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Genetic diversity and metal tolerance of two marine species: a comparison between populations from contaminated and reference sites

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Abstract

Long-term contamination of the marine environment surrounding a lead smelter offered a unique opportunity to examine how pollutants might have acted to alter genetic characteristics of populations of organisms in the receiving system. This study used random amplified polymorphic DNA analysis to compare the genetic diversity of populations of the prawn, *Leander intermedius*, and the isopod, *Platynympha longicaudata* from the smelter discharge site with reference populations. The genetic diversity of the prawn population from the smelter discharge site (Port Pirie) was lower than that found in one reference population, and not significantly different from the other two reference populations. Genetic diversity of the Port Pirie population of isopods was found to be significantly lower than that of all reference populations. Prawns and isopods were also exposed to metal mixtures in the laboratory in a similar ratio to that found in seston near the smelter effluent discharge site. Both pre-exposed and reference populations of prawns tolerated elevated levels of metals and exhibited no significant difference in response. This contrasted with the isopods, with the pre-exposed isopod population showing greater tolerance to elevated metal levels compared with the reference population. These results highlight the need to include a number of reference populations for comparative purposes in genetic diversity studies, and the need to assess the influence of pollution on the genetic diversity of more than one species if genetic diversity analyses are to be used to gauge remediation success.

Keywords: Genetic diversity; Metal tolerance; Metal pollution; RAPDs; Prawn; Isopod

1. Introduction

Commonly used indicators of pollution effects, such as species diversity and population densities, will often return to normal shortly after removal of the pollutant(s), but do not reflect altered population gene pools (Bickham et al., 2000). Such traditional methods of assessing pollutant effect could potentially generate erroneous conclusions as to the health of the system by assuming that diversity and densities alone indicate return to a relatively natural condition. There is a growing interest among ecotoxicologists in the extent to which contaminant exposure can alter allelic variation in populations (Nevo et al., 1986; Benton and Guttman, 1992; Bickham and Smolen, 1994; Grunwald et al., 1999; Bickham et al., 2000). This alteration, which often results in reduction of genetic diversity, may occur by selection for toxicant resistance, selection on traits not related to toxicant resistance (secondary selection), or founder effects resulting from re-establishment of extinct populations (Nadig et al., 1998). Reduction in the genetic diversity of exposed populations can result in significant long term biological consequences as populations with reduced genetic diversity may be less efficient at utilizing resources and may be at a greater risk of extinction in fluctuating environments (Nadig et al., 1998; Krane et al., 1999). The number of studies that have assessed genetic diversity of populations as a biomarker is still limited and a greater understanding is required to determine whether genetic diversity can be used routinely as a reliable indicator of population exposure to toxicants.

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This study compared the genetic diversity of a prawn population (*Leander intermedius*), and an isopod population (*Platynympha longicaudata*) collected from near a lead smelter effluent outfall, with that of reference populations, using the molecular technique random amplified polymorphic DNA (RAPDs) (Williams et al., 1990). RAPDs analysis is one molecular technique for assaying population genetic variation that has recently been used successfully in ecotoxicological applications (Fore et al., 1995; Nadig et al., 1998; Grunwald et al., 1999; Krane et al., 1999). Recent studies by Krane et al. (1999) and Nadig et al. (1998) used RAPDs to assess the genetic diversity in aquatic animal populations exposed to anthropogenic contaminants. Both studies identified the RAPDs technique to be a valuable alternative to, or augmentation of, more standard assessments of contaminant impact. In addition, both studies found that compared with other molecular techniques, assessing genetic diversity of a population using RAPDs is advantageous in terms of cost, time, required background knowledge and expertise.

1.1. Study site

The smelter at the study site is the largest lead smelting operation in the world. Situated at Port Pirie, on the Upper Spencer Gulf, South Australia, it has been a major source of metal pollution since it began operating in 1889 (Ward et al., 1982). While there has been a reduction in the load of metals entering the Gulf through aerial sources (Bell, 1983), the waste-water effluent continues to be a significant source of trace metals, including lead, zinc, cadmium, copper and manganese (Ward and Young, 1981; Ross and Bidwell, 2001).

