# THE UPTAKE AND DISTRIBUTION OF SELECTED HEAVY METALS IN THE FRESHWATER CRAB, *POTAMONAUTES PERLATUS* (MILNE EDWARDS), IN THE EERSTE RIVER, WESTERN CAPE

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# DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: _____ Date: 25 / 11 / 1996

# ABSTRACT

A number of studies on the general physico-chemical character of the Eerste River, Western Cape, had previously been done, but the problem of heavy metal pollution had, by 1993, not been addressed. A study was therefore undertaken from 1993-1995 in order to investigate these aspects. Since several researchers have shown that freshwater crabs accumulate certain heavy metals in their bodies and may therefore be used as monitors of environmental heavy metal pollution, the present study concentrated mainly on metal concentrations (Mn, Zn, Cu, Pb and Cd) in the local freshwater crab species, *Potamonautes perlatus*, and its possible use as biomonitor in the Eerste River.

Two localities in the Eerste River were chosen, in order to make comparisons, namely a relatively uncontaminated site in the Assegaaibosch Nature Reserve, Jonkershoek, and a visibly polluted site downstream from Stellenbosch, behind Stellenbosch Farmers' Winery (SFW). Crabs, water and sediment samples were collected seasonally at both localities, and metal concentrations thereof determined by atomic absorption spectrophotometry.

The results showed that the Eerste River down to the SFW locality is still relatively unpolluted in terms of heavy metals. It was, however, clear that runoff from the Stellenbosch municipal, industrial and agricultural areas do have an influence on other physico-chemical features of the river. The concentrations of heavy metals in whole crabs, tissues and carapace showed that Zn concentration was well regulated in *P. perlatus* from both localities, Mn and Cu were accumulated in individuals from SFW, and Pb and Cd accumulated in both populations. Compartmentalization of heavy metals was shown to occur in *P. perlatus*: the carapace was found to be the most important storage site for Mn, Zn and Pb, the carapace and gonads equally important for Cd storage, and the digestive gland the most important site for Cu storage. Whereas gender was shown, generally, to be of little importance in heavy metal uptake in *P. perlatus*, crab body size and seasonality were both shown to influence heavy metal uptake to some extent. However, only summer peaks in whole crab, carapace and tissue manganese concentrations were shown to correlate with peaks in environmental Mn concentrations.

It was concluded that *P. perlatus* would possibly only be a suitable monitor of environmental Mn, Pb and Cd pollution, although there is no guarantee that the crab body would accurately reflect environmental concentrations. It was also ascertained that, since a study of the sperm ultrastructure of *P. perlatus* showed a significantly larger number of abnormal spermatozoa in male crabs from SFW, and since these observed differences could possibly be related to heavy metal exposure, the sperm of this species might be a more reliable indicator of heavy metal pollution. It was finally concluded that more intensive research need to be undertaken on various aspects, especially the use of the spermatozoon as indicator of environmental heavy metal pollution, and that the results of the present study could serve as a basis for future studies.

# UITTREKSEL

'n Aantal studies oor die algemene fisiese en chemiese eienskappe van die Eersterivier, Wes-Kaap, is voorheen gedoen, maar die probleem van swaarmetaalbesoedeling is, teen 1993, nog nie aangeraak nie. 'n Studie is dus onderneem, van 1993-1995, om hierdie aspekte te ondersoek. Aangesien verskeie navorsers bevind het dat varswaterkrappe sekere swaarmetale in hul liggame akkumuleer en dus moontlik as monitors van omgewingsbesoedeling i.t.v. swaarmetale kan dien, het die huidige studie hoofsaaklik gekonsentreer op metaalkonsentrasies (Mn, Zn, Cu, Pb en Cd) in die plaaslike varswaterkrapspesie, *Potamonautes perlatus*, asook sy moontlike gebruik as biomonitor in die Eersterivier.

Twee lokaliteite in die Eersterivier is gekies om vergelykings te kan tref, naamlik 'n relatief ongekontamineerde plek in die Assegaaibosch Natuurreservaat, Jonkershoek, en 'n ooglopend besoedelde plek stroomaf van Stellenbosch, agter Stellenbosch Boere Wynmakery (SBW). Krappe, water- en sedimentmonsters is seisoenaal by albei lokaliteite versamel, en metaalkonsentrasies daarvan bepaal d.m.v. atoomabsorpsie spektrofotometrie.

Die resultate het getoon dat die Eersterivier tot by die SBW lokaliteit nog relatief onbesoedel is i.t.v. swaarmetale. Dit was egter duidelik dat afloop vanaf die Stellenbosch munisipale, industriële en landbougebiede wel 'n invloed het op ander fisiese en chemiese eienskappe van die rivier. Die swaarmetaalkonsentrasies in heel krappe, weefsels en die karapaks, het getoon dat Zn konsentrasie goed gereguleer is in *P. perlatus* eksemplare van albei lokaliteite, Mn en Cu geakkumuleer is in eksemplare van SBW, en Pb en Cd geakkumuleer is in beide bevolkings. Kompartementalisasie van swaarmetale is waargeneem in *P. perlatus*: die karapaks was die belangrikste bergingsplek van Mn, Zn en Pb, die karapaks en gonades ewe belangrik vir Cd berging, en die spysverteringsklier die belangrikste bergingsplek vir Cu. Terwyl dit geblyk het dat geslag nie baie belangrik is in die opneem van swaarmetale in *P. perlatus* nie, het krap liggaamsgrootte en seisoenaliteit wel geblyk om, tot 'n mate, 'n invloed te hê op die opneem van swaarmetale. Slegs die somer pieke vir mangaankonsentrasies in heel krap, karapaks en weefsel het egter gekorreleer met pieke in omgewingskonsentrasies.

Daar is tot die gevolgtrekking gekom dat *P. perlatus* moontlik slegs 'n paslike monitor van omgewingsbesoedeling met Mn, Pb en Cd sal kan wees. Daar is egter geen waarborg dat die krapliggaam omgewingskonsentrasies akkuraat sal weerspieël nie. Dit is ook bevind dat, aangesien 'n studie van die sperm ultrastruktuur van *P. perlatus* 'n beduidend groter getal abnormale spermselle in manlike krappe van SBW opgelewer het, en aangesien die waargenome verskille moontlik herlei kan word na metaalblootstelling, die spermsel van hierdie spesie moontlik 'n meer betroubare indikator van swaarmetaalbesoedeling kan wees. Die finale gevolgtrekking was dat meer intensiewe navorsing oor verskeie aspekte onderneem moet word, veral oor die gebruik van die spermatosoön as indikator van omgewingsbesoedeling met

swaarmetale, en dat die resultate van die huidige studie sal kan dien as basis vir toekomstige studies.

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Dedicated to my Heavenly Father

*"...Who is able to do immeasurably more than all we ask or imagine, according to His power that is at work within us, to Him be glory..."*

Ephesians 3:20-21

and to my parents, Genie and the late Chris Snyman, my role-models.

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# CHAPTER 1

## INTRODUCTION

Water is one of mankind's most precious resources and needs to be protected and maintained in a healthy state (Roux et al., 1996). Unfortunately, in South Africa, according to Davies et al. (1993), we are already facing a water resources crisis of appalling dimensions. They state that South African rivers are not only threatened by uneven rainfall and extraordinarily high evaporation rates, but that few rivers in this region have not been affected by over-exploitation, degradation, pollution and regulation by impoundments.

The destruction of our freshwater ecosystems due to poor management, inconsiderate use and the needs of a rapidly-expanding human population, is placing tremendous pressure on conservation bodies to develop new conservation strategies, wherein stricter control of human activities in and around lakes, dams and rivers, can be implemented. Rivers, especially, are very vulnerable, since runoff from factories, industries, homes and farms, are dumped directly into them or reach them through ground water seepage, while human activities such as swimming, picnicking, boating, etc., cause serious litter problems.

The criteria used to evaluate the degree of pollution in a river are normally those which only give an indication of organic pollution, such as the amounts of litter and bacteria in and around the river and the concentrations of oxygen, carbon dioxide, phosphates and nitrates in the water. This, however, does not present the full picture. A riverine ecosystem might seem relatively unpolluted, but even small volumes of effluent of industrial, household and agricultural origin can contain high concentrations of inorganic pollutants, especially heavy metals and pesticides such as polychlorinated biphenyls (PCB's).

Another problem that arises is that even after it has been established that a river contains relatively low concentrations of these pollutants, the long term effects on the entire ecosystem (fauna included) might still be great and should be investigated.

Many animal species are not apparently adversely affected by sublethal levels of pollutants. Various are known to regulate the concentrations of heavy metals and PCB's in their bodies, whilst other species, including plants, bioaccumulate these pollutants in their bodies, to relatively high concentrations (e.g. Bryan, 1968; de Wet et al., 1990 and Steenkamp, 1992). Reinecke (pers. comm.) defines a bioaccumulator as an organism which accumulates xenobiotic substances over time in its body, thus reflecting exposure levels and bioavailability and possible threat to other participants in the food chain.

The question arises whether and to what extent these relatively high concentrations affect the animals in some other way, for example morphologically, physiologically or behaviourally. A very popular research topic nowadays involves the effects of pollutants on the sperm morphology of the animal. For instance, Ackerman (1995), Reinecke et al. (1995) and Reinecke & Reinecke (1996 in press) have all illustrated the negative effects of heavy metals and pesticides on the sperm morphology of their study animals.

Several authors have studied the relationship between the concentrations of heavy metals in aquatic plant species and the degree of water pollution, e.g. de Wet et al. (1990), Manny et al. (1991) and van der Merwe et al. (1990). Others, however, have concentrated on aquatic animals such as invertebrates (Bryan, 1971 & 1976; Burrows & Whitton, 1983; Dixit & Witcomb, 1983; Kiffney & Clements, 1993; Klump et al., 1987; Lynch & Popp, 1988; Sanders & Chandler, 1972 and Timmermans et al., 1989) and fish (Bezuidenhout et al., 1990; Seymore et al., 1995 and Stouthart et al., 1996).

A number of these studies were carried out over a period of several years and the species investigated were, therefore, used as biomonitors, e.g. Burrows & Whitton (1983), Kiffney & Clements (1993) and Lynch & Popp (1988). Van Straalen & Verkleij (1993) defines a biomonitor as an indicator organism which is used repeatedly in order to establish trends in environmental quality. Reinecke (pers. comm.) describes an indicator organism, on the other hand, as one whose responses at various levels can be reliably, specifically and causally linked to environmental factors.

Since magnification of certain heavy metals and pesticides can occur in the food chain, scientists are becoming increasingly concerned about the effects on birds and mammals and several authors, therefore, expanded their ecotoxicological research to include these groups (Mason et al., 1986; Mason & Macdonald, 1986; Mason & Sullivan, 1993; Scheuhammer, 1987 and Van Eeden & Schoonbee, 1993).

However, the invertebrates remain the best studied group. Extensive research on Crustacea-pollutant interactions have been undertaken, in the field as well as in the laboratory. Topics range from the metabolic requirements in crustaceans for heavy metals (Depledge, 1989), to environmental studies and laboratory tests on the accumulation and regulation of heavy metals by crustaceans (Bryan, 1968; Johns & Miller, 1982; Rainbow & White, 1989 and Yan et al., 1989) and to the toxic effects of heavy metals and pesticides on these animals (Corner & Sparrow, 1956 and Weis et al., 1992).

The marine crustaceans, especially, have received much attention. Not only have investigations been made into heavy metal accumulation, regulation and distribution in these animals, but also

into the toxicity of heavy metals and pesticides to various species, as well as their degree of tolerance to these pollutants.

Among the species studied are the lobster *Homarus vulgaris* (Bryan, 1965), shrimps such as *Crangon crangon* (Rasmussen et al., 1995) and *Palaemon elegans* (Rainbow & Nugegoda, 1984 and Rainbow & White, 1989), as well as several species of crab: *Portunus pelagicus* (Hilmy et al., 1988), *Scylla serrata* (Arumugam & Ravindranath, 1983; 1987), *Carcinus mediterraneus* (Devescovi & Lucu, 1995), *Carcinus maenas* (Rasmussen et al., 1995; Pedersen & Bjerregaard, 1995 and Rainbow, 1985), the *Cancer* species, *C. pagurus* (Lind et al., 1995) and *C. irroratus* (Martin, 1974), *Chasmognatus granulata* (Rodriguez & Lombardo, 1991), the blue crab *Callinectes sapidus* (Engel & Brouwer, 1987) and a number of *Uca* species: *U. annulipes*, *U. triangularis* (Devi, 1987) and *U. uruguayensis* (Rodriguez & Lombardo, 1991).

During the past two decades numerous publications have appeared on freshwater crustaceans and pollutants. Several are field and laboratory studies on the accumulation and regulation of heavy metals, while others discuss the toxic effects of various pollutants on body processes. The decapod crustaceans, particularly the freshwater crayfish and crab, have been given the most publicity. Examples include the crayfish species *Orconectes propinquus* (Roldan & Shivers, 1987), *O. virilis* (Bendell Young & Harvey, 1991; France, 1987 and Anderson & Brower, 1978), *Austropotamobius pallipes* (Lyon, 1984) and two *Cambarus* species, namely *C. bartoni* and *C. robustus* (Bendell Young & Harvey, 1991). Rajeswari et al. (1988), Rafi et al. (1991) and Radhakrishnaiah (1987), have all studied the freshwater field crab *Oziotelphusa senex senex*, whereas Tulasi et al. (1987) and Tulasi & Ramana Rao (1988) brought out publications on *Barytelphusa guerini*.

Only one freshwater crab species, *Potamonautes warreni*, has been studied intensively in South Africa. Van Eeden & Schoonbee (1991), Steenkamp (1992), Steenkamp et al. (1993; 1994a; 1994b; 1995) and du Preez et al. (1993), investigated the bioaccumulation of selected heavy metals in this species. Preliminary results show that *P. warreni* accumulates certain heavy metals to high levels, whereas the concentrations of others are regulated well in their bodies. It has also become clear that some tissues and organs are subjected to higher metal loads than others. It was concluded that *P. warreni* can serve as a biomonitor, though only when studying certain heavy metals.

The freshwater crab species *Potamonautes perlatus* belongs to the family Potamonautidae and occurs in rivers in the south-western parts of the Western Cape Province (Barnard, 1950). Freshwater crabs are known to be the largest naturally occurring invertebrates inhabiting southern African rivers and are likely to play an important rôle in the processing of organic material (Hill & O'Keeffe, 1992). Apart from a report on respiratory exchange in *P. perlatus* by Hogben & Zoond (1930), a general description of the species and its distribution by Barnard

(1950), its ecology, studied by Hill & O'Keeffe (1992) and the sperm ultrastructure, discussed by Jamieson (1993), very little is known about this species. A full description of its reproductive cycle is lacking and, until 1993, no ecotoxicological studies on *P. perlatus* had been done.

With the findings of Van Eeden & Schoonbee (1991) and Steenkamp (1992) as basis, a study was undertaken from 1993 to 1995, in the Eerste River near Stellenbosch, using *P. perlatus* as a biomonitor. Even though there are no heavy industries on the banks of this river or of its tributaries, there has been growing concern for its future condition, as shown by the implementation of the Stellenbosch Rivers Interest Group. It was thus found necessary to investigate the levels of inorganic pollutants, which might originate from the existing factories, wineries and farmlands in the vicinity, in this ecosystem.

The study was of an ecotoxicological nature and dealt with the accumulation, regulation and distribution of selected heavy metals in the body, as well as the possible sublethal effects of these heavy metals on the sperm ultrastructure of the species. The selected heavy metals tested for were manganese, zinc, copper, lead and cadmium.

## Aims of the study

· To determine the effects of industries, farms and wineries on the Eerste River ecosystem, through a comparison between the concentrations of heavy metals and other chemical factors at two different locations in the river, namely upstream and downstream from Stellenbosch (Assegaaibosch Nature Reserve and Stellenbosch Farmers' Winery respectively).

· To establish whether and to what extent *Potamonautes perlatus* accumulates heavy metals, as well as the specific tissues and organs to which these metals are distributed, i.e. either the digestive gland, gills, gonads, muscles or exoskeleton (carapace).

· To investigate the relationships between size, gender, seasonality and the concentrations of heavy metals in the animal.

· To evaluate the possible use of *P. perlatus* as a monitor of heavy metal pollution, i.e. to determine to what extent the species satisfies the definition of a biomonitor.

· To ascertain whether sublethal levels of heavy metals affect the sperm ultrastructure of the species, through (a) an electron microscopic study of its sperm ultrastructure, as well as through

(b) comparisons of the total number of sperm and number of abnormal sperm per grid block (see Chapter 3), per individual, from the two chosen localities.

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# CHAPTER 2

## STUDