eThekwini Mussel Watch Programme

Surveys made in 2015




Final Report

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1. Introduction

The eThekwini Mussel Watch programme uses mussels as sentinel organisms to monitor nearshore marine water quality along the eThekwini shoreline. Mussel Watch programmes[1] have been implemented in many parts of the world for this purpose (*e.g.* Monirith *et al.* 2002, Andral *et al.* 2004, Liu and Kueh 2005, Mubiana *et al.* 2005, Wei *et al.* 2006, Isobe *et al.* 2007, Kimbrough *et al.* 2008).

The basis for using bivalves (*e.g.* mussels, oysters) as sentinel organisms for pollution monitoring is their ability to bioaccumulate contaminants in their tissue to a degree that is proportional to the contaminants bioavailability. Many contaminants that bivalves accumulate undergo minimal metabolic transformation in their tissue, unlike in other organisms such as fish where substantial metabolic transformation may occur. This and the fact that bivalves are sessile make them good indicators of 'recent' (one to a few months) local contamination of surrounding waters (Roesijadi *et al.* 1984, Sericano 1993, Kimbrough *et al.* 2008).

There are other reasons why bivalves are useful sentinel organisms. First, they are usually widely distributed, albeit not necessarily the same species. This makes them valuable indicators of local conditions and for broader-scale comparison when they are collected from multiple locations. Second, they are usually abundant and easy to collect and identify. Third, they bioaccumulate contaminants in their tissue to far higher concentrations than present in the water column. This makes the detection and measurement of contaminant concentrations in the laboratory easier. Fourth, contaminant concentrations in the water column are usually highly variable due to water column turbulence/mixing (*e.g.* currents) and variable anthropogenic inputs. The chemical analysis of water samples thus provides a snapshot of coastal water quality at the time of monitoring and important contamination events may be missed. Bivalves, in contrast, provide a more temporally integrated measure of contamination since they achieve equilibrium with the surrounding water and take time to depurate contaminants.

The monitoring of contaminant concentrations in bivalves is also useful for assessing possible risks to the health of humans that consume these organisms.

This report analyses and discusses the findings of the 2015 survey of the eThekwini Mussel Watch programme. The Mussel Watch Programme has two primary objectives. The first is to determine if concentrations of chemicals in the tissue, and bacteria in the mantle cavity of mussels provide evidence for nearshore coastal water quality impairment along the eThekwini shoreline. The second is to determine if concentrations of chemicals in the tissue and bacteria in the mantle cavities of the mussels pose a potential risk to the health of human consumers.

2. Materials and Methods

2.1. Sentinel organism

The brown mussel, *Perna perna*, is used as the sentinel organism for the eThekwini Mussel Watch programme for several reasons. First, *P. perna* is distributed along much of the South African shoreline and is adapted to local conditions. Second, *P. perna* is easier to collect and measure morphometrically (*e.g.* length) in the laboratory compared to other potential sentinel organisms (*e.g.* oysters), which are either present at lower densities and/or are harder to collect and measure morphometrically (*e.g.* due to irregular shell shape).

Oysters tend to bioaccumulate copper and zinc to higher concentrations in their tissue than mussels, while mussels tend to bioaccumulate higher concentrations of chromium and lead in their tissue, but there is generally little difference between mussels and oysters in terms of the bioaccumulation of other chemicals (Kimbrough *et al.* 2008). It is thus assumed that the status of coastal water quality and human health risks associated with the consumption of oysters and other bivalves is addressed by the use of *P. perna*,

[1] While the term Mussel Watch is used to describe this type of monitoring, mussels are not the only organisms used as sentinels. Oysters are widely used for this purpose, because mussels are not always present at all locations of interest and/or because mussels and oysters accumulate chemicals/contaminants to different degrees in their tissue.

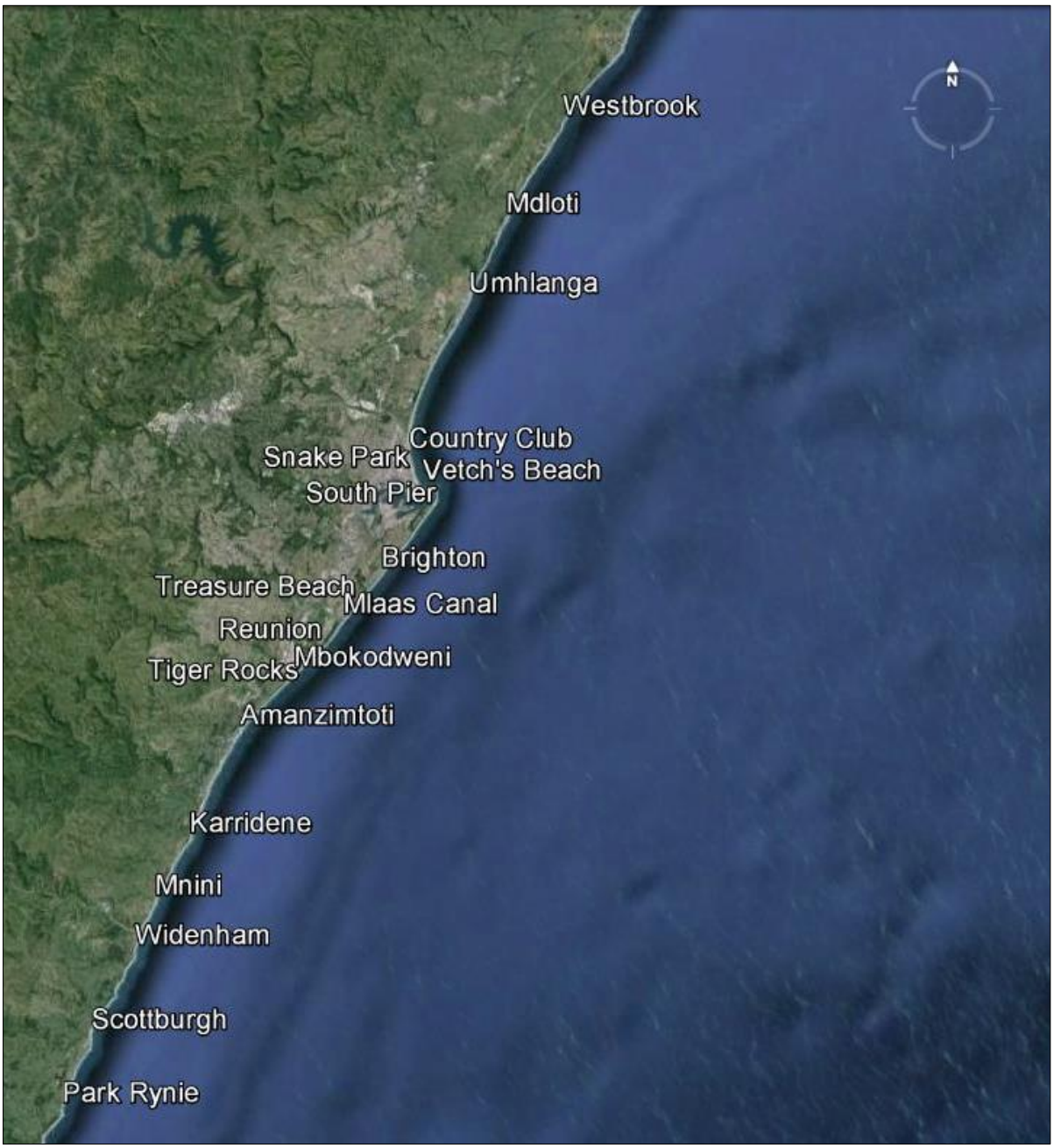

Figure 1. Aerial view the eThekwini area of KwaZulu-Natal showing the positions of Mussel Watch sampling locations.

with the probable exception of copper and zinc. Third, studies have shown that *P. perna* has the propensity to bioaccumulate contaminants in its tissue and is thus a suitable sentinel organism for coastal water quality status and trends monitoring (*e.g.* Banaoui *et al*. 2004, Sokolowski *et al.* 2004, Francioni *et al.* 2007). Lastly, *P. perna* is collected and consumed by South Africans, in some cases on more or less subsistence basis.

2.2. Fieldwork

In August of 2015, CSIR scientists collected mussels by hand from the rocky intertidal at 20 locations between Westbrook Beach in the north and Park Rynie in the south (Figure 1). About 40 - 120 mussels were collected at each location depending on their size. The mussels were wrapped in aluminium foil and held on ice until return to the laboratory. Mussels destined for chemical analysis were frozen pending further processing. Mussels destined for microbiological analysis were processed immediately on return to the laboratory.

2.3. Sample analysis

2.3.1. Mussel processing

Mussels destined for chemical analysis were partially thawed and their total length (anterior umbo to posterior growing lip) measured using

vernier callipers. Mussels between 60 - 80 mm length were targeted. Mussels of this length were, however, not always available at the different collection locations. Mussels with a total length as small as 44.34 mm and as large as 105.04 mm were consequently analysed.

The mussels were shucked whilst partially thawed, their byssus threads trimmed, and the tissue wet weight of each individual measured. The tissue of 23 - 75 mussels depending on their size were composited in acid and solvent rinsed glass jars with foil lined lids, and then frozen until analysis.

All apparatus used to clean, shuck and macerate the mussels was comprised of non-contaminating material (*e.g.* stainless steel knives), and was cleaned between the processing of mussels from different locations by sequentially rinsing in 10% nitric acid, hexane and deionised water.

2.3.2. Laboratory analyses

2.3.2.1. Chemicals analysed[2]

The suite of chemicals that were analysed for in mussel tissue is provided in Table 1. The chemicals were identified based on the fact that many are common contaminants of fish and shellfish tissue in other parts of the world, many present known or strongly suspected risks to the health of humans (*e.g.* are known or suspected carcinogens), and many have been responsible for the issuance of fish and shellfish consumption advisories in other parts of the world. Many of the chemicals have been identified as priority pollutants by the United States Environmental Protection Agency. The production and use of some of the chemicals (*e.g.* DDT, polychlorinated biphenyls) is banned or restricted under conditions of the Stockholm Convention on Persistent Organic Pollutants, because of the ecological and human health risks they pose.

2.3.2.2. Tissue moisture content

The moisture content of mussel tissue was determined by drying a known mass of wet tissue in an oven at 80^{o}C for 24 hrs. The moisture content was taken as the difference in weight before and after drying.

Table 1. Suite of chemicals analysed in the tissue of mussels collected in Augsut 2015 for the eThekwini Mussel Watch programme.

Metals	Polycyclic aromatic hydrocarbons	Organochlorine pesticides	Polychlorinated biphenyls
Aluminium	Naphthalene	Heptachlor	PCB 8
Iron	Acenaphthylene	Heptachlor epoxide	PCB 18
Arsenic	Acenaphthene	Aldrin	PCB 28
Cadmium	Fluorene	γ-BHC	PCB 44
Copper	Phenanthrene	α-BHC	PCB 52
Chromium	Anthracene	β-BHC	PCB 66
Manganese	Fluoranthene	δ-BHC	PCB 77
Mercury	Pyrene	trans-Chlordane	PCB 101
Nickel	Benz(a)anthracene	cis-Chlordane	PCB 105
Lead	Chrysene	Oxychlordane	PCB 118
Selenium	Benzo(b) & (k)fluoranthene	Dieldrin	PCB 126
Zinc	Benzo(a)pyrene	p'p'-DDE	PCB 128
	Indeno(1,2,3-cd)pyrene	p'p'-DDD	PCB 138
	Dibenzo(a,h)anthracene	p'p'-DDT	PCB 153
	Benzo(g,h,i)perylene	Endrin	PCB 169
		Endrin aldehyde	PCB 170
		Endrin ketone	PCB 180
		α -Endosulfan	PCB 187
		β-Endosulfan	PCB 195
		Endosulfan sulfate	PCB 206
		Methoxychlor	PCB 209

[2] Chemical concentrations are presented on a wet weight basis unless otherwise stated.

2.3.2.3. Metal analysis

The mussel tissue was freeze dried and ball-milled. Approximately 1 g of the tissue was then transferred to high-pressure microwave extraction vessels containing 10 ml of HNO_3. The tissue was digested with microwave assistance. The digestate was filtered, diluted to volume, and the concentrations of trace metals were detected and quantified using Inductively Coupled Plasma Mass Spectrometry and Inductively Coupled Plasma-Atomic Emission Spectrometry, or using Cold Vapour Atomic Absorption Spectrometry for mercury.

2.3.2.4. Organic chemical analysis

Samples were extracted using dichloromethane. The extracts were cleaned-up using Gel Permeation Chromatography or silica column clean-up. The final extracts were then analysed by Gas Chromatography with Electron Capture Detection (dual column) and confirmed using Gas Chromatograph Tandem Mass Spectrometry.

2.3.2.5. Microbiology

About 5 - 6 mussels collected at each location were shucked fresh. Depending on the test about 10 - 25 g of the tissue was added to 90 or 250 ml of peptone salt solution and mechanically stomached for about one minute. Suspensions from the stomached samples were then used for *Escherichia coli* and Salmonella determination, as per SANS (2007) and SANS (2008).

2.3.2.6. Quality assurance and quality control

Various procedures were followed to assess laboratory analytical performance (Tables 2 and 3). For metals, extraction efficiency was assessed against a certified reference material. For organic chemicals, matrix spike samples, laboratory control samples, duplicate samples and method blanks were analysed with batches of samples. Samples were also spiked with a surrogate to determine recovery. Acceptable recoveries for metals were 75 - 120% and for polycyclic aromatic hydrocarbons, organochlorine pesticides and polychlorinated biphenyls were 50 - 150%.

For microbiological analyses, positive, negative and sterility samples were run for quality control and quality assurance purposes.

2.4. Risk assessment

2.4.1. General

Risk assessment is a process by which the degree and nature of a risk is characterised. The outcome of a risk assessment determines if there is a need for risk management, that is, whether prevention and control measures or options can and should be implemented to reduce the risk. In the context of seafood consumption this may include a ban on fishing or shellfish collection or an advisory on the consumption of particular seafood species caught or collected in a waterbody.

The risk assessment approach followed in this study is for all intents and purposes identical to the approach recommended by the United States Environmental Protection Agency (USEPA, 2000a,b) for evaluating human health risks arising from exposure to chemicals through the consumption of fish and shellfish (*i.e.* a dietary pathway). The risk assessment process comprises four stages, namely:

- Hazard identification,
- Dose-response assessment,
- Exposure assessment,
- Risk characterisation.

2.4.2. Hazard identification

Screening Values (sometimes called Action Levels), which represent concentrations of chemicals in fish and shellfish tissue that are of potential human health concern were calculated for two components of the South African population, namely subsistence and recreational consumers (these components are discussed further below). Concentrations of some chemicals such as arsenic in mussels collected at all locations along the eThekwini shoreline exceeded Screening Values for carcinogenic health risks for recreational and subsistence fishers and thus indicated the need for a detailed assessment of potential health risks to these consumers.

2.4.3. Dose-response assessment

The quantitative relationship between a chemical

dose and the incidence of carcinogenic and non-carcinogenic health effects in humans was assessed using toxicity data from the United States Environmental Protection Agency Integrated Risk Information System (IRIS).

2.4.4. Exposure assessment

The goal of exposure assessment is to identify populations that might be exposed to chemicals of concern, the pathway through which they may be exposed, and the variables for the exposure assessment that allow the chemical dose to be quantified. The degree to which a risk assessment represents an exposed population depends on various assumptions. Unfortunately, many of the variables required to calculate exposure have not as far as the scientists that prepared this report could establish been quantified for the South

Table 2. Extraction efficiency of metals from certified reference material.

	Extraction Efficiency (%)		
Analyte	**Mean**	**Max**	**Min**
Aluminium	-	-	-
Arsenic	105.2	107.8	102.7
Cadmium	93.2	94.6	91.8
Chromium	106.2	111.6	100.8
Copper	94.9	96.4	93.4
Iron	101.4	102.2	100.6
Lead	96.2	100.2	92.1
Manganese	94.2	94.2	94.2
Mercury	103.5	104.6	102.4
Nickel	93.5	94.3	92.8
Selenium	91.1	91.3	91
Zinc	93.5	97.1	89.9

Table 3. Relative Percent Difference between duplicate sample analysis, and percentage recovery (%) of analytes from laboratory control samples and matrix spikes of mussel tissue.