The shorelines of the Upper Spencer Gulf are fringed by extensive mangroves, intertidal mudflats and seagrass beds (Ward et al., 1982). These ecosystems are vitally important as they provide habitat, nursery, feeding and breeding grounds for commercially valuable fish and invertebrates (Short and Neckles, 1999). Decreased abundance and distribution of the faunal communities has been found in the vicinity of the discharge site compared with non-affected reference sites (Ward and Hutchings, 1996; Cook, 1999; Ross and Bidwell, 2001). However, the site still supports a diverse range of seagrasses and invertebrate species. Notably the prawn, *L. intermedius*, and the isopod, *P. longicaudata*, are abundant species in the vicinity of the smelter (Ward and Hutchings, 1996; Cook, 1999; Ross and Bidwell, 2001). Long-term history of exposure to metal toxins and the existence of geographically distant, non-exposed populations suggested that these species were ideal for use in assessing the utility of genetic diversity assessment as a biomarker of pollution effects in the Upper Spencer Gulf system. Genetic diversity of the Port Pirie prawn and isopod populations were compared with that of three other non-exposed populations from reference sites.

It is widely accepted in toxicology that different individuals of one species will not react in an identical manner to a toxicant. Likewise, it is then reasonable to suppose that different populations of one species may also not react in an identical manner to a pollutant (Moriarty, 1988). In order to determine whether there were differences in response to metals between the Port Pirie prawn and isopod populations and reference populations, tolerance of the two species to metals in food was assessed. Prawns and isopods were exposed to metal mixtures in the laboratory in a similar ratio to that found in seston near the smelter effluent discharge site. If a selective shift related to metal tolerance had occurred in the Port Pirie population, it was hypothesized that individuals from the contaminated area would survive exposure to higher concentrations of metals than those from unpolluted sites.

In order to provide a more complete evaluation of prawn response to metal pollution in the Port Pirie population, metallothionein levels were also measured in field-collected prawns from the smelter and reference populations. Metal-specific induction of metallothionein in crustacean species in response to both single and mixed metal exposures has been demonstrated in both laboratory (Brouwer et al., 1995; del Ramo et al., 1989; Pederson et al., 1996) and field studies (Francesconi et al., 1998).

2. Materials and methods

2.1. Sampling sites

Prawns and isopods were collected from four sites, one contaminated with trace metals (Port Pirie), and three reference sites. The three reference sites were located at Middle Beach, Edithburgh and Kangaroo Island (Fig. 1). These reference sites have previously been reported to have low metal concentrations (Zann, 1995; Ward et al., 1984). All sites are located along the coastline of South Australia and are ecologically and topographically similar, having shallow water, seagrass meadows (*Zostera* sp. and *Posidonia* sp.) and similar communities of organisms.

2.2. Sampling methods

Prawns and isopods were collected at low tide by repeatedly sweeping the seagrass beds with a net (2 mm mesh). Prawns (30–35 mm) and isopods (10–12 mm) were selected, sorted and transported back to the laboratory where they were held in 20 l aerated tanks for 48 h prior to toxicity tests, or washed with Milli RO® water

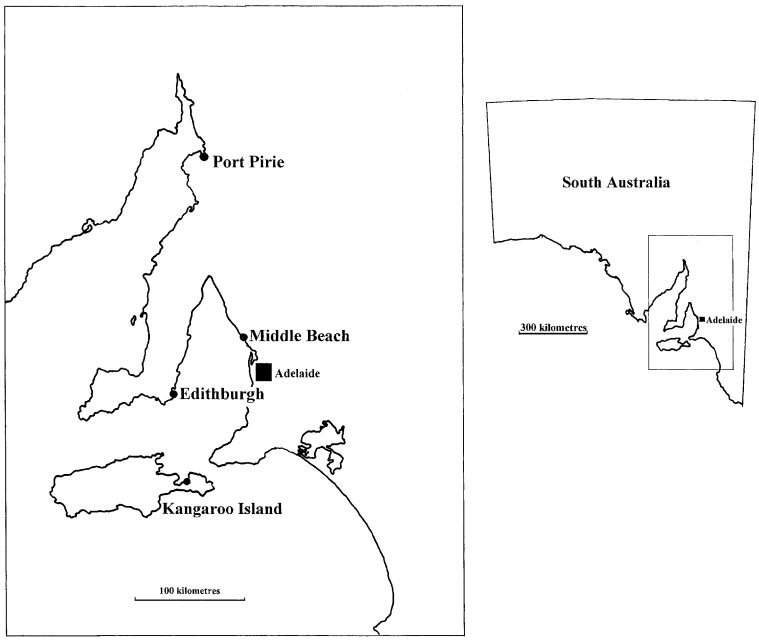

Fig. 1. Map indicating organism collection sites.

and frozen for DNA extraction, and metallothionein measurement.

2.3. Genetic diversity assessment

DNA extraction followed the methods described by Singer-Sam et al. (1989) and Walsh et al. (1991). Amplification of DNA was performed using the method described by Williams et al. (1990) although volumes of reagents were modified. Amplification reactions for *L. intermedius* were carried out in 20 μl volumes (made up with sterile water) containing: 5 ng DNA template, 1 pmol primer (Operon Technologies®, California), 0.5 μM each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 20 mM tris-HCl, 50 mM KCl and 0.5 U Taq® polymerase. Amplification reactions for *P. longicaudata* were carried out in 20 μl volumes (made up with sterile water) containing: 75 ng DNA template, 2 pmol primer (Operon Technologies®, California), 0.5 μM each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 20 mM tris-HCl, 50 mM KCl and 0.5 U Taq® polymerase. Prawn DNA was amplified using a PCR Eppendorf Mastercycler Personal (Eppendorf®, Germany) with PCR times empirically adjusted for optimum amplification of prawn DNA. PCR parameters were: 4 min at 94 °C, followed by 40 cycles of 94 °C for 1 min, 35 °C for 1 min and 72 °C for 2.5 min. Isopod DNA was amplified using a PCR Sprint thermocycler (Hybaid Ltd., UK) with PCR times adjusted for optimum amplification of isopod DNA. PCR parameters were: 3 min at 94 °C followed by 45 cycles of 94 °C for 1 min, 35 °C for 1 min and 76 °C for 2.5 min. Following amplification, the solutions were stored at 4 °C until electrophoresis and visualization.

RAPD-PCR products were electrophoresed in 1.5% agarose gels in TBE (8.9 mM tris-acetate, 8.9 mM boric acid and 0.2 mM EDTA) buffer. Gels were run at 100 V for 1 h and DNA fragments were visualized using a Foto-UV Transilluminator system, and recorded by photography. Ethidium bromide (0.5 μl of 10 mg/ml stock solution) was added to each amplification reaction prior to loading into the gels. Double-stranded DNA molecular weight standards (100 bp ladder; Geneworks®, Australia) were run in the first and last lanes of every gel in order to estimate and score the size of the PCR fragments. Positive and negative controls were run to determine reproducibility and to establish the presence of primer and other PCR artifacts (non-specific primer derived amplification products). Positive controls used DNA pooled from several prawns and this composite DNA mix was run with each gel. The same technique was used with the isopod.

Amplified RAPD fragment bands were scored '1' for their presence and '0' for their absence for each DNA sample. Data were recorded in spreadsheets that tabulated individual banding patterns for each population. Data were analyzed using a program devised to calculate genetic diversity within each of the four populations (Cooper, 2000).

Two indices to estimate genetic diversity were calculated: the band sharing index (BSI) and the matching index (MI) (Lynch, 1990). The BSI calculates the fraction of bands that are shared between individuals in a population to approximate the average identity-in-state for all possible pairs of individuals using the formula:

$$\mathrm{BSI} = 2 \times \mathrm{NAB}/(\mathrm{NA} + \mathrm{NB}),$$

where NAB is the number of molecular weight bands present in both samples and NA and NB are the bands present in the first sample and second sample, respectively.

The value of BSI may range between 0 and 1 with a value of 0 indicating that all individuals are completely different and 1 indicating that they are completely alike with reference to their banding patterns.

The MI calculates the proportion of all identified bands for which two samples match in state, either both present or both absent, using the formula:

$$MI = NAB/NT,$$

where NAB is the number of bands for which two samples match in state, and NT is the total number of bands identified. As with BSI, MI varies between 0 and 1. Two purely random data sets would have a MI of 0.5.

To determine whether adequate numbers of individuals were assessed from each population, individual, cumulative BSI and MI values were graphed, and further individuals assayed until the resultant curve appeared linear for both measures. To ensure adequate numbers were assessed, further individuals ($N = 9$–10) were assayed after the line of apparent visual linearity was reached. Significant differences between population genetic diversity were determined using Students *t*-test: $(BSI_1 - BSI_2)/[\mathrm{var}(BSI_1) + \mathrm{var}(BSI_2)]^{1/2}$, with infinite degrees of freedom (Theodarkis et al., 2001), and variance (var) of the BSI calculated according to Lynch (1990) using a custom designed program (Cooper, 2001).

2.4. Metal tolerance assessment

The tolerance of prawns and isopods from Port Pirie to a metal mixture was compared with that of a reference population (Middle Beach). Initially prawns were exposed to a mixture of the major metals (Zn, Pb, Cd, Cu and Mn) at concentrations and ratios found in the Port Pirie area (Ward et al., 1984; Cook, 1999) in aqueous solution, although it was found that the concentrations required to cause mortality were at the solubility limit of Pb, Zn, Cu, and Mn (MINTEQA2, 1999). The experimental protocol was redesigned and both prawns and isopods were fed food bound trace metals to simulate the most likely field exposure route of trace metals. Prawns and isopods were fed metal mixtures added to dry cat food biscuits in varying concentrations ranging from 0.14 to 26 times that found in the seston at the Port Pirie study site. Final concentrations of trace metals in the food pellets were verified through acid digestion and atomic absorption spectroscopy (Table 1). One food pellet (0.07 ± 0.02 g) was added to beakers filled with 300 ml filtered seawater, each containing 10 prawns or isopods, with four replicates per concentration. Organisms were exposed to metals for 96 h, following a 48 h depuration period. The tests were continually aerated and dead prawns or isopods were removed and recorded every 24 h. 96 h LC50 values were calculated using the trimmed Spearman–Karber method (Hamilton et al., 1977).

Table 1
Concentrations of trace metals in food pellets (μg/g)

Metal concentration as a proportion of seston metals	Pb	Zn	Cu	Cd	Mn
0	0	0	18.9	7.8	0
0.14	47.3	388	35.1	17.0	66.2
0.62	1633	2114	48.4	83.6	396.3
0.85	1520	3736	74.9	118.4	718.0
1.1	3212	4214	84.8	120.8	668.9
2.6	7133	12146	201.6	275.4	1555
6.0	6892	29097	473.8	1149	5333
10	14067	50255	953.7	1574	9161
17	26123	91120	1488	2386	13577
26	34552	136197	1975	4580	22359

2.5. Metallothionein levels

Environmental exposure to metals was also evaluated by measuring the levels of metallothionein in prawns collected from Port Pirie and Middle Beach. Metallothionein levels were determined using the ^{109}Cd saturation method described by Eaton and Toal (1982), which estimates total metallothionein (nmol/g wet weight). Prawns from both sites (collected over different seasons) were assessed for metallothionein induction. Significant differences between population metallothionein levels were assessed using ANOVA followed by Tukey's HSD (Zar, 1984).

3. Results

3.1. Genetic diversity

Forty five primers from the OPB, OPK, OPF, OPN and OPH kits (Operon Technologies®) were screened to isolate those primers amplifying polymorphic, distinct DNA fragments in *L. intermedius* and *P. longicaudata*. Notably, a number of primers generated bands in the negative control—non-specific amplification products under the PCR conditions used—these primers were therefore excluded. The primer OPK-04 (5′-CCGCCCAAAC-3′) was selected for the prawn survey, and the OPB-04 (sequence: 5′-GGACTGGAGT-3′) for the isopods study since these primers yielded reproducible, clear and sharp, polymorphic banding patterns.

3.1.1. Genetic diversity—*L. intermedius*

A total of 107 prawns were assayed from four locations ($N = 25$ from Port Pirie, 29 from Middle Beach, 28 from Edithburgh and 25 from Kangaroo Island). RAPD

analysis using the OPK-04 primer generated a total of 10 bands in all individuals for all populations, all of which were polymorphic to some degree. However, several bands (at 700 and 1000 bp) were common to most individuals in all populations. One band, at 600 bp, was apparently nearly fixed in the Middle Beach population, although was less common in the other three populations. Both BSI and MI values (0.82 and 0.89, respectively) were highest (lowest genetic diversity) in the Port Pirie population. Kangaroo Island had the highest diversity (lowest BSI and MI values) of the four populations, with BSI and MI values of 0.75 and 0.84 respectively. Middle Beach and Edithburgh prawns fell between Port Pirie and Kangaroo Island in both measures of genetic diversity (Fig. 2). Statistically significant differences between populations were detected only between the Kangaroo Island and Port Pirie populations ($p < 0.05$).

3.1.2. Genetic diversity—P. longicaudata

A total of 31 individual, polymorphic bands were identified and scored in isopod samples using the OPB-04 primer. One hundred and 45 individuals in total were assayed for genetic analysis (Port Pirie: $N = 39$, Middle Beach: $N = 48$, Edithburgh: $N = 32$, and Kangaroo Island: $N = 26$). The isopod BSI values for the three reference sites indicate that genetic diversity between these populations (BSI values of 0.45, 0.41, and 0.42 for Middle Beach, Edithburgh and Kangaroo Island respectively) was not significantly different ($p < 0.05$). There was a statistically significant ($p < 0.05$) difference in diversity between the Port Pirie isopod population (BSI = 0.85) and all three reference populations.

The second measure of genetic diversity, the MI, was also calculated to estimate genetic similarities among the four isopod populations. As with the BSI, the MI value determined for the Port Pirie population (MI = 0.95) was closer to one than that of other populations (0.84, 0.83 and 0.84 for Middle Beach, Edithburgh and Kangaroo Island respectively) (Fig. 2). The MI of 0.95 also indicates that the isopods from the contaminated population have a higher level of similar RAPD banding patterns than those individuals within the three reference populations and differ significantly in genetic diversity from reference populations ($p < 0.05$).

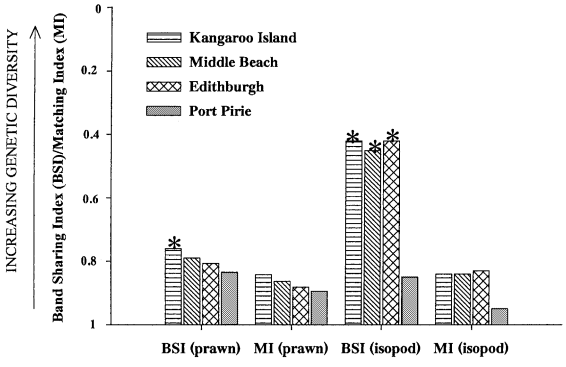

Fig. 2. BSI and MI values for *L. intermedius* and *P. longicaudata* from four populations (∗ indicates statistically significant difference from metal exposed population (Port Pirie)—BSI only).

