, to broaden emphasis from local food supply towards commercial export into mainstream seafood markets. The range of species being trailed for development can potentially meet these diverse benefits. Clams and oysters can be grown for cultural uses and nutritional benefit and can also be exported to Darwin seafood markets for local distribution. Ranching of sea cucumber for export to China through industry partners is seen by Indigenous people as a way to improve employment opportunities in communities, particularly for the young, and develop local businesses to manage the stock production and seafood processing operations.

Oyster farming and wild oyster harvest activities appear to fit people's development aspirations because they align with traditional marine harvesting practices for shellfish and other intertidal species. If oyster farming is developed in a way that emulated and enhanced traditional wild caught oyster harvesting activities, it may offer a culturally-aligned, contemporary employment opportunity for remote coastal communities.

In addition to the cultural significance of oyster harvesting for Indigenous coastal people, the elder members of the community on Goulburn Island have strong and positive memories of the Methodist mission era that ran from 1916 to about 1974. A range of seafood, agricultural enterprises and wild harvest activities were managed by the missionaries to engage local people in work (paid in rations), as well as strive for self-sufficiency in food production and generate funds through sales (of trepang, oysters, mussels, dugong, turtle and fish) into Darwin markets (Fleming *et al.* 2015). Older people reminisced fondly of 'mission times' when fresh oysters and fish were exported to the mainland, and a range of fresh foods were produced locally, such as eggs, milk, beef and bread. One senior elder told a favourite story about the times when '*Goulburn Island oysters were famous all the way to Tennant Creek'*. Some people talked proudly of these past oyster farming activities and were very optimistic and hopeful about aquaculture enterprises in relation to these times (Fleming *et al.*, 2015).

Preliminary market assessment of tropical oysters

To date tropical edible oyster aquaculture (referring to non pearl oyster species) has not been commercialised in Australia. In the northern waters of Queensland, there has been a history of harvesting wild milky and blacklip oysters from rocky foreshore areas (Beattie, 2001). For example, during 1999/2000 16 tonne of both species were harvested by 109 operators who each worked 600m of foreshore (Nell, 2001).

In 2011 Mr Ziko Ilac of the Darwin Fish Markets P/L assessed the market acceptance of blacklip oysters by providing live samples to a number of chefs in top-end Darwin restaurants. The chefs were positive and saw potential in developing a 'local and unique dining experience' where oysters would be place on hot coals at the dining table to open naturally (their irregular shape would make manual opening difficult and time consuming for the kitchen staff). The likelihood of low volumes and irregularity of supply from remote communities (flown in weekly when seasonally available) was seen as a positive by the chefs. The infrequent inclusion on the menu of locally grown oysters was seen as an attractive marketing strategy, adding to the Indigenous fair trade, exclusively tropical NT dining experience for national and international visitors to Darwin. Based on the learnings from other wildlife enterprises in remote Indigenous communities (Fleming, 2015), such regionally local markets (short supply chain) that seek small, irregular/seasonal volumes of product are more likely to be economically viable.

Production techniques for tropical edible oysters

During the 1980s work was done across the Asia-Pacific region, including Australia, on the culture techniques for native tropical oyster species and a number of species were investigated for their mariculture potential (see Southgate & Lee, 1998 for a review of the literature). Despite this early work, today relatively little information is available on aspects of the biology of these species and hatchery techniques remain unreliable.

Hatchery rearing trials of the blacklip oyster in northern Queensland produced small numbers of spat (Southgate and Lee, 1998; Beattie, 2001), although poor larval survival was considered a constraint to commercial development until further research identified optimal rearing conditions. Reports of the successful collection of several thousand spat from trials done in Thailand (Anon., 1988) suggest collection of natural spat may be a viable alternative to hatchery production.

The Darwin Aquaculture Unit is currently investigating reliable hatchery production techniques for the blacklip oyster. This research is supported by tropical oyster experts (from Paspaley P/L) and a temperate oyster species expert (Dr Wayne O'Conner of NSW DPI).

Current oyster production trials in the NT

To date the growout trials on blacklip tropical oyster on both the Goulburn and Tiwi Islands have identified the most suitable sites, growout structures and management methods for remote communities. Initially oysters were held just offshore in baskets secured to racks on the sea floor (Figs. 1 & 2) and were maintained during low tide periods. But the limited access for maintenance and monitoring (due to the infrequency of suitably low tides during the daytime) led to the adoption of a floating system that can be accessed any time from a boat (Figs. 3 & 4).

Fig. 1. Attached rack system during low tide at Mardbalk Bay, Goulburn Island. Baskets are tied to a rack that is secured to the sea floor with four star pickets.

Fig. 2. Attached rack system during low tide at Mardbalk Bay, Goulburn Island. Stock management is limited to periods of low tide by walking from the shore.

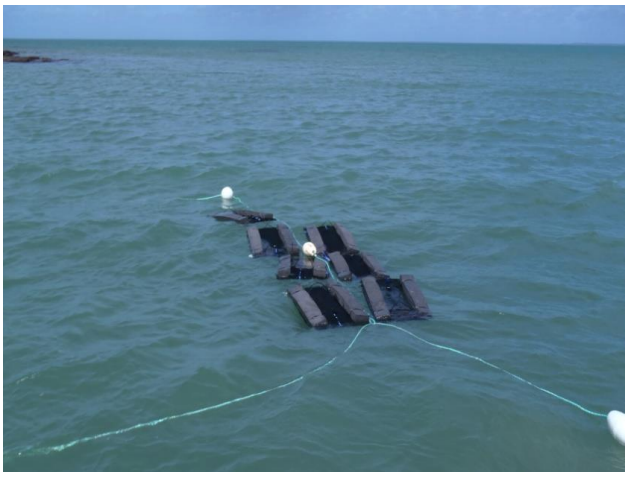

Fig. 3. Floating oyster baskets at Fletchers Point, Goulburn Island. Baskets can be easily accessed from a boat at any time for stock management and harvest.

Fig. 4. Floating system during low tide at Mardbalk Bay, Goulburn Island showing anchorage points.

Growth rates, survival and fouling of the shell varied between sites around South Goulburn Island, indicating some sites were more suitable than others for oyster farming based on production considerations (Fig. 5-6, see Fig. 7 for location of sites). Growth data indicates an average growout period of about 18 months to market size (10-15 cm) for blacklip oysters under these conditions.

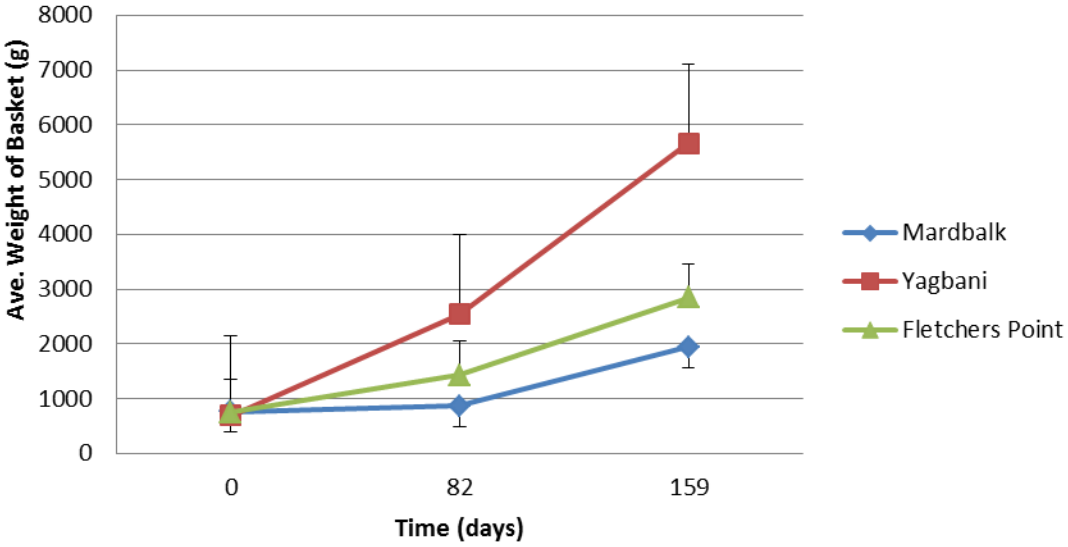

Fig. 5. Average weight of batches of blacklip oysters held in 3 baskets (about 470 animals per basket) secured to the seafloor in the intertidal at three sites (Fletchers Point, Yagbani and Mardbalk) on South Goulburn Island. Data shows average weight of oysters in each basket between 0-82 days (Oct 12 - Feb13) and 83-159 days (Mar 13 - July13). Initial individual oyster weight was about 0.9g and length was about 10mm.

Figure 6. Blacklip edible oysters grew well (with minimal fouling) at Mardbalk Bay, Goulburn Island after 7 months at sea.

Health risks associated with oyster consumption

Oysters are filter feeders, extracting phytoplankton, bacteria and suspended organic and inorganic particles from the surrounding water. Oyster growing waters may be subjected to pollution from a range of human activities, including discharges of untreated or poorly treated human waste, direct discharges of industrial wastes and runoff from urban and agricultural areas.

Oysters also bio-accumulate organic and inorganic particles that may be present due to the mineralogy of the area and the natural geological processes, such as leaching of heavy metals into marine systems from benthic sediments and coastline erosion. These elements can be taken up by oysters via the metals associated with the seawater, via sediements suspended in the seawater and via those accumulated in phytoplankton.

Quality assurance programs to monitor health risks

As a consequence of their ability to bio-accumulate pathogens, chemicals and toxins derived from contaminated growing waters, and because they are often eaten raw or only lightly cooked with the gastrointestinal tract intact, oysters have been associated with numerous outbreaks of human disease. As a consequence of the risk to human health, oyster farmers must monitor potential contaminants to minimise the risk to human health.

Maximum permissible concentrations of high-risk chemicals are specified in the FSANZ Food Standards Code. Food or health authorities in southern Australian states (and Queensland) have developed a body of guidelines governing the growing, processing and marketing of oysters to ensure these food standards are maintained for all commercially farmed oysters in Australia. The Australian Shellfish Quality Assurance Program (ASQAP), a national program modelled on the National Shellfish Sanitation Program of the United States, requires that shellfish harvest areas be classified on the basis of a sanitary survey and the results of an ongoing water-sampling program. This program has been applied to wild shellfish harvest and aquaculture shellfish growing areas in Tasmania, NSW, South Australia, and Western Australia and to one harvest area in Queensland.

Preliminary data on levels of heavy metals in oysters

In December 2011 samples of oyster flesh opportunistically taken at two sites off Goulburn Island (West Arnhem Land, Northern Territory) showed high levels of cadmium and arsenic; both above the MLs for these elements. Other studies have shown similar results. For instance, the Environmental Chemistry and Microbiology Unit (ECMU) of Charles Darwin University has advised that their data and those of Peerzada *et al.* (1993) also showed elevated cadmium and arsenic in oysters collected along the northern Australian coastline. However not all sites recorded elevated levels and Peerzada *et al.* (1993) implicated the occurrence of phytoplankton as a key source of elevated metals.

The implications of these data for Indigenous organisations planning to sell tropical oysters into Australian markets was unknown at the time. A more extensive assessment of the occurrence of heavy metals in tropical edible oysters was needed to assess the risk to human health and identify possible management strategies to ensure oyster product grown in the NT meets food safety standards. Before informed decisions about the future of this very promising enterprise could be made, an accurate measure of heavy metals in replicated oyster samples over the wet and dry seasons and across sites was needed. From a management perspective, knowledge on the source of heavy metals was beneficial, and so we also sought to determine if there is an association with metal levels in water and/or phytoplankton.

Objectives

1. Conduct a sampling survey of the spatial and temporal variability of heavy metals in tropical oysters (blacklip and milky) in the West Arnhem region
2. Assess the implications of results on the development strategy of the oyster enterprise and the sale of tropical oysters into the Australian seafood market
3. Employ Indigenous partners to conduct the shellfish monitoring outlined in this project to develop Indigenous capacity in fisheries sciences and an additional employment steam for Indigenous people

Methods

The study site

The research was carried out on South Goulburn Island, located 280km northeast of Darwin and 3km off the west Arnhem coast (Fig. 7). The southern coast of South Goulburn Island faces the mainland and is exposed to annual rainfall runoff during the wet season from November to April. The northern coastline is exposed to the open oceanic currents of the Arafura Sea. No commercial industries operate in the region. The only known discharge into the sea comes from a small sewage treatment plant on the NW side of the island. Heavy metals occur naturally in NT waters as a result of the mineralogy of the region and the natural geological processes, such as leaching of heavy metals into marine systems from benthic sediments and erosion of coastline formations (Munksgaard & Parry, 2001; 2002).

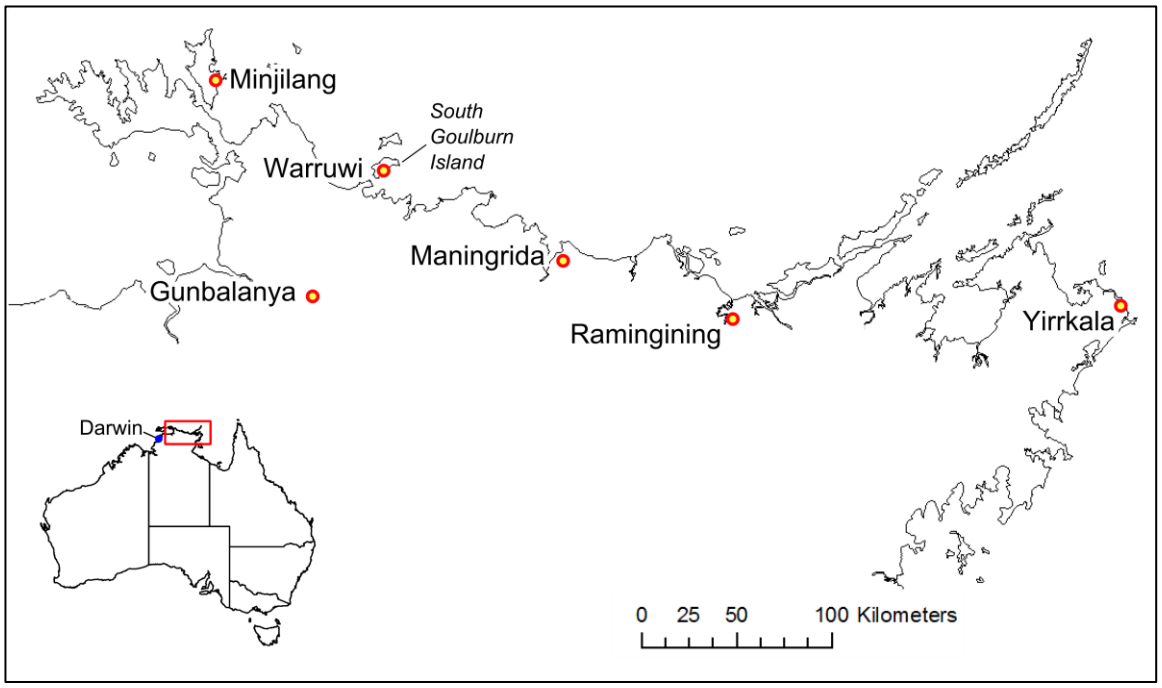

Figure 7. Location of South Goulburn Island, Northern Territory

Four sampling sites were selected on Goulburn Island (Figures 8-9a&b; see Appendix 1 for site coordinates). Three of these locations (sites 1-3) where previously selected by the Aquaculture Unit of NT Fisheries as suitable sites for oyster growout trials as they offered reletavely sheltered conditions and supported natural populations of oysters. These trials were still underway during this research. Site 4 was more exposed to oceanic conditions and extensive populations of milky oysters were predominant on rocky outcrops.

To investigate seasonal effects on heavy metal content of oysters and seawater, samples were collected twice in the dry season (September 17-20, 2012 and September 9-10, 2013) and once in the wet season (February 12-15, 2013).

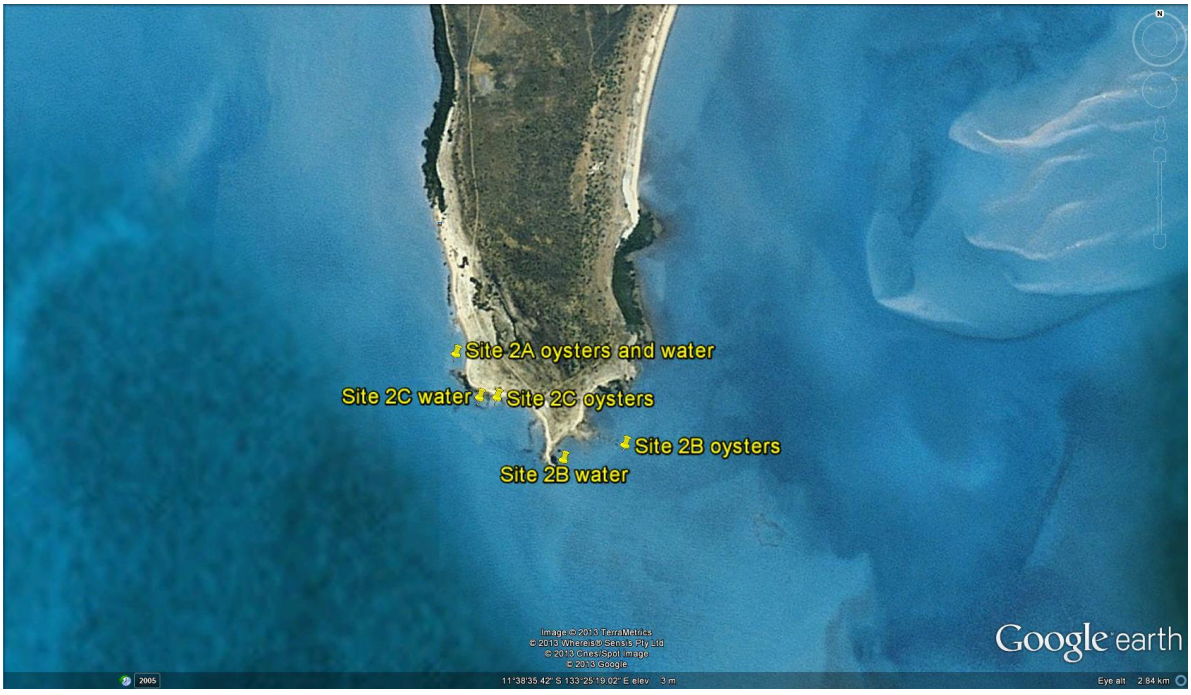

Figure 9a: Enlarged view of sites 1 and 2 showing locations of oyster and water sub-samples

Figure 9b: Enlarged view of sites 3 and 4 showing locations of oyster and water sub-samples

The local Indigenous aquaculture support team

The project was conducted in partnership with the local Indigenous aquaculture team on Goulburn Island. This team consists of about 10 men who are working towards gaining a vocational training certificate in aquaculture (VETII), through the CDU's Vocational Education and Training School. During 3-day sampling trips between 3-5 men were assigned by the team leader to assist Fisheries staff in sample collection. An Indigenous team leader was responsible for acting as liaison officer and contact person for field trip planning, organisation and logistical and team support.

The technical work was integrated into the training program so that students understood the reason for the survey, its practical application in the future oyster enterprise, the reason for following scientific procedures accurately and to reinforced skill development in survey methods, and handling and processing samples.

Sampling and initial sample processing

Wild milky oysters (*Saccostrea mordax*) and blacklip wild oysters (*Saccostrea mytiloides*) were collected at three sampling locations (where possible) within each of the four sites around South Goulburn Island (Apendices 1-3). For trips 2 and 3, the numbers of oysters collected at each sample location varied based on availability. During trip 1 insufficient oyster samples were collected due to the low occurrence of wild blacklip oysters at some sampling locations. To address this, farmed blacklip oysters were subsequently deployed in September 2012 at the sites indicated in Appendices 2 and 3. They were then sampled at trip 2 (five months after deployment) and trip 3 (12 months after deployment) for trace element analysis in the same manner as wild harvest oysters. Mean size and total number of oysters collected per site, oyster type and farmed status are shown in Table 1. At each site, 1 litre of surface seawater was collected for heavy metal, total suspended solid and chlorophyll a analysis. The unopened oysters were placed in zip-lock plastic bags and immediately placed on ice for transport to the laboratory.

Table 1: Mean size and total number of oysters collected per site, oyster type and farmed status

	Oyster type	**Not farmed**				**Farmed**		
		Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 1**	**Site 2**	**Site 3**
Mean size (sd) #Total oysters collected	Mordax	5.0 (0.6) #43	3.9 (0.6) #63	3.6 (0.6) #32	5.0 (1.2) #68	NA	NA	NA
	Blacklip	9.4 (2.1) #16	9.6 (1.7) #21	NA	9.6 (0.9) #8	4.3 (0.2) #21	4.7 (NA) #12	4.0 (0.1) #21

(sd) indicates standard deviation.

During the September 2013 dry season trip, a phytoplankton bloom occurred, so an additional 1 litre of surface seawater was opportunistically sampled at all four sites. Samples were collected in plastic bottles and a sub-sample was fixed in Lugol's solution to a final volume of 250 mLs for phytoplankton identification.

Seawater sample preparation and trace metal analysis

Immediately on arrival at the laboratory (an average of 6 hrs from the time of collection), a sample of seawater (125 mLs) was filtered using a 0.4 µm syringe filter. This filtered sample and an unfiltered sample (125 mLs) were prepared for elemental analysis (USEPA method 1638 (1995) and USEPA method 6020 CLP-M version 7.0). Briefly, the samples were acidified to pH < 2 with ultrapure analytical grade concentrated nitric acid. The unfiltered acidified water samples were digested overnight at 60°C to release metals from particulate matter. The following elements were measured in the acidified filtered and unfiltered samples by inductively coupled plasma mass spectrometry (ICPMS, Agilent 7500ce): S, Mg, K, Ca, Al, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb and U. Reference samples were run as quality controls in all ICPMS runs (See Appendices 4-6).

Oyster sample preparation and trace metal analysis

Upon arrival at the laboratory, oysters were weighed and their dimensions measured. The shells were opened under clean-room conditions and the soft tissue extracted. A pooled oyster tissue sample of, on average, 6 individual oysters (exact numbers are shown in

Appendices 7-9) were homogenised using an Ultra Turrax macerator then digested with Nitric acid in a microwave oven (15 min ramp to 200^{o}C, hold for 15 min). Sediment samples were digested in nitric + perchloric acids in open digestion tubes at 200^{o}C for 4 hours. Instrumental analysis of metal and As concentrations in acid digests was carried out by Inductively Coupled Plasma Mass Spectrometry (ICPMS). The moisture content of oyster tissue samples was determined gravimetrically. Quality control measures included analysis of blank digests, certified reference materials, replicate digests and metal-spiked digests.

Measuring total suspended solids (TSS)

To measure total suspended solids (TSS), 0.6-1 L of seawater samples were filtered through 0.45 µm filters (Pall) in the laboratory. The filters were weighed pre and post filtering on a UMX2 Ultramicrobalance (Mettler Toledo). The difference in weight pre and post filtering was used to calculate the TSS in samples. The TSS method was developed in house at Charles Darwin University.

Bioavailable trace metal analysis in TSS

To determine the bioavailability of the trace metals within TSS, filters were digested in 2 mL of 1N HCl and 2 mL of high pure water using a microwave digestion technique. The microwave was run at 400W and 200 °C for 15 min, before a 30 min cool down. The resulting digests were analysed to determine the concentrations of Fe, Mn, Co, Ni, Cu, Zn, As, Cd, Pb and U by ICP-MS (Agilent 7500ce).

Phytoplankton identification

Water samples were sent to Dalcon Environmental Pty Ltd (in Malaga, WA) to identify and quantify algal composition. For each species, cell density, percentage of total cells and presence of potentially toxic or harmful species were recorded.

Bioavailable trace element analysis in phytoplankton

Samples were digested in 2 mL of 1N HCl and 2 mL of high pure water using a microwave digestion technique. The microwave was run at 400W and 200 °C for 15 min, before a 30min cool down. The resulting digests were analysed for the concentrations of Fe, Mn, Co, Ni, Cu, Zn, As, Cd, Pb and U by ICP-MS (Agilent 7500ce). ICP-MS quality control included duplicates of the following certified reference materials: DORM-2, AGAL-3 and 1566b oyster (Institute for National Measurement Standards, National Research Council of Canada).

Chlorophyll a measurements

Fluorometric analysis (Trilogy Model, Turner Instruments, Sunnyvale CA, USA) was used to measure chlorophyll *a* (chl. *a*) in phytoplankton samples filtered from 1 L seawater following a modified version of the EPA method 4450 (Arar & Collins, 1997).

Statistical analyses

Data were analysed using PRIMER 6 (Plymouth Routines In Multivariate Ecological Research) and the PERMANOVA+ add-on (PRIMER-E Ltd, 2007 UK) and using Stata IC13 (www.stata.com).

Results

Data related to the FSANZ maximum level guidelines

Collection locations, oyster sample numbers collected (for both wild and farmed) and site descriptions are given for each sampling trip in Appendices 1–3. Trace element data (for Al, P, V, Fe, Co, Ni, Cu, Zn, As, Mo, Cd and Pb) are reported for seawater samples in Appendices 4-6 and for oyster samples in Appendices 7-9. The range of metals tested were chosen based on previous national residue surveys in seafood across the NT (and our preliminary screening of the study site) that indicated likely contaminants. For example, in this study mercury was not tested as the preliminary screening test done on South Goulburn Island indicated mercury to be low (0.005-0.007 mg/kg; ML 0.5mg/kg) and previous extensive heavy metal testing done by various national surveys along the NT coastline over the last few decades reported consistently low levels of mercury in various seafood products.

Of particular relevance to this study is the data collected for those trace elements listed in the Australian and New Zealand Food Standards Code for safe human consumption of seafood (FSANZ, 2002). These guidelines list the maximum level of As (inorganic), Cd, Pb, Cu, Zn and Hg recommended for safe consumption of molluscs (Table 2). In particular, the guidelines state the maximum levels (MLs) of arsenic, cadmium and lead permissible for safe consumption of molluscs. Oyster stocks sold commercially in Australia must be shown to comply with these MLs. Most states with an oyster industry have adopted the Australian Shellfish Quality Assurance Program (ASQAP) guidelines as a minimum standard. These guidelines use the Food Standards to set the maximum permissible levels in product. ASQAP guidelines do not prescribe what should be tested but rather proposes shoreline surveys to assess possible contaminant sources and risks. Consequently, the results and discussion of this study will focus primarily on the data generated for arsenic (As), cadmium (Cd) and lead (Pb) as these were found to be at eleveated levels in oyster samples in preliminary tests at the study site. Results will be discussed in terms of the implication for future oyster farming enterprises in the NT, and possible management strategies. The generally expected levels (GELs) for copper (Cu) and zinc (Zn) are included in Table 2 for comparison and interest, but there are now no maximum allowable limits set for these elements. In recent years they were removed from the food standards when safety assessments indicated a low risk to consumers. These and other trace elements will be reported in this study for interest and discussion on potential biochemical interactions between key elements that may influence the heavy metal content of oysters. It must be noted that due to the small size range of oysters collected, data presented here is likely to underrepresent that levels of heavy metals in commercial sized (10-15 cm) animals. As such, caution needs to be used when making management recommendations based on these results.

Table 2: Maximum Levels of contaminants and natural toxicants and additional guidelines for Generally Expected Levels. Excerpt taken from "Table to clause 2" from the Australian and New Zealand Food Standards Guide Standard 1.4.1.

Contaminant	Food	Maximum level (mg/kg)	Generally Expected levels (mg/kg)
Arsenic (inorganic)	Molluscs	1.0	
Cadmium	Molluscs (excl dredge/bluff oysters & queen scallops)	2.0	
Lead	Molluscs	2.0	
Copper	Molluscs		30
Zinc	Oysters		290
Mercury	Molluscs	0.5	

Trace elements in seawater

Trace element levels in seawater at all sites for each trip did not exceed the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC, 2000) and at most sites levels of Cd, Zn and Cu were close to or below the detection limit (see Appendices 4-6 for data).

Trace elements in oyster tissue - comparing sites and species

Mean size and total number of oysters collected per site, oyster type and farmed status as well as mean Cd, Zn and Cu concentrations are shown in Table 3.

Table 3: Oyster sizes and numbers by site and type and farmed status as well as mean Cd, Zn and Cu concentrations

	Oyster type	Not farmed				Farmed		
		Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3
Mean size (sd) #Total oysters collected	Mordax	5.0 (0.6) #43	3.9 (0.6) #63	3.6 (0.6) #32	5.0 (1.2) #68	NA	NA	NA
	Blacklip	9.4 (2.1) #16	9.6 (1.7) #21	NA	9.6 (0.9) #8	4.3 (0.2) #21	4.7 (NA) #12	4.0 (0.1) #21
Mean Cd (sd) mg/kg	Mordax	1.5 (0.4)	3.6 (0.8)	1.1 (0.2)	1.0 (0.2)	NA	NA	NA
	Blacklip	4.0 (1.0)	5.1 (1.3)	NA	3.3 (0.8)	2.0 (0.0)	2.5 (NA)	1.4 (0.0)
Mean Zn (sd) mg/kg	Mordax	21.3 (6.0)	9.8 (2.3)	266.8 (47.8)	24.9 (6.0)	NA	NA	NA
	Blacklip	21.6 (8.0)	14.0 (5.5)	NA	30.3 (11.5)	19.6 (1.4)	18.2 (NA)	77.7 (7.2)
Mean Cu (sd) mg/kg	Mordax	13.8 (2.6)	7.7 (1.6)	42.0 (15.2)	12.7 (1.7)	NA	NA	NA
	Blacklip	16.6 (3.7)	13.8 (3.8)	NA	13.3 (1.7)	9.8 (1.1)	9.4 (NA)	16.3 (0.8)

(sd) indicates standard deviation.

Accounting for trips, sites and type of oyster in a multivariate regression, the farmed (blacklip) oysters were on average half the size of wild oysters (P<0.001) and wild Blacklip were on average 2.2 times bigger than wild Mordax oysters (P<0.001).

Comparisons between sites (Table 4) were based on multivariate regressions with the outcome metals natural log transformed. All regressions accounted for size, trips, sites and oyster type (see Appendix for regression analyses, P values of <0.05 were considered significant).

Table 4: Site comparisons and significant metal differences

Comparisons between	Significantly different metal levels between groups (each at fixed levels of sites, trips, type of oyster, farmed and size)
Site 2 vs Site 1	less Al, V, Fe, Ni, Cu, Zn, As more Cd, Co
Site 3 vs Site 1:	less Cd, Al, As more Cu, Zn, Pb
Site 4 vsSite 1	less Cd, Al, V, Fe, Mo, Pb
Trip 2 vs Trip 1	less Ni, Pb more P, V, Mo
Trip 3 vs Trip 1	more V, Ni
Farmed vs not farmed	less Cd, Co more P
Blacklip vs Mordax	less P more Cd, Co, Ni, Mo, Pb
Increasing size of oyster	More Cu, As (with other variables fixed incl. farmed and type of oyster)

At a fixed oyster size, on average 82% more Cd was found at site 2 in oyster tissue as compared to site 1 and 32-36% less at sites 3 and 4 (P=0.001). There was on average 61% less Cd in oyster tissue in farmed oysters (P<0.001) and 2.9 times more Cd in blacklip as compared to mordax (P<0.001).

Pearson correlates between metals were used to show positive or negative associations between metals (Table 5). Fe and V showed a Pearson correlation of 0.8 while Zn and Cu showed the strongest positive correlation of 0.9.

Table 5: Pearson correlates between metals

	Al	P	V	Fe	Co	Ni	Cu	Zn
Al	1.00							
P	-0.07	1.00						
V	0.60	0.04	1.00					
Fe	0.66	-0.06	0.80	1.00				
Co	0.28	-0.19	0.30	0.08	1.00			
Ni	0.33	-0.40	0.23	0.15	0.77	1.00		
Cu	-0.12	-0.10	0.04	0.09	-0.07	-0.01	1.00	
Zn	-0.17	-0.10	-0.16	-0.03	-0.12	-0.07	0.90	1.00
As	-0.06	0.21	0.19	0.04	0.07	0.27	-0.11	-0.32
Mo	0.35	0.19	0.59	0.19	0.42	0.04	-0.07	-0.25
Cd	-0.02	-0.20	0.23	-0.04	0.43	0.05	-0.27	-0.43
Pb	0.50	-0.32	0.44	0.43	0.36	0.20	0.37	0.31
	As	**Mo**	**Cd**	**Pb**				
As	1.00							
Mo	0.12	1.00						
Cd	0.14	0.55	1.00					
Pb	-0.26	0.38	0.31	1.00				

The high correlations between Fe and V and between Zn and Cu are illustrated below in Figure 10. The scatter plots between these metals show that the high correlations are also due to mordax oysters having particularly high Fe and V levels from site 1b (Figure 10 left) or particularly high Cu and Zn levels from sites 3a, b and c.

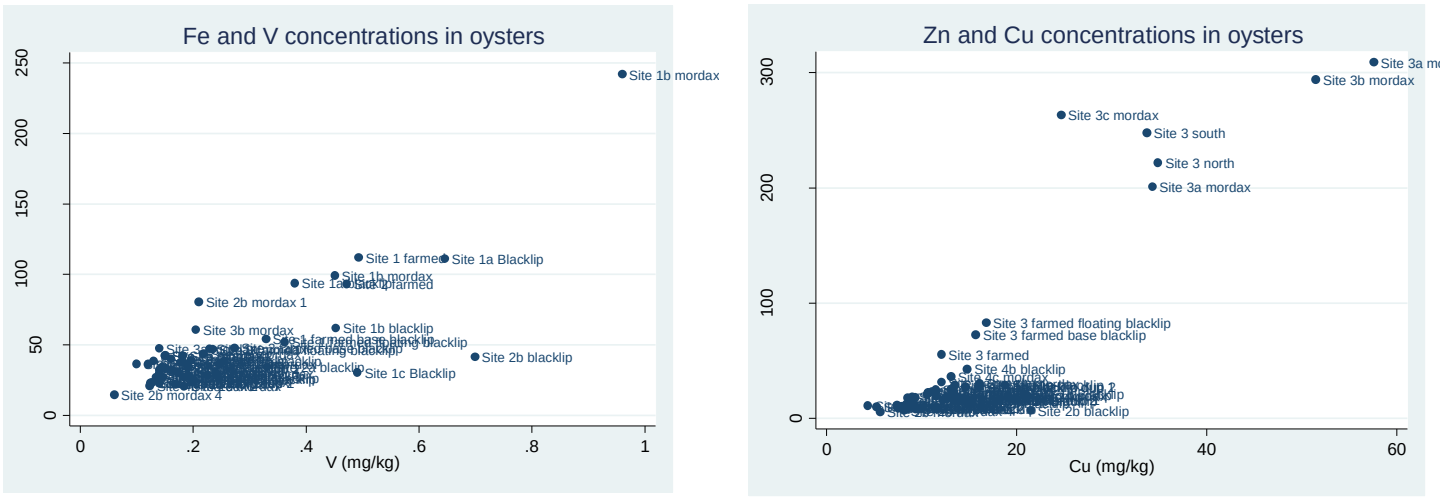

Figure 10: Scatter plots of Fe and V (left) and Zn and Cu (right) levels from Mordax and Blacklip oysters.

Trace element levels in oyster tissue and quality control data are shown in Appendices 7 - 9.

Analysis of metal profiles and oysters – taking into account size

The following analysis was performed in Primer-7, metals of oyster tissue and oyster size were normalized and an Euclidean distance matrix was calculated based on the normalized metal and size data.

Principal Component Analysis (PCA) of metals in oyster tissue and oyster size

Figures 11-14 show metal and oyster size profiles of all samples. (The closer two triangles, the more related their metal and size profile). The vectors show the direction and size contributions of the metal and oyster size variables for the eigenvectors of the PCA ordination.

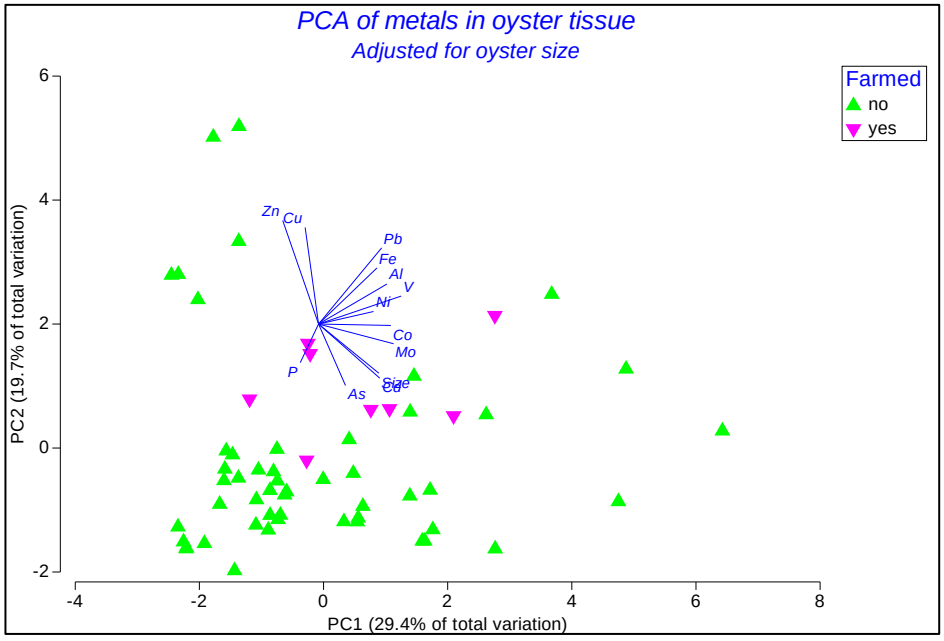

Figure 11: PCA ordination of metals in oysters – farmed vs wild

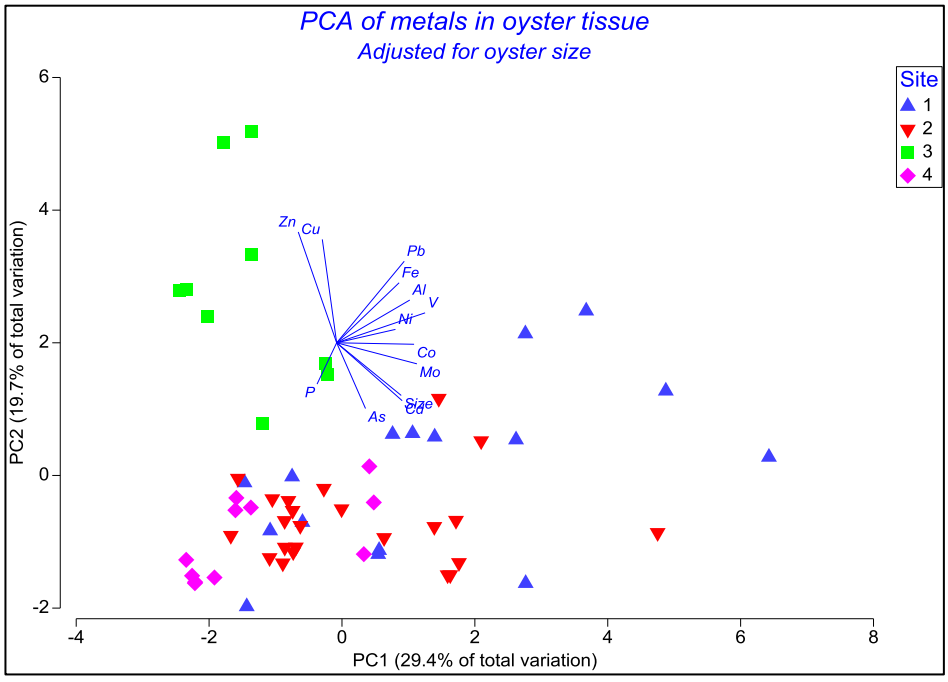

Figure 12: PCA ordination of metals in oysters – sites

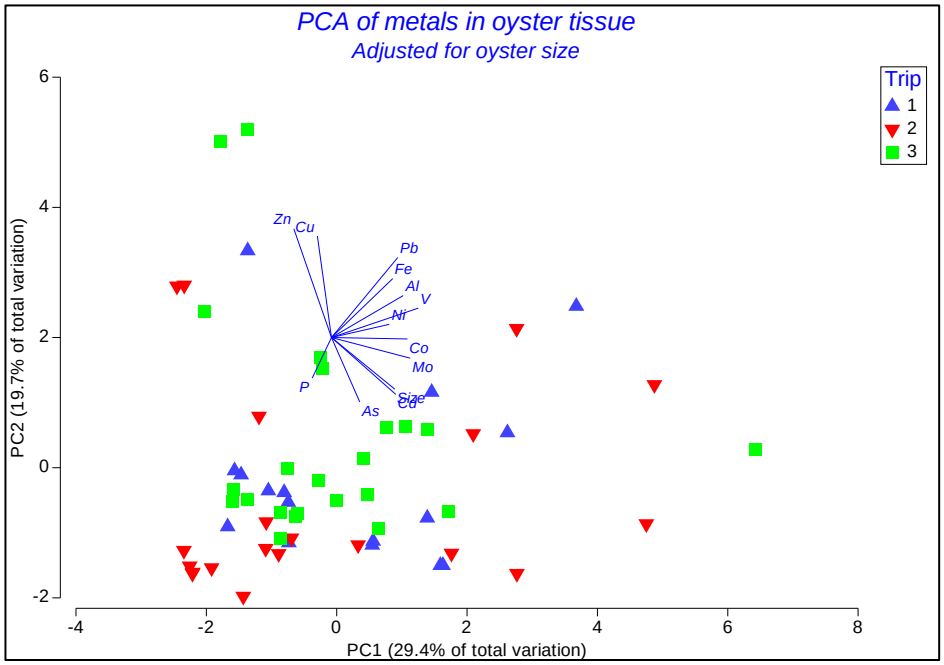

Figure 13: PCA ordination of metals in oysters – trips

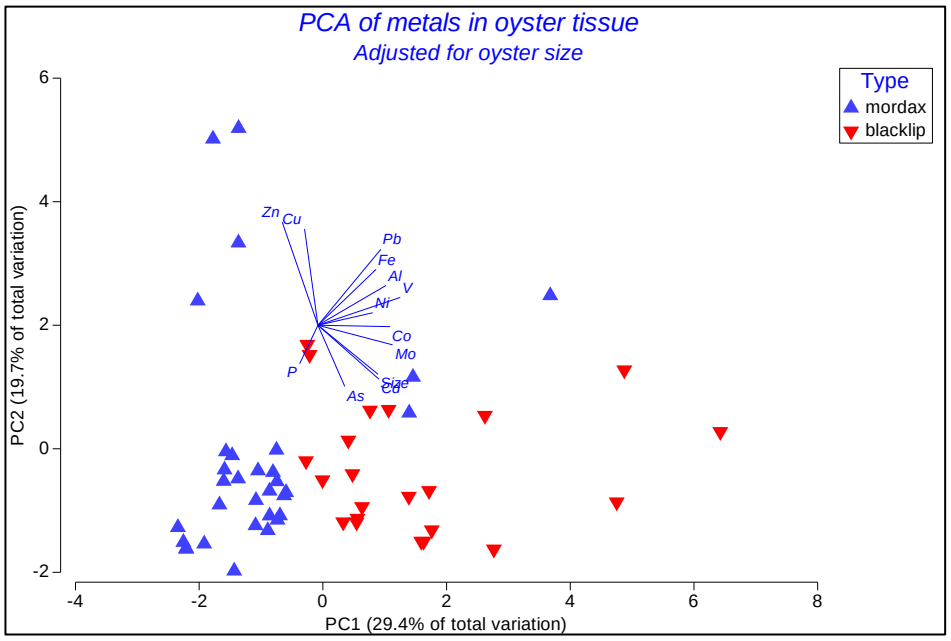

Figure 14: PCA ordination of metals in oysters – Species

Permanova analysis

A PERMANOVA analysis was performed to analyse whether the metal and oyster size profiles were different between samples of different types of oyster, sites and trips. A cross-design was chosen and all predictors (i.e. oyster type, sites and trips) were fixed factors.

Table 6 shows the groups between which the metal and size profiles of the oysters were different at P<0.05.

Table 6: Main comparisons with P<0.05 adjusted for sites, trips and oyster type

Groups	Pseudo-F (df)	P value (permutations)	Square root of estimate of component of variation
Sites*	6.6 (2)	0.001 (998)	2.2
Oyster type	10.5 (1)	0.001 (999)	1.8
Sites x type	2.3 (4)	0.023 (999)	1.3
Trips x type	4.2 (2)	0.001 (999)	1.6

* Pairwise comparisons adjusted for sites, trips and oyster type showed a significant difference of metals and oyster size between sites 2 vs 3 (t=3.38, P=0.001) and sites 2 vs 4 (t=3.43, P=0.001)

Table 7 shows pairwise comparisons which also include farmed vs wild in a cross design with sites, trips and oyster type.

Table 7: Pairwise comparisons with P<0.01, adjusted for sites, trips, oyster type as well as farmed

Groups	T statistic	P value (permutations)
Sites 1 vs 4	3.02	0.002 (998)
Sites 2 vs 4	3.47	0.001 (999)
Trips 1 vs 3	2.39	0.009 (999)
Farmed (blacklips)	3.29	0.001 (998)
Oyster types*	5.00	0.001 (999)

*not farmed

Distance linear model and dbRDA

The dbRDA (Figure 15) based on the metal profiles without oyster size is presented as an alternative approach where in this case the metal profiles were compared against oyster size, type, sites, trips and number of oysters collected. The dbRDA shows the combination of predictors which best explained the metal profiles (based on Akaike information criterion AIC and stepwise selection). Accounting for oyster types and farmed, oyster size no longer added any significant information to explain the metal profiles.

The first 2 axes of the dbRDA explained 35.2 % of the total variation in the metal profiles.

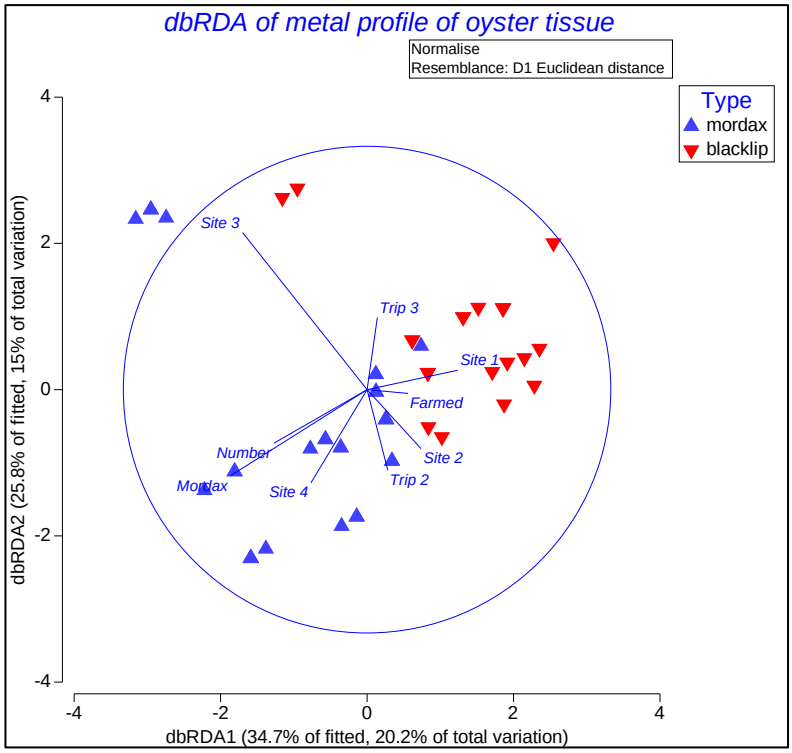

Figure 15: dbRDA of metals in oysters and predictors which best explained the metal profiles

Table 8 shows the marginal tests of variables which significantly explained parts of the metal profile variability. For instance, site 3 on its own explained 15.7% of the data variability while Mordax vs Blacklip explained 12.9% of the metal profile. Oyster size explained 9.9%.

Table 8: marginal tests of variables which significantly explained parts of the metal profile variability

Variable	Pseudo-F	P value	Proportion explained
Site 3	10.62	0.001	0.157
Mordax	8.47	0.001	0.129
Size	6.31	0.001	0.099
Site 1	5.96	0.001	0.094
Trip 2	5.40	0.001	0.086
Site 2	5.13	0.001	0.082
Site 4	4.25	0.004	0.069
Number	3.88	0.001	0.063
Trip 3	3.10	0.003	0.051
Farmed	2.73	0.022	0.045

Supporting statistics and diagnostics for the analyses above (pages 21-26) are given in Appendix 10.

Composition of the September 2013 phytoplankton bloom

During trip 3 conducted at the end of the dry season (September 2013) a phytoplankton bloom was observed in the waters off Goulburn Island (Figure 16). This bloom was sampled opportunistically near sites 1 and 2 and was found to be dominated by *Trichodesmium erythraeum* (Table 9), a filamentous cyanobacterium typically commonly present in nutrient poor tropical and subtropical ocean waters. *T. erythraeum* colonies are visible to the naked eye and sometimes form blooms, which can be extensive on surface waters. This large bloom presented as extensive surface scum (saw dust like appearance) extending some 500m offshore from sampled sites. *T. erythraeum* is common along the NT coastline from September to November as warmer waters stimulate its proliferation offshore (Smit pers. comm.). We do not know how long this bloom had persisted in the region prior to sampling. Although potentially problematic taxa (in terms of presence of toxins) such as *Nitzschia* spp, *Rhizosolenia setigera, Rhizosolenia striata* and Pseudo-nitzschia seriata group were present in many of the samples analysed, these species were a minor constituent and not present in any noteworthy bio-volume. Similarly, the Dinophyceae species detected are of unknown toxicity but the low concentrations recorded in the bloom suggest a low likelihood of toxicity.

Figure 16: MODIS Goulburn Island image for 0913 (Courtesy NASA and CDU Remote Sensing group) shows dark green to black plumes indicative of the phytoplankton bloom around the islands and extending north, a little to the east and far to the west.

Table 9. Species list of phytoplankton samples collected at four sites during trip 3 (September 2013) and percentage of cells per sample counted for taxa present.

	Genus species	**Site 1**	**Site 2**	**Site 3**	**Site 4**
Bacillariophyceae		**% of sample**			
	Bacillaria paxillifera	0.0	0.29	0.0	0.0
	Bellerochea sp. 003	0.0	0.0	0.0	0.09
	Coscinodiscus spp.	0.0	0.03	0.0	0.0
	Cocconeis spp.	0.09	0.0	0.0	0.0
	Cylindrotheca closterium	0.09	0.08	0.0	0.0
	Diatom 101	0.09	0.0	0.0	0.0
	Detonula sp. 002	0.0	0.0	1.54	0.0
	Navicula spp.	3.46	0.53	0.05	0.38
	Nitzschia spp.	0.33	0.03	0.16	0.05
	Pseudo-nitzschia "seriata group"	0	0.03	0.0	0.0
	Rhizosolenia setigera	0.09	0.05	0.0	0.09
	Rhizosolenia striata	0	0.03	0.0	0.05
	Thalassionema frauenfeldii	0.14	0.11	0.16	0.05
	Thalassionema nitzschioides	1.7	4.55	2.47	2.36
	Thalassiosira pseudonana	0.71	0.13	1.75	0.14
	Totals	**6.723**	**5.845**	**6.14**	**3.21**
Cyanobacteria					
	Beaded cyanobacteria filament	11.17	*5.71*	0.0	
	Trichodesmium erythraeum	81.82	88.44	93.86	96.79
	Totals	**92.992**	**94.115**	**93.86**	**96.79**
Dinophyceae					
	Ceratium fusus	0.05	0.0	0.0	0.0
	Dinophysis sp. 005	0.05	0.0	0.0	0.0
	Gymnodinium spp.	0.05	0.0	0.0	0.0
	Heterocapsa sp. 001	0.09	0.0	0.0	0.0
	Protoperidinium sp. 016	0.05	0.0	0.0	0.0
	Totals	**0.284**	**0.0**	**0.0**	**0.0**

	Potentially toxic species
	Potentially harmful (non-toxic) species

Chlorophyll a levels in seawater

Chlorophyll a levels in seawater (Table 10) collected during trip 2 were significantly less than those collected during trip 3 (Pseudo F=165.09; P=0.001), but were not significantly different (Appendix 12).

Table 10: Chlorophyll *a* levels in seawater at the four sampling sites during trips 2 (Wet season – 12-15 February 13) and 3 (Dry season - 9-10 September 13).

	Chl *a* ug/L	
Location	**Trip 2 (Feb-13)**	**Trip 3 (Sep-13)**
Site 1a	0.48	1.93
Site 1b	0.40	1.74
Site 1c	0.41	1.86
Site 2a	0.88	2.60
Site 2b	0.66	2.43
Site 2c	0.77	2.41
Site 3a	0.77	1.87
Site 3b	1.16	1.16
Site 3c	0.86	2.28
Site 4a	0.79	3.84
Site 4b	0.47	2.32
Site 4c	0.72	2.42

TSS levels in seawater

TSS levels in seawater for trip 3 (dry season- 9-10 September 13 during a phytoplankton bloom event) were on average 6X higher than for the previous two trips (Table 11). TSS levels were analysed by PERMANOVA and results indicated no significant difference between sites (Pseudo F=1.95; P=0.172) but a significant difference between trips (Pseudo F=259.31; P=0.001). TSS levels from trip 3 were significantly higher compared to the other two sampling times (Appendix 12).

Table 11: Seawater TSS levels for each sampling event. ND = no data.

	TSS mg/L		
Location	**Trip 1**	**Trip 2**	**Trip 3**
Site 1a	3.2	5.5	24.3
Site 1b	2.8	3.9	20.2
Site 1c	ND	4.8	20.6
Site 2a	7.4	6.5	28.2
Site 2b	4.6	4.5	29.7
Site 2c	ND	6.8	29.4
Site 3a	3.6	4.2	25.5
Site 3b	3.7	4.9	19.6
Site 3c	7.7	3.9	29.2
Site 4a	ND	5.8	28.0
Site 4b	ND	1.1	30.9
Site 4c	ND	1.8	29.3

Trace elements in TSS

Bioavailable element levels in TSS (Appendix 11) were analysed by PERMANOVA and results indicated no significant difference between sites (Pseudo F=1.18; P=0.355) but a significant difference between trips (Pseudo F=8.39; P=0.001). When these associations were analysed further by Permutational MANOVA, element levels in TSS from trip 3 (dry season- 9-10 September 13 during a phytoplankton bloom event) were significantly higher compared to samples collected during trips 1 and 2, however element levels in TSS samples collected during trips 1 and 2 were not significantly different (Appendix 12). The difference in TSS trace metal levels between trip 3 and the two other trips was evident in the CAP analysis (Figure 17). The trip 3 cluster with representatives from all sites was associated with elevated Cd and Zn. Elements with a Spearman $\geq$ 0.6 correlation included Al, As, Cu, Fe and Pb - all of which were lower in trip 3 TSS samples compared to the other trips.

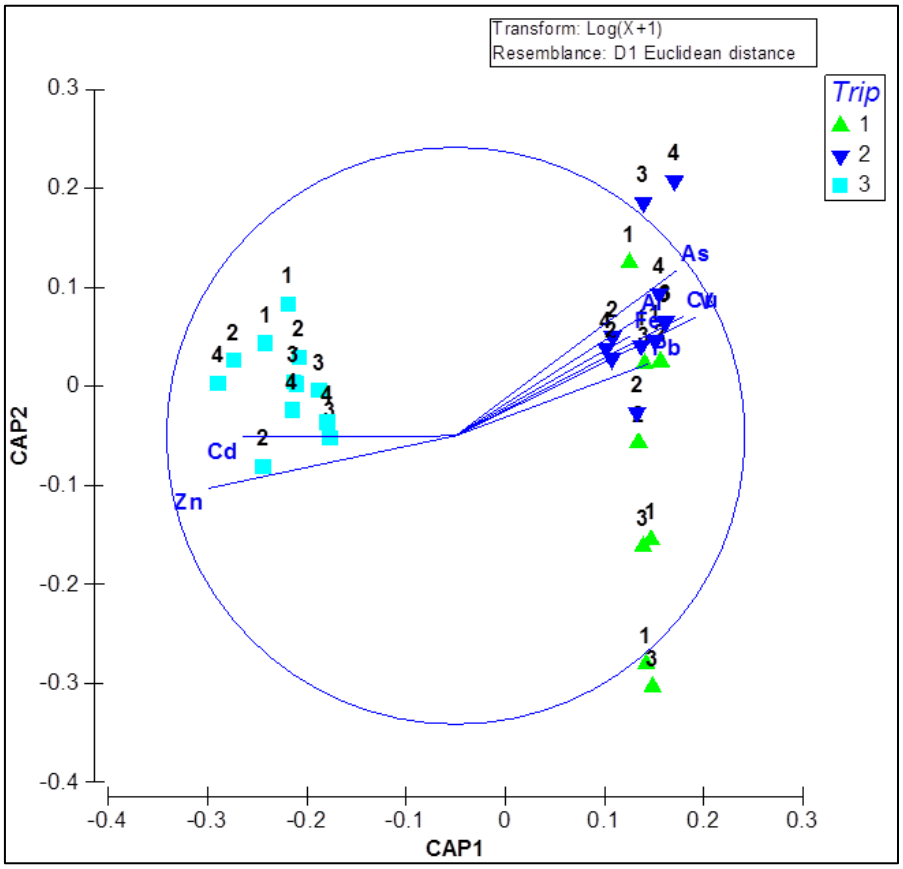

Figure 17: Canonical Analysis of Principal Coordinates (CAP) of trace elements in TSS at each sampling location, for each sampling time. Numbers above the symbols refer to the four sampling locations. Vector lengths indicate the strength of relationships with the indicated element. The circle simply orients the viewer.

Bioavailable trace element values (Appendix 11) showed that although TSS levels were low in trips 1 and 2, there was still sufficient material to detect metals and metalloids, such as Al, As, Cu, Fe and Pb.

Cd and Zn was below detectable levels for trips 1 and 2 (Appendix 11), however they were detectable in TSS for trip 3 (Table 5).

Cd and Zn levels in TSS and the bloom

Cd and Zn levels in TSS were analysed by PERMANOVA and results indicated no significant difference between sites (Pseudo F=0.78; P=0.608) but a significant difference between trips (Pseudo F=15.47; P=0.001). When these associations were analysed further by Permutational MANOVA, Cd and Zn levels from trip 3 (dry season- 9-10 September 13 during a phytoplankton bloom event) were significantly higher than the other two trips (Appendix 12). Bloom samples taken near sites 1 and 2 during trip 3 had detectable levels of bioavailable Cd but not Zn (Table 12). Other trace elements that were detectable in the bloom samples are shown in Appendix 11.

Table 12: Bioavailable concentrations of Zn and Cd in TSS and in the phytoplankton bloom sampled opportunistically during the bloom event trip 3. The bloom samples were scooped from the water near site 1 and site 2 where it was clearly observed floating on the water surface.

	Trip 3 TSS		Trip 3 Bloom	
	Bioavailable 1N HCl			
Location	Zn mg/kg	Cd mg/kg	Zn mg/kg	Cd mg/kg
Site 1a	15.8	0.2		
Site 1b	12.0	0.2	<	0.15
Site 1c	11.2	0.2		
Site 2a	12.2	0.2		
Site 2b	42.0	0.3	<	0.15
Site 2c	41.9	0.2		
Site 3a	14.8	0.1		
Site 3b	7.9	0.2		
Site 3c	11.0	0.1		
Site 4a	23.5	0.1		
Site 4b	7.6	0.1		
Site 4c	13.0	0.2		

The local Indigenous aquaculture team

The engagement of the aquaculture team during the course of the project was challenging at times, but improved over time. This work was carried out during a period when a number of tensions existed within the community, exacerbated by some non-Indigenous personnel were vocal in calling for local people to be better paid for Fisheries work. Support and leadership from the local governance body - Yagbani Aboriginal Corporation - was limited as they were going through an uncertain time regarding funding and security of the manager's position. This created some mistrust of Fisheries staff by some of the local aquaculture team. Negotiation and formalisation of payment for hours worked improved trust and participation. This method of payment is now routine across all aquaculture projects - where the team is paid through the local employment scheme for routine work done between visits by Fisheries staff, and they are paid by the hour, through research project funds, for support in the field when staff are on site. Such arrangements are critical in establishing trust and a productive working arrangement between communities and external facilitators.

Despite payments being introduced, the aquaculture team could not be routinely relied upon to be present for work when requested, conduct support activities prior to or following on from field trips nor conduct research work independently. Clearly further work was needed to

improve regular communication, trust building and mentoring between the local aquaculture team and Fisheries staff. A strategy to address these ongoing issues during the project involved creating a dedicated staff position within the Aquaculture Unit as liaison officer between the community, Yagbani, aquaculture support teams and Fisheries. A very capable person filled this position, but she left soon after and the position was cut during a budget review.

Discussion

Introductory comments

The first objective in this study was to survey spatial and temporal variability of trace (heavy) metals in tropical oysters (blacklip and milky) in the West Arnhem region. The second objective was to assess the implications of results on the development strategy of the oyster enterprise and the sale of tropical oysters into the Australian seafood market. We have addressed both of these objectives in the discussion below. The third objective, which was to employ Indigenous partners to assist in conducting the shellfish monitoring is addressed in both the final section of the discussion and in the subsequent section on further developments arising from this project.

Seawater trace element levels in the oyster harvest areas

Seawater from the four sampling sites had trace element levels that were well below ANZECC guidelines.

Trace elements in oyster tissue - overview

Despite the low levels of heavy metals in the seawater, there was evidence that both oyster species were accumulating trace elements from their environment. Possible sources are discussed below. Copper (Cu) levels in tissue samples of both oyster species did not exceed FSANZ maximum levels (MLs) at any sites or sampling times. In contrast, at certain sites and sampling times, the levels of cadmium (Cd) and zinc (Zn) exceeded FSANZ MLs for one or both oyster species. Oyster species differed in their heavy metal signature, even at the same site, indicating they bioaccumulate heavy metal elements differently. In particular, there seems to be a clear difference between the two species in their response to Zn and Cu. Milky oysters bioaccumulated higher levels of Zn and Cu at site 3 (above the ANZFS MLs) compared to blacklip oysters at this site. Levels of heavy metals in both oyster species differed markedly between different sites. Variation of heavy metal signatures between wet and dry sampling times were associated with higher nickel (Ni) and P content during the wet season. Accounting for oyster types and farmed, oyster size did not add any significant information to explain the metal profiles. If metals were analysed separately, more Cu and As were found in bigger oysters after accounting for oyster type, farmed status, sites and trips.

The ANZFS ML for arsenic (As) reports inorganic As levels only. The percentage of inorganic As present in mollusc tissue is generally <10% with the remainder present as organo-arsenic compounds, which are considered non-toxic to humans. Since the total As concentrations of oysters sampled in this study exceeded the inorganic As MLs by up to a factor of 3.5, the inorganic As would have to constitute approximately 29% of total As in order for the ML for inorganic As to be exceeded. Such a high proportion of inorganic As is considered unlikely but further As speciation analysis is needed to confirm this. Based on this, it appears likely the levels of As in oysters sampled were below the ANZFS ML.

Cd levels in both oyster species exceeded ANZFS MLs and differences occurred between sample sites for both oyster species. We also found differences in uptake between oyster species. Since the Cd exceedence may potentially impact on the commercial sale of oysters for human consumption from Goulburn Island, the remainder of this discussion focuses on this and associated elements (Zn, Cu).

Trace elements in milky oysters

The trace element signature in milky oyster tissue was different between all sites except 1 and 4. The milky oysters from site 2 had a distinctive trace element signature that was driven by elevated levels of cadmium (Cd). In fact at this site 2, Cd levels exceeded the Australian Food Standards ML (FSANZ, 2012). There was no Cd exceedence at the other sites except for one sample at site 1. At site 3 near the town centre where there was no Cd exceedence, Zn and Cu levels were elevated in milky oyster tissue. Our analysis showed that Cd and Zn levels in milky oysters were significantly negatively correlated.

Trace elements in wild harvest blacklip oysters

The trace element signatures in blacklip did not show the distinct site clustering that was evident for milky oysters. Cd levels in wild harvest blacklip exceeded the ANZFS ML at sites 1, 2 and 4 for each of the three trips (wild blacklip of a suitable size were not present at site 3). The blacklip oyster association with Zn was not as strong as that for milky oysters, which may account for the difference in Cd exceedences. There was no significant difference in Zn levels in blacklip oysters between sites 1 and 2 however zinc levels at site 4 were higher and although these differences were significant between sites 1 and 4, it was not sufficient to affect the Cd exceedence.

Trace elements in farmed blacklip oysters

The farmed blacklip were placed at sites 1, 2 and 3 in September 2012 and tested after five months (Feb 2013) and 12 months (Sept 2013). Unlike wild harvest blacklip, Cd levels in farmed blacklip did not exceed the ANZFS ML at site 1. Similar to wild harvest blacklip and milky oysters, they exceeded at site 2 but did not exceed at site 3 near the town where Zn and Cu levels were elevated in the farmed oyster tissue. The Cd exceedences were small and in fact the differences between sites and trips were not significant. This could be a reflection of the short time of deployment. In contrast, Zn levels were significantly different between sites but not between trips. This suggests that sufficient time had elapsed for the farmed oysters to accumulate Zn.

The bloom and associated elevated TSS as a source of Cd and Zn?

Trips 1 and 3 coincided with end of the dry season (September 2012 and 2013) and Trip 2 was in the wet season (February 2013). On trip 3 there was a phytoplankton bloom, which was sampled opportunistically near sites 1 and 2. Total suspended solids (TSS) in the water column were higher for trip 3 than for trips 1 and 2. The bloom event is observed in the region every year between September and October (Smit, pers. comm.) so presumably oysters are exposed to this annually. When bioavailable trace metal levels were measured for the TSS, trip 3 TSS samples were different from the other two trips and differentiated by detectable levels of bioavailable Zn and Cd. Bioavailable Zn and Cd levels for trips 1 and 2 may have been below detectable levels (30 and 1.5 mg/kg for Zn and Cd respectively) because the yield of suspended solids was low. For trip 3 ranges of 7-42 mg/kg Zn and 0.12-0.25 mg/kg Cd were recorded so one interpretation is that Zn and Cd levels were elevated as a concentration in sediment in trip 3. However a more likely interpretation is that because TSS levels were higher there would most likely have been more Zn and Cd available per volume of water. Either way the oysters had access to more bioavailable Zn and Cd during trip 3.

For trips 1 and 2, the TSS were clustered and associated with Al, As, Cu, Fe and Pb, all of which were lower in trip 3 TSS samples compared to the other trips. So although TSS levels were low in trips 1 and 2, there was still sufficient material to analyse and detect metals and metalloids such as Al, As, Cu, Fe and Pb.

So, while bioavailable Cd and Zn were below detectable levels for trips 1 and 2 they were detectable in TSS and in the bloom samples for trip 3. It is possible therefore that oysters accumulate this bioavailable Cd and Zn from TSS and the bloom annually. However since

there was no significant difference in Cd and Zn levels in TSS between sites, there must be some other explanation for the Cd exceedence by milky oysters at site 2.

Although we do not know the age of wild harvest oysters, we know that the farmed oysters were deployed in September 2012 before that year's bloom event. This means that when they were sampled five months later in February 2013 they had already been exposed to the bloom. Ideally if we could sample farmed oysters that had not been exposed to a bloom, and then again some time after a bloom event we could determine if those sampled before the bloom event were bio-accumulating Cd and Zn from some other source. As it currently stands we can only speculate that TSS and the bloom are a source of Cd and Zn.

There was no evidence that trace element signatures in oysters between trips was related to the bloom event in trip 3 and this may simply reflect the fact that oysters exposed to annual 'doses' of TSS/bloom Cd and Zn do not clear these metals from their system. Alternatively there may be multiple sources not related to the bloom. For example, in milky oysters there was a significant difference in the trace element signature between trips 1 and 2, and between trips 2 and 3, but not between trips 1 and 3. For blacklip there was no difference between trips 1 and 3, or between 2 and 3, but there was a difference between trips 1 and 2. In fact the difference in oyster trace element signatures between trips appeared to be more influenced by season. For both species there was a separation between the wet season trip 2 and the dry season trips 1 and 3. For both species the cluster at trips 1 and 3 was associated with elevated nickel (Ni), and the trip 2 cluster was weakly associated with elevated phosphorous (P). Although oysters can accumulate Ni (Zaroogian & Johnson, 1984) there are no MLs for Ni and P and therefore this result has little bearing on food safety.

Cd-Zn-Cu bioaccumulation and the Zn-Cd antagonism in oysters and other bivalves

Wild milky oysters had fewer Cd exceedences than wild blacklip and had a stronger capacity to bioaccumulate Zn at site 3 near the town. We cannot rule out a genetic basis for this difference, however there may be multiple mechanisms of accumulation in operation because farmed blacklip showed the same patterns of Cd exceedence as milky oysters. This could however simply reflect the fact that the farmed blacklip were deployed in the field for a shorter time. Filter feeders such as oysters have been shown to bio-accumulate essential or non-essential heavy metals. This bioaccumulation occurs via uptake from dissolved phase (water column) and particulate phase (food). As trace elements, some heavy metals such as Cu and Zn are essential to maintain the metabolism of aquatic animals. However at higher concentrations they can become toxic. Other heavy metals, non-essential e.g. Cd can be toxic at very low levels. In the natural environment, Zn concentrations in water are generally higher than those of Cd. Oysters have been shown to have a high potential for cadmium bioaccumulation (Frazier, 1979). As a non-essential metal, Cd may be accumulated without excretion or with some excretion, however no regulation process has been found (Daka, 2005). As an essential-metal, Zn can be regulated and accumulated with or without excretion (Rainbow, 2002). Generally, bioaccumulated concentrations of Cd are lower than Zn concentrations and we found this to be the case in this study.

In this study we did not measure sediment element levels because, although sediments play an important role for metal storage in marine environments, no significant correlations have been observed between total concentrations of metals in superficial sediments (easily resuspended in the water column) and concentrations in oysters (normally exposed to sediment-bound metal). This is probably due to the non-bioavailability of the metals captured in sediment (Chong & Wang, 200). Hédouin *et al.* (2010) have also found a low bioavailability of sediment-bound metals. On the contrary, a strong correlation exists between dissolved metal concentration and bioaccumulated concentration, which is a good argument that the dissolved fraction of a metal is the most bioavailable for filter-feeders (Amiart *et al.,* 2007). Regarding Cd uptake, the most common route seems to occur from the dissolved phase (Lim *et al.*, 1998). To complicate the search for sources of bioaccumulated metals, Blackmore and Wang (2004) found that metal uptake in oysters was not directly proportional to metal concentrations in the water and the uptake of Zn was higher than that of Cd.

It has been shown that Zn and Cd have high chemical similarities and tend to bind with proteins (Wang and Fisher, 1999). As a result of their chemical affinities, Cd and Zn may share similar uptake pathways into organisms and use the same carriers for their transport (Rainbow, 1997). Different scenarios have been observed in metal interaction. Amiard-Triquet and Amiard (1998) have observed that in many mollusc species, exposure to Cd had no effect on Zn accumulation, whereas exposure to Zn had an antagonistic effect on Cd accumulation.

The source of Zn and Cd in the natural environment is not well understood. Shi and Wang (2004) have studied Cd and Zn bioaccumulation in different populations of marine clams (*Mactra veneriformis* and *Ruditapes philippinarum*) with different Cd contaminations levels. They observed that for both species, the population with a higher Cd tissue concentration accumulated Cd and Zn more efficiently from the dietary phase. While this provides evidence that diet has a role to play, in our study there were too many variables because it was a natural setting. We could not find published work on TSS and natural blooms as a source of Cd and Zn.

To add complexity, the influence of trace metal exposure on metal accumulation may be explained by the induction of specific metal-blinding ligands like metallotheioneins (Wang and Rainbow, 2005). There may also be multiple mechanisms that operate at different stages of the growth cycle of shellfish. For example, studying bioaccumulation in populations of *Littorina saxatilis*, Daka (2009) measured an increase in Cd accumulation with increasing Zn concentration in the tissue showing a synergistic relationship at low concentrations. However this relationship reverses at the highest Zn concentration, showing an antagonistic effect of Zn on Cd accumulation. On the same species, Daka and Hawkins (2006) also found that in interactions between Cd and Zn. Zn accumulation was higher in a mixed solution of Zn and Cd than from a solution of Zn alone.

A full literature review on this topic is provided in Appendix 13.

Engagement of the local Indigenous aquaculture team - lessons learnt

The project was conducted in partnership with the local Indigenous aquaculture team on Goulburn Island. This team consists of about 10 men (and at the time of writing 2 women) who are working towards gaining a vocational training certificate in aquaculture (VETII), through the CDU's Vocational Education and Training School. The indigenous team supported Fisheries and CDU staff in conducting the sampling program in the field. The technical work was integrated into the training program so that students understood the reason for the survey, its practical application in the future oyster enterprise and to reinforced skill development in survey methods, and handling and processing samples. Men were paid an hourly wage for work done, through this research project.

Results showed that better strategies were needed to improve regular communication, trust building and mentoring between the local aquaculture team and Fisheries staff.

The lessons learnt in this project and subsequent work (investigating broader quality assurance needs) has provided further insight and detail into the support processes needed to build Indigenous science capacity. Support and mentoring needs to gradually build understanding, familiarity, confidence and skills in local support teams. The process followed to date involves:

During this project:

1) one-on-one training by CDU trainers in scientific techniques with appropriate background theory and explanation followed by -

2) application of recently learnt skills in field work supported by Fisheries staff,

Subsequent to this project:

3) gradually transitioning to a period where the Indigenous trainees work independently of external agents, but are supported locally by an Aquaculture Coordinator employed by the Aboriginal Corporation (Yagbani). Samples are now collected according to the protocol provided, and sent to Fisheries staff for processing in testing laboratories. Fisheries staff report back that samples meet the standards as set out in the protocols and, if needed, offer additional advice.

This transition to independent research activities without external support is a key step in achieving the community's aspirations to be in control of their own affairs and to take pride in supporting external scientists. In this way the community is a full participant in co-developing fisheries opportunities that allow communities to operate and work in local fisheries-based businesses.

The following table presents the key aspects used to date to build Indigenous capacity to independently conduct field-based science on Goulburn Island. Future work will seek to identify effective processes and strategies to achieve these elements of local ownership and responsibility.

AIM for Indigenous participants: To develop skills and gradually transfer sense of ownership and responsibility for local science work to Indigenous support teams on communities. **AIM for external support agents:** To build trusting relationships, mutual respect (i.e. for opinion and advice) and effective communication processes to ensure a culturally appropriate and effective learning environment for Indigenous trainees.				
OUTCOME: Indigenous fisheries teams have the skills, confidence, pride, responsibility, work ethic, and mutual respect for effectively developing fisheries-based opportunities on community. **OUTCOME:** Support agents demonstrate a respect for culture, Indigenous people in the way they support trainees.				
Elements to achieving local science capacity	**Community Roles**		**External Roles**	
	Indigenous Aquaculture Teams	**Yagbani/Land Councils**	**NT Fisheries staff**	**CDU VET training providers**
Develop capacity	Commitment to study and attend lessons.	Provide fisheries coordinator to support learning and study activities	Support for reinforcing learning new skills in the field	Local training that is tailored to skill needs and provides quality training programs.
Trust building	Commitment to agreed field trip plans. Showing up when agreed to. Understanding repercussions for non-shows. Understanding fairness and mutual respect of arrangements.	Provide leadership and support for trainees. Reinforce messages about commitment, fairness, mutual respect and responsibility.	Follow through on commitments made. Ensure open and constant communication. Explain repercussions for not turning up - not selected next time. Explain fairness and mutual respect for all parties in these arrangements. Listen to and respect local opinions and advice (both local trainees and local coordinators).	Follow through on commitments made. Ensure open and consistent communication. Explain repercussions for not turning up. Explain fairness and mutual respect for all parties in these arrangements.
Transferring a sense of ownership of local science activities	Understand the key role local teams need to take in achieving successful fisheries programs	Fisheries coordinator key in acting as a conduit for transfer of ownership.	Make ownership of research apparent via communication and continued but gradual transfer of responsibilities.	Support and encourage local ownership and individual's aspirations.

Build sense of responsibility	Understand the key roles local teams need to take in achieving successful fisheries programs	Fisheries coordinator key in acting as a conduit for building and supporting responsibility.	Provide opportunities to test local team's capacity to self-manage activities. Collectively workshop the event and identify learnings for next time.	Support and encourage responsibility. Acting as role models for local trainees.
Showing cultural respect	Understanding and acknowledging cultural differences; two-way communication.	Providing advice to Fisheries where appropriate	Follow cultural ways within reason (i.e. working with male and female teams separately)	Follow cultural ways within reason (i.e. working with male and female teams separately)
Building pride and work ethic	Taking pride in commitment to their personal development.	Encouraging and supporting the personal development of local team members. Acting as a role model.	Encouraging and supporting the personal development of local team members. Payments for services provided in the field.	Encouraging and supporting the personal development of local team members. Acting as a role model.

Conclusion & Implications

Our analysis of trace metals in tropical oysters (blacklip and milky) in the West Arnhem region has shown that wild harvest blacklip accumulate Cd levels that exceed the food safety standards at all sites collected. Milky oysters only exceeded at site 2 as did farmed blacklip deployed for up to 12 months. These exceedences occurred at each of the three occasions tested over two years.

The implications of these results for a development strategy of an oyster enterprise and the sale of tropical oysters into the Australian seafood market are:

1. Of the four sites used for wild harvest and farm oyster deployment, site 2 should be avoided.
2. Only milky oysters should be wild harvested except near the town site (3) where either species can be harvested.
3. Farm blacklip can be deployed and grown-out for 12 months at any site except site 2. Additional studies are needed to test accumulation of heavy metals over the average growout period of 18 months for blacklip oysters to reach market size (about 10-15 cm).
4. It should be noted however that there is still an element of risk for Cd exceedence. Even milky oysters came close to the MLs at sites other than 2 (one exceedence at sampling site 1b) and there is a possibility that a site that is recorded as being below the ML for some years, could then be subject to some unknown influence which then causes the ML to be exceeded thereafter. Ultimately all the sites were positioned in close proximity to each other, and we don't know why oysters differed in the metal accumulation between these sites.
5. In some cases the FSANZ makes exceptions for seafood types shown to be naturally high in some metals. It is recommended that NT Fisheries discuss with FSANZ the possibility of an exemption to the Cd standard.

Our third objective was to employ Indigenous partners to conduct the shellfish monitoring outlined in this project to develop Indigenous capacity in fisheries sciences and an additional employment stream for Indigenous people.

The CDEP Aquaculture Team supported fieldwork as part of their employment program and Vet II training and in so doing gained skills in oyster management and water quality assessment. Importantly the work reported here on developing effective processes for communication and relationship building provided a solid foundation to gradually build upon in future programs. Indeed, since this project, substantial gains have been made in mapping out a framework that captures the key elements of transferring local ownership and responsibility for scientific field work, as reported in the Discussion. This knowledge base will continue to be developed to improve Fisheries staffs' working relationship with Indigenous partners.

Importantly, the work reported here has provided more insight into the Cd exceedence and as a result we have been able to suggest options to continue this enterprise. This is particularly helpful since the initial results for Cd suggested no clear path forward. As a result of our work we now know that avoiding some sites and relying on farmed blacklip will significantly reduce the risk of exceedence. While this gives a cautious green light to this indigenous enterprise it should be noted however that there is still an element of risk for Cd exceedence. While some sites seem better than other, they are still geographically close and site characteristics could change. Ultimately a formal enterprise will be coupled with a Quality Assurance program so any change would be identified in ongoing monitoring.

While we reported that total arsenic levels were such that the inorganic component to which the guidelines relate are not likely to have been exceeded, we stress that further As speciation analysis would be needed to confirm this.

This work has also raised awareness that this oyster enterprise will involve not just the sale of the final product, but water quality testing and the development of an NT Shellfish Quality Assurance Program (NTSQAP). Our work showing a phytoplankton bloom comprising the potentially toxic cyanobacteria *Trichodesmium erythraeum* also adds urgency to the need for toxin testing as part of an NTSQAP.

In year 2 of the project the trip 1 and 2 data were used as part of a body of evidence that underpinned a successful bid for funding to the North Australia Marine Research Alliance (NAMRA) for $60,000 to fund a project called: Further Developing Indigenous Capacity-Water Quality Testing for Shellfish QA Program. This program is building on the initial community based enterprise development to develop Indigenous capacity in fisheries sciences and an additional employment stream for Indigenous people. In December 2014 the Indigenous aquaculture team and the Aquaculture Unit of NT Fisheries began this more extensive water quality monitoring survey (and associated sanitary survey) to establish a 1-year database of potential contaminants (indicator bacteria, algal toxins, etc.) in oysters and surrounding waters. This survey, collection and analysis protocols were developed under the guidance of QA experts and will be used to develop an NTQAP for oyster monitoring in the Goulburn Island region.

There are also several opportunities to employ Indigenous partners to conduct further research. One obvious area of research centres on the implications of the bloom event as a source of Cd and Zn for oysters. This would develop research skills and would provide a short-term additional employment steam for Indigenous people.

Recommendations and Further Developments

Management recommendations to avoid Cd exceedence are relatively straightforward and have been given above. It is recommended that NT Fisheries discuss with FSANZ the possibility of an exemption to the Cd standard.

The unknown issue is the role of the annual algal bloom and the associated role of an increase of TSS in Cd accumulation and toxin levels in oysters flesh. Both of these aspects should be the subject of further focussed research using farmed blacklip and involving the Darwin Aquaculture Centre.

While the mechanism of Cd accumulation and the interactions between Cd and Zn would be an interesting follow-up study, at this stage not knowing underlying mechanisms does not prevent a management solution. However if the opportunity arose to study this further, possibly in tank trials at the DAC and *in situ*, we'd propose to study the role of molecules such as metallothionein (MTs), ie low molecular weigh proteins that have the ability to bind heavy metals (such as Cd, Cu and Zn). They can be induced by metals and are involved in uptake, transport and regulation of heavy metals (Roesijadi, 1994). Also, the capacity of MTs to bind metals is suspected to provide protection against metal toxicity (Roesijadi, 1996). The relative activity of such proteins and their genes in milky oysters vs blacklip following metal challenges would be an interesting study – as would a transcriptomic study to measure changes at the whole genome level.

Extension and Adoption

In year two of this study we collaborated with Dr Linda Ford, The Northern Institute, Charles Darwin University on a project that will support extension and adoption of our results. This project funded by the FRDC, is called *Warruwi Fisheries and Aquaculture Knowledge Partnerships Project (2013-2014)*. Through this project Dr Ford (Project Leader), Dr Fleming and Professor Gibb are developing a knowledge partnership with the Warruwi Traditional Owner Authorities, Yagbani Aboriginal Corporation and Elders to prepare fisheries and aquaculture communication and education materials that draws on their knowledge systems, languages, cultural practices, heritage, beliefs, past fisheries experiences and values. These materials will be used to facilitate understanding, communication and engagement in sea farming enterprises by Warruwi community members.

Materials will consist of:

- Life cycles of sea farmed animals
- Sea farming seasonal calendar and maps
- 'Good eating' cues seasonal calendar.

This last 'Good eating' cues seasonal calendar links to this FRDC project in the sense that we seek to bring together 'Good eating' and 'Safe eating' by linking the outputs of Dr Ford's project with the outputs from this FRDC project. The way we seek to do this is illustrated in Figure 15a&b and it is essentially a blending of both Traditional and Science Knowledge.

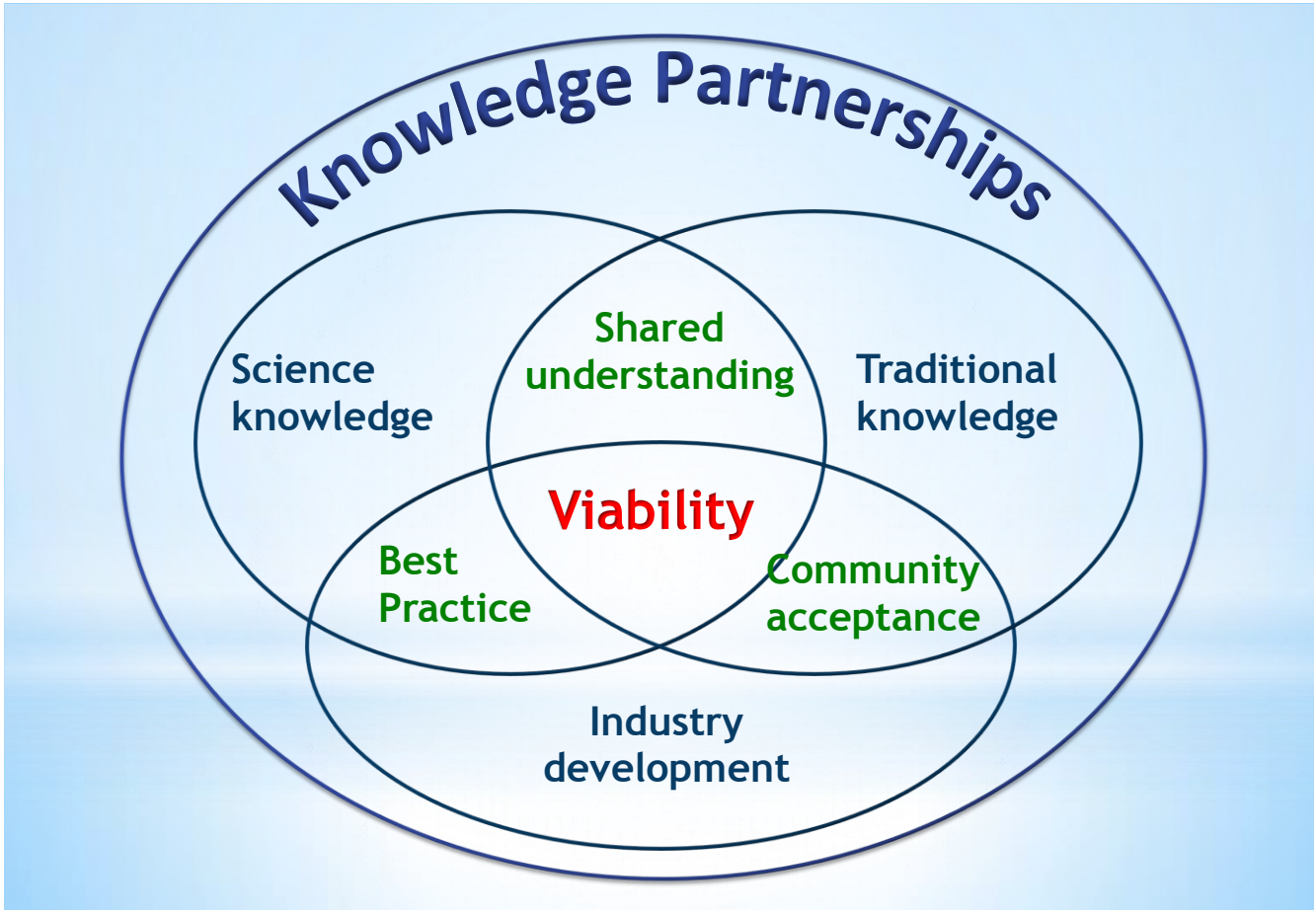

Figure 15a: Key elements to a *Knowledge Partnership* as developed in the 'Good eating' project.

Figure 15b: How these key elements specifically relate to the oyster-trace element study.

As part of this parallel project Dr Ford is preparing a website that will include outcomes from this FRDC project through the *Good eating = Safe Eating* calendar. Materials produced for this website will acknowledge the FRDC and we will seek prior approval. Further outcomes from this FRDC project will be communicated via the website and through DAC staff involved in the North Australian Marine Research Alliance project: *Further Developing Indigenous Capacity- Water Quality Testing for Shellfish QA Program*.

Publication

In addition to the website mentioned above, we are preparing a scientific paper from these data and will lodge an accepted version of the manuscript with the FRDC.

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Includes references for Appendix 13: Literature review on metal bioaccumulation in marine invertebrates

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Appendices

Appendix 1: Trip 1 collection details (Dry season - 17-20 September 12)

Site description	Number	Sample	Milky # indiv. collected	Blacklip # indiv. collected	Latitude S	Longitude E
Site 1						
Rock spur near little mangroves	1a	Oysters	4	4	11 40 24.61	133 23 11.21
Rocks leading to island Island	1b	Water	Yes	Yes	11 40 22.90	133 23 14.63
		Oysters	13	6		
	1c	Oysters	0	0	11 40 29.58	133 23 17.74
Site 2						
Yagbani, inside Wigu	2a	Oysters	13	7	11 38 36.74	133 25 05.95
Rock spur	2b	Water	0	0	11 38 46.19	133 25 15.75
		Oysters	9	8		
First rock island outcrop on flat	2c	Oysters	0	0	11 38 44.86	133 25 21.36
Site 3						
Rocky shore in front of council accommodation	3a	Oysters	8	0	11 38 55.87	133 23 29.60
In front of police station	3b	Water	Yes	Yes	11 39 00.43	133 23 30.33
Boat ramp behind school	3c	Water	Yes	Yes	11 38 43.67	133 23 26.65

Appendix 2: Trip 2 collection details (Wet season – 12-15 February 13)

Site description	Number	Sample	Milky# indiv.	Blacklip # indiv.	Latitude S	Longitude E
Site 1						
Rock spur near little mangroves	1a	Water	Yes	Yes	11 40 28.6	133 23 08.5
		Oysters	9	4		
Rocks leading to island	1b	Water	Yes	Yes	11 40 29.98	133 23 17.73
		Oysters	5	2		
	1c	Water	Yes	Yes	11 40 27.5	133 23 19.6
		Oysters	9	3		
Farmed blacklip - floating basket system		Oysters	-	8		
Site 2						
Yagbani, inside Wigu	2a	Water	Yes	Yes	11 38 38.3	133 25 05.7
		Oysters	9	3		
Rock spur	2b	Water	Yes	Yes	11 38 51	133 25 33
		Oysters	10	1		
Farmed blacklip - floating basket system		Oysters	-	8		
First rock island outcrop on flat	2c	Water	Yes	Yes	11 38 45.1	133 25 13.4
			9	0		
Site 3						
Rocky sore in front of council accommodation	3a	Water	Yes	Yes	11 38 55.2	133 23 29.4
		Oysters	10	No		
In front of police station	3b	Water	Yes	Yes	11 38 59.7	133 23 30.2
		Oysters	9	No		
Boat ramp behind school	3c	Water	Yes	Yes	11 38 44.24	133 23 27.03
		Oysters	9	No		
Farmed blacklip - floating basket system		Oysters	-	11		
Site 4						
NIGPIN	4A	Water	Yes	Yes	11 35 36.3	133 27 46.2
		Oysters	9	0		
Angalamuwarn	4B	Water	Yes	Yes	11 35 32.3	133 27 47.6
		Oysters	9	0		
Angalamuwarn east	4C	Water	Yes	Yes	11 35 28.8	133 27 59.3
		Oysters	10	2		

Appendix 3: Trip 3 collection details (Dry season- 9-10 September 13)

Site description	Number	Sample	Milky # indiv.	Blacklip # indiv.	Latitude S	Longitude E
Site 1						
Rock spur near little mangroves	1a	Water	Yes	Yes	11 40 28.6	133 23 08.5
		Oysters	9	No		
Rocks leading to island	1b	Water	Yes	Yes	11 40 29.98	133 23 17.73
		Oysters	5	2		
Rocks leading to island	1c	Water	Yes	Yes	11 40 27.5	133 23 19.6
		Oysters	9	No		
Farmed blacklip - floating basket system		Oysters	-	11		
Farmed blacklip - rack system attached to substrate		Oysters	-	11		
Site 2						
Yagbani, inside Wigu	2a	Water	Yes	Yes	11 38 38.3	133 25 05.7
		Oysters	9	3		
Rock spur	2b	Water	Yes	Yes	11 38 51	133 25 33
		Oysters	10	3		
First rock island outcrop on flat	2c	Water	Yes	Yes	11 38 45.1	133 25 13.4
		Oysters	9	3		
Farmed blacklip - floating basket system		Oysters	-	12		
Site 3						
Rocky shore in front of council accommodation	3a	Water	Yes	Yes	11 38 55.2	133 23 29.4
		Oysters	10	No		
In front of police station	3b	Water	Yes	Yes	11 38 59.7	133 23 30.2
		Oysters	9	No		
Boat ramp behind school	3c	Water	Yes	Yes	11 38 44.24	133 23 27.03
		Oysters	9	No		
Farmed blacklip - floating basket system		Oysters	-	11		
Farmed blacklip - rack system attached to substrate		Oysters	-	10		
Site 4						
Nigpin	4a	Water	Yes	Yes	11 35 36.3	133 27 46.2
		Oysters	9	3		
Angalamuwarn	4b	Water	Yes	Yes	11 35 32.3	133 27 47.6
		Oysters	9	3		
Angalamuwarn east	4c	Water	Yes	Yes	11 35 28.8	133 27 59.3
		Oysters	10	No		

Appendix 4: Trace element data for seawater at all sites for trip 1 (Dry season - 17-20 September 12)

Environmental Chemistry and Microbiology Unit

ICPMS analysis of elemental composition of Goulburn Island sea water samples trip 1

Sample name	Al µg/L	P µg/L	V µg/L	Fe µg/L	Co µg/L	Ni µg/L	Cu µg/L	Zn µg/L	As µg/L	Mo µg/L	Cd µg/L	Pb µg/L
F GI Site 1a	2.03	<	2.03	<	<	<	<	<	1.42	10.9	<	<
F GI Site 1b	1.88	<	1.96	<	<	<	<	<	1.54	10.8	<	<
F GI Site 2a	2.37	<	2.05	<	<	<	<	0.50	1.47	10.9	<	<
F GI Site 2b	1.77	<	2.13	<	<	<	<	<	1.59	11.0	<	<
F GI Site 3a	2.65	<	2.08	<	0.03	<	<	<	1.51	11.2	<	<
F GI Site 3b	2.83	<	2.22	<	<	<	<	0.74	1.55	11.1	<	<
F GI Site 3c	1.92	<	2.06	<	0.03	0.20	<	<	1.56	11.2	<	<
UF GI Site 1a	25.6	<	1.97	35.9	0.03	<	<	<	1.45	10.6	<	<
UF GI Site 1b	24.7	<	2.08	29.0	<	<	<	<	1.56	10.8	<	<
UF GI Site 2a	96.7	<	2.47	135	0.0	0.2	<	<	1.66	10.1	<	0.05
UF GI Site 2b	50.7	<	2.32	71.4	0.04	0.20	<	<	1.76	10.9	<	0.03
UF GI Site 3a	102	<	2.45	95.6	0.04	0.22	0.24	<	1.66	10.4	<	0.06
UF GI Site 3b	49.2	<	2.35	64.0	0.04	0.23	<	<	1.56	10.9	<	0.03
UF GI Site 3c	64.0	<	2.30	88.3	0.04	0.21	<	0.43	1.60	10.8	<	0.04
Reporting limit	0.30	3.00	0.40	2.90	0.03	0.20	0.20	0.30	0.30	1.20	0.03	0.02
CASS-5	0.51	2.36	1.32	<	0.08	0.32	0.37	0.76	1.49	9.83	<	<
CASS-5 certified	nc	nc	1.32	1.44	0.10	0.33	0.38	0.72	1.24	9.80	0.02	0.01
QUASI 158	1.53	<	3.00	<	0.41	1.76	5.08	14.4	4.09	11.0	0.76	0.89
QUASI 158 certified	nc	nc	nc	2.81	0.40	1.85	5.03	13.7	3.72	nc	0.72	0.95
Alc ref	65.7	<	34.5	<	<	0.20	0.31	0.53	3.49	12.5	<	<
Alc ref CDU average	63.3	nc	34.0	nc	0.01	0.21	0.34	0.63	3.43	12.5	0.013	nc
MRM Ref	23.5	3.17	22.9	20.7	4.17	20.2	20.2	20.3	22.4	15.1	3.89	3.80
MRM Ref CDU average	23.0	nc	22.5	20.8	4.24	20.3	20.5	20.3	21.3	15.3	3.94	3.92
Detection limit	0.30	3.00	0.40	2.90	0.03	0.20	0.20	0.30	0.30	1.20	0.03	0.02

<: less than detection limit; nc: not certified; na: not analysed

Notes: ICPMS analyses used an octapole reaction system to limit matrix interferences; small interference errors estimated at 0.1-1 µg/L for V, Cr, Fe, Co, Ni, Cu, Zn, As and Se may remain uncorrected

Appendix 5: Trace element data for seawater at all sites for trip 2 (Wet season – 12-15 February 13)

Environmental Chemistry and Microbiology Unit

ICPMS analysis of elemental composition of Goulburn Island sea water samples trip 2

Sample name	Al µg/L	P µg/L	V µg/L	Fe µg/L	Co µg/L	Ni µg/L	Cu µg/L	Zn µg/L	As µg/L	Mo µg/L	Cd µg/L	Pb µg/L
F GI Site 1a	1.59	<	1.96	<	0.02	<	<	<	1.40	10.7	<	<
F GI Site 1b	1.31	<	1.97	<	<	<	<	0.31	1.52	10.5	<	<
F GI Site 1c	1.73	<	2.04	<	<	<	<	0.34	1.60	10.7	<	<
F GI Site 2a	1.21	<	2.17	<	0.02	0.20	<	<	1.75	11.1	<	<
F GI Site 2b	1.15	<	2.08	<	0.02	<	<	<	1.74	10.9	<	<
F GI Site 2c	1.18	<	2.16	<	0.02	<	<	<	1.60	10.6	<	<
F GI Site 3a	1.83	<	2.10	<	0.03	0.20	<	<	1.60	10.7	<	<
F GI Site 3b	2.35	<	1.79	<	0.02	<	0.20	0.34	1.51	10.4	<	<
F GI Site 3c	1.50	<	2.21	<	<	<	<	<	1.76	11.1	<	<
F GI Site 4a	2.28	<	1.93	<	0.02	<	<	<	1.63	10.9	<	<
F GI Site 4b	1.63	<	1.94	<	<	<	<	<	1.70	11.0	<	<
F GI Site 4c	2.45	<	2.04	<	0.02	<	<	<	1.69	11.1	<	<
UF GI Site 1a	14.1	<	2.10	26.3	0.02	<	<	<	1.73	10.8	<	<
UF GI Site 1b	12.6	<	2.11	24.1	0.03	0.20	<	<	1.61	10.6	<	<
UF GI Site 1c	13.6	<	2.07	17.0	0.03	<	<	<	1.62	10.8	<	<
UF GI Site 2a	21.2	<	2.28	50.5	0.03	0.22	<	<	1.82	11.2	<	0.02
UF GI Site 2b	10.0	<	2.20	18.6	0.02	0.20	<	0.51	1.83	11.0	<	<
UF GI Site 2c	43.2	<	2.46	99.5	0.03	0.24	<	<	1.81	10.5	<	0.05
UF GI Site 3a	25.5	<	2.34	36.5	0.04	0.20	0.21	<	1.72	10.8	<	<
UF GI Site 3b	26.6	<	1.94	58.2	0.04	<	0.23	<	1.53	10.4	<	0.04
UF GI Site 3c	20.3	<	2.42	22.2	0.04	0.23	0.22	<	1.84	11.1	<	0.02
UF GI Site 4a	11.9	<	2.05	15.7	0.02	<	<	<	1.70	10.9	<	<
UF GI Site 4b	5.96	<	2.19	7.89	0.02	<	<	<	1.80	11.0	<	<
UF GI Site 4c	7.15	<	2.17	6.81	0.03	<	<	<	1.72	11.1	<	<
Reporting limit	0.40	31.0	0.30	2.00	0.02	0.20	0.20	0.30	0.30	1.30	0.03	0.02
CASS-5	0.54	<	1.48	<	0.09	0.36	0.39	0.81	1.45	10.1	<	<
CASS-5 certified		nc	1.32	1.44	0.10	0.33	0.38	0.72	1.24	9.80	0.02	0.01
QUASI 158	1.29	<	3.03	4.01	0.40	1.67	4.87	13.9	4.06	11.4	0.70	0.92
QUASI 158 certified		nc		2.81	0.40	1.85	5.03	13.7	3.72		0.72	0.95
Alc ref	58.8	<	31.6	<	<	0.25	0.33	0.61	3.50	13.1	<	0.02
Alc ref CDU average	63.0	nc	33.9	0.58	0.01	0.22	0.33	0.59	3.41	12.7	0.01	0.02
MRM Ref	21.2	<	20.3	20.1	3.96	18.5	19.0	18.8	21.5	15.5	3.69	3.77
MRM Ref CDU average	22.8	nc	22.3	20.7	4.22	20.2	20.4	19.5	21.6	15.4	3.91	3.93
Reporting limit	0.40	31.0	0.30	2.00	0.02	0.20	0.20	0.30	0.30	1.30	0.03	0.02

<: less than detection limit; nc: not certified; na: not analysed

Notes: ICPMS analyses used an octapole reaction system to limit matrix interferences; small interference errors estimated at 0.1-1 µg/L for V, Cr, Fe, Co, Ni, Cu, Zn, As and Se may remain uncorrected

Appendix 6: Trace element data for seawater at all sites for trip 3 (Dry season - 9-10 September 13). Values highlighted in yellow exceed FSANZ food standards.

Environmental Chemistry and Microbiology Unit

ICPMS analysis of elemental composition of Goulburn Island sea water samples trip 3

Sample Name	Al µg/L	P µg/L	V µg/L	Fe µg/L	Co µg/L	Ni µg/L	Cu µg/L	Zn µg/L	As µg/L	Mo µg/L	Cd µg/L	Pb µg/L
F GI Site 1a	0.949	<	1.94	<	0.035	<	<	0.29	1.46	10.8	<	<
F GI Site 1b	0.976	<	1.87	<	0.020	0.22	<	0.26	1.41	10.4	<	<
F GI Site 1c	1.415	<	1.89	<	0.015	<	<	0.24	1.40	10.7	<	<
F GI Site 2a	1.026	<	1.96	<	0.020	0.21	<	<	1.60	10.7	<	<
F GI Site 2b	<	<	1.98	<	0.025	0.21	<	0.32	1.42	10.6	0.022	<
F GI Site 2c	1.235	<	1.94	<	0.026	0.21	<	1.66	1.53	10.6	<	<
F GI Site 3a	1.402	<	1.87	<	0.028	0.23	<	0.27	1.37	10.5	<	<
F GI Site 3b	1.326	<	1.99	<	0.028	<	<	0.35	1.27	10.4	<	<
F GI Site 3c	1.235	<	1.97	<	0.019	0.22	<	0.21	1.23	10.4	0.026	<
F GI Site 4a	<	<	2.01	<	0.017	0.22	<	2.43	1.54	10.6	<	<
F GI Site 4b	<	<	1.96	<	0.019	0.20	<	<	1.48	10.7	<	<
F GI Site 4c	<	<	2.12	<	0.009	0.20	<	<	1.45	10.9	0.022	<
							<					
UF GI Site 1a	113	<	2.20	125	0.046	0.28	0.21	0.30	1.48	10.4	0.020	0.050
UF GI Site 1b	98.9	<	2.12	107	0.046	0.24	0.22	0.29	1.61	10.4	0.027	0.037
UF GI Site 1c	129	<	2.04	150	0.050	0.33	<	0.29	1.53	10.1	0.027	0.064
UF GI Site 2a	172	<	2.18	199	0.058	0.37	<	0.36	1.51	10.2	<	0.073
UF GI Site 2b	160	<	2.20	186	0.064	0.28	0.24	0.56	1.50	10.5	0.023	0.075
UF GI Site 2c	141	<	2.12	158	0.045	0.28	0.22	0.31	1.55	10.4	<	0.064
UF GI Site 3a	132	15.3	2.04	158	0.055	0.27	0.21	0.29	1.49	10.1	0.021	0.057
UF GI Site 3b	147	<	2.19	163	0.049	0.32	0.20	0.26	1.62	10.2	<	0.053
UF GI Site 3c	210	<	2.25	235	0.059	0.31	0.21	0.46	1.49	10.1	0.025	0.085
UF GI Site 4a	143	<	2.09	147	0.057	0.27	<	0.35	1.56	10.4	<	0.057
UF GI Site 4b	179	<	2.13	183	0.064	0.27	<	<	1.63	10.4	<	0.074
UF GI Site 4c	145	<	2.11	147	0.051	0.28	<	0.29	1.74	10.4	<	0.058
Reporting Limit	0.80	15.0	0.750	1.50	0.001	0.20	0.20	0.20	0.20	0.30	0.020	0.030
CASS-5	<	<	1.42	<	0.086	0.34	0.39	0.80	1.53	9.87	0.023	0.033
CASS-5 Certified	nc	nc	1.32	1.44	0.095*	0.330	0.380	0.719	1.24	9.8	0.0215	0.011
Q158	1.11	<	2.83	1.79	0.37	1.58	4.94	13.0	4.01	11.10	0.72	0.92
Quas 158 Certified	nc	nc	2.89	2.81	0.40	1.85	5.03	13.7	3.72	nc	0.72	0.95
SW12 Ref Av	60.0	<	33.2	<	0.011	0.24	0.34	0.48	3.45	12.6	<	<
SW12 Ref CDU Long Term Value	62.6	5.95	33.9	1.01	0.012	0.22	0.33	0.63	3.40	12.3	0.014	0.020
ECMU SW Ref Av	20.1	15.8	21.0	18.6	3.95	18.7	18.7	17.3	20.1	14.3	3.81	3.69
ECMU SW Ref CDU Long Term Val	20.7	nv	20.7	19.3	4.09	18.8	18.8	19.2	19.8	14.4	3.80	3.72
Reporting Limit	0.80	15.0	0.750	1.50	0.001	0.20	0.20	0.20	0.20	0.30	0.020	0.030

* information value only

<: less than detection limit; nc: not certified; na: not analysed

Notes: ICPMS analyses used an octapole reaction system to limit matrix interferences; small interference errors estimated at 0.1-1 µg/L for V, Cr, Fe, Co, Ni, Cu, Zn, As and Se may remain uncorrected

Appendix 7: Trace element data for oyster tissue at all sites for trip 1 (Dry season - 17-20 September 12). Values highlighted in yellow exceed FSANZ food standards. Those highlighted green are high compare to other sites. Note: Mordax refers to milky oysters.

ICPMS analysis of elemental composition of Goulburn Island oyster soft tissue trip 1

Microwave digestion in HNO3

Concentrations in wet weight

Sample:	Oyster size (cm)	Al	P	V	Fe	Co	Ni	Cu	Zn	As	Mo	Cd	Pb
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Site 1a mordax	4.0, 4.5, 3.0, 4.0	16.1	823	0.15	42.5	0.02	0.15	13.5	17.3	4.10	0.08	1.50	0.01
Site 1a blacklip	8.5, 11.0, 12.0	40.8	756	0.38	93.7	0.09	0.30	18.8	28.6	5.77	0.13	3.37	0.03
Site 1b mordax	5.0, 4.0, 5.0, 5.0	30.6	988	0.96	242	0.03	0.17	19.1	19.4	6.59	0.12	2.17	0.03
Site 1b blacklip dup 1	10.0, 9.0, 7.5, 10.0	11.9	722	0.14	22.9	0.07	0.20	16.0	27.8	7.16	0.20	3.23	0.02
Site 1b blacklip dup 2		10.4	719	0.14	22.9	0.07	0.21	16.1	27.8	7.21	0.21	3.24	0.02
Site 2a mordax	4.0, 3.5, 3.0, 4.0	10.0	663	0.12	36.1	0.06	0.07	8.54	11.0	2.22	0.08	2.23	0.01
Site 2a Blacklip	9.0, 13.0, 12.0, 7.5	23.5	922	0.16	40.1	0.09	0.15	13.4	23.5	5.49	0.26	3.76	0.03
Site 2b mordax	3.0, 4.0, 4.5, 5.0	22.0	841	0.13	38.5	0.04	0.06	4.37	11.0	2.54	0.21	2.88	0.02
Site 2b mordax 1	5.5	55.8	843	0.21	80.5	0.09	0.10	5.26	9.99	2.39	0.19	2.55	0.04
Site 2b mordax 2	3.5	5.91	678	0.15	42.2	0.06	0.09	8.59	8.69	3.87	0.08	4.25	0.02
Site 2b mordax 3	3.0	8.35	582	0.14	29.0	0.09	0.11	9.38	7.99	2.89	0.07	3.63	0.01
Site 2b mordax 4	4.0	1.88	555	0.06	14.7	0.05	0.07	8.13	6.56	2.90	0.09	4.00	0.01
Site 2b mordax 5	3.0	8.94	1321	0.10	36.6	0.07	0.09	9.25	11.3	4.70	0.10	5.10	0.02
Site 2b blacklip dup 1	10.0, 10.0, 11.5, 12.(	12.9	1046	0.15	33.7	0.10	0.16	14.4	16.2	6.11	0.25	5.88	0.03
Site 2b blacklip dup 2		12.7	1048	0.15	32.9	0.10	0.16	14.1	16.0	6.06	0.24	5.87	0.03
Site 3a mordax	5.5, 4.5, 4.5, 3.5	12.3	700	0.14	47.5	0.05	0.11	34.3	201	3.05	0.07	1.08	0.04
Reporting limti		0.03	0.60	0.00	0.070	0.00	0.00	0.01	0.04	0.01	0.00	0.00	0.00
Digest blank av.		0.03	-0.44	0.00	0.097	0.00	0.00	0.00	-0.02	0.01	0.00	0.00	0.00
Oystef Reference 1566b av.		83.6	6280	0.51	181	0.31	0.91	71.9	1328	7.33	0.18	2.29	0.28
certified value		197	nc	0.58	206	0.37	1.04	71.6	1424	7.65	nc	2.48	0.31
ANZFA MPC food standard		nv	nv	nv	nv	nv	nv	nv	nv	1.000 inorganic	nv	2.000	2.000

Notes:

<: less than detection limit, nc: not certified; nv: no value

Data accurate to 2-3 digits only

Al is not quantitatively extracted in HNO3 digestion

Appendix 8: Trace element data for oyster tissue at all sites for trip 2 (Wet season – 12-15 February 13). Values highlighted in yellow exceed FSANZ food standards. Those highlighted green are high compare to other sites. Note: Mordax refers to milky oysters.

ICPMS analysis of elemental composition of Goulburn Island oyster soft tissue trip 2
Microwave digestion in HNO3
Concentrations in wet weight

Sample name	Oyster size cm	Al mg/kg	P mg/kg	V mg/kg	Fe mg/kg	Co mg/kg	Ni mg/kg	Cu mg/kg	Zn mg/kg	As mg/kg	Mo mg/kg	Cd mg/kg	Pb mg/kg
Site 1a Blacklip	6.0, 8.0, 8.0, 10.0	51.2	786	0.646	111	0.131	0.156	18.0	15.0	3.58	0.583	4.87	0.038
Site 1a mordax	4.5, 5.0, 6.0, 6.0, 6.0, 6.0, 6.0	24.2	1630	0.218	43.7	0.037	0.070	11.5	22.5	4.52	0.178	1.18	<
Site 1 farmed	3.5, 3.5, 4.0, 4.0, 4.0, 4.0, 4.0, 5.0	73.5	1190	0.493	112	0.068	0.098	10.7	21.6	2.70	0.360	1.95	0.037
Site 1c Blacklip	10.0, 13.0, 14.0	9.86	1670	0.491	30.6	0.096	0.105	20.9	20.9	6.14	0.505	5.67	0.025
Site 1c mordax	4.0, 4.5, 5.0, 5.5, 5.5, 5.5, 6.0	9.91	2190	0.199	23.7	0.028	0.039	12.1	31.1	7.48	0.240	1.19	<
Site 2a blacklip	7.0, 8.0, 9.0	12.9	1670	0.331	34.3	0.091	0.049	12.6	17.9	4.31	0.539	5.41	0.024
Site 2a mordax	3.0, 3.5, 4.0, 4.0, 4.0, 5.5, 6.0,	5.63	1480	0.201	25.3	0.076	0.022	7.40	11.2	3.55	0.271	3.22	0.015
Site 2 farmed	4.0, 4.0, 4.0, 4.0, 4.0, 4.5, 4.5, 4.5	51.2	3080	0.472	93.1	0.083	0.066	8.55	17.6	3.72	0.378	2.56	0.037
Site 2b blacklip	12	18.7	1300	0.699	41.5	0.136	0.120	21.5	6.89	5.27	0.686	6.56	0.036
Site 2b mordax	3.0, 4.0, 4.0, 4.0, 4.0, 4.5, 4.5, 6.0	10.1	1850	0.240	29.4	0.049	0.010	5.63	5.15	4.41	0.126	3.59	0.017
Site 2c mordax	3.0, 3.5, 3.5, 4.0, 4.0, 4.0, 4.0	3.44	1660	0.184	20.6	0.072	0.014	8.40	12.1	3.85	0.226	3.32	0.013
Site 3 north	3.0, 3.5, 3.5, 4.0, 4.0, 4.0, 4.0	10.2	1350	0.145	34.3	0.052	0.032	34.9	222	2.78	0.054	0.711	0.022
Site 3 south	2.5, 3.5, 4.0, 4.0, 4.0	8.59	1400	0.140	32.3	0.060	0.035	33.7	248	3.14	0.062	1.05	0.024
Site 3 farmed	2.5, 2.5, 3.0, 3.0, 3.0, 3.0, 3.0, 3.0, 3.0, 3.5	16.4	1420	0.181	42.4	0.066	0.068	12.1	55.5	3.11	0.071	1.40	0.028
Site 4A mordax	6.0, 6.0, 6.0, 6.0, 6.0 6.5, 7.0, 7.5	7.06	2500	0.124	23.0	0.029	0.073	16.1	30.2	5.65	0.062	0.897	<
Site 4b mordax 1	5.0, 5.0, 5.0, 6.0, 6.0, 6.0, 6.0, 6.0,	6.11	2150	0.127	23.2	0.030	0.065	12.7	17.5	5.78	0.065	0.945	<
Site 4b mordax 2	4.0, 5.0, 5.0, 6.0, 6.5, 7.5, 7.5	5.27	1850	0.123	21.1	0.027	0.058	13.1	25.2	6.21	0.059	0.947	<
Site 4c blacklip	7.0, 14.0	8.88	868	0.191	34.3	0.109	0.147	11.5	19.7	3.80	0.097	4.26	0.013
Site 4c mordax 1	4.5, 5.0, 5.5, 6.0, 6.0, 7.5, 7.5, 7.5	11.1	2200	0.161	31.9	0.033	0.071	10.7	22.3	5.66	0.058	0.836	<
Site 4c mordax 2	3.0, 3.5, 4.0, 5.0, 5.0, 5.0, 6.0, 6.0	6.66	2150	0.134	27.1	0.035	0.063	13.6	19.2	5.11	0.054	0.879	<
Reporting limit		0.011	15.5	0.013	0.052	0.002	0.003	0.005	0.60	0.034	0.037	0.009	0.012
Blank digest average		<	<	<	<	<	<	<	<	<	<	<	<
Oyster ref 1566b average		70.4	9110	<	192	0.321	0.753	69.8	1340	7.49	0.164	2.24	0.273
Oyster ref 1566b certified		197	nc	0.577	206	0.371	1.04	71.6	1424	7.65	nc	2.48	0.308
Reporting limit		0.011	15.5	0.013	0.052	0.002	0.003	0.005	0.60	0.034	0.037	0.009	0.012
ANZFA MPC food standard		nv	nv	nv	nv	nv	nv	nv	nv	1.000 inorganic	nv	2.000	2.000

Appendix 9: Trace element data for oyster tissue at all sites for trip 3 (Dry season - 9-10 September 13). Values highlighted in yellow exceed FSANZ food standards. Those highlighted green are high compare to other sites. Note: Mordax refers to milky oysters.

ICPMS analysis of elemental composition of Goulburn Island oyster soft tissue trip 3
Microwave digestion in HNO3
Concentrations in wet weight

Sample Name	oyster size cm	Al mg/kg	P mg/kg	V mg/kg	Fe mg/kg	Co mg/kg	Ni mg/kg	Cu mg/kg	Zn mg/kg	As mg/kg	Mo mg/kg	Cd mg/kg	Pb mg/kg
Site 1a mordax	4.0, 5.0, 5.0, 5.5, 5.5, 5.5, 5.	24.6	800	0.229	47.2	0.031	0.202	14.6	27.3	4.93	0.072	1.26	0.009
Site 1b blacklip	6.0, 8.0	44.4	655	0.453	61.9	0.420	1.04	10.0	9.28	6.13	0.292	3.44	0.029
Site 1b mordax	3.0, 4.0, 6.0, 6.0, 6.0	52.5	916	0.451	99.2	0.033	0.228	13.5	15.8	6.56	0.100	1.73	0.017
Site 1c mordax	5.0, 5.0, 5.0, 5.0, 5.0, 6.0, 6.	21.0	743	0.247	36.7	0.029	0.170	12.3	15.8	6.11	0.098	1.80	0.009
Site 1 farmed floating blacklip	3.0, 3.0, 4.0, 4.5, 4.5, 5.0, 5.	41.5	860	0.362	52.0	0.059	0.232	10.6	20.6	4.32	0.218	1.98	0.026
Site 1 farmed base blacklip	3.5, 4.0, 4.0, 4.0, 4.0, 4.0, 4.	41.4	862	0.329	54.0	0.066	0.209	9.00	18.6	4.41	0.126	1.96	0.027
Site 2a blacklip	8.0, 8.0, 13.0	14.6	777	0.225	28.4	0.089	0.199	11.7	8.63	4.22	0.084	4.40	0.021
Site 2a mordax	4.0, 4.0, 4.0, 4.0, 4.0, 4.0, 4.	8.33	732	0.172	31.0	0.056	0.100	8.95	10.9	4.31	0.081	3.48	0.017
Site 2b blacklip	9.0, 9.0, 10.0	27.0	774	0.255	38.6	0.081	0.177	15.2	13.0	5.70	0.094	6.14	0.033
Site 2b mordax	3.0, 3.0, 4.0, 4.0, 4.0, 4.0, 4.	3.78	811	0.168	22.0	0.060	0.121	7.53	8.74	4.40	0.083	4.42	0.016
Site 2c blacklip	5.0, 7.0, 9.0	11.2	668	0.246	25.9	0.071	0.188	7.87	10.2	4.07	0.106	2.70	0.023
Site 2c mordax	3.5, 4.0, 4.0, 4.0, 4.0, 4.0, 4.	7.80	726	0.191	28.1	0.073	0.121	8.99	13.0	4.37	0.086	4.00	0.017
Site 2 farmed blacklip	4.0, 4.0, 4.0, 4.0, 4.5, 4.5, 4.	12.5	844	0.183	26.9	0.067	0.163	9.35	18.2	4.34	0.150	2.54	0.028
Site 3a mordax	3.0, 3.0, 3.5, 4.0, 4.0, 4.0, 4.	9.95	764	0.178	37.7	0.050	0.166	57.6	309	3.52	0.078	0.982	0.037
Site 3b mordax	2.5, 2.5, 2.5, 3.0, 3.0, 3.5, 3.	12.9	722	0.205	60.7	0.061	0.161	51.5	294	2.97	0.072	0.910	0.040
Site 3c mordax	2.5, 2.5, 3.0, 3.0, 3.0, 3.0, 3.	5.15	765	0.173	30.1	0.054	0.160	24.7	263	3.80	0.093	1.26	0.019
Site 3 farmed floating blacklip	3.5, 3.5, 3.5, 4.0, 4.0, 4.0, 4.	22.1	904	0.234	46.8	0.049	0.177	16.8	82.8	3.91	0.082	1.43	0.040
Site 3 farmed base blacklip	3.5, 3.5, 3.5, 4.0, 4.0, 4.0, 4.	29.4	891	0.273	47.6	0.051	0.183	15.7	72.6	3.65	0.084	1.36	0.032
Site 4a blacklip	9.0, 10.0, 10.0	14.7	662	0.188	36.5	0.073	0.269	13.5	28.7	4.80	0.098	2.80	0.024
Site 4A mordax	2.0, 3.0, 3.0, 4.0, 4.0, 4.0, 4.	14.9	1044	0.140	29.8	0.029	0.222	11.5	25.0	4.34	0.093	1.07	0.007
Site 4b blacklip	8.0, 9.0, 9.0	20.8	686	0.196	38.8	0.083	0.269	14.8	42.5	3.73	0.095	2.83	0.022
Site 4b mordax	4.0, 4.0, 4.0, 4.5, 4.5, 4.5, 4.	10.5	849	0.147	26.9	0.042	0.261	11.1	23.2	4.45	0.089	1.22	0.007
Site 4c mordax	3.0, 3.0, 3.0, 3.0, 3.0, 3.0, 3.	11.4	1040	0.142	25.9	0.027	0.243	13.1	36.3	5.55	0.084	1.22	0.007
Reporting limit		0.037	15.0	0.030	0.090	0.000	0.013	0.008	0.045	0.018	0.002	0.002	0.001
Blank average Goulburn Island		<	<	<	<	<	<	<	<	<	<	<	<
Oyster Ref 1566b average		77.6	6774	0.563	191	0.325	1.03	70.0	1384	8.16	0.175	2.33	0.285
Oyster Ref 1566b certified		197	nc	0.577	205.8	0.371	1.04	71.6	1424	7.65	nc	2.48	0.308
Reporting limit		0.037	15.0	0.030	0.090	0.000	0.013	0.008	0.045	0.018	0.002	0.002	0.001
Reporting limit		0.0037	1.50	0.0030	0.01	0.0000	0.0013	0.0008	0.0045	0.0018	0.0002	0.0002	0.0001
ANZFA MPC food standard		nv	nv	nv	nv	nv	nv	nv	nv	0.10 inorganic	nv	0.20	0.20

Appendix 10: Additional statistics for oysters

Comparison of size (natural log transformed) between trips, sites, farmed status and type of oysters

```
. regress lnsize i.trip farmed type i.site

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  7,    44) =   35.15
       Model |  7.22697172     7  1.03242453           Prob > F      =  0.0000
    Residual |  1.29244262    44  .029373696           R-squared     =  0.8483
-------------+------------------------------           Adj R-squared =  0.8242
       Total |  8.51941434    51   .16704734           Root MSE      =  .17139

------------------------------------------------------------------------------
      lnsize |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        trip |
          2  |   .1254504   .0691367     1.81   0.076    -.0138855    .2647863
          3  |  -.0592616   .0649604    -0.91   0.367    -.1901806    .0716574
             |
      farmed |  -.6792926   .0982013    -6.92   0.000    -.8772043   -.4813808
        type |   .7675395   .0550105    13.95   0.000     .6566731    .8784058
             |
        site |
          2  |  -.1161951   .0601069    -1.93   0.060    -.2373325    .0049423
          3  |  -.1776997    .088676    -2.00   0.051    -.3564144    .0010151
          4  |   .0103487   .0735226     0.14   0.889    -.1378264    .1585238
             |
       _cons |   .7402922   .0958769     7.72   0.000      .547065    .9335193
------------------------------------------------------------------------------
```

Multivariable regressions with outcome metals and predictors site, type of oyster, trip, farmed and size.

<u>In depth analysis of Cd levels</u>

The association between Cd levels and predictors*

```
. regress lncd i.site i.farmed i.type size if sample~="Site 2c blacklip"

      Source |       SS       df       MS              Number of obs =      51
-------------+------------------------------           F(  6,    44) =   45.39
       Model |  17.2076037     6  2.86793396           Prob > F      =  0.0000
    Residual |  2.78010651    44  .063184239           R-squared     =  0.8609
-------------+------------------------------           Adj R-squared =  0.8419
       Total |  19.9877102    50  .399754205           Root MSE      =  .25136

------------------------------------------------------------------------------
        lncd |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        site |
          2  |   .5962768   .0895571     6.66   0.000     .4157864    .7767672
          3  |  -.4515325   .1311088    -3.44   0.001    -.7157649   -.1873001
          4  |  -.3880804   .1036554    -3.74   0.001    -.5969841   -.1791766
             |
    1.farmed |   -.940871   .2253944    -4.17   0.000    -1.395124   -.4866185
      2.type |   1.068722    .197078     5.42   0.000      .671537    1.465906
        size |  -.0559798   .0342694    -1.63   0.109    -.1250453    .0130857
       _cons |   .7616463   .1754214     4.34   0.000     .4081076    1.115185
------------------------------------------------------------------------------
```

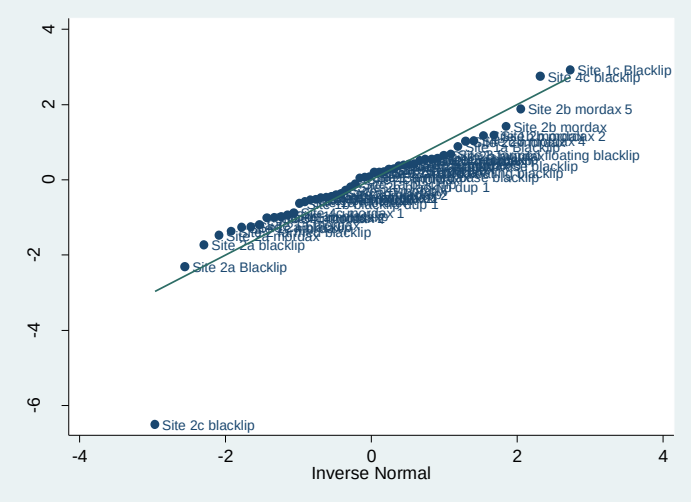
*trip was not included as P>0.8 and the inclusion did not change the coefficients of the other predictors

Diagnostics

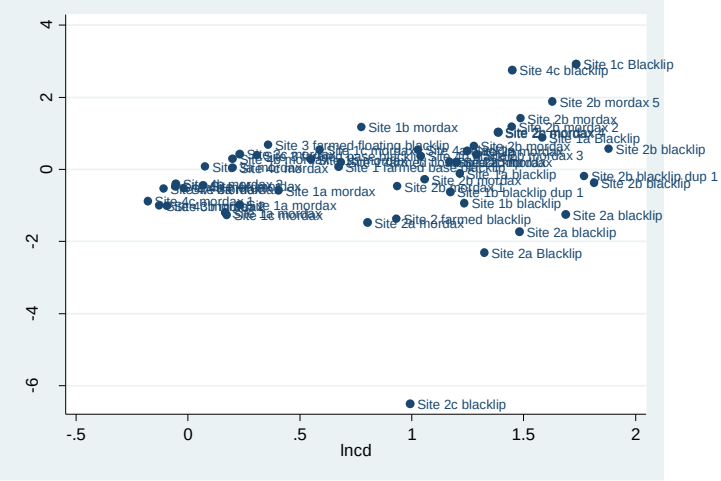
Breusch-Pagan test for heteroskedasticity - still weak evidence with a P=0.03; however, largely improved after exclusion of outlier (Site 2c blacklip)

```
. estat hettest

Breusch-Pagan / Cook-Weisberg test for heteroskedasticity
         Ho: Constant variance
         Variables: fitted values of lncd

         chi2(1)      =     4.59
         Prob > chi2  =   0.0322
```

Site 2c Blacklip was excluded based on below normal quantile plot of regression residuals



Regression residuals over Outcome Cd levels (log transformed) – again, Site 2c blacklip is a strong outlier and was therefore excluded from the regression analysis



Regression analyses with remaining metals

Pb

```
. regress lnpb size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =   14.51
       Model |  48.1780134     8  6.02225168            Prob > F      =  0.0000
    Residual |  17.8508963    43  .415137123           R-squared     =  0.7297
-------------+------------------------------           Adj R-squared =  0.6794
       Total |  66.0289097    51   1.2946845           Root MSE      =  .64431

------------------------------------------------------------------------------
        lnpb |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |  -.0578946    .092027    -0.63   0.533    -.2434847    .1276955
             |
        site |
          2  |   .4213455   .2303236     1.83   0.074    -.0431464    .8858374
          3  |   .9303734   .3381633     2.75   0.009      .248402    1.612345
          4  |  -.8230046   .2767512    -2.97   0.005    -1.381126   -.2648827
             |
    1.farmed |  -.8689276   .5625379    -1.54   0.130    -2.003393    .2655381
      2.type |   1.460447    .512957     2.85   0.007     .4259706    2.494923
             |
        trip |
          2  |  -.8744942   .2652942    -3.30   0.002    -1.409511   -.3394776
          3  |   .0137823   .2498053     0.06   0.956    -.4899981    .5175627
             |
       _cons |  -4.315736   .4930954    -8.75   0.000    -5.310158   -3.321314
```

Mo

```
. regress lnmo size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =    9.57
       Model |  13.1152926     8  1.63941158            Prob > F      =  0.0000
    Residual |  7.36252911    43  .171221607           R-squared     =  0.6405
-------------+------------------------------           Adj R-squared =  0.5736
       Total |  20.4778217    51  .401525916           Root MSE      =  .41379

------------------------------------------------------------------------------
        lnmo |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |  -.0257074   .0591015    -0.43   0.666     -.144897    .0934821
             |
        site |
          2  |  -.1126901   .1479183    -0.76   0.450    -.4109958    .1856157
          3  |   -.431497   .2171751    -1.99   0.053    -.8694723    .0064783
          4  |  -.8550481    .177735    -4.81   0.000    -1.213485   -.4966113
             |
    1.farmed |  -.4293049   .3612728    -1.19   0.241    -1.157881    .2992711
      2.type |   .7683211    .329431     2.33   0.024     .1039601    1.432682
             |
        trip |
          2  |   .5838775   .1703771     3.43   0.001     .2402794    .9274757
          3  |  -.0658057   .1604298    -0.41   0.684    -.3893433    .2577319
             |
       _cons |  -2.062988   .3166755    -6.51   0.000    -2.701625   -1.424351
------------------------------------------------------------------------------
```

As

```
. regress lnas size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =    4.84
       Model |   1.8965997     8  .237074962           Prob > F      =  0.0003
    Residual |  2.10769351    43  .049016128           R-squared     =  0.4736
-------------+------------------------------           Adj R-squared =  0.3757
       Total |  4.00429321    51  .078515553           Root MSE      =   .2214

------------------------------------------------------------------------------
        lnas |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |   .0739023    .031622     2.34   0.024     .0101305    .1376741
             |
        site |
          2  |  -.2665813    .079143    -3.37   0.002    -.4261883   -.1069744
          3  |  -.3634857   .1161984    -3.13   0.003    -.5978222   -.1291493
          4  |  -.1413252   .0950962    -1.49   0.145     -.333105    .0504546
             |
    1.farmed |   .1826566   .1932972     0.94   0.350    -.2071643    .5724775
      2.type |  -.2677454   .1762604    -1.52   0.136    -.6232083    .0877176
             |
        trip |
          2  |   .0943002   .0911594     1.03   0.307    -.0895403    .2781406
          3  |    .160864   .0858372     1.87   0.068    -.0122432    .3339711
             |
       _cons |   1.224096   .1694356     7.22   0.000     .8823962    1.565795
------------------------------------------------------------------------------
```

Zn

```
. regress lnzn size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =   32.94
       Model |  37.4053489     8  4.67566861           Prob > F      =  0.0000
    Residual |  6.10400146    43  .141953522           R-squared     =  0.8597
-------------+------------------------------           Adj R-squared =  0.8336
       Total |  43.5093503    51  .853124516           Root MSE      =  .37677

------------------------------------------------------------------------------
        lnzn |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |   .0525505   .0538137     0.98   0.334    -.0559751    .1610761
             |
        site |
          2  |  -.6104821   .1346839    -4.53   0.000    -.8820981    -.338866
          3  |   2.262203   .1977442    11.44   0.000     1.863413    2.660992
          4  |   .2480467   .1618329     1.53   0.133    -.0783204    .5744138
             |
    1.farmed |  -.1386911   .3289494    -0.42   0.675    -.8020807    .5246986
      2.type |  -.1711308   .2999565    -0.57   0.571    -.7760507    .4337891
             |
        trip |
          2  |  -.1748083   .1551333    -1.13   0.266    -.4876644    .1380478
          3  |   .0247358    .146076     0.17   0.866    -.2698546    .3193261
             |
       _cons |   2.789636   .2883422     9.67   0.000     2.208138    3.371133
------------------------------------------------------------------------------
```

Cu

```
. regress lncu size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =   14.99
       Model |  8.72891385     8  1.09111423           Prob > F      =  0.0000
    Residual |  3.12919769    43  .072772039           R-squared     =  0.7361
-------------+------------------------------           Adj R-squared =  0.6870
       Total |  11.8581115    51  .232511991           Root MSE      =  .26976

------------------------------------------------------------------------------
        lncu |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |   .0868679   .0385302     2.25   0.029     .0091643    .1645715
             |
        site |
          2  |  -.3924563   .0964329    -4.07   0.000    -.5869317   -.1979809
          3  |   1.002305   .1415836     7.08   0.000      .716774    1.287835
          4  |   -.132808   .1158713    -1.15   0.258    -.3664848    .1008688
             |
    1.farmed |  -.2888477   .2355257    -1.23   0.227    -.7638305    .1861351
      2.type |  -.1394246    .214767    -0.65   0.520    -.5725435    .2936942
             |
        trip |
          2  |  -.0059542   .1110745    -0.05   0.957    -.2299572    .2180488
          3  |   .0599198   .1045895     0.57   0.570    -.1510051    .2708446
             |
       _cons |   2.151127   .2064512    10.42   0.000     1.734779    2.567476
------------------------------------------------------------------------------
```

Ni

```
. regress lnni size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =   30.15
       Model |  26.315881     8  3.28948512           Prob > F      =  0.0000
    Residual |  4.6909218    43  .109091205           R-squared     =  0.8487
-------------+------------------------------           Adj R-squared =  0.8206
       Total |  31.0068028    51  .607976525           Root MSE      =  .33029

------------------------------------------------------------------------------
        lnni |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |   .0201873   .0471753     0.43   0.671    -.0749507    .1153253
             |
        site |
          2  |  -.7356255   .1180695    -6.23   0.000    -.9737353   -.4975158
          3  |  -.2901987   .1733507    -1.67   0.101    -.6397937    .0593963
          4  |    .053413   .1418693     0.38   0.708    -.2326938    .3395198
             |
    1.farmed |  -.4678538   .2883705    -1.62   0.112    -1.049408    .1137008
      2.type |   .6292755   .2629542     2.39   0.021     .0989779    1.159573
             |
        trip |
          2  |  -1.078507   .1359962    -7.93   0.000     -1.35277   -.8042447
          3  |   .2988097   .1280562     2.33   0.024     .0405596    .5570597
             |
       _cons |  -1.936725   .2527726    -7.66   0.000     -2.44649    -1.42696
------------------------------------------------------------------------------
```

Co

```
. regress lnco size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =    9.74
       Model |  9.69981833     8  1.21247729           Prob > F      =  0.0000
    Residual |  5.35040114    43  .124427933           R-squared     =  0.6445
-------------+------------------------------           Adj R-squared =  0.5784
       Total |  15.0502195    51  .295102342           Root MSE      =  .35274

------------------------------------------------------------------------------
        lnco |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |  -.0972213   .0503823    -1.93   0.060    -.1988269    .0043844
             |
        site |
          2  |   .2716413   .1260961     2.15   0.037     .0173443    .5259384
          3  |   .1379893   .1851355     0.75   0.460     -.235372    .5113506
          4  |   -.196456   .1515139    -1.30   0.202     -.502013     .109101
             |
    1.farmed |  -1.061205   .3079746    -3.45   0.001    -1.682295   -.4401151
      2.type |   1.325586   .2808304     4.72   0.000     .7592374    1.891934
             |
        trip |
          2  |   .1357591   .1452415     0.93   0.355    -.1571484    .4286666
          3  |   .0141589   .1367618     0.10   0.918    -.2616476    .2899653
             |
       _cons |    -2.8243   .2699567   -10.46   0.000     -3.36872   -2.279881
------------------------------------------------------------------------------
```

Fe

```
. regress lnfe size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =    3.15
       Model |  4.46245968     8   .55780746           Prob > F      =  0.0068
    Residual |  7.61763357    43  .177154269           R-squared     =  0.3694
-------------+------------------------------           Adj R-squared =  0.2521
       Total |  12.0800933    51  .236864574           Root MSE      =   .4209

------------------------------------------------------------------------------
        lnfe |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |   .0045867   .0601167     0.08   0.940    -.1166502    .1258236
             |
        site |
          2  |  -.5778171   .1504591    -3.84   0.000    -.8812469   -.2743874
          3  |  -.1902109   .2209055    -0.86   0.394    -.6357093    .2552875
          4  |  -.5507553   .1807879    -3.05   0.004     -.915349   -.1861617
             |
    1.farmed |  -.0567859   .3674784    -0.15   0.878    -.7978768    .6843049
      2.type |   .1122623   .3350896     0.34   0.739    -.5635104     .788035
             |
        trip |
          2  |  -.3284424   .1733037    -1.90   0.065    -.6779425    .0210578
          3  |  -.2074359   .1631855    -1.27   0.211    -.5365309    .1216591
             |
       _cons |   4.112365    .322115    12.77   0.000     3.462758    4.761971
------------------------------------------------------------------------------
```

V

```
. regress lnv size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =    6.99
       Model |  7.62651095     8  .953313868           Prob > F      =  0.0000
    Residual |  5.86146279    43  .136313088           R-squared     =  0.5654
-------------+------------------------------           Adj R-squared =  0.4846
       Total |  13.4879737    51  .264470073           Root MSE      =  .36921

------------------------------------------------------------------------------
         lnv |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |   .0325492   .0527337     0.62   0.540    -.0737985    .1388968
             |
        site |
          2  |  -.4829487    .131981    -3.66   0.001    -.7491138   -.2167836
          3  |  -.3850471   .1937758    -1.99   0.053    -.7758332     .005739
          4  |  -.8483373   .1585851    -5.35   0.000    -1.168155   -.5285199
             |
    1.farmed |  -.0714863   .3223478    -0.22   0.826    -.7215627    .5785901
      2.type |   .2274886   .2939368     0.77   0.443    -.3652915    .8202687
             |
        trip |
          2  |   .4405262    .15202     2.90   0.006     .1339487    .7471038
          3  |   .3608526   .1431445     2.52   0.015     .0721742     .649531
             |
       _cons |  -1.719679   .2825556    -6.09   0.000    -2.289507   -1.149851
------------------------------------------------------------------------------
```

P

```
. regress lnp size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =   18.08
       Model |     6.41122     8    .8014025           Prob > F      =  0.0000
    Residual |  1.90603366    43  .044326364           R-squared     =  0.7708
-------------+------------------------------           Adj R-squared =  0.7282
       Total |  8.31725366    51  .163083405           Root MSE      =  .21054

------------------------------------------------------------------------------
         lnp |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |   .0554619   .0300712     1.84   0.072    -.0051824    .1161062
             |
        site |
          2  |   .0239761   .0752617     0.32   0.752    -.1278035    .1757557
          3  |  -.0375868   .1104999    -0.34   0.735    -.2604311    .1852574
          4  |   .1132792   .0904325     1.25   0.217    -.0690953    .2956538
             |
    1.farmed |   .5483711   .1838176     2.98   0.005     .1776676    .9190746
      2.type |   -.475566   .1676163    -2.84   0.007    -.8135965   -.1375355
             |
        trip |
          2  |    .653904   .0866888     7.54   0.000     .4790793    .8287286
          3  |  -.0094162   .0816276    -0.12   0.909    -.1740339    .1552015
             |
       _cons |   6.482284   .1611262    40.23   0.000     6.157342    6.807226
------------------------------------------------------------------------------
```

Al

```
. regress lnal size i.site i.farmed i.type i.trip

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  8,    43) =    5.19
       Model |  14.0229679     8  1.75287098           Prob > F      =  0.0001
    Residual |   14.515395    43  .337567325           R-squared     =  0.4914
-------------+------------------------------           Adj R-squared =  0.3967
       Total |  28.5383628    51  .559575742           Root MSE      =  .58101

------------------------------------------------------------------------------
        lnal |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        size |   .0610151    .082985     0.74   0.466    -.1063401    .2283703
             |
        site |
          2  |  -.8309472   .2076935    -4.00   0.000    -1.249801   -.4120934
          3  |  -.6932569   .3049376    -2.27   0.028    -1.308222   -.0782917
          4  |   -.741738   .2495594    -2.97   0.005    -1.245022   -.2384536
             |
    1.farmed |   .4455865   .5072665     0.88   0.385    -.5774139    1.468587
      2.type |   .1896662   .4625572     0.41   0.684    -.7431692    1.122502
             |
        trip |
          2  |  -.4173554   .2392281    -1.74   0.088    -.8998048    .0650939
          3  |   .0375757    .225261     0.17   0.868    -.4167065    .4918578
             |
       _cons |   2.802093    .444647     6.30   0.000     1.905377    3.698809
------------------------------------------------------------------------------
```

Appendix 11: Bioavailable metal concentrations in TSS for all trips

ICPMS analysis of elemental comosition of Goulburn Island total suspended solids, 1M HCl digest. Trip 1 and trip 2

Sample Name	Al mg/kg	P mg/kg	V mg/kg	Fe mg/kg	Co mg/kg	Ni mg/kg	Cu mg/kg	Zn mg/kg	As mg/kg	Mo mg/kg	Cd mg/kg	Pb mg/kg
GI Site 1a trip 1	<	<	<	<	<	<	5.05	<	<	<	<	<
GI Site 1b trip 1	2707	<	49.0	7032	<	2.90	4.03	<	12.2	<	<	4.39
GI Site 2a trip 1	4522	<	64.9	11772	1.63	4.52	5.76	<	16.5	<	<	6.65
GI Site 2b trip 1	4119	<	44.0	11312	1.51	4.55	4.68	<	10.6	<	<	5.65
GI Site 3a trip 1	2934	<	47.6	7391	<	2.52	6.91	<	8.54	<	<	8.65
GI Site 3b trip 1	3794	<	47.2	10271	1.51	2.66	4.26	<	9.61	<	<	7.92
GI Site 3c trip 1	3804	<	55.6	10046	1.62	4.15	6.69	<	14.7	<	<	7.77
GI site 1a trip 2	3939	<	52.2	11271	1.79	7.10	7.67	<	13.4	<	<	6.03
GI site 1b trip 2	4003	<	44.9	11222	1.86	4.65	9.43	<	12.6	<	<	6.29
GI site 1c trip 2	3849	<	48.9	11042	1.81	4.90	5.79	<	13.1	<	<	5.80
GI site 2a trip 2	5633	<	45.9	15979	2.30	4.72	5.78	<	12.4	<	<	7.65
GI Site 2b trip 2	4751	<	43.2	13267	1.96	5.84	6.20	<	10.1	<	<	6.34
GI site 2c trip 2	5050	<	46.5	14555	1.92	3.91	4.27	<	13.0	<	<	6.79
GI Site 3a trip 2	4755	<	78.5	15992	2.37	3.64	9.34	<	16.5	<	<	9.58
GI Site 3b trip 2	5446	<	95.6	19105	3.03	3.53	8.20	<	19.8	<	<	12.2
GI Site 3c trip 2	5172	<	52.6	14510	2.36	3.81	6.80	<	13.9	<	1.79	8.00
GI site 4a trip 2	1785	<	30.8	4177	<	1.62	4.03	<	11.8	<	<	2.76
GI site 4b trip 2	4733	<	66.5	11458	1.99	11.7	17.8	<	19.3	<	<	7.30
GI site 4c trip 2	2474	<	65.7	6908	<	2.00	8.53	<	19.2	<	<	4.26
Reporting limit	72.0	600	5.50	350	1.50	1.00	3.00	30.0	5.50	10.5	1.50	2.00
New filter blank average	<	<	<	<	<	<	<	<	<	<	<	<
MESS-3	1620	873	27.3	8380	4.04	8.02	16.3	52.4	5.29	0.14	0.18	13.9
CDU HCL long term ave	1570	802	23.8	8059	3.72	7.26	16.1	49.0	4.89	0.26	0.19	14.4
MESS-3 certified	85900	nc	243	43400	14.4	46.9	33.9	159	21.2	2.78	0.24	21.1
Reporting limit	72.0	600	5.50	350	1.50	1.00	3.00	30.0	5.50	10.5	1.50	2.00

ICPMS analysis of elemental comosition of Goulburn Island total suspended solids, 1M HCl digest. Trip 3

Sample	Al mg/kg	P mg/kg	V mg/kg	Fe mg/kg	Co mg/kg	Ni mg/kg	Cu mg/kg	Zn mg/kg	As mg/kg	Mo mg/kg	Cd mg/kg	Pb mg/kg
Site 1a	1144	<	5.63	3912	0.65	0.72	2.40	15.8	3.92	<	0.16	2.37
Site 1b	1385	<	6.70	4839	0.78	0.66	1.88	12.0	3.91	<	0.18	2.41
Site 1c	1469	<	7.88	5470	0.81	0.56	1.54	11.2	5.46	<	0.24	2.64
Site 2a	1667	<	7.70	5954	0.93	1.12	2.26	12.2	4.52	<	0.17	2.78
Site 2b	1775	<	8.07	6397	1.00	3.21	3.73	42.0	5.35	<	0.25	3.33
Site 2c	2225	<	10.7	7901	1.23	1.78	2.73	41.9	5.13	<	0.20	3.60
Site 3a	1475	<	8.90	5646	0.75	0.39	1.62	14.8	5.07	<	0.12	2.54
Site 3b	1318	<	8.60	5127	0.67	<	0.78	7.92	4.37	<	0.15	2.27
Site 3c	1658	<	10.5	6534	0.84	0.74	1.43	11.0	5.75	<	0.13	2.86
Site 4a	1382	<	5.40	4878	0.81	0.89	1.84	23.5	4.05	<	0.13	3.18
Site 4b	1289	<	5.10	4628	0.76	1.90	1.60	7.61	3.44	<	0.12	2.88
Site 4c	1733	<	7.66	6075	0.99	12.8	6.50	13.0	4.19	<	0.15	4.14
Bloom 1	27	<	<	128	0.031	0.57	0.20	<	6.26	<	0.15	0.09
Bloom 2	24	<	<	115	0.027	0.50	0.17	<	5.71	<	0.15	0.09
Lugols 1	<	<	<	<	<	0.35	<	<	<	<	<	<
Lugols 2	<	<	<	<	<	0.34	<	<	<	<	<	<
Reporting limit	9.50	340	1.00	10.0	0.020	0.080	0.070	0.80	0.20	7.00	0.05	0.060
TSS filter blank average	<	<	<	<	<	0.34	<	<	<	<	<	<
Low weight MESS similar to samples												
MESS-3 average	3457	695	31.6	14816	6.01	15.1	20.2	74.1	9.34	<	<	16.0
MESS-3 CDU HCL dige	1565	802	23.8	8185	3.72	7.26	16.1	49.0	4.89	<	0.19	14.4
MESS-3 certified	85900	nc	243	43400	14.4	46.9	33.9	159	21.2	<	0.24	21.1
0.2g of MESS												
Average	3058	839	30.7	13116	5.35	13.1	18.9	71.4	6.26	<	0.14	15.4
MESS-3 CDU HCL dige	1565	802	23.8	8185	3.72	7.26	16.1	49.0	4.89	<	0.19	14.4
MESS-3 certified	85900	nc	243	43400	14.4	46.9	33.9	159	21.2	<	0.24	21.1
Reporting limit	9.50	340	1.00	10.0	0.020	0.080	0.070	0.80	0.20	7.00	0.05	0.060

Appendix 12: PERMANOVA Permutational MANOVA Pair-wise tests – chlorophyll a and trace elements in TSS and bloom

Chlorophyll a levels in seawater

When chlorophyll *a* levels in seawater were compared between sites there were no significant pairwise site differences.

Sites	t	P(perm)
1, 2	7.76	0.084
1, 3	0.336	0.888
1, 4	2.3731	0.106
2, 3	1.951	0.092
2, 4	0.72631	0.9
3, 4	1.9194	0.106

TSS levels in seawater

TSS levels in seawater were compared between trips using Permutational MANOVA, and levels from trip 3 were significantly different from each of the other two trips.

Trips	t	P(perm)
1, 2	0.19	0.863
1, 3	16.29	0.001
2, 3	16.10	0.001

Bioavailable trace elements in TSS

Pairwise comparisons between trips analysed by Permutational MANOVA, showed that bioavailable trace elements in TSS from trip 3 were different from both trips 1 and 2, however trip 1 and 2 levels were not significantly different.

Trips	t	P(perm)
1, 2	1.23	0.261
1, 3	2.24	0.003
2, 3	10.49	0.001

Cd and Zn levels in TSS

Pairwise comparisons between trips analysed by Permutational MANOVA, showed that Cd and Zn levels from trip 3 were significantly different from both of the other two trips.

Trips	t	P(perm)
1, 2	0.93	0.408
1, 3	16.00	0.001
2, 3	4.07	0.001

Appendix 13: Literature review on metal bioaccumulation in marine invertebrates

Author: Nathalie Mauraud (CDU)

1. Bioaccumulation in marine bivalves

Metal contamination is an environmental issue in many coastal areas. In regards of environmental risk assessment and seafood safety it is important to understand the metal accumulation and toxicity in aquatic animals.

Filter-feeders such as oysters have been shown to bio-accumulate essential or non-essential heavy metals. This bioaccumulation occurs via uptake from dissolved phase (water column) and particulate phase (food). As trace elements, some heavy metals (e.g. Copper (Cu) and Zinc (Zn)) are essential to maintain the metabolism of aquatic animals. However at higher concentrations they can become toxics. Other heavy metals, non-essential (e.g. Cadmium (Cd)) can be toxics at very low levels.

A huge variability in bioaccumulation and in metal body burden (Total amount of metal present in the body) exists among different invertebrate species, even between taxonomically very closely related species (Wang and Rainbow, 2008). Physiological properties of oysters, physico-chemical properties of the metals, routes of uptake and environmental conditions are important parameters to assess their metal bioaccumulation (Apeti *et al*., 2005; Wang and Rainbow, 2005).

As contaminants, metals have particular properties: they are non-degradable and may be concentrated rather than diluted in marine organisms (Frazier, 1979).

In natural environment, Zn concentrations in water are generally higher than those of Cd. However in environments impacted by anthropogenic activities, Cd concentrations can be very high. Along with this, oysters have been shown to have a high potential for cadmium bioaccumulation (Frazier, 1979). As a non-essential metal, cadmium may be accumulated without excretion or with some excretion, however no regulation process have been found (Daka, 2005). As an essential-metal, zinc can be regulated and accumulated with or without excretion (Rainbow, 2002). Generally, bioaccumulated concentrations of Cd are lower than Zn concentrations.

2. Bioavailability of metals

Metals are present in different compartment of the environment (water column, sediments and organisms). Bioaccumulation, via the transfer from a compartment to another, is possible if the metal is in a form available for the accumulation (bioavailable) by marine organisms.

Sediments play an important role for metal storage in marine environments. However no significant correlations have been observed between total concentrations of metals in superficial sediments (easily resuspended in the water column) and concentrations in oysters (normally exposed to sediment-bound metal). This is probably due to the non-bioavailability of the metals captured in sediment (Chong and Wang, 200). Hédouin *et al.* (2010) have also found a low bioavailability of sediment-bound metals. On the contrary, a strong correlation exists between dissolved metal concentration and bioaccumulated concentration, which is a good argument that the dissolved fraction of a metal is the most bioavailable for filter-feeders (Amiart *et al.,* 2007).

3. Assimilation pathways and patterns

Metal concentration in a marine organism is the net result of uptake, storage, transformation and elimination and varies between type of metals, species and individuals.

Metal uptake from dissolved or particulate phases is variable among marine organisms and a great variety of assimilation pathways and patterns have been found.

Heavy metal uptake occurs through the gills (from the dissolved phase) and the alimentary absorption (from the particulate phase) (Lim, 1998). However the proportion of each route depends on the metal, the organism studied and the environmental conditions (Rainbow, 1997). Understanding metals pathways and patterns is important to understand how they accumulate in marine bivalves.

Blackmore and Wang (2004) have quantified the metal uptake from the dissolved phase in oysters. They have found that this uptake was not directly proportional to metal concentrations in the water and the uptake of Zn was higher than this of Cd. They also observed that the dissolved uptake rate in the oysters was higher than those of other species of marine bivalves. Hédouin *et al.* (2010) have observed that the uptake of Cd, Cu and Zn in two species of oysters was faster and more efficient than those of other metals (Chromium (Cr) and Cobalt (Co)) and that oysters can also store a large amount of these three metals.

Regarding Cd uptake, the most common route seems to occur from dissolved phase (Lim *et al.*, 1998).

Different accumulation patterns have been discovered in invertebrates. They depend on the physiology of the species under study, the metals, and wether the metals are used for an essential metabolic purpose or not, regulated, excreted or stored in the body.

For essential metals, accumulation patterns can be the regulation of body metal concentration, the accumulation without extraction and the accumulation with some extraction. For non-essential metals, patterns can be accumulation with or without extraction and for both kind of metals, they are either concentrated as metabolically available or stored detoxified (Rainbow, 2002).

The transfer of trace metal from the environment or the food to the cell compartment can be made possible by the help of carriers. A trace metal has the ability to bind to a molecule with an affinity for that metal (Rainbow, 2002), it has an affinity for sulphur and nitrogen and proteins contain sulphur and/or nitrogen. First metabolically bioavailable, the trace metal is detoxified when binding to a metallothionein (MTs). MTs are low molecular weigh proteins and have the ability to bind heavy metals (such as Cd, Cu and Zn). They can be induced by metals and are involved in uptake, transport and regulation of heavy metals (Roesijadi, 1994). Also, the capacity of MTs to bind metals is suspected to provide protection against metal toxicity (Roesijadi, 1996).

In the carrier-mediated facilitated transport model (involving MTs), trace metals are first sequestered by a membrane protein ligand, and then by an intracellular protein ligand with a higher binding stability. Binding with protein ligands influences metal transport through biological membranes and metals binding with protein ligands (such as Ag (Silver), Cd and Zn) have the highest dissolved uptake rate in marine mussels (Wang *et al*., 1996).

Other accumulation patterns have been observed, for example Wang and Fischer (1999) have found that in aquatic invertebrates, the Calcium channels can be involved in transporting metals.

4. Metal Interactions

For decades metals contaminations have been studied in focusing on one metal at a time. However effects of metals interactions have been demonstrated and can play an important role in metal uptake and accumulation (Rainbow, 2002).

The interaction between two metals or more can vary from synergistic (i.e. the concurrent presence of one metal enhances the bioaccumulation of the other) to antagonistic (i.e. the concurrent presence of one metal causes a reduction in the bioaccumulation of the other) (Rainbow *et al.,* 2000; Daka, 2005).

The first mechanism potentially involved in the metal-metal interaction is the competitions for MTs (carriers) binding sites. Some metals are known to use the same (MTs) for their extra and intra-cellular transport (Magos *et al*., 1978).

5. Interactions Zinc/Cadmium

It has been shown that Zn and Cd have high chemical similarities and tend to bind with proteins (Wang and Fisher, 1999). As a result of their chemical affinities, Cd and Zn may share similar uptake pathways into organisms and use the same carriers for their transport (Rainbow, 1997).

Understanding the metal interactions in bioaccumulation requires defining when and where the interactions take place. More specifically, has the exposition to different metals occurred at the same time? Has a pre-exposure to a particular metal happened? And where were the populations studied from?

Different scenarios have been observed in metal interaction. Amiard-Triquet and Amiard (1998) have observed that in many mollusc species, exposure to Cd had no effect on Zn accumulation, whereas exposure to Zn had an antagonistic effect on Cd accumulation. However Otitoloju and Don-Pedro (2006) have observed that the gastropod *Tympanotonus fuscatus* tends to accumulate smaller levels of Zn, Cu and Cd when exposed to a mix solution compared to when exposed to a single solution of each metal.

Under natural conditions, animals are simultaneously or alternately exposed to different metals from different sources (dissolved and particulate phases). The effect of exposure to one metal on the uptake of other metals is important to define and understand metal-metal interactions. Depending on which factor is measured the effect of one metal on the other is different.

Shi and Wang (2004) have studied Cd and Zn bioaccumulation in different populations of marine clams (*Mactra veneriformis* and *Ruditapes philippinarum*) with different Cd contaminations levels. They observed that for both species, the population with a higher Cd tissue concentration accumulated Cd and Zn more efficiently from the dietary phase. The influence of trace metal exposure on metal accumulation may be explained by the induction of specific metal-blinding ligands like MTs (Wang and Rainbow, 2005).

In aquatic invertebrates, it has been shown that a pre-exposure of Cd produces a major change in the subcellular distribution of Cd (due to the production of MTs) and an increase of Cd assimilation efficiency (AE: ie the fraction of ingested food that is absorbed and used in metabolism) (Wang and Rainbow, 2005). At the same time a high concentration of Cd may lead to a facilitated Zn AE, due to an induction of MTs providing available binding sites (Blackmore and Wang, 2002). Populations living in contaminated areas may be able to adapt to metal stress in modifying their physiological and chemical responses.

In natural populations of oysters from a Cd-rich environment, the metal composition of MTs indicates reciprocal binding of Cd and Zn. However, in individuals with higher cytosolic Cd concentrations, MTs have bound more atoms of Cd and less atoms of Z, but they have seemed to firstly bind to Zn (Roesijadi, 1996).

Studying bioaccumulation in populations of *Littorina saxatilis*, Daka (2009) has observed an increase of Cd accumulation with increasing Zn concentration in the tissue showing a synergistic relationship at low concentrations. However this

relationship reverses at the highest Zn concentration, showing an antagonistic effect of Zn on Cd accumulation. On the same species, Daka and Hawkins (2006) have found that in interactions between Cd and, Zn accumulation was higher in a mix solution of Zn and Cd than from a solution of Zn alone. Zn had an antagonistic effect on Cd accumulation.

On the other hand, a pre-exposure to dissolved Zn in the green mussels *Perna viridis* has led to a decrease of dissolved uptake for Zn and could be explained by a regulation of Zn body burden in response to elevated concentration in the environment (Blackmore and Wang, 2002).

These results can be explained by the fact that, as shown in several other studies, marine bivalves are able to develop various strategies in metal detoxification and storage under metal stress conditions (Shi and Wang, 2004).

6. Zinc/Cadmium interactions in other groups

Metal-metal interactions have been studied in other groups and have revealed similar processes. The analysis of metal interaction in the Freshwater shrimp *Paratya tasmaniensis* revealed that the interaction between Zn and Cd appeared not to be additive at low concentrations, it is possible that one of the metals may have an antagonistic effect on the other one (Thorp and Lake, 1974).

Studying metal interaction, Kargin and Cogun (1999) have measured that Cd accumulation in the tissues of fish exposed to Cd+Zn mixtures were lower when compared with those exposed to Cd only. However Zn accumulation in the tissues of fish exposed to high levels of Cd+Zn mixture increased when compared to those exposed to high level of Zn only. Zn had an antagonistic action on the uptake of Cd on the freshwater fish *Tilapia nilotica.* This action can be partially explained by the competition for binding sites on MTs.

In a freshwater ecosystem, the presence of Zn have reduced the accumulation of Cd in the clam *Anondota cygnea* by half in whole animals compared to values measured in Cd exposure alone, Zn have exerted an antagonistic effect on Cd uptake (Hemelraad *et al.*,1987). They have also discovered that the effect of Zn on Cd uptake was different depending on what organ was analysed (concentration in gills, mantle and labial paps was really reduced, by contrast accumulation in mid-gut gland and kidney was hardly affected). Moreover they observed that Cd is transported more rapidly to internal organs at high Zn concentrations.

7. Cadmium/Copper and Zinc/Copper interactions

Other metal-metal interactions have been identified in oysters and can play a role in Zn and Cd accumulation. Potential synergistic effects of Ag and Cu on Cd accumulation have been identified in the oyster *Crassostrea gigas* (Amiard *et al.,* 2004). By contrast exposition to Cu concentrations in the gastropod *Littorina saxatilis* have been shown to reduce Zn accumulation (Daka and Hawkins, 2005*).*

8. Impacts of Environmental factors: Temperature and Salinity

Studies on metal bioaccumulation revealed that metal concentrations accumulated in marine molluscs may be affected by environmental factors. Regional and seasonal differences (based on local water concentration, population biology and weather) have been found in oysters (Frazier, 1979).

It has been observed that the tissue metal concentration in molluscs is influenced by the salinity. Uptake and concentration of metals are generally inversely related to salinity (Frazier, 1975; Wang and Fisher, 1999 and Wright, 1995).

The analysis of the effect of salinity on metal uptake in the mussel *Perna viridis* and the clam *Ruditapes philipinarum* has shown that the metal influx rates of Cd and Zn increased when the salinity was reduced (Chong and Wang, 2001; Luoma and Rainbow, 2005).

Different explanations have been proposed, first a change in metal speciation (increasing salinity can lead to a reduction of metal bioavailability), then physiological changes such the permeability of gill surface (facilitating the transport of metals) and finally a change in Calcium (Ca) concentration (decrease of competition with Ca for Calcium channels allow more metals to be transported) (Chong and Wang, 2001).

Studying the interaction of Cd and Zn on the Freshwater shrimp *Paratya tasmaniensis*, Thorp and Lake (1974) have observed seasonal variation in sensitivity to Cd. The effect of temperature in metal bioaccumulation has been reported by Frazier (1979). He highlighted that under a certain limit (depending of the species and population studied) the accumulation is reduced or stopped. He showed that a species/ population dependent threshold exist, under which the accumulation is greatly reduced or stopped, and on the contrary highest water temperatures could enhance metal bioaccumulation (in particular Cd).

9. Conclusion and perspectives

Metal bioaccumulation is a complex and variable process and a lot of different sources of variations have been identified over the past decades.

Bioaccumulation greatly varies at both inter and intra levels (with specifics assimilation patterns and pathways). The life history of a given population (i.e. its possible environmental adaptation) has been shown to influence rates of metal accumulation. Moreover, the bioaccumulation patterns are metal dependant and what is known for one metal cannot be applied to another. Metal accumulation depends on metal concentration in the environment, the kind of metal (essential or non-essential to metabolic activities), the metal involved (some heavy metals are more bioaccumulated than others, i.e. Zn and Cd), metals interactions (synergistic or antagonistic effects may greatly modify metal uptake in marine bivalves) and metal speciation (which determines its bioavailability). Environmental factors can also influence metal bioaccumulation. Temperature and salinity are known to modify metal uptake in marine organisms but there might be other sources of variation such as light or pH. Finally anthropogenic inputs (linked to weather conditions or localisation) can also affect the quantity of metal present in the environment and thus can influence the bioaccumulation processes.

Explaining the metal concentrations found in a given species or population, from a specific location at a specific time is very complex and integrating these different parameters is challenging when studying bioaccumulation. However determining the factors influencing bioaccumulation seems important to understand a particular metal contamination.