	Relative Percent Difference (%)			Laboratory Control Sample (%)			Matrix Spike Recovery (%)		
Analyte	**Mean**	**Max**	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**	**Min**
Organochlorine pesticides									
Heptachlor	-	-	-	84.0	107.0	61.0	141.0	145.0	137.0
Aldrin	-	-	-	109.0	110.0	108.0	101.0	110.0	92.0
gamma-BHC	-	-	-	97.0	102.0	92.0	142.0	143.0	141.0
Dieldrin	-	-	-	115.0	118.0	112.0	124.0	125.0	123.0
p'p'-DDE	-	-	-	55.0	108.0	2.0	138.5	140.0	137.0
p'p'-DDD	-	-	-	134.5	139.0	130.0	89.0	89.0	89.0
p'p'-DDT	25	-	-	87.0	110.0	64.0	77.5	92.0	63.0
Endrin	-	-	-	109.5	114.0	105.0	111.5	120.0	103.0
Polycyclic aromatic hydrocarbons									
Naphthalene	-	-	-	92.5	100	85	52.0	50	54
Fluorene	-	-	-	88.5	100	77	44.0	77	11
Phenanthrene	7.7	-	-	95.0	105	85	125.5	126	125
Fluoranthene	4.1	4.1	4.1						
Pyrene	-	-	-	115.5	122	109	133.5	150	117
Benz[a]anthracene	-	-	-	100.0	100	100	97.5	106	89
Chrysene				112.5	114	111	90.5	102	79
Benzo[a]pyrene	41.0	18	64	104.5	108	101	81.0	112	50
Dibenz[a,h]anthracene	-	-	-	92.5	100	85	52.0	50	54
Surrogate: TER-D14	-	-	-	88.5	100	77	44.0	77	11
Polychlorinated biphenyls									
PCB # 52	-	-	-	134.0	136	132	103.0	110	96
PCB # 118	-	-	-	130.0	133	127	133.0	137	129
PCB # 153	-	-	-	115.0	117	113	103.5	104	103
PCB # 180	-	-	-	112.5	117	108	101.0	104	98

African population. It was thus necessary to make informed assumptions or to use default values prescribed by the United States Environmental Protection Agency (USEPA, 2000a,b).

Whether these assumptions are valid for recreational and subsistence consumers in the eThekwini area is uncertain.

2.4.4.1. Identification of exposed populations

As mentioned previously, two exposed populations were identified, namely subsistence and recreational consumers. These populations are distinguished by the amount of seafood they consume. Subsistence consumers, through socio-cultural practices or necessity (*e.g.* economic reasons) consume larger amounts of seafood compared to recreational consumers and are, therefore, potentially at greater risk of exposure to chemicals accumulated by fish and shellfish. Recreational consumers consume seafood at a lower rate, but which is nevertheless considered to exceed the rate for the general population.

The United States Environmental Protection Agency (USEPA, 2000a,b) recommends consideration of sensitive and insensitive segments of exposed populations. Sensitive segments are defined as infants, children and females of childbearing age, while insensitive segments are adult males and adult females beyond their childbearing years. These segments were not considered for this risk assessment as the consumption rate was considered to be proportional to body weight. Assessing the risk posed to an average adult thus concurrently caters for the risk posed to females of childbearing age, children and infants.

2.4.4.2. Exposure pathway

Although several exposure pathways could conceivably result in human exposure to the chemicals of concern, for the purposes of this risk assessment the consumption of mussels was considered the only source of exposure. This is an obvious simplification of the real-world situation since many foodstuffs contain metals and polycyclic aromatic hydrocarbons amongst other chemicals. Other exposure pathways besides consumption, such as inhalation, may also result in exposure.

2.4.4.3. Quantification of exposure

An individual's exposure through a seafood consumption pathway depends upon several factors, including the concentration of contaminants in seafood, the amount of seafood consumed, how often and for how long seafood is consumed, and the consumer's body weight. Because exposure occurs over time the total exposure is divided by a time period of interest to obtain an average exposure rate per unit time. When this is expressed as a function of body weight the exposure rate is referred to as the Chemical Specific Daily Intake (CDI). The Chemical Specific Daily Intake of chemicals in mussels was calculated as:

$$CDI = (C \times CR \times EF \times ED)/(BW \times AT) \qquad \text{Equation 1}$$

Where:
CDI = Chemical Specific Daily Intake ($mg.kg^{-1}$-day),
C = Chemical concentration in mussel tissue ($mg.kg^{-1}$),
CR = Consumption rate (kg per day),
EF = Exposure frequency (days per year),
ED = Exposure duration (years),
BW = Body weight (kg),
AT = Averaging time for exposure duration (30 years × 365 days per year for non-carcinogens and 70 years × 365 days per year for carcinogens).

2.4.4.4. Chemical concentrations

The concentrations of chemicals in mussel tissue (variable C in Equation 1) are provided in Appendices 1 - 4. Laboratory methods and instruments do not allow for the accurate measurement of chemicals below a certain concentration, known as the method detection limit. The method detection limit is instrument and method specific. Chemical concentrations reported as below the method detection limit are usually referred to as non-detects. Because there is no certainty that a chemical reported as below the method detection limit was not present in the tissue of mussels a decision must be made on how to treat non-detects. The most conservative approach is to substitute non-detects with a concentration equivalent to one-half the method

detection limit rather than a value of zero. This is consistent with United States Environmental Protection Agency risk assessment guidance (USEPA, 2000a,b). However, there may be problems associated with this approach if the method detection limit is not sufficiently low. This is because the total concentrations of some chemicals are used to assess risk, but the chemicals are comprised of 'chemical building blocks'. For example, the total polychlorinated biphenyl concentration is the sum of 'chemical building blocks' called congeners. If the method detection limit is not sufficiently low then replacing the concentration of each 'chemical building block' with a concentration equivalent to one-half the method detection limit may result in the total concentration being identified as posing a risk to human consumers even though the concentrations of all congeners were below the method detection limit. For the purposes of this risk assessment, chemical concentrations below the method detection were substituted with a concentration equivalent to zero.

Inorganic and organic forms of arsenic are present in the tissue of fish and shellfish however only the inorganic forms of arsenic are considered toxic (carcinogenic) to humans. Analysing the inorganic forms of arsenic is complicated and expensive. Thus for the purpose of this report a similar approach used by most laboratories were used, namely to analyse for total arsenic and thereafter assume that a scientific literature defined proportion of total arsenic is of an organic form. Scientific literature sues that inorganic arsenic usually comprises between 1 - 10% of the total arsenic concentration in fish and shellfish (Goessler *et al.*, 1997; Donohue and Abernathy, 1999, Schoof *et al*., 1999a,b; Morrisey and Abernathy, 1999; De Gieter et al., 2002; Li *et al*., 2003; Fabris et al., 2006; Peshut *et al*., 2008). In order to avoid overstating a health risk, a 10% adjustment factor is usually used as an estimate for the proportion of inorganic arsenic present in fish and shellfish. This approach is consistent with recommendations of the United States Environmental Protection Agency (USEPA 2000). Nevertheless, recent studies have indicated that inorganic arsenic concentrations in fish and shellfish are usually below 5%. Inorganic arsenic concentrations analysed in the tissue of mussels collected along the eThekwini shoreline in previous surveys (2013 and 2014) showed that inorganic arsenic concentrations contributed a median of 2.2% to the total arsenic concentration. For comparative purposes risk was also characterised for contributions of inorganic arsenic to total arsenic at 5% and 2.2%.

Mercury is present in fish and shellfish tissue in two predominant forms, namely elemental mercury and methylmercury. The most toxic form is methylmercury. Analysing for methylmercury is expensive and the approach followed in this study was to analyse for elemental mercury and assume that all of this mercury was present as methylmercury. This approach is valid in that the contribution of methylmercury to total mercury in fish and shellfish typically exceeds 90% (>95% - Bloom, 1992; >96% - Kim, 1995; 90 to 100% - USEPA 2000a, 2009; 98% - Hammerschmidt and Fitzgerald, 2006; >95% - Senn *et al*., 2010), although the contribution can be variable (45 to 124% - Kannan *et al*., 1988; 43 to 76% - Forsyth *et al*., 2004; 60 to 100% - Storelli *et al*., 2005). Assuming that all mercury is present as methylmercury is thus a conservative approach.

A number of polycyclic aromatic hydrocarbon isomers were detected in mussel tissue. Of these only benzo(a)pyrene has a Cancer Slope Factor (latter term discussed below). To estimate the risk of exposure to polycyclic aromatic hydrocarbons in the tissue of mussels a Toxic Equivalency Factor approach was followed. This involved expressing the carcinogenic potency of six isomers relative to benzo(a)pyrene and then summing the potencies and that for benzo(a)pyrene to derive the Toxic Equivalency Factor (USEPA, 2000a,b).

2.4.4.5. Consumption Rate

The consumption rate (variable CR in Equation 1) is critical for calculating the Daily Intake. As far as the scientists that prepared this report could determine a quantitative study of seafood consumption rates by recreational and subsistence consumers in the eThekwini area of KwaZulu-Natal has not been performed. Nel and Steyn (2002) provide average per capita fresh and canned fish consumption rates

for South Africans of 1 - 5 years, 6 - 9 years, and 10 years and older as 6.7, 7.2 and 11.77 - 15.13 g per day respectively (two approaches were used to define intake for the 10 years and older cohort). However, only a low proportion of the study participants reported consuming fish and shellfish. If only study participants consuming fish and shellfish are considered then the consumption rates increased to 89.8, 85.1 and 113.8 or 125.28 g per day respectively. For the purposes of this risk assessment the consumption rate for recreational consumers was taken as the 90th percentile of the average per capita consumption of fish and shellfish in the United States of America, at 17.5 g per day. This consumption rate slightly exceeds the average consumption rate for South Africans of 10 years and older and thus provides a conservative estimate of risk. The consumption rate for subsistence consumers was taken as the 99th percentile of the average per capita consumption of fish and shellfish in the United States of America, at 142.4 g per day. This consumption rate slightly exceeds the consumption rate of South Africans of 10 years and older that reported they consume fish and shellfish in the survey by Nel and Steyn (2002) and thus also provides a conservative estimate of risk.

Since consumers usually find it difficult to determine their consumption rate it is worthwhile placing the abovementioned consumption rates into context. The typical weight of (frozen) packaged fish fillets (*e.g.* hake) purchased in stores in South Africa is about 100 - 120 g. A standard can of tuna purchased from retail outlets weighs 170 g including packing liquid (oil or water), and about 120 g after draining. A 'large' can of foodstuff typically weighs 410 g. In a South African restaurant, a fish serving is typically of the order of 180 - 280 g in wet weight. The United States Environmental Protection Agency (USEPA, 2000a,b) considers the average size of a fish or shellfish meal for adults of 70 kg weight in the United States of America to be 227 g before cooking. This equates to a meal of about two fish fillets purchased in South African stores and about the average fish meal size in a restaurant. The same meal size has been assumed for this study. At a meal size of 227 g the ingestion rates equate to a little more than two meals a month and 28 meals a year for recreational consumers and about 19 meals a month and 229 meals a year for subsistence consumers.

2.4.4.6. Chemical absorption

It was assumed the entire concentration of the chemicals analysed in mussel tissue ingested by humans is absorbed across the intestinal tract.

2.4.4.7. Exposure frequency

An exposure frequency of 365 days per year was assumed for the chemical specific Daily Intake calculation, a standard practice for human health risk assessment.

2.4.4.8. Exposure duration

The exposure duration is the period over which exposure occurs at the concentration and ingestion rate specified. As is the case for other variables in Equation 1 the period that subsistence and recreational consumers might consume mussels collected in the eThekwini area is unknown. Bradshaw *et al*. (2011) estimated the average life expectancy at birth for males and females in South Africa in 2011 at 57.2 and 62.8 years respectively. This provides an average life expectancy for South Africans of 60 years. Since the general approach in risk assessment is to overestimate risk by using conservative values for variables in risk equations and a large proportion of South Africans can be expected to have a lifespan longer than 60 years, a life expectancy of 70 years was used in this risk assessment. This assumes an individual will live in the same area for a 70 year period and will consume mussels contaminated at or above the level of concern during this period. Additional motivation for using this exposure period is that the United States Environmental Protection Agency's Integrated Risk Information System (IRIS) assumes a 70 year lifetime for the derivation of cancer slope factors. The use of a 70 year life expectancy thus avoids the need to adjust cancer slope factors to a shorter life expectancy.

An exposure period of thirty years was used to assess non-carcinogenic risk. This default value is recommended by the United States Environmental Protection Agency (USEPA, 2000a,b).

2.4.4.9. Averaging time

As discussed earlier, exposure to contaminants in seafood occurs over time. Therefore, the total exposure is divided by the time period of interest to obtain an average exposure rate per unit time. When this rate is expressed as a function of body weight the resulting exposure rate is referred to as the Daily Intake expressed in milligrams of a chemical taken into the body per kilogram body weight per day. The averaging time for estimating carcinogenic risk was 25 550 days, the number of days in a 70 year exposure period. The averaging time for assessing non-carcinogenic risk was 10 950 days, the number of days in a 30 year exposure period. This assumes that fishers will consume mussels collected from the same location for these periods.

2.4.4.10. Body weight

There is conflicting information on the average body weight of South Africans. The South African Demographic and Health Survey for 2003 (DOH, 2007) provides the average bodyweight for South African's of 15 years and older at 66 kg for males and 68 kg for females. The average body weight is thus 67 kg. The South African National Health and Nutrition Examination Survey (Shisana *et al*., 2013) provides the average bodyweight for South African's of 15 years and older at 67.3 kg for males and 72.2 kg for females. The average body weight is thus 69.8 kg. For the purposes of this study an average body weight of 70 kg was used as it provides a conservative estimate of risk. The United States Environmental Protection Agency's Integrated Risk Information System (IRIS) also assumes a 70 kg adult body weight for the derivation of Cancer Slope Factors.

2.4.4.11. Cooking loss of contaminants

Cooking can lead to the loss of certain chemicals from the tissue of fish and shellfish (*e.g.* Armbruster *et al*., 1987; Zabik *et al*., 1996; Salama *et al*., 1998), with a concomitant lowering of the risk profile. This is significant for organic chemicals, such as polychlorinated biphenyls, which are usually associated with lipids that are commonly lost during the cooking process. Chemicals may also be volatised during the cooking process. However, cooking loss will not result if a stew-type meal is prepared, that is, the lost lipids are not allowed to 'escape'. The situation is slightly different for metals, which can become concentrated in fish and shellfish tissue due to fluid loss, although their bioaccessibility may decrease. This is in spite of the fact that a significant proportion of the metal content in fish and shellfish may be lost during the cooking process, although this is metal specific (Metian *et al*., 2009). However, several workers have reported no loss of contaminants during cooking of eels and fish (*e.g.* Trotter *et al*., 1989; Moya *et al*., 1998).

Cooking loss was not incorporated into this risk assessment given the incomplete information on how each chemical is affected by cooking. This is the most conservative approach and is in agreement with United States Environmental Protection Agency (USEPA, 2000a,b) guidance on fish and shellfish consumption advisories, which recommends that cooking loss should only be considered if there is information on how methods of preparation influence chemical concentrations in fish and shellfish tissue. However, some agencies recommend reducing chemical concentrations by up to 50% for polychlorinated biphenyls and similar chemicals that have a high octanol/water partition coefficient (K_{ow} > 3) and are thus concentrated in fatty tissue rather than muscle.

2.4.5. Risk Characterisation

Risk characterisation integrates the results of the exposure assessment with chemical toxicity information to derive estimates of risk. Non-carcinogenic and carcinogenic risk estimates are calculated separately because of fundamental differences in their critical toxicity values.

2.4.5.1. Non-carcinogenic risk

In general humans that consume contaminated seafood are exposed to low concentrations of chemicals over an extended period. This type of exposure rarely results in acute toxicity, that is, exposure to a single high dose of a chemical. However, long-term exposure may result in chronic toxicity. The potential for chronic, non-carcinogenic health effects was thus evaluated by calculating the ratio of chemical exposure to an Oral Reference Dose (RfD). This ratio of exposure to toxicity for an

individual chemical, referred to as a Hazard Quotient (HQ), was calculated as:

HQ = CDI/RfD Equation 2

Where:
HQ = Chemical specific hazard quotient (unitless),
CDI = Chemical specific daily intake ($mg.kg^{-1}$-day),
RfD = Chemical specific reference dose ($mg.kg^{-1}$-day).

The oral reference dose is an estimate, with an uncertainty spanning perhaps an order of magnitude (a tenfold difference), of the daily oral exposure of a population, including sensitive subpopulations, to a potentially hazardous material that is likely to be without an appreciable risk of deleterious non-carcinogenic effects over a lifetime (USEPA, 2000b). The underlying assumption of a reference dose is that there is a threshold dose below which there is a negligible risk that certain toxic effects will occur.

Because of uncertainty associated with toxicity data 'safety factors' are included, resulting in a lower and more protective reference dose. If a Hazard Quotient exceeds a value of one (*i.e.* exceeds the Oral Reference Dose) then individuals may be at risk. The magnitude of the risk can be inferred from the degree to which the reference dose is exceeded. If the Hazard Quotient is only slightly above a value of one then the dose will likely fall below the toxic effect level because of the abovementioned safety factors. However, a Hazard Quotient is not linear, with the result that a Hazard Quotient of four does not imply a four times greater risk compared to a Hazard Quotient of one. Rather, the United States Environmental Protection Agency (USEPA, 2000b) suggests that a Hazard Quotient of less than one should be interpreted as 'no cause for concern' whereas a Hazard Quotient exceeding one should indicate some cause for concern.

To estimate the cumulative potential for non-carcinogenic effects due to simultaneous exposure to multiple chemicals in mussel tissue, Hazard Quotients for all chemicals and health effects were summed to derive a Hazard Index. The Hazard Index is interpreted in the same manner as the Hazard Quotient, that is, a Hazard Index less than one should be interpreted as no cause for concern whereas a Hazard Index exceeding one should indicate some cause for concern. Although many workers re-investigate Hazard Indices exceeding a value of one by then only considering groups of toxicologically similar chemicals (*i.e.* with similar health effects or that affect the same organ), this approach was not followed in this risk assessment.

2.4.5.2. Carcinogenic risk

The potential health risk posed by chemicals identified as (probable) carcinogens was estimated as the incremental probability of an individual developing cancer over a lifetime of exposure. The United States Environmental Protection Agency (USEPA, 2000b) assumes that a threshold dose does not exist for carcinogens and that any dose can contribute to carcinogenic health risk. In other words, there is never a zero probability of cancer risk when exposed to carcinogenic chemicals. Carcinogenic risk was calculated as an Excess Cancer Risk (ECR), as:

$ECR = CDI \times CSF$ Equation 3

Where:
ECR = Excess Cancer Risk (unitless),
CDI = Chemical specific daily intake ($mg.kg^{-1}$-day),
CSF = Chemical specific cancer slope factor ($mg.kg^{-1}$-day)

The Cancer Slope Factor (CSF) is an upper-bound estimate, approximating 95% confidence limits, of the probability an individual will develop cancer over a lifetime as a consequence of exposure to a given dose of a specific carcinogen (USEPA, 2000b). Current regulatory practice suggests there is no 'safe dose' of a carcinogen and that a very small dose of a carcinogen will give a very small cancer risk. Cancer risk estimates are, therefore, not yes/no answers, but measures of probability. Such measures, however uncertain, are useful in determining the magnitude of a cancer threat because any level of a carcinogenic contaminant carries an associated risk. The interpretation of Excess Cancer Risk thus requires that an acceptable increase in cancer risk be defined. This is referred to as the acceptable risk level. There is no universally accepted acceptable risk level. The

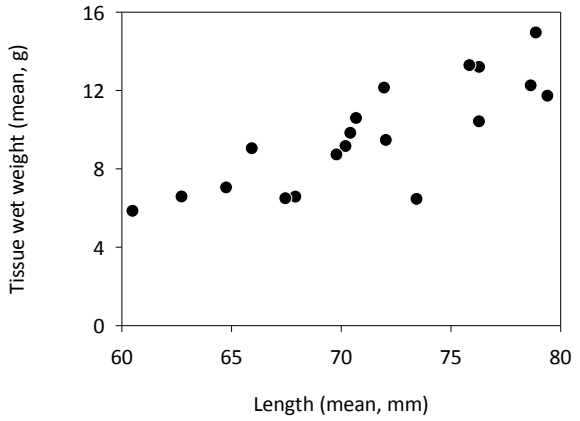

Figure 2. Relationship between the total length and wet tissue weight of mussels collected along the eThekwini shoreline in August 2015.

United States Environmental Protection Agency (USEPA, 2000b) considers risk levels between 10^{-4} (one excess case of cancer for every 10 000 persons) and 10^{-6} (one excess case of cancer for every 1 000 000 persons) to be acceptable for the purpose of issuing fish and shellfish consumption advisories. Acceptable risk levels of 1×10^{-5} or 1×10^{-6} are most commonly used. However, because of the well-documented health benefits of consuming fish and shellfish some jurisdictions consider a risk level of 1×10^{-4} as acceptable. Risks above 1×10^{-4} are nearly always considered unacceptable. For this risk assessment the acceptable risk level was defined as 1×10^{-5}. Where risks fall between 1×10^{-5} and 1×10^{-4} this was considered as warranting further investigation. Risks exceeding 1×10^{-4} were generally considered unacceptable and warranting some form of action or management to reduce the risk.

To estimate the cumulative cancer risk due to simultaneous exposure to multiple chemicals in mussel tissue the Excess Cancer Risk for individual chemicals was summed to calculate a total Excess Cancer Risk.

2.4.6. Meal limits

As discussed below, the Hazard Indices for mussels collected at some locations exceeded a value of one and/or the Excess Cancer Risk for arsenic and the Total Cancer Risk exceeded an acceptable risk level of 1×10^{-5}. This does not necessarily mean the mussels cannot be consumed but rather that care should be taken in the number of meals consumed per defined period. Thus, the number of meals that can safely be consumed per month was calculated. For this purpose meal size was set at 142.4 g.

3. Results and Discussion[3]

3.1. Mussel length, wet weight and moisture content

The average total length and wet tissue weight of mussels was strongly positively correlated ($r = 0.858$, $p < 0.001$), (Figure 2). The average moisture content of mussel tissue was 87.2 %, with a range of 82.0 - 91.5%.

3.2. Chemical concentrations in mussel tissue

3.2.1. Metals

All metals that were analysed in the tissue of mussels collected at all sites along the eThekwini shoreline in August 2015 were at a concentration exceeding the method detection limit (Figure 3). This was not surprising considering all biological tissue naturally contains metals, albeit usually at low concentrations. Certain metals, including copper, selenium and zinc, are in fact required for the normal physiological functioning of biological tissue, but only at trace concentrations. However, even metals that have no known biological function (*e.g.* mercury) can be expected to occur naturally in biological tissue, again at low concentrations. This is because metals are naturally present in seawater, sediment and food. The mere presence of metals in the tissue of mussels does not, therefore, necessarily mean they have been exposed to contaminated seawater and food. It is, therefore, necessary to discriminate between metal concentrations in the tissue of mussels that reflect exposure to natural metal concentrations and concentrations that reflect exposure to metal contaminated seawater and food. Several approaches were followed in the eThekwini Mussel Watch programme for this purpose.

There were no obvious or consistent spatial trends evident in a comparison of metal concentrations in the tissue of mussels collected at different sites along the eThekwini shoreline (Figure 3). Thus,

[3] Raw data are presented as appendices to this report.

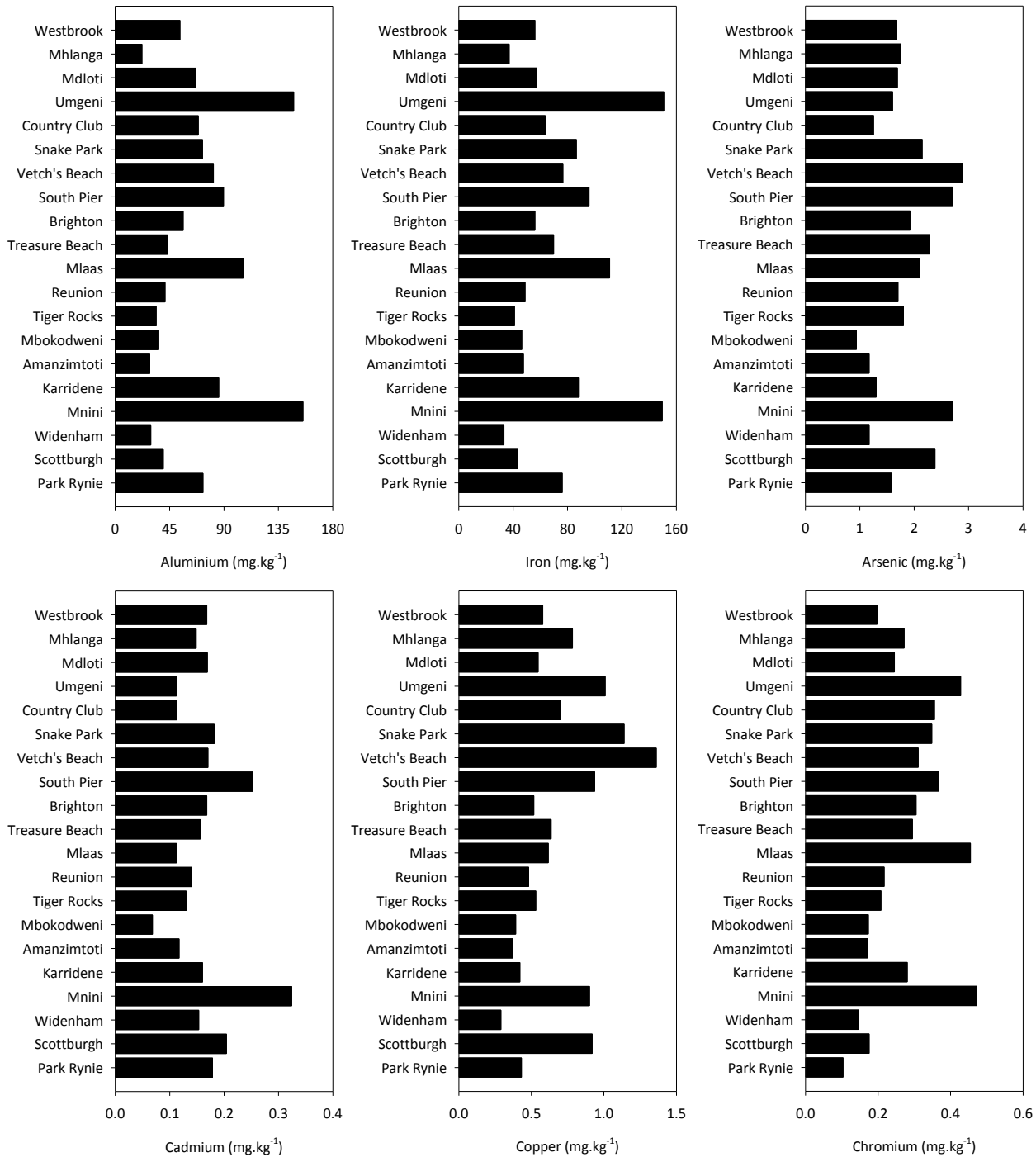

Figure 3. Metal concentrations (wet weight) in the tissue of mussels collected along the eThekwini shoreline in August 2015.

although mussels at some sites had elevated concentrations of certain metals in their tissue, this was usually isolated to a site and mussels at neighbouring sites that usually had concentrations comparable to those for mussels at other sites. Also, mussels at some sites had elevated concentrations of only a single, or at most two metals in their tissue. Notable exceptions were for mussels collected at Umgeni and Mnini, which had higher, or amongst the highest concentrations of numerous metals in their tissue as compared to mussels at other sites (Figure 3).

The bioaccumulation of metals by mussels can be influenced by their physiology. Some investigators have reported a non-linear increase in metal concentrations with increasing mussel length and/ or wet tissue weight (*i.e.* with age; *e.g.* Wang and Fisher, 1997; Mubiana *et al*., 2006), while others have reported an inverse relationship (*e.g.* Burger

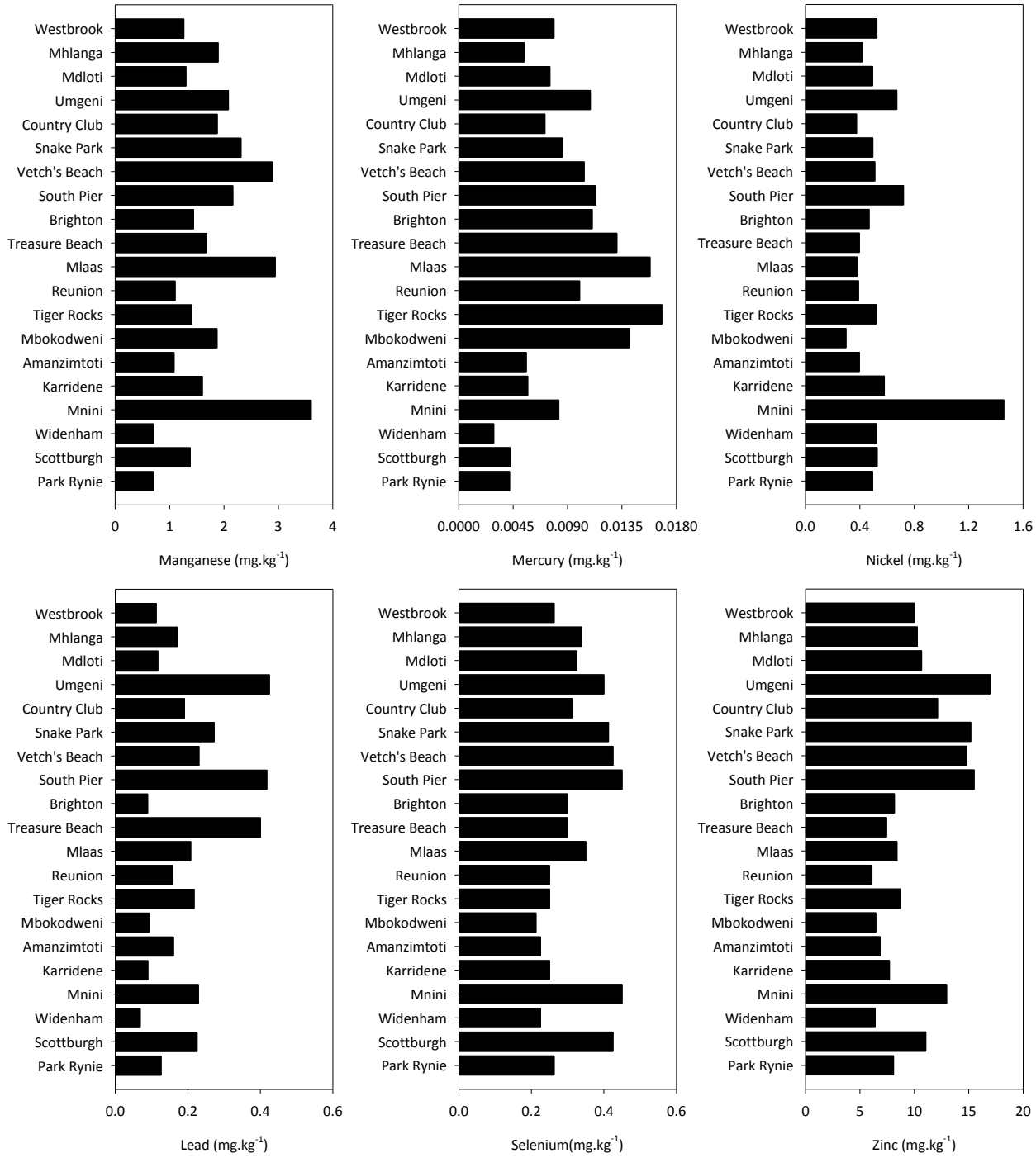

Figure 3 continued. Metal concentrations (wet weight) in the tissue of mussels collected along the eThekwini shoreline in August 2015.

and Gochfield, 2006) or no relationship at all (*e.g.* Saavedra *et al.*, 2004; Burger and Gochfield, 2006).

The first approach in attempting to determine if mussels at different sites along the eThekwini shoreline had accumulated metals in their tissue to excessive concentrations as a result of exposure to contaminated food and water was to evaluate the relationship between the average total length and wet tissue weight of mussels, and metal concentrations in their tissue. In all cases the relationships could not be adequately modelled through any form of regression (*i.e.* the regressions were weak and not statistically significant). There was thus no unifying relationship between the average total length and wet tissue weight of mussels, and the concentrations metals in their tissue. This does not mean there are no relationships between the total length and wet tissue weight and metal concentrations in the tissue of mussels along the eThekwini shoreline,

but rather that if strong relationships exist they are likely to be site specific. The sampling design of the eThekwini Mussel Watch programme does not permit the analysis of such relationships at each collection site because the size range of mussels analysed per site is too narrow (but this range was specifically targeted).

Metals generally occur in geological material (*e.g.* rocks, sediment) in a particular geographical area in a proportionate manner. When metals are released from rocks and soil through weathering they may be available for uptake by mussels in a similarly proportionate manner. If so, there should be a strong linear relationship between metal concentrations in mussel tissue. Two approaches were followed to investigate if this was the case for mussels along the eThekwini shoreline. First, the strength of the relationship between aluminium, which is the second most abundant element in the earth's crust, and other metals was explored. Aluminium is commonly used as a normaliser of metal concentrations for geochemical baseline studies. Second, the strength of relationships between all metals was explored.

Relationships between metal and aluminium concentrations in the tissue of mussels collected along the eThekwini shoreline were generally weak (Figure 4), but there were exceptions. The relationship between concentrations of aluminium and iron ($r = 0.97$, $p < 0.001$), aluminium and manganese provided one outlier was trimmed ($r = 0.74$, $p < 0.001$), and aluminium and chromium provided one outlier was trimmed ($r = 0.86$, $p < 0.001$) was strong to very strong. There were statistically significant relationships between metal concentrations in mussel tissue, as follows: arsenic and copper ($r = 0.75$, $p < 0.001$), arsenic and cadmium ($r = 0.70$, $p < 0.001$), arsenic and selenium ($r = 0.82$, $p < 0.001$), copper and selenium ($r = 0.89$, $p < 0.001$), copper and zinc ($r = 0.88$, $p < 0.001$), nickel and cadmium ($r = 0.83$, $p < 0.001$), iron and manganese ($r = 0.78$, $p < 0.001$), iron and chromium ($r = 0.88$, $p < 0.001$), selenium and zinc ($r = 0.86$, $p < 0.001$), manganese and chromium ($r = 0.85$, $p < 0.001$), and manganese and selenium ($r = 0.71$, $p < 0.001$) (Figure 5). Many metals thus appear to have been proportionally bioaccumulated by mussels, which either reflects their proportional availability in seawater and food or that they have a similar uptake preference. The weak relationships between other metals may reflect contamination, or simply that no relationship should be expected because of their variable rates of bioaccumulation (sites specific availability) by mussels.

The third approach was to examine the cumulative distribution of metal concentrations in the tissue of mussels collected along the eThekwini shoreline for marked inflections and gaps. Cumulative distributions that approximated linearity were taken as representing concentrations belonging to a single non-contaminated 'population', with the difference in concentrations across the distribution taken as representing natural variability in metal bioaccumulation as a result of variable mussel size and/or physiology, and metal bioavailability between locations.

The cumulative concentration distributions for iron, arsenic, selenium and zinc approximated linearity (Figure 6). For other metals there were inflections and gaps in the concentration distribution, although these were only marked for aluminium, cadmium, nickel and lead. These gaps and inflections were taken as the discriminating point between two 'populations'. Concentrations to the right of inflections and gaps were taken as being anomalously high and possibly reflecting bioaccumulation due to exposure to metal contaminated seawater and food. The anomalous concentration of a single metal in the tissue of mussels at a particular location is unlikely to be the result of bioaccumulation due to exposure to metal contaminated seawater and food. This is because it is uncommon for seawater and food to be contaminated by a single metal. Rather, point and non-point sources of contaminants to coastal waters are typically characterised by multiple contaminants. It is thus more likely that mussels exposed to contaminated seawater and food will have anomalous concentrations of several metals in their tissue. The proximity of mussel collection locations to potential anthropogenic sources of metals thus also needs to be considered when attempting to determine if anomalous metal concentrations are likely to reflect bioaccumulation

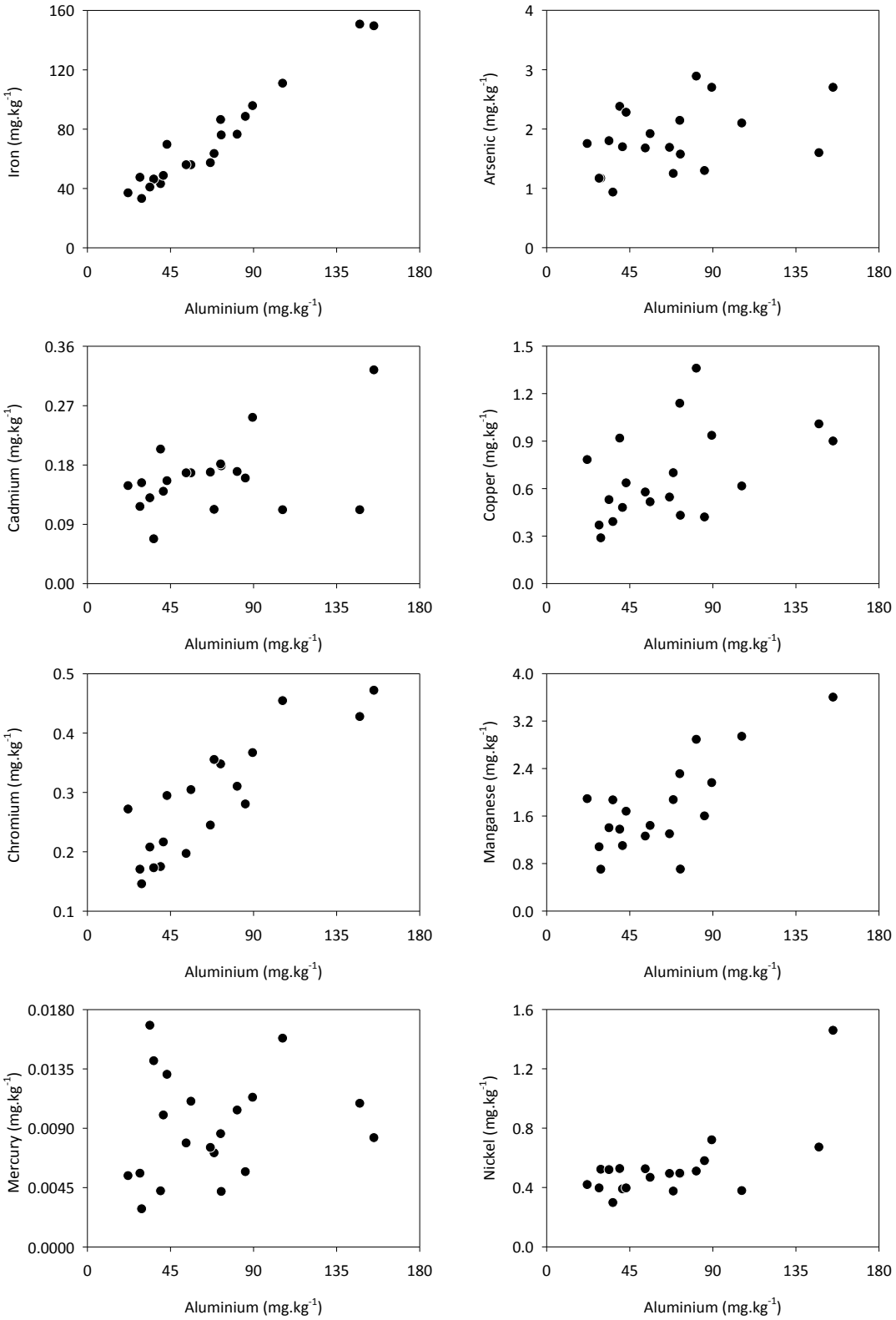

Figure 4. Relationships between the concentrations (wet weight) of metals and aluminium in the tissue of mussels collected along the eThekwini shoreline in August 2015.

due to excessive exposure to contaminated seawater and food.

Mussels collected at Mnini had the most metals at anomalous concentrations in their tissue, namely aluminium, cadmium, chromium, manganese and nickel (Figure 6). Mussels at Mlaas Canal, Umgeni, Vetches Beach and South Pier also had metals at anomalous concentrations in their tissue, but fewer than at Mnini. Mnini is situated far from major anthropogenic sources of metals and it thus seems unlikely the high metal concentrations in the tissue

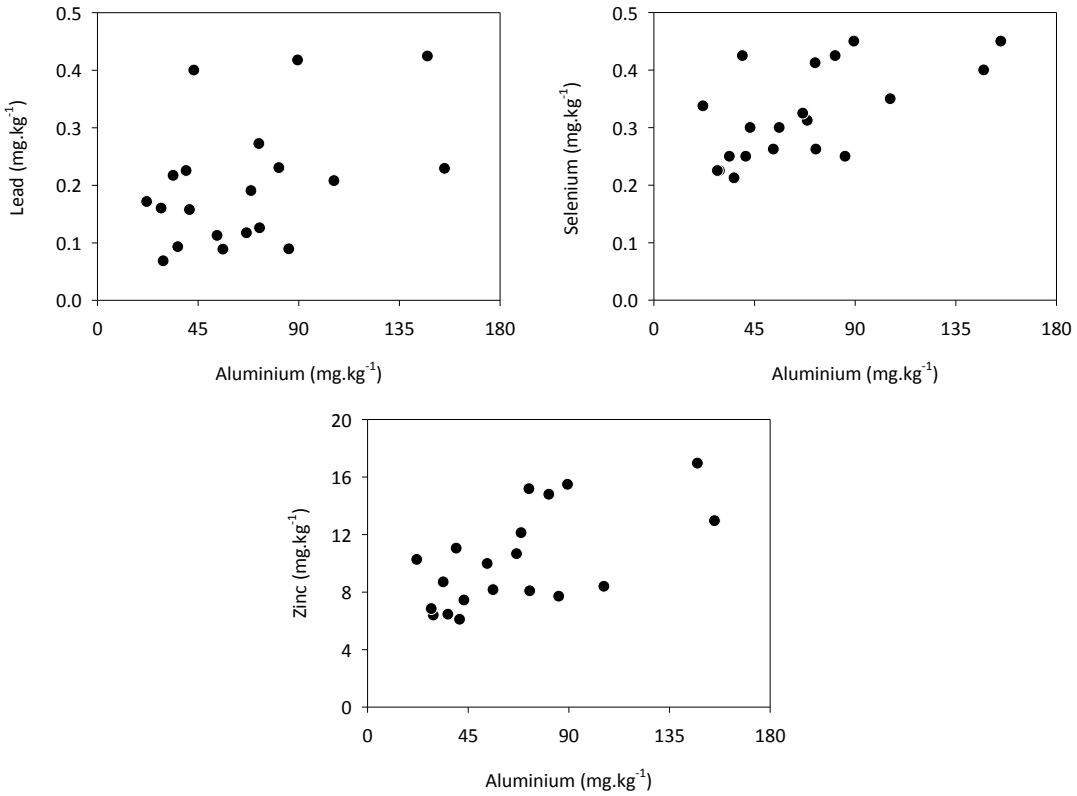

Figure 4 continued. Relationships between the concentrations (wet weight) of metals and aluminium in the tissue of mussels collected along the eThekwini shoreline in August 2015.

of mussels at this site were a result of exposure to metal contaminated seawater and food.

The strongest likelihood for the bioaccumulation of anthropogenic metals was mercury in mussels at Tiger Rocks, Mlaas Canal, Mbokodweni and Treasure Beach. Mussels at these sites have had anomalous mercury concentrations in their tissue in previous Mussel Watch surveys. These sites are all situated in 'close' proximity to one another and to the Isipingo industrial area, which is a possible source of the mercury. This said, mercury concentration in the tissue of mussels collected at these sites were not markedly higher than at other sites, and if there was contamination evident then this was of a 'mild' nature.

Previous Mussel Watch surveys reported generally higher cadmium concentrations in mussels from sites south of Durban, but no obvious spatial trends in cadmium concentrations were evident for the 2015 survey.

3.2.2. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons were at a concentration exceeding the method detection limit in the tissue of mussels at all sites apart from Brighton Beach, Treasure Beach, Tiger Rocks and Park Rynie (Figure 7). The highest total polycyclic aromatic hydrocarbon concentration was in mussels collected at Widenham, followed by mussels at Mhlanga, South Pier and Mlaas Canal (Figure 7). Total concentrations in mussels were spatially variable, presumably reflecting spatially variable inputs of these chemicals to the nearshore marine environment along the eThekwini shoreline. Based on the categorisation of total polycyclic aromatic hydrocarbon concentrations in mussels proposed by Baumard *et al.* (1998), total polycyclic aromatic hydrocarbon concentrations in mussels at all but one site along the eThekwini shoreline in August 2015 fall into the low pollution category (0 - 100 µg.kg^{-1} dry weight). The total polycyclic aromatic hydrocarbon concentration (121 µg.kg^{-1} dry weight) in mussels collected at Widenham falls marginally into the moderate pollution category (100 - 1000 µg.kg^{-1} dry weight).

The presence of polycyclic aromatic hydrocarbons in mussels collected at virtually all sites along the eThekwini shoreline was expected since recent (unpublished) research by the scientists from the

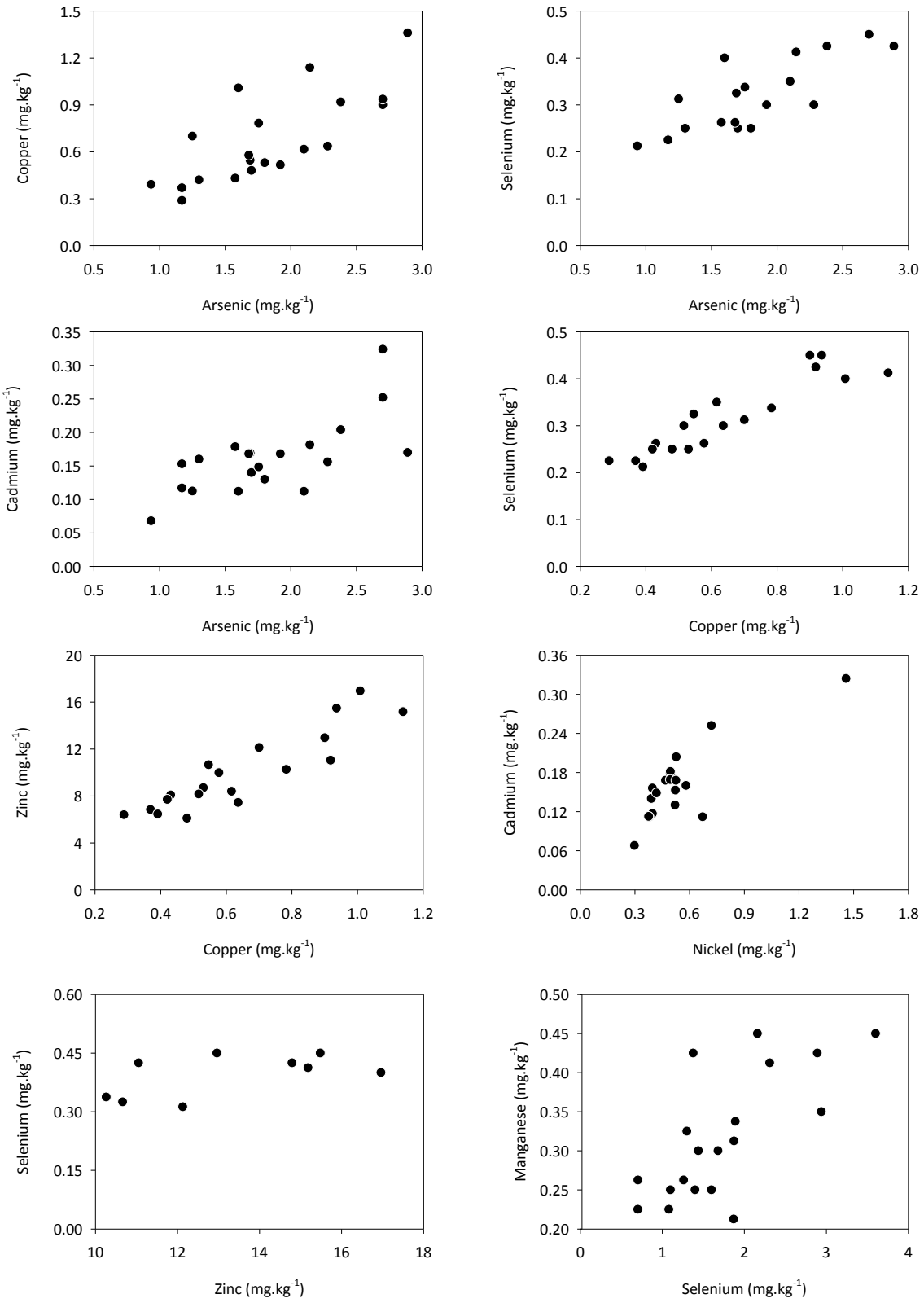

Figure 5. Relationships between the concentrations (wet weight) of selected metals in the tissue of mussels collected along the eThekwini shoreline in August 2015.

Coastal Systems research group of the CSIR has shown that these chemicals are ubiquitous in sediment in rivers, estuaries and canals in eThekwini area. However, this does not necessarily mean they are present as contaminants since polycyclic aromatic hydrocarbons have numerous natural sources, including bush fires. This said, anthropogenic sources are most significant sources of these chemicals to aquatic ecosystems.

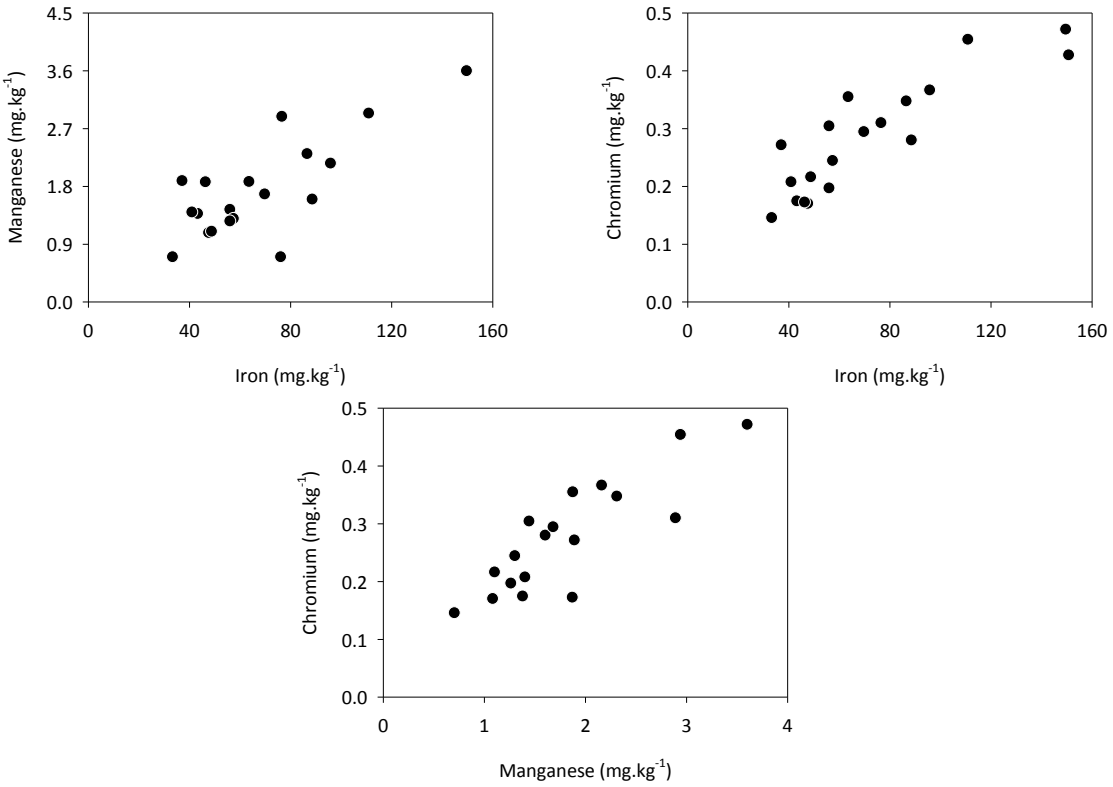

Figure 5 continued. Relationships between the concentrations (wet weight) of selected metals in the tissue of mussels collected along the eThekwini shoreline in August 2015.

The correlation between total polycyclic aromatic hydrocarbon concentrations and the average length and wet tissue weight of mussels was weak. Polycyclic aromatic hydrocarbon bioaccumulation by mussels was thus likely to have been influenced by localised exposure rather than physiological differences between mussels at different sites. As stated previously the highest total polycyclic aromatic hydrocarbon concentration was in mussels collected at Widenham, where exposure to seawater and food contaminated by these chemicals would not ordinarily be expected. This may, however, reflect other anthropogenic sources of polycyclic aromatic hydrocarbons in this area, such as the combustion of sugarcane. The next highest concentrations were for mussels collected at Mhlanga, South Pier and Mlaas Canal, which is more easily explained since these mussels were collected near a stormwater outfalls or contaminated river discharges.

3.2.3. Organochlorine pesticides

Six organochlorine pesticides were at a concentration exceeding the method detection limit in the tissue of mussels collected at numerous sites along the eThekwini shoreline in August 2015 (Figure 8). This is in stark contrast to the 2014 survey, when organochlorine pesticides were never detected in mussels. These pesticides have, however, been detected sporadically in previous surveys. DDT and its derivatives were most frequently detected, in mussels at nine sites (Figure 8). One or more endosulfan derivatives were detected in mussels at six sites while dieldrin and endrin were detected in mussels at one site each. The presence of DDT and its derivatives in mussels was not entirely unexpected given that DDT is a widespread contaminant of sediment in rivers and estuaries in the eThekwini area (CSIR, unpublished data). The actual source of the DDT is uncertain. One theory (Batterman *et al*., 2008) is that the DDT is transported atmospherically from that malaria belt in northern KwaZulu-Natal, where DDT is used to control mosquitoes. However, relatively high concentrations of DDT and its derivatives in sediment in some rivers and estuaries in the eThekwini area hint at localised sources. An air quality study provided evidence for the presence of numerous chlorinated pesticides present in the atmosphere over Durban, including all of the pesticides detected in this study (Batterman *et al*., 2008).

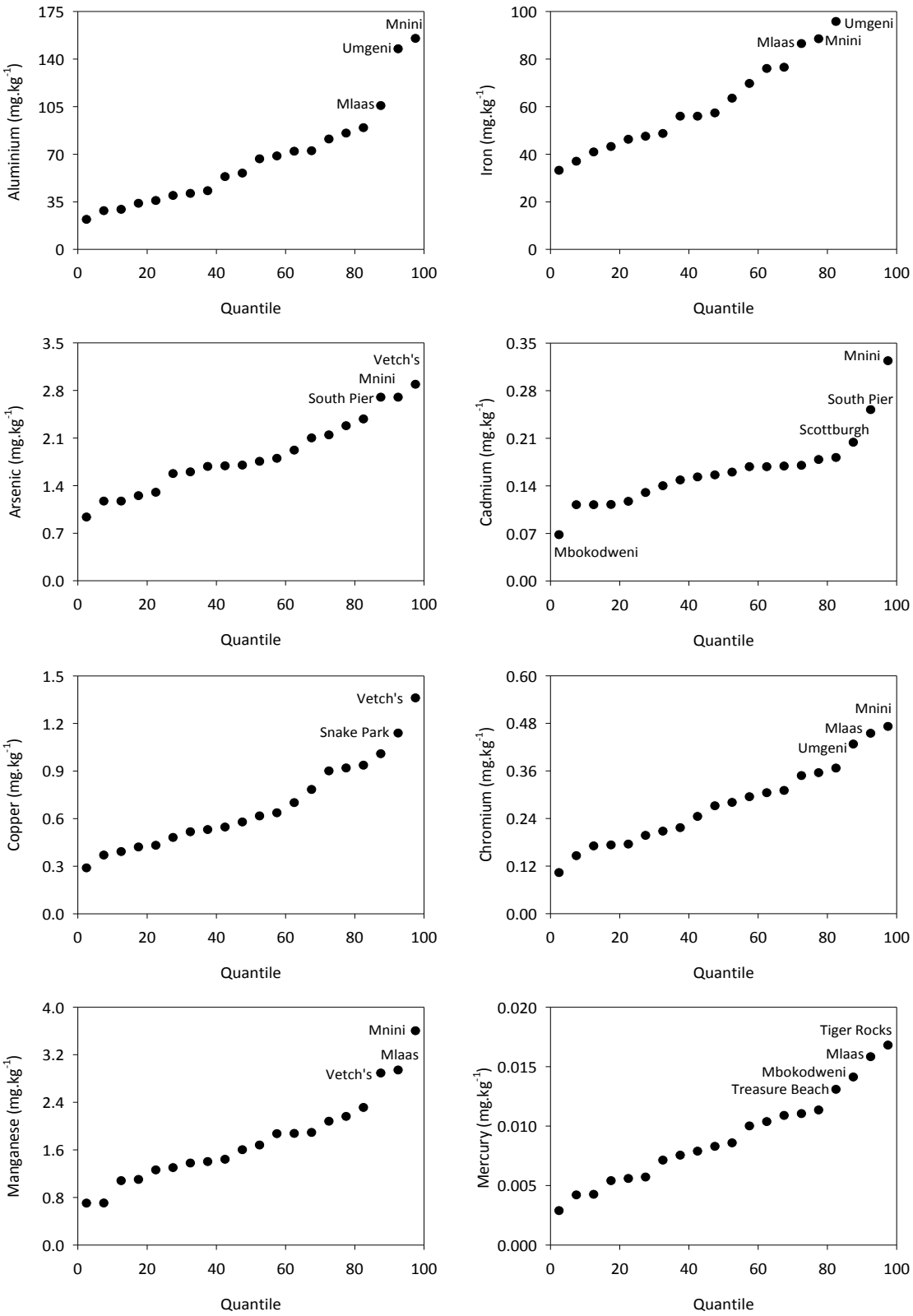

Figure 6. Cumulative concentrations (wet weight) distributions of metal concentrations in the tissue of mussels collected along the eThekwini shoreline in August 2015. Anomalous metal concentrations are highlighted by location identifiers.

3.2.4. Polychlorinated biphenyls

Polychlorinated biphenyls were at a concentration exceeding the method detection limit in the tissue of mussels collected at eight sites along the eThekwini shoreline in August 2015 (Figure 9). This is also in stark contrast to the 2014 survey, when polychlorinated biphenyls were never detected in mussels. Polychlorinated biphenyls have, however, been detected sporadically in previous surveys. Only a few polychlorinated biphenyl congeners

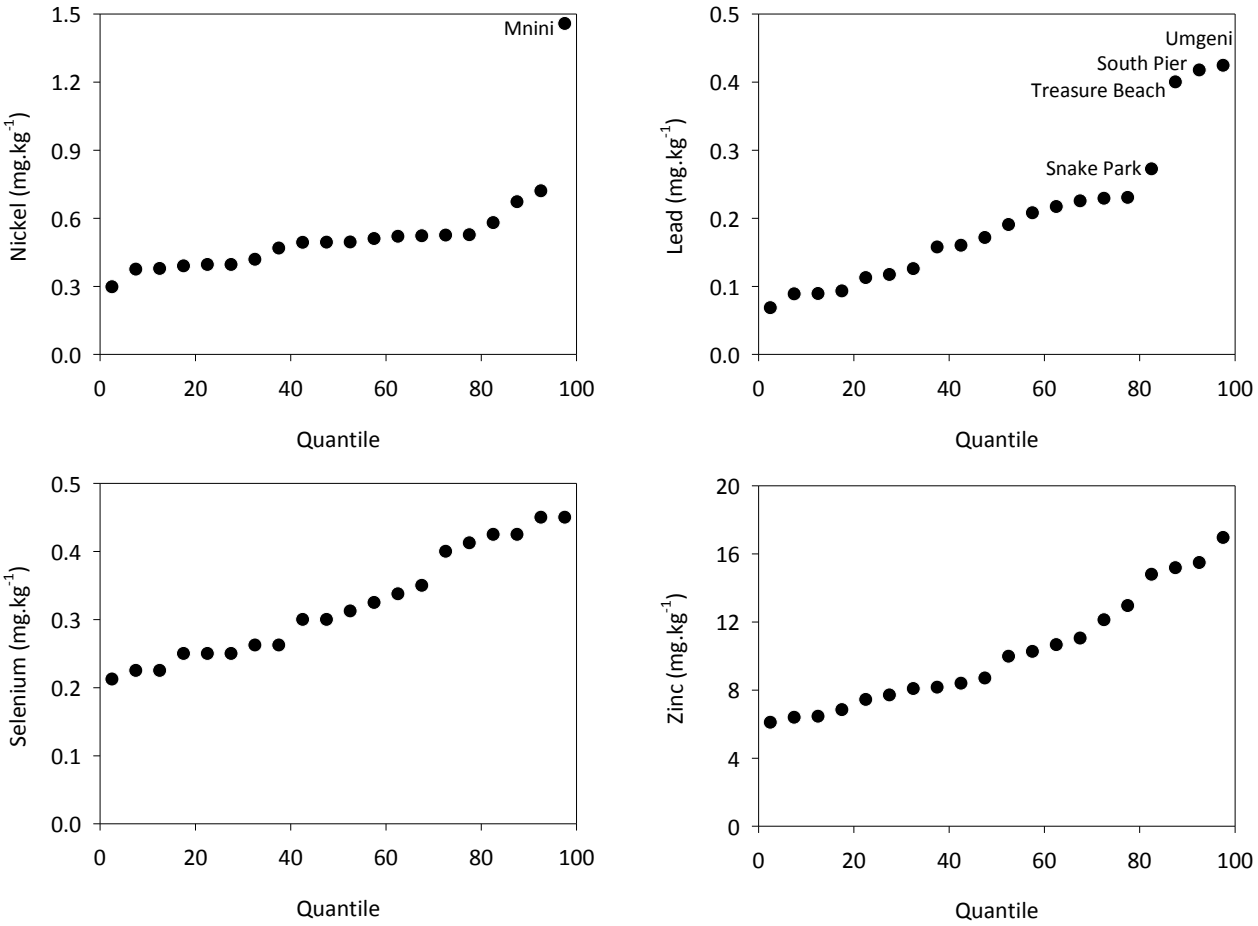

Figure 6 continued. Cumulative concentrations (wet weight) distributions of metal concentrations in the tissue of mussels collected along the eThekwini shoreline in August 2015. Anomalous metal concentrations are highlighted by location identifiers.

(PCB 28, 44, 52, 66, 101, 126) were detected in mussels in 2015. Thus, the total polychlorinated biphenyl concentration in mussels at Westbrook, Mhlanga, Country Club, Vetch's Beach, Treasure Beach, Tiger Rocks and Mnini was represented by a single congener, and by two congeners in mussels at Scottburgh. Although the presence of polychlorinated biphenyl congeners in mussels collected near the more industrialised and urbanised parts of eThekwini (such as Mhlanga, Country Club, Vetch's Beach, Treasure Beach and Tiger Rocks) makes sense, the presence of these chemicals at Westbrook, Mnini and Scottburgh is difficult to explain. Previous research by the scientists from the Coastal Systems research group of the CSIR has shown that polychlorinated biphenyls are frequent contaminants of sediment in rivers and estuaries in the greater Durban area, but not in 'rural' rivers and estuaries (CSIR, unpublished data). This suggests these chemicals are transported by currents to remote areas after their introduction to the nearshore marine environment.

3.3. Risk assessment

3.3.1. Non-carcinogenic risk

Hazard Indices and Hazard Quotients for recreational consumers did not exceed a value of one. In other words, mussels collected along the eThekwini shoreline can be safely consumed as per this consumer scenario. For subsistence consumers, the Hazard Quotient for arsenic slightly exceeded a value of one for mussels collected at 15 sites (Figure 10). If the contribution of inorganic arsenic to the total arsenic concentration is taken as 5% then the number of sites where the Hazard Quotient exceeds a value of one does not change, although the quotient values are lower. If the contribution of inorganic arsenic is taken as 2.2% then Hazard Quotients fall below one at all sites.

Hazard Indices for mussels collected at all sites as per the subsistence consumer scenario, exceed a value of one (Figure 11). Thus, the consumption of mussels at all sites as per this consumption scenario

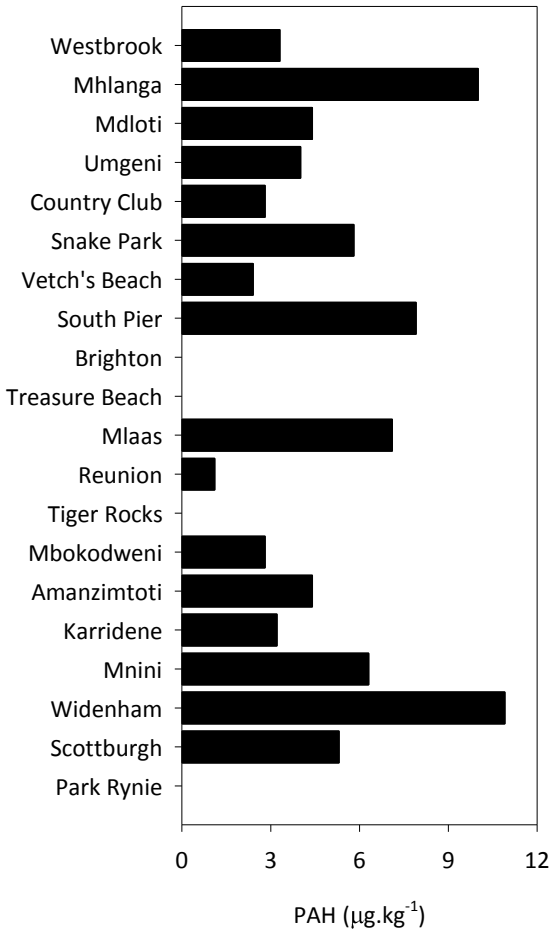

Figure 7. Total polycyclic aromatic hydrocarbon (PAH) concentrations (wet weight) in the tissue of mussels collected along the eThekwini shoreline in August 2015.

poses a potential chronic health risk due to exposure to chemicals in the tissue of mussels. This said, the Hazard Index for Country Club, Mbokodweni, Amanzimtoti, Karridene and Widenham only slightly exceeded a value of one and it is unlikely consumers will experience health risks due to exposure to chemicals in these mussels. Scrutiny of the chemicals that resulted in Hazard Indices exceeding a value of one shows that arsenic was by far the largest contributor (>60% for mussels at all but one site if the inorganic arsenic concentration contribution to the total arsenic concentration is assumed to be 10%). If the contribution of inorganic arsenic to the total arsenic concentration is assumed to be 2.2% then the Hazard indices for all but eight sites fall below a value of one, and in the latter instances only slightly exceed a value of one (Figure 11).

3.3.2. Carcinogenic risk

The Excess Cancer Risk for as per the recreational consumer scenario exceeded 1×10^{-5} for mussels at all sites if the contribution of inorganic arsenic to the total arsenic concentration is taken as 5 or 10% (Figures 12 and 13), but was below 1×10^{-5} at Widenham, Amanzimtoti and Mbokodweni if the contribution is taken as 2.2% (Figure 12). For subsistence consumers the Excess Cancer Risk exceeded 1×10^{-4} for mussels at all sites regardless of whether the contribution of inorganic arsenic to the total arsenic concentration is taken as 2, 5 or 10% (Figure 13). Total Excess Cancer Risk ranged between 3.51×10^{-5} to 1.08×10^{-4} for recreational consumers and 2.85×10^{-4} to 8.82×10^{-4} for subsistence consumers.

Total Cancer Risk for recreational consumers exceeded 1×10^{-5} for mussels at all sites regardless of whether the contribution of inorganic arsenic to the total organic arsenic concentration is taken as 2.2, 5 or 10% (Figures 14 and 15). For subsistence consumers the Total Cancer Risk even exceeded 1×10^{-4} for mussels at all sites regardless of whether the contribution of inorganic arsenic to the total organic arsenic concentration is taken as 2.2, 5 or 10%. Mussels presenting the highest Total Cancer Risk were at South Pier for both recreational (1.16×10^{-4}) and subsistence (9.42×10^{-4}) consumers. Total Cancer Risk for recreational consumers ranged between 4.02×10^{-5} to 1.16×10^{-4} and for subsistence consumers between 3.27×10^{-4} to 9.42×10^{-4}. At all sites inorganic arsenic was either the sole, or by far the most significant contributor to acceptable risk level exceedance.

Based on the contribution of arsenic to Excess Cancer Risk and Total Cancer Risk the obvious question that arises is whether mussels along the eThekwini shoreline are accumulating excessive concentrations of arsenic in their tissue due to the widespread contamination of nearshore coastal waters by this metal. There is unfortunately relatively little data in the scientific literature on arsenic concentrations in mussels along the South African shoreline for comparative purposes. Where such data is available it is often presented in graphs, making it difficult to directly compare the data to the findings of this study.

Mills (2005) provides metal concentrations for mussels (*P. perna*) collected at Sheffield Beach and

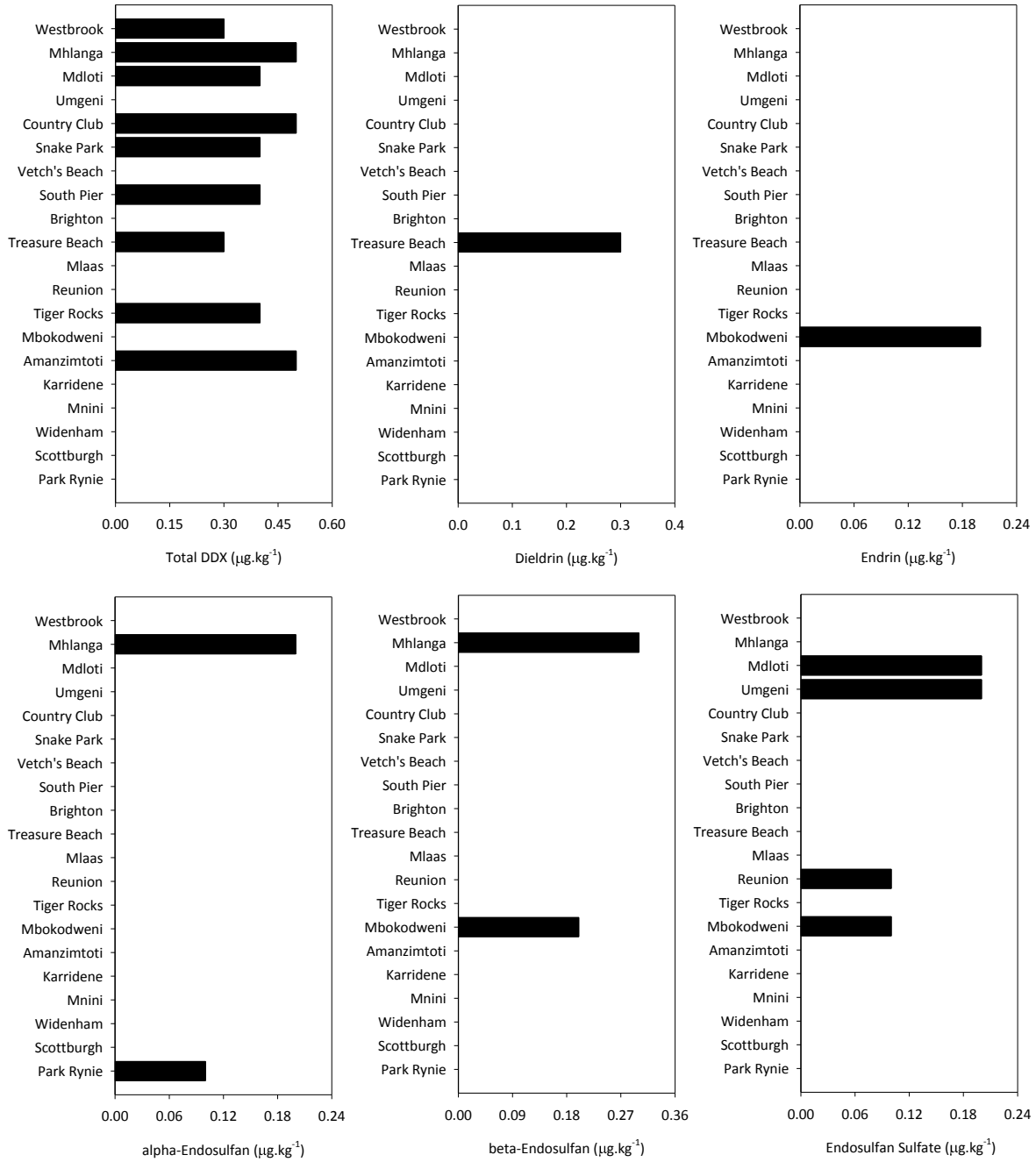

Figure 8. Organochlorine pesticide concentrations (wet weight) in the tissue of mussels collected along the eThekwini shoreline in August 2015.

Dawson's Rocks (control sites), and in the Port of Richards Bay in KwaZulu-Natal. Arsenic concentrations in mussels collected at Sheffield Beach and Dawson's Rocks were below the method detection limit, while the average concentration in mussels collected in Richards Bay was 13.13 mg.kg^{-1} dry weight. The latter concentration is broadly comparable to arsenic concentrations in mussels collected along the eThekwini shoreline in 2015 (average concentration of 14.33 mg.kg^{-1} dry weight). The concentrations in mussels collected at Sheffield Beach and Dawson's Rocks are only slightly lower. However, in the same study Mills (2005) provides arsenic concentrations that varied seasonally between 2.23 - 3.70 mg.kg^{-1} (dry weight) for mussels collected at Sheffield Beach and between 1.06 - 2.60 mg.kg^{-1} (dry weight) for mussels collected in Richards Bay. These concentrations are considerably lower than in mussels collected along the eThekwini shoreline in 2015. It is uncertain why arsenic concentrations varied so significantly between mussels collected at different times at the same locations in the study by Mills (2005).

Figure 9. Total polychlorinated biphenyl (PCB) concentrations (wet weight) in the tissue of mussels collected along the eThekwini shoreline in August 2015.

Degger (2010) provides metal concentrations in mussels (*P. perna*) collected in several ports along the South African shoreline. Based on the figures provided in this study it is apparent that metal concentrations varied considerably between mussels collected in 2008 and 2009. In 2008 the mean arsenic concentration ranged between about 15 - 35 mg.kg^{-1} (dry weight), with generally little difference in the mean concentration between ports (*i.e.* concentrations were generally near the lower end of the latter range). In contrast, in 2009 the mean arsenic concentration in mussels in all ports was not only considerably lower, between about 0.1 - 1.5 mg.kg^{-1} (dry weight), but also showed more variability between ports. As is the case with the study by Mills (2005) it is uncertain why arsenic concentrations varied so significantly between mussels collected at different times at the same location.

Greenfield *et al.* (2014) provide metal concentrations for mussels (*P. perna*) transplanted to and resident in the Port of Richards Bay and in mussels (*P. perna*) collected at a control site at Sheffield Beach in 2006 and 2009. Metal concentrations in mussels at all locations differed considerably between 2006 and 2009. Based on estimates from figures provided by Greenfield *et al.* (2014) the mean arsenic concentration in mussels collected in 2009 was about 10 - 13 mg.kg^{-1} (dry weight) compared to about 1 - 3 mg.kg^{-1} (dry weight) in 2006. Once again it is uncertain why arsenic concentrations varied so significantly between mussels collected at different times at the same location. Greenfield *et al.* (2014) were of the opinion the differences reflected the remobilisation of arsenic (and other metals) during a capital dredging programme in the Port of Richards Bay in 2005. However, this is unlikely considering maintenance dredging occurs annually in the port yet similar changes were not evident for mussels collected at other times, and more importantly that the capital dredging programme focussed on a part of the port where there is little to no evidence the sediment is metal contaminated, and particularly not by arsenic. The difference in metal concentrations for mussels collected at Sheffield Beach also cannot be accounted for by the dredging induced mobilisation of metals, as this site is situated far from Richards Bay.

Comparison of arsenic concentrations in mussels collected along the eThekwini shoreline in August 2015 to concentrations reported by Mills (2005), Degger (2010) and Greenfield *et al.* (2014) thus does not resolve the question on whether arsenic concentrations in mussels along the eThekwini shoreline are anomalously high due to their exposure to seawater and food contaminated by this metal.

Based on a comparison to arsenic and inorganic arsenic concentrations reported for mussels in other parts of the world, including for *P. perna* in other parts of Africa and in Brazil, it appears that *P. perna* along the eThekwini shoreline have slightly higher tissue arsenic concentrations than mussels in these parts of the world. However, the concentrations are not markedly higher and the comparison is influenced by whether the concentrations are presented on a wet or dry weight basis. Arsenic concentrations in *P. perna* collected along the eThekwini shoreline are slightly higher than concentrations in mussels in the conterminous United States of America. For example, O'Connor (2002) reported the 15th, 50th

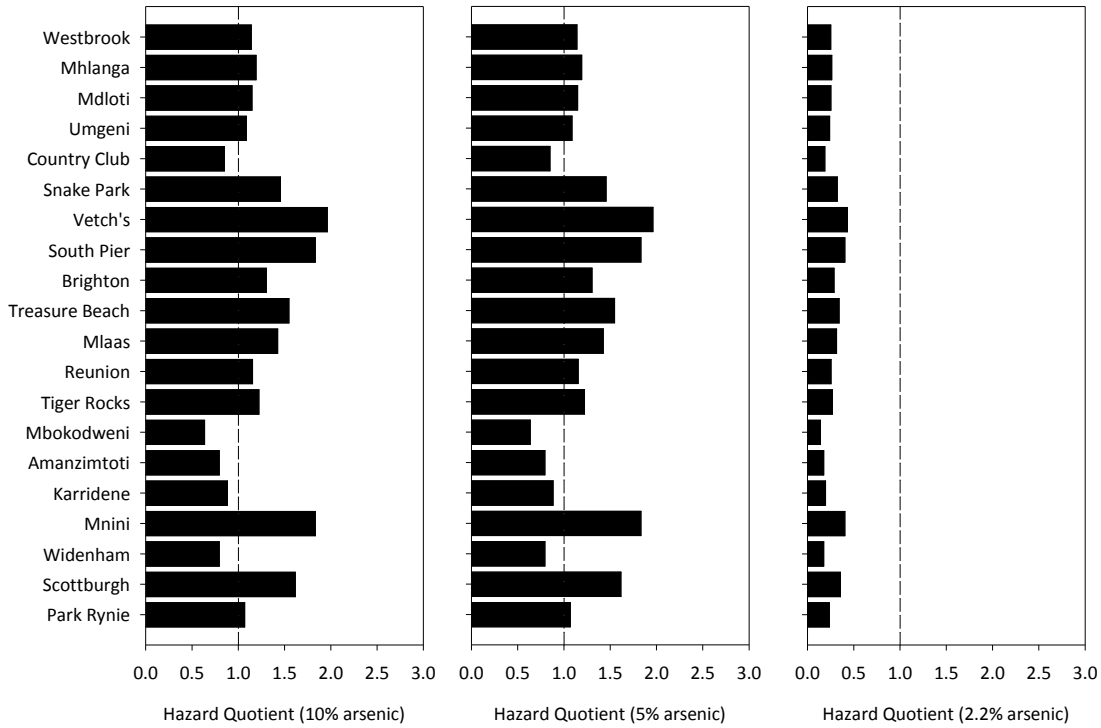

Figure 10. Hazard Quotients for subsistence consumers for mussels collected along the eThekwini shoreline in August 2015, based on different proportions of inorganic to total arsenic concentrations.

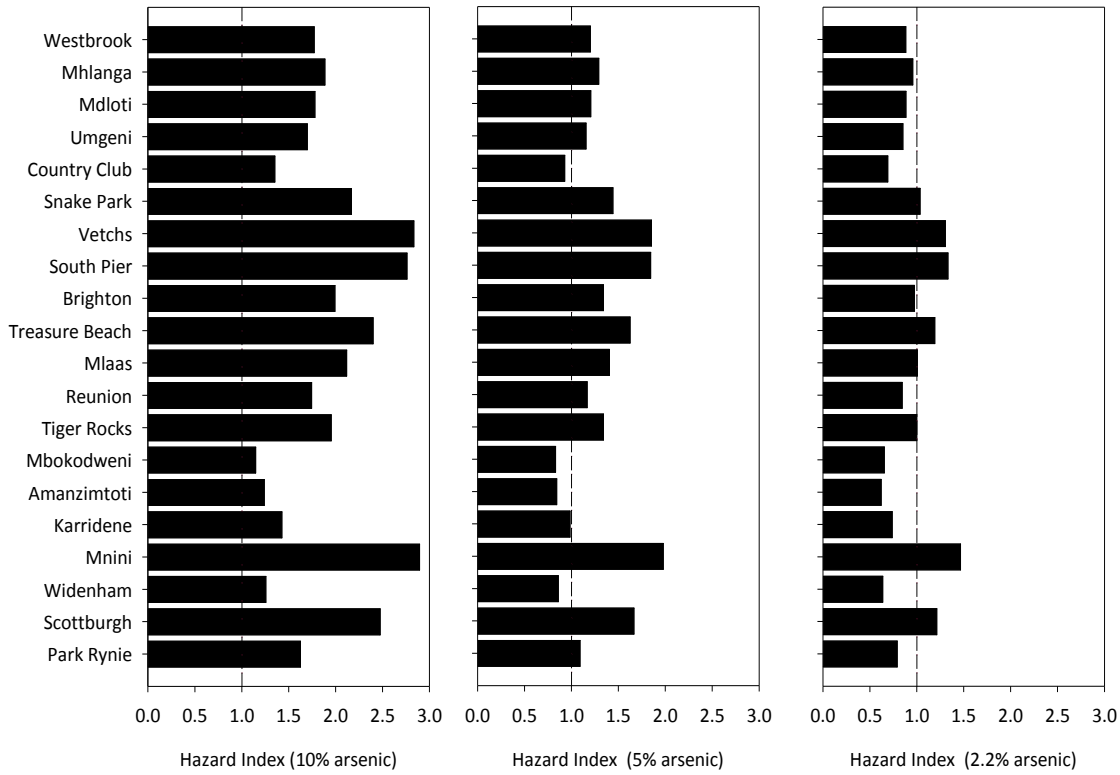

Figure 11. Hazard Indices for subsistence consumers for mussels collected along the eThekwini shoreline in August 2015, based on different proportions of inorganic to total arsenic concentrations.

and 85th percentiles of arsenic concentrations (dry weight) in mussels and oysters from the conterminous United States coastline as 6, 9.5 and 16 µg.g^{-1}. For mussels collected along the eThekwini shoreline in August 2015 the percentiles were 12.7, 14.5 and 17.0 µg.g $^{-1}$ (dry weight), which are slightly higher than in the United States. In the most recent data summary for the Mussel Watch programme in the United States (Kimbrough *et al.*, 2008) three arsenic concentration ranges were identified for

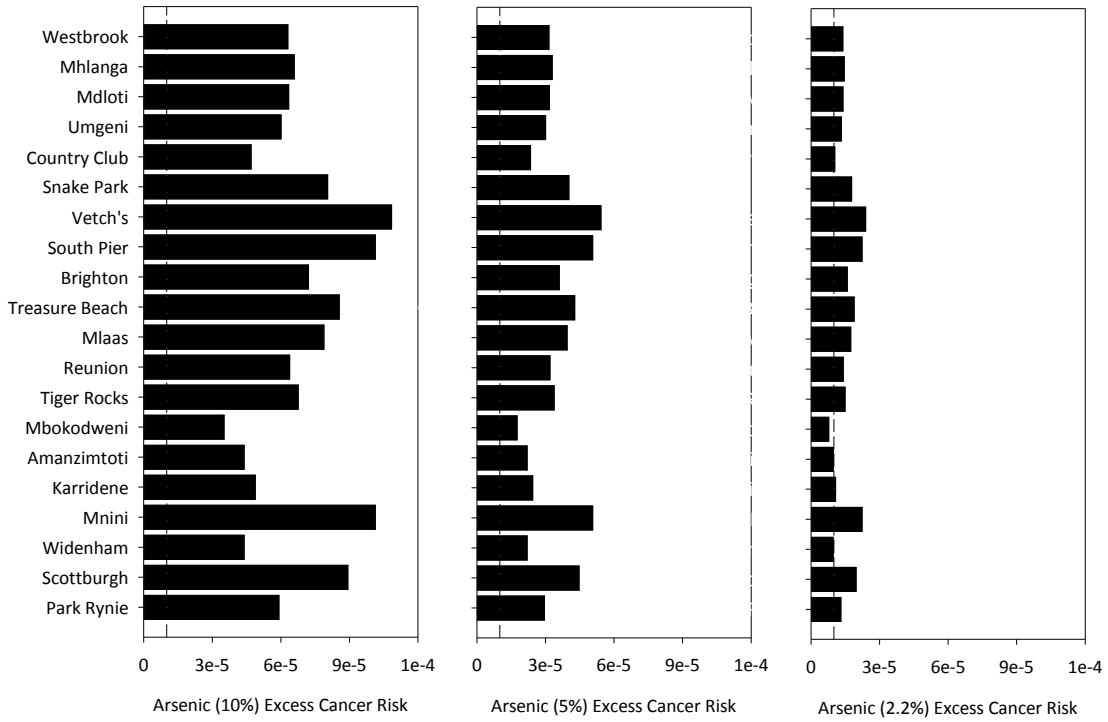

Figure 12. Arsenic Excess Cancer Risk for recreational consumers for mussels collected along the eThekwini shoreline in August 2015.

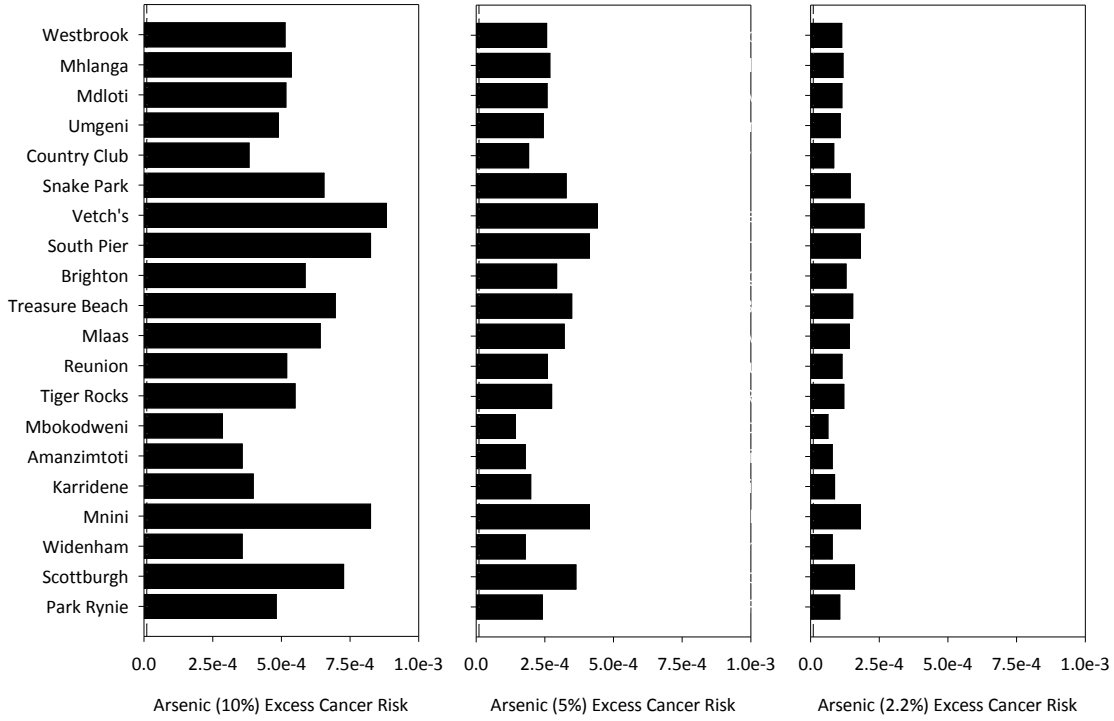

Figure 13. Arsenic Excess Cancer Risk for subsistence consumers for mussels collected along the eThekwini shoreline in August 2015.

mussels of the genus *Mytilus*, namely 5 - 11 (Low), 12 - 22 (Medium), and 23 - 41 mg.kg^{-1} dry weight (High). Arsenic concentrations in *P. perna* at all but three locations along the eThekwini shoreline fall into the Medium range, the exceptions (Mbokodweni, Country Club and Umgeni) falling into the Low range.

The contribution of inorganic arsenic to total arsenic in *P. perna* along the eThekwini shoreline is also not anomalously high, ranging between 1.4 - 4.2%, with an average and median contribution of 2.3% and 2.2% respectively (on a dry weight basis). The scientific literature suggests that inorganic

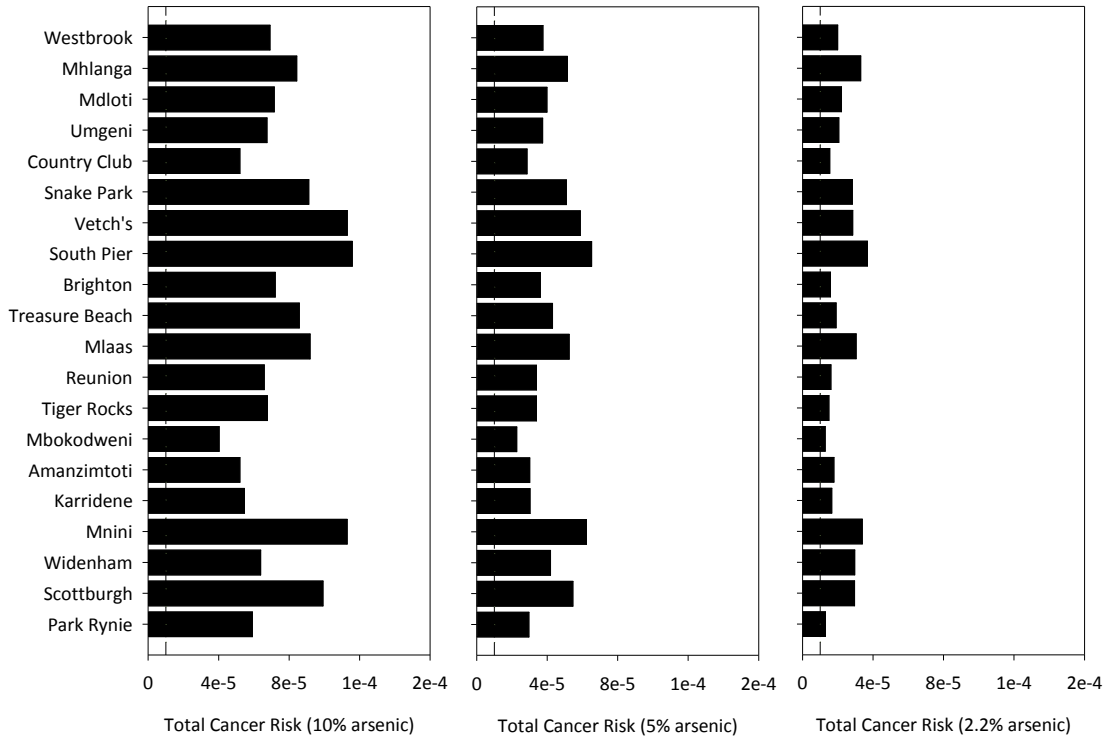

Figure 14. Total Cancer Risk for recreational consumers for mussels collected along the eThekwini shoreline in August 2015.

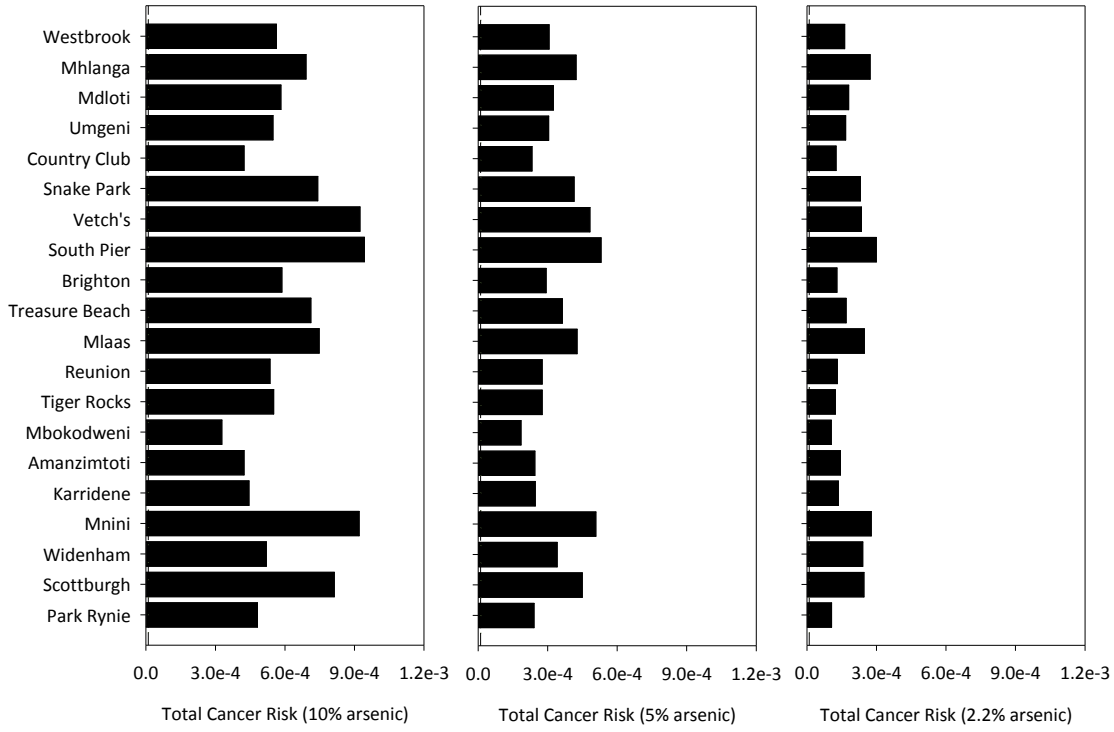

Figure 15. Total Cancer Risk for subsistence consumers for mussels collected along the eThekwini shoreline in August 2015.

arsenic usually comprises between 1 - 10% of the total arsenic concentration in fish and shellfish (Goessler *et al.* 1997, Donohue and Abernathy 1999, Schoof *et al.* 1999a,b, Morrissey and Abernathy 1999, Muñoz *et al.* 2000, De Gieter *et al.* 2002, Li *et al.* 2003, Fabris *et al.* 2006, Liu *et al.* 2006, Peshut *et al.* 2008). As discussed previously there was no pronounced spatial trend for arsenic in *P. perna* collected along the eThekwini shoreline in August 2015 (see Figure 3), a trend also prevalent in previous surveys of the eThekwini Mussel Watch programme. This lack of a spatial

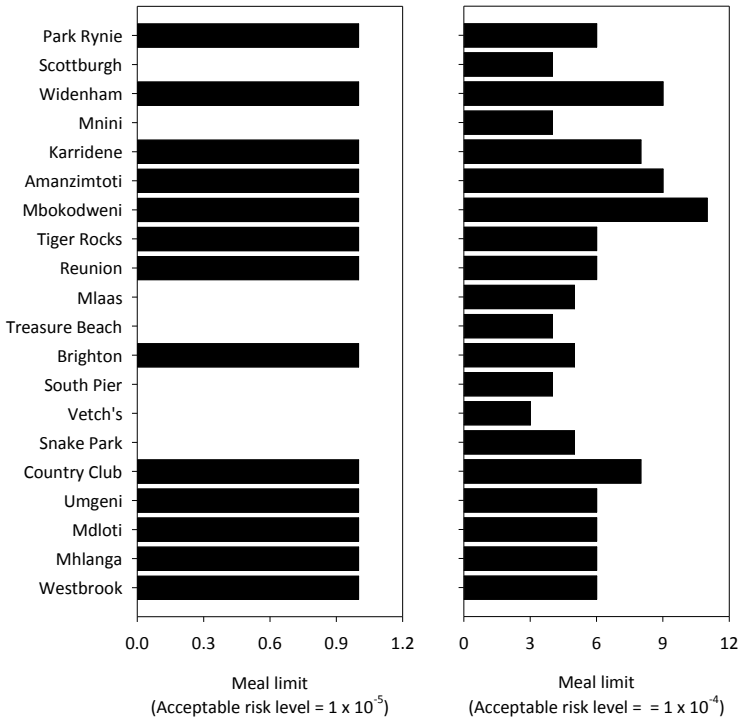

Figure 16. Meal limits based on different levels of acceptable risk for mussels collected along the eThekwini shoreline in August 2015.

trend would not be expected if there were significant anthropogenic sources of arsenic to eThekwini coastal waters, since many of the collection sites were remote from significant anthropogenic sources of metals. The presumption, therefore, is that arsenic concentrations in *P. perna* along the eThekwini shoreline are not anomalously high due to exposure to arsenic contaminated water and food, but are naturally high enough to trigger the identification of a potential risk posed by this metal to humans through a mussel consumption pathway.

Given this it may be prudent to consider if an acceptable risk of 1×10^{-4} rather than 1×10^{-5} should be considered. This said, even then the Excess Cancer Risk for arsenic and the Total Cancer Risk for subsistence consumers exceeds this risk level, although usually only marginally. There are no exceedances for recreational consumers.

3.3.3. Meal consumption limits

Since the Excess Cancer Risk for arsenic, and Total Cancer Risk for mussels at all sites exceeded acceptable risk levels the implication is that consumers should reduce their intake of mussels to reduce exposure to chemicals in the mussels. Based on a meal size of 142.4 g and a risk level of 1×10^{-5}, one or fewer meals per month of mussels collected at the majority of sites along the eThekwini shoreline should be consumed (Figure 16). However, if a risk level of 1×10^{-4} is considered for reasons discussed above then the number of meals that can be consumed increases to between three to 11 (Figure 16).

The above finding on exceedance of an acceptable risk level of 1×10^{-5} theoretically calls for the issuance of an advisory on the number of meals of mussels that should be consumed by subsistence and recreational consumers per month. However, certain assumptions made for this risk assessment might not be valid for recreational and subsistence consumers in the eThekwini area of KwaZulu-Natal (see further discussion in following section). Also, as far as the scientists that prepared this report could determine there are no documented cases in the literature of arsenic toxicity in humans and other mammals after the consumption of large amounts of seafood. Lastly, although there is a potential for risk over a lifetime of mussel consumption, arsenic concentrations are not high enough to pose an acute toxic risk (*i.e.* through the consumption of a single meal).

3.3.4. Risk assessment uncertainties

Although the risk assessment component of this study identified concentrations of arsenic in mussels collected at various locations along the eThekwini shoreline in August 2015 as posing a potential risk to the health of human consumers it is important to take cognisance of uncertainties associated with the assessment. These arise due to the lack of information on certain variables required to calculate risk and the consequent need to make assumptions on the values of these variables.

First, risks were identified based on exposure to concentrations of chemicals analysed in mussels collected in August 2015. The assumption is that the concentrations measured will persist through the 30 or 70 year exposure periods that are used for chronic and carcinogenic health risk assessment purposes. With the possible exception of arsenic, which occurs naturally in the environment and which has been shown in previous eThekwini Mussel Watch surveys to pose a potential risk to the health of human consumers, it is highly unlikely the concentrations of organic chemicals that contribute to risk will remain unchanged for these periods. As discussed previously, the comparison of data between surveys for the eThekwini Mussel Watch programme has in fact showed that concentrations of organic chemicals in mussels have varied temporally at and between collection locations in an unpredictable manner.

It is also unknown if recreational and subsistence consumers have historically, or will in future consume mussels from each location studied at the stipulated intake rates for the 30 year exposure period for chronic health risks and the 70 year exposure period for carcinogenic health risks.

The second uncertainty relates to mussel consumption rate. There is no information on seafood consumption rates for subsistence and recreational consumers in KwaZulu-Natal, nor indeed for other parts of South Africa, albeit that there is information on seafood consumption for the South African population at large (*e.g.* Nel and Steyn, 2002). However, it seems unlikely the latter consumption rates apply to recreational and subsistence fishers, who are likely to consume more seafood than the average South African. This creates uncertainty on the degree of risk since consumption rate is an important determinant of risk. The consumption rates used in this study are those recommended for the population of the United States of America and appear to exceed consumption rates for the South African population at large as provided by Nel and Steyn (2002).

Based on these uncertainties the findings of the risk assessment component of this study should not be construed as absolute but rather as a conservative indicator of possible risks to the health of humans that consume mussels collected along the eThekwini shoreline, through exposure to contaminants in the tissue of the mussels.

3.4. Microbiology

Because of their filter feeding lifestyle, bivalve shellfish may accumulate and concentrate bacteria and viruses in their mantle cavity. The amount of bacteria in the mantle cavity not only provides an understanding of the microbiological quality of the surrounding water but also the potential health risk posed to humans that consume inadequately cooked mussels.

Escherichia (E.) coli bacteria were detected in mussels collected at two sites (Snake Park and Reunion) in August 2015 (Figure 17), but Salmonella was never detected. The presence of *E. coli* does not always indicate a human faecal input since other mammal and bird faeces also contain *E. coli*. Snake Park is situated in central Durban and the mussels collected there are exposure to outflows from the Snake Park stormwater outfall which drains the city. Reunion is located near the Isipingo industrial. Considering the locations of these sites there is a strong likelihood the *E. coli* were derived from an anthropogenic source.

As far as the scientists that prepared this report are aware there are no microbiological guidelines for shellfish collected for personal consumption in South Africa. However, the South African Molluscan Shellfish Monitoring and Control Programme provides guidelines for *E. coli* and Salmonella in mussels harvested for commercial purposes from aquaculture facilities. Three classes are defined as

Figure 17. *E. coli* colony forming unit count per 100 g of wet tissue for mussels collected along the eThekwini shoreline in August 2015.

follows (taken verbatim from guideline document):

Class A

Shellfish harvested from an approved (Class A) area shall comply with the following conditions: The *E. coli* most probable number may not exceed 230 *E. coli* per 100 g of flesh and intravalvular liquid in 80% of the samples. No sample may exceed 700 *E. coli* per 100 g of flesh and intravalvular liquid.

Class B

Shellfish harvested from a restricted (Class B) area is one in which the sanitary survey indicates a limited degree of microbial pollution. Limited pollution is defined as: The *E. coli* most probable number may not exceed 4 600 *E. coli* per 100 g of flesh and intravalvular liquid in 90% of the samples. No sample may exceed 14 000 *E. coli* per 100 g of flesh and intravalvular liquid. No shellfish may be harvested for direct human consumption from restricted areas at any time. Shellfish from restricted areas can only be harvested for depuration or relaying. However, Department of Agriculture, Fisheries and Forestry may consider the issuing of a special permit to harvest shellfish of which the *E. coli* count of the flesh and intravalvular fluids are below 4 600/100 g flesh, on condition that it is sterilised in hermetically sealed containers or subject to an approved heat treatment and frozen.

Class C

Shellfish shall not be harvested from a prohibited (Class C) area for either direct human consumption, depuration, relaying or further processing. An area will be classified as Prohibited when any of the following conditions exist: There is no current sanitary survey or annual evaluation report. The sanitary survey indicates levels of microbiological pollution exceeding the restricted area limits. Areas adjacent to sewage outfalls and other waste discharges of public health significance shall be classified as prohibited.

Based on these guidelines mussels collected at all sites along the eThekwini shoreline in August 2015 would be classified as falling into an approved area based on *E. coli* bacteria colony forming unit counts in their mantle cavities.

4. Conclusions

The eThekwini Mussel Watch programme has two primary objectives. The first is to determine if concentrations of chemicals and bacteria in the mussels provide evidence for significant and widespread sources of these contaminants to nearshore coastal waters along the eThekwini shoreline. The second is to determine if concentrations of chemicals and bacteria in the mussels pose a risk to the health of human consumers.

There was no evidence for the significant or widespread accumulation of metals derived from anthropogenic sources by mussels collected along the eThekwini shoreline in August 2015. Polycyclic aromatic hydrocarbons were detected in mussels collected at all sites and attest to widespread sources of these chemicals to eThekwini nearshore coastal waters. Although there are natural sources of polycyclic aromatic hydrocarbons, anthropogenic sources are considered to be the most significant source of thee contaminants in the environment, and are most likely the source of polycyclic aromatic hydrocarbons accumulated by mussels. The concentrations were, nevertheless, typically

low to very low.

Organochlorine pesticides were detected at concentrations exceeding the method detection limit in the tissue of mussels collected from numerous sites along the eThekwini shoreline. DDX was present in the tissue of mussels collected at nine sites. Endosulfan derivatives were detected in mussels at six sites, and dieldrin and endrin in mussels at one location each. The presence of DDX and its derivatives in mussel tissue was not unexpected given that this DDT and its derivatives are widespread contaminants of sediment in rivers and estuaries in the eThekwini area.

Polychlorinated biphenyls were detected in the tissue of mussels collected at eight sites.

Risk assessment identified inorganic arsenic concentrations in mussels at all sites as posing a potential carcinogenic risk to the health of recreational and subsistence consumers and in theory calls for the issuance of a consumption advisory. However, as discussed above there are numerous uncertainties that influence the outcome of the risk assessment. Furthermore, there is no evidence to suggest that arsenic in the tissue of mussels along the eThekwini shoreline is derived from anthropogenic sources.

Escherichia (E.) coli bacteria were detected in mussels collected at two locations in August 2015 but Salmonella was never detected. According to South African Molluscan Shellfish Monitoring and Control Programme guidelines, mussels at all locations fall into an approved collection area based on *E. coli* bacteria colony forming unit counts in their mantle cavities.

5. Recommendations

The public should be warned not to consume shellfish collected along the eThekwini shoreline if the shellfish have not been depurated (*i.e.* allowed to get rid of material in their guts, by leaving them in clean seawater that is renewed for about 6 hours) and properly cooked. The public should also be warned to avoid consuming shellfish collected near any stormwater outfall or river discharge.

A study is required to identify fish and shellfish consumption patterns for subsistence and recreational consumers in the greater eThekwini area. This research will provide information for a key unknown in risk assessment, namely consumption rate. However, the commissioning of such a study is not necessarily the responsibility of the eThekwini Municipality.

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7. Appendices

Appendix 1. Average moisture content (%), total length (cm) and wet tissue weight, and metal concentrations (mg.kg^{-1} dry weight) in the tissue of mussels collected at locations between Westbrook Beach and Park Rynie in August 2015.

Location	Moisture	Length	Tissue weight	Aluminium	Arsenic	Cadmium	Chromium	Copper	Iron	Lead	Manganese	Mercury	Nickel	Selenium	Zinc
Westbrook	89.50	71.95	12.14	690	15	1.8	2.6	5	831	1.3	20	0.05	8.1	2.5	72
Mhlanga	83.00	78.87	14.95	233	14	1.6	2.8	4.2	885	0.9	16	0.06	5.8	2.5	77
Mdloti	91.00	70.67	10.58	326	13	1.3	1.9	4.1	528	1.8	12	0.06	4.4	2.5	76
Umgeni	82.00	60.48	5.85	862	15	0.8	2.0	4.6	544	1.1	22	0.17	3.5	2.5	76
Country Club	90.00	67.91	6.57	855	13	1.3	2.1	5.3	409	2.2	14	0.17	5.2	2.5	87
Snake Park	91.00	65.92	9.04	316	13	1.4	2.2	4.8	487	1.6	11	0.10	3.9	2.5	61
Vetch's Beach	91.50	73.43	6.46	422	11	0.8	3.2	4.4	792	1.5	21	0.11	2.7	2.5	60
South Pier	90.00	64.75	7.04	338	18	1.3	2.5	5.3	581	3.3	14	0.11	3.3	2.5	62
Brighton	90.00	79.4	11.72	411	17	1.4	2.5	4.3	466	0.7	12	0.09	3.9	2.5	68
Treasure Beach	86.00	78.64	12.25	755	15	1.4	2.0	5.2	532	2.3	12	0.06	4	2.5	86
Mlaas	88.00	70.19	9.15	359	19	1	1.8	8	450	1.4	17	0.06	3	2.5	87
Reunion	88.00	72.03	9.46	467	16	1.1	2.1	6.9	524	1.7	14	0.05	3	2.5	92
Tiger Rocks	82.00	67.45	6.49	497	15	0.9	2.8	5.6	508	1.5	15	0.06	3	2.5	97
Mbokodweni	83.00	76.28	13.19	477	17	0.7	2.7	6.3	942	2.7	13	0.07	4.2	2.5	106
Amanzimtoti	83.50	62.72	6.58	437	13	1.3	1.9	4.2	441	0.9	10	0.06	3.8	2.5	82
Karridene	87.50	69.78	8.72	549	10	1.1	2.0	5.8	274	1.3	14	0.04	3.1	2.5	76
Mnini	84.00	48.62	2.66	922	10	1.6	1.9	5.5	533	1.1	12	0.08	5	2.5	95
Widenham	87.00	76.27	10.41	512	13	1.8	2.6	5	831	1.3	20	0.05	8.1	2.5	72
Scottburgh	86.50	75.84	13.28	163	13	1.6	2.8	4.2	885	0.9	16	0.06	5.8	2.5	77
Park Rynie	89.50	70.41	9.83	509	16	1.3	1.9	4.1	528	1.8	12	0.06	4.4	2.5	76

Appendix 2. Polycyclic aromatic hydrocarbon concentrations (mg.kg^{-1} dry weight) in the tissue of mussels collected at locations between Westbrook Beach and Park Rynie in August 2015. < = denotes concentration below relevant Method Detection Limit, as illustrated by value following this symbol.

Location	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benz(a)anthracene	Chrysene	Benzo(b)&(k)fluoranthene	Benzo(a)pyrene	Indeno(1,2,3-cd)pyrene	Dibenzo(a,h)anthracene	Benzo(g,h,i)perylene
Westbrook	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.015	<0.01	0.016	<0.02	<0.01	<0.01	<0.01	<0.01
Mhlanga	0.019	<0.01	<0.01	<0.01	0.031	<0.01	<0.01	0.024	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Mdloti	<0.01	<0.01	<0.01	<0.01	0.034	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Umgeni	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.025	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Country Club	<0.01	<0.01	<0.01	<0.01	0.022	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Snake Park	<0.01	<0.01	<0.01	<0.01	0.018	<0.01	<0.01	0.017	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Vetch's Beach	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
South Pier	<0.01	<0.01	<0.01	<0.01	0.025	<0.01	<0.01	0.019	<0.01	<0.01	0.022	<0.01	<0.01	<0.01	<0.01
Brighton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Treasure Beach	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.027	<0.01	<0.01	<0.01	<0.01
Mlaas	<0.01	<0.01	<0.01	<0.01	0.027	<0.01	<0.01	0.024	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Reunion	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.011	<0.01	<0.01	<0.01	<0.01
Tiger Rocks	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.026	<0.01	<0.01	<0.01	<0.01
Mbokodweni	<0.01	<0.01	<0.01	<0.01	0.033	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Amanzimtoti	<0.01	<0.01	<0.01	<0.01	0.034	<0.01	<0.01	0.015	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Karridene	<0.01	<0.01	<0.01	<0.01	0.019	<0.01	<0.01	0.013	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Mnini	<0.01	<0.01	<0.01	<0.01	0.016	<0.01	<0.01	0.019	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
Widenham	<0.01	<0.01	<0.01	<0.01	0.034	<0.01	<0.01	<0.01	<0.01	<0.01	0.032	<0.01	<0.01	<0.01	<0.01
Scottburgh	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	0.041	<0.01	<0.01	<0.01	<0.01
Park Rynie	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01

Appendix 2. Organochlorine pesticide concentrations (mg.kg^{-1} dry weight) in the tissue of mussels collected at locations between Westbrook Beach and Park Rynie in August 2015. < = denotes concentration below relevant Method Detection Limit, as illustrated by value following this symbol.

Location	HCB	Heptachlor	Heptachlor epoxide	Aldrin	γ-BHC	α-BHC	β-BHC	δ-BHC	trans-Chlordane	cis-Chlordane	Oxychlordane	Dieldrin	p'p'-DDE	p'p'-DDD	p'p'-DDT	Endrin	Endrin Aldehyde	Endrin Ketone	α-Endosulfan	β-Endosulfan	Endosulfan Sulfate	Methoxychlor
Westbrook	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0032	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Mhlanga	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0039	<0.001	<0.001	<0.001	0.0017	0.0022	<0.001	<0.001	
Mdloti	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0032	<0.001	<0.001	<0.001	<0.001	<0.001	0.0013	<0.001	
Umgeni	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0011	<0.001	
Country Club	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0038	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Snake Park	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0025	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Vetch's Beach	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
South Pier	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0023	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Brighton	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Treasure Beach	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0021	<0.001	<0.001	0.0029	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Mlaas	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Reunion	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0013	<0.001	
Tiger Rocks	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Mbokodweni	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0019	<0.001	<0.001	<0.001	0.0021	0.0016	<0.001
Amanzimtoti	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0036	0.0021	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Karridene	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Mnini	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Widenham	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Scottburgh	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Park Rynie	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0013	<0.001	<0.001	<0.001	

Appendix 3. Polychlorinated biphenyl concentrations (µg.kg^{-1} dry weight) in the tissue of mussels collected at locations between Westbrook Beach and Park Rynie in August 2015. < = denotes concentration below relevant Method Detection Limit, as illustrated by value following this symbol.

Location	PCB 8	PCB 18	PCB 28	PCB 44	PCB 52	PCB 66	PCB 77	PCB 101	PCB 105	PCB 118	PCB 126	PCB 128	PCB 138	PCB 153	PCB 169	PCB 170	PCB 180	PCB 187	PCB 195	PCB 206	PCB 209
Westbrook	<2	<2	<2	<2	<2	<2	<2	2.1	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Mhlanga	<2	<2	<2	<2	<2	<2	<2	10	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Mdloti	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Umgeni	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Country Club	<2	<2	<2	4	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Snake Park	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Vetch's Beach	<2	<2	<2	<2	8.5	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
South Pier	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Brighton	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Treasure Beach	<2	<2	11	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Mlaas	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Reunion	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Tiger Rocks	<2	<2	2.3	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Mbokodweni	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Amanzimtoti	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Karridene	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Mnini	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	2.6	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Widenham	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Scottburgh	<2	<2	<2	<2	7.6	3.2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Park Rynie	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2

Appendix 4. Counts (colony forming unit counts per 100 g of tissue) of *Escherichia coli* and Salmonella in the mantles of mussels collected at locations between Westbrook Beach and Park Rynie in August 2015.

Location	*Escherichia coli*	Salmonella
Westbrook	0	0
Mhlanga	0	0
Mdloti	0	0
Umgeni	0	0
Country Club	0	0
Snake Park	60	0
Vetch's Beach	0	0
South Pier	0	0
Brighton	0	0
Treasure Beach	0	0
Mlaas	0	0
Reunion	60	0
Tiger Rocks	0	0
Mbokodweni	0	0
Amanzimtoti	0	0
Karridene	0	0
Mnini	0	0
Widenham	0	0
Scottburgh	0	0
Park Rynie	0	0

Glossary

Acceptable risk: the maximum level of individual lifetime carcinogenic level risk considered 'acceptable' by risk managers.

Anthropogenic(ally): deriving from a human source.

Bioaccumulation: general term describing a process by which certain chemicals are taken up by a plant or animal either directly from exposure to a contaminated medium (soil, sediment, water) or by eating food containing the chemical. Compounds of a certain type can accumulate in living things when they are taken up and stored faster than they are broken down (metabolized) or excreted. Certain compounds are easily broken down and do not bioaccumulate.

Bioavailable: able to be absorbed by living organisms.

Biomagnification: sequence of processes in an ecosystem by which higher concentrations are attained in organisms at higher trophic levels (at higher levels in the food web); at its simplest, a process leading to a higher concentration of a substance in an organism than in its food.

Bivalve: any mollusc having two valves or shells that are hinged together, as in mussels and clams

Byssus threads: the fine fibres or bundle of silky threads secreted by a gland found in the foot of some bivalves by which they attach themselves permanently to rocks or other solid objects.

Cancer slope factor (CSF): a value assigned to a cancer causing chemical that is used to estimate its ability to cause cancer in humans.

Carcinogen: an agent capable of inducing a cancer response.

Chronic: multiple exposures occurring over an extended period of time, or a significant fraction of the organism's life-time; effects from chronic exposure, or long-term effects from high short-term exposures.

Contaminant: a substance that is either present in an environment where it does not belong or is present at levels that might cause harmful (adverse) health effects

Dose: the amount of a substance to which a person is exposed over some time period. Dose is a measurement of exposure. Dose is often expressed as milligram (amount) per kilogram (a measure of body weight) per day (a measure of time) when people eat or drink contaminated water, food, or soil. In general, the greater the dose, the greater the likelihood of an effect.

Dose-response: the relationship between the amount or magnitude of exposure (dose) and the biological response or toxic injury produced by the chemical.

Exposure: contact made between a chemical, physical, or biological agent and the outer boundary of an organism. Exposure is quantified as the amount of an agent available at the exchange boundaries of the organism (*e.g.* skin, lungs, gut).

Exposure assessment: an identification and evaluation of the human population exposed to a toxic agent that describes its composition and size and the type, magnitude, frequency, route, and duration of exposure.

Exposure pathway: the physical course a chemical or pollutant takes from its source to the organism exposed.

Exposure scenario: A combination of facts, assumptions, and inferences that define a discrete situation where potential exposures may occur. These may include the source, the exposed population, the time frame of exposure, microenvironment(s), and activities. Scenarios are often created to aid exposure assessors in estimating exposure.

Meal consumption limits: recommended restrictions on the frequency of fish meals based on chemical concentrations found in fish tissue. Meal consumption limits are set to keep amounts of chemicals eaten in fish at or below levels believed to cause no adverse health effects.

Non-carcinogen: a chemical or substance that causes non-cancer health effects

Organochlorine pesticides: Pesticides with a chlorine based structure.

Polycyclic aromatic hydrocarbons: substances that occur through incomplete burning of organic substances such as wood, and are also manufactured and used in medicines or to make

dyes, plastics and pesticides.

Polychlorinated biphenyls: industrial chemicals once widely used in electrical equipment, heat exchangers, hydraulic systems and several other specialized applications. Although banned since 1985, they do not readily break down and may remain in the environment for a very long time.

Reference dose (RfD): an estimate (with uncertainty spanning perhaps and order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

Risk: is the chance of something happening that will have a (generally adverse) impact on plants, animals, ecosystems or humans. It is measured in terms of likelihood.

Sensitivity: the condition whereby adverse health effects that occur from exposure to a chemical contaminant are determined by quantitative differences; a chemical can produce the same effect in infants, children, or adults, but the magnitude of effect differs.

Stockholm Convention: an international convention established to address global concerns about persistent organic pollutants. It aims to reduce/eliminate production, use, and/or release of key persistent organic pollutants under the support of the United Nations Environment Programme (UNEP).

Susceptibility: the condition whereby adverse health effects from exposure to a chemical contaminant are due to qualitative differences; such as, unique processes of growth and development in the exposed organism, particularly in young, not fully matured individuals, changes due to aging, state of health, nutritional status, or genetic predisposition to harm.

Uncertainty: uncertainty occurs because of a lack of knowledge. It is not the same as variability. For example, a risk assessor may be very certain that different people drink different amounts of water but may be uncertain about how much variability there is in water intakes within the population. Uncertainty can often be reduced by collecting more and better data, whereas variability is an inherent property of the population being evaluated. Variability can be better characterized with more data but it cannot be reduced or eliminated. Efforts to clearly distinguish between variability and uncertainty are important for both risk assessment and risk characterization.