3.2. Metal tolerance assessment

Prawns were collected from Middle Beach and Port Pirie on three occasions. One water borne metal exposure test was conducted, with identical responses for Middle Beach and Port Pirie populations, indicating no difference in metal tolerance between the populations. However, as mentioned above, since the solubility limit for Pb, Zn, Cu and Mn in this combination had been reached (as determined by MINTEQA2, 1999), a different exposure regime was developed using food exposures. As with the water borne metal exposures, metal tolerance was not significantly different between populations for food borne exposures (Fig. 3). Both populations tolerated high levels of metals in their food, with LC50 values of ~10 times the field levels of metals found in the seston at Port Pirie (roughly 14,000 μg/g Pb, 50,000 μg/g Zn, 950 μg/g Cu, 1500 μg/g Cd and 9000 mg/g Mn). These results indicate that both the Middle Beach and Port Pirie populations can tolerate similar levels of metals in food borne acute exposures. This contrasts with the results from the isopod populations. Isopods were collected from Port Pirie and from a reference site at Middle Beach on six occasions. Toxicity of the metals to the isopods was significantly different between population collection locations ($p < 0.05$) (Fig. 3). For both the reference and contaminated sites, the toxicity of metals was significantly higher (between 1.3 and 1.8 times the seston metal concentration for Middle Beach and 2.3 and 4.3 times the seston metals concentration for Port Pirie) immediately following the

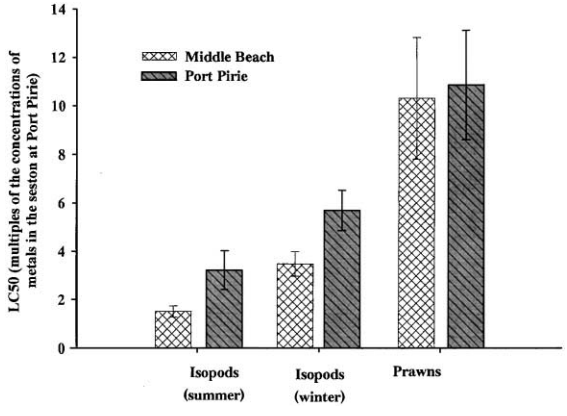

Fig. 3. Response to metals of the pre-exposed (Port Pirie) and reference (Middle Beach) *L. intermedius* and *P. longicaudata* populations to metals in their food in laboratory exposures (LC50 values are multiples of the levels of lead, zinc, cadmium, copper and manganese associated with seston at Port Pirie).

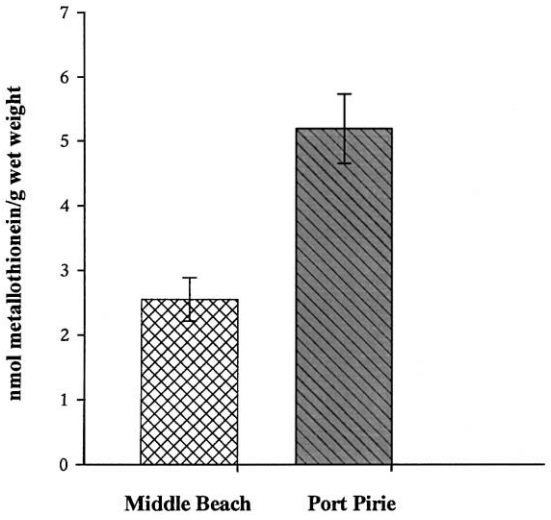

Fig. 4. Metallothionein levels in prawns from the Port Pirie and reference (Middle Beach) *L. intermedius* populations (nmol/g wet weight).

summer period compared with the winter and post-winter samples (between 2.7 and 4.3 times the seston metal concentration for Middle Beach and 4.1 and 7.8 times the seston metal concentration for Port Pirie). Independent of season the Port Pirie isopods exhibited significantly higher LC50 values compared with that of the reference site.

Water quality parameters were measured for exposure tests and fell within acceptable limits for dissolved oxygen (6.7–8.9 ppt), pH (7.9–8.3), salinity (32–34 ppt) and temperature (21–23 °C).

3.3. Metallothionein levels

Metallothionein levels in the field-collected prawns from Port Pirie were significantly higher than in prawns from Middle Beach ($p < 0.01$). Metallothionein levels of 5.2 (+/−0.54) nmol/g wet weight were detected in the Port Pirie prawns while those in Middle Beach prawns were 2.6 (+/−0.33) nmol/g wet weight (Fig. 4). Isopod metallothionein was below the detection limits using this method, so only *L. intermedius* data are reported here.

4. Discussion

Assessing the genetic diversity of populations could be a valuable addition to more traditional tools for determining the effects of environmental pollution on aquatic ecosystems (Nevo et al., 1986; Bickham and Smolen, 1994; Nadig et al., 1998). Following removal of pollution input, alteration of genetic diversity may exhibit effects over a longer time scale than is seen for reduction of population numbers, due to the slow process of incorporation and spread of new alleles through populations (Krane et al., 1999; Bickham et al., 2000). Therefore, in terms of assessing remediation following pollution impact, incorporation of genetic diversity assessment into the suite of assessment parameters may allow greater insight into recovery success.

In this study, the two species gave rise to differing results, both in terms of comparative genetic diversity, and with respect to metal tolerance of the pre-exposed populations compared with reference populations. As discussed below, there are a number of possible reasons for our results.

4.1. Long term metal pollution

Long term metal pollution in the Spencer Gulf may have acted as a cause of the reduction in genetic diversity in the Port Pirie prawn population compared with the Kangaroo Island population, and the lower genetic diversity of the pre-exposed isopod population compared with all reference populations. The main micro-evolutionary forces determining the patterns of genetic structure are genetic drift, gene flow and selection. The observed reduction in genetic diversity could have occurred through several possible mechanisms. Metal pollution may have reduced genetic diversity by causing large-scale mortality in a non-genotype specific manner, producing a random loss of allelic variation associated with population bottlenecks (an extreme form of genetic drift) (Hartl, 1981; Merrel, 1981). Given a lack of historical information on *L. intermedius* and *P. longicaudata* populations in the Spencer Gulf, it is also possible that the Port Pirie populations are the result of past extinction followed by colonization from an outside source populations and subsequent re-expansion (another form of genetic drift). Under this hypothesis the population from Port Pirie would have been formed by few individuals from (an) unknown source population(s) possessing a biased sample of alleles and an associated decrease in allelic variation (Merrel, 1981). Both hypotheses would produce similar results in terms of within population genetic diversity, and although there is no biological evidence to favor either hypothesis, neither can be completely discounted as being contributing factors to the reduced genetic diversity found within the Port Pirie prawn and isopod populations. It should be noted that further biochemical, physiological and genetic studies are required to explain these speculative causal factors.

Reduction in species diversities and densities have previously been reported at the metal contaminated study site in studies by Cook (1999) and Ross and Bidwell (2001). When these data are considered in conjunction with our observations of reduced genetic diversity in the metal exposed isopod population, these results support results published by Krane et al. (1999), Nadig et al. (1998) and Grunwald et al. (1999). The assumption that metal exposure is responsible for the

decreased genetic diversity in the Port Pirie isopod population is further supported by the results of the metal exposure tests. The observation that isopods from the exposed site were more resistant to metals than isopods from a non-exposed population could be the result of selection for metal resistant individuals in the population. Though acclimation cannot be ruled out as the cause of increased resistance, this selection-based hypothesis would account for both increased resistance and the observed decrease in genetic diversity revealed by RAPD analysis.

4.2. Metal tolerance

The Port Pirie and reference *L. intermedius* populations showed similar responses in acute exposure to metals, both in food and water. Both populations tolerated very high levels of metals in laboratory toxicity experiments. Nearly identical tolerance to high levels of metals in adult individuals from both Port Pirie and Middle Beach suggests that there has not been direct selection at the Port Pirie site for metal tolerance. These results agree with those of Kovatch et al. (2000), who reported no difference between a pre-exposed population and a reference population of copepods in their response to metals and PAHs.

The results of the prawn laboratory exposure tests raised the possibility that the tolerance of the reference population to metals may have been a result of current metal exposure and resulting metal tolerance comparable to the Port Pirie population. To test this hypothesis, metallothionein levels were measured for both populations to establish exposure. Many studies have shown that metallothionein is a reliable biomarker for metal exposure (Brouwer et al., 1995; del Ramo et al., 1989; Pederson et al., 1994; Pederson et al., 1996; Francesconi et al., 1998). Metallothionein levels were significantly higher in the Port Pirie population compared with those of the Middle Beach population. These results are consistent with those of Moksnes et al. (1995), who reported an increase of 180% in metallothionein levels in shrimp exposed to cadmium. This suggests that, at least in terms of recent exposure history, the Port Pirie prawns were exposed to significantly higher metals concentrations than those from Middle Beach. The high tolerance to metals in their food by both populations could be the result of a number of mechanisms. These include the use of metal binding enzymes or proteins (such as metallothionein), high toxin excretion efficiency, and the ability to sequester toxins in a 'safe' place within the body. These mechanisms have been shown to be activated under elevated metal concentrations in a number of organisms (Stubblefield et al., 1999), and the results from this study suggest that both populations (and possibly all individuals of this species) possess efficient mechanisms to survive elevated metal concentrations.

4.3. Random amplified polymorphic DNA technique

In our prawn study, using a single primer, RAPDs were able to detect 50 bp or greater differences, sufficient to discriminate divergence of the Port Pirie population from one reference population. Greater degrees of population differentiation of RAPD amplification products in another prawn species (*Penaeus stylirostris*) were reported recently by Aubert and Lightner (2000), possibly as a result of different fragment visualizing techniques. That our study detected only 10 polymorphic RAPD fragments in *L. intermedius* is unlikely to be the result of anonymous primer amplification of conserved regimes within the prawn genome. Over 40 primers were screened and the OPK-04 primer found to generate the most polymorphic clear, reproducible bands. The relatively low number of bands and polymorphisms detected in the prawns, compared with previous studies is unlikely to be an artifact of the primer used, as the many other primers screened did not produce more polymorphic banding patterns. The results may be due to overall low levels of polymorphism in *L. intermedius* or simply interspecies genomic differences among prawns.

4.4. Population genetic drift

Gene frequencies will not remain static from one generation to the next since there will be chance fluctuations in gene frequency (Shorrocks, 1978). Therefore the difference in genetic diversity between the Port Pirie and Kangaroo Island prawn population and the Port Pirie isopod population and all reference populations may be the result of random genetic drift. However, it is generally agreed that selection is a more powerful (and directional) evolutionary force, since large populations are not very susceptible to genetic drift and tend to maintain their original degree of genetic variance (Shorrocks, 1978; Merrel, 1981).

4.5. Geographic distribution of populations

Genetically isolated populations can exhibit lower degrees of variation due to the lack of migration into the populations (Wallace, 1981). The results found in this study could be due to greater isolation of the Port Pirie population compared with the reference populations. Isopod females brood the fertilized eggs in their marsupium, giving rise to postlarva organisms. While details of the reproductive strategies for *L. intermedius* are limited, the prawn *Palaemon lamarrei* (of the same family) have been observed clinging to the mother after hatching (Rebach and Dunham, 1983). This suggests that both the prawn and isopod populations in this study are likely to be discrete, as larval dispersal would be expected to be minimal. Notwithstanding, geographic influence on gene flow cannot be discounted. However,

it should be noted that absence of migration into a population does not necessarily mean low within-population genetic variation, if allelic diversity is already intrinsically high.

5. Conclusions

While a lower level of genetic diversity was detected in the prawns from the metal contaminated site compared with the Kangaroo Island reference site, and in the isopods compared with all reference sites, an immediate, specific, causal mechanism, such as direct selection acting on loci relevant to metal tolerance cannot be established by our data. Nor can the results of this study discriminate between types of mechanisms—direct or indirect selection, genetic drift or bottleneck effects—responsible for the observed genetic diversity results. Notably, this study indicates that long term exposure to metal pollution does not necessarily result in decreased genetic diversity and that a number of reference sites need to be included in genetic diversity analyses. The tolerance of both *L. intermedius* populations exposed in the laboratory to metals suggests selection for metal tolerance may not have occurred in the prawn population from Port Pirie. The tolerance also suggests that all individuals of the species may possess mechanisms to cope with elevated metals in their environment. This contrasts with the isopods, with clear genetic diversity differences possibly resulting from selection under long term metal contamination. This indicates that comparisons of genetic diversity of populations from polluted sites with reference sites should include more than one species, particularly if genetic diversity is being used to determine cause/effect relationships, or to gauge remediation success.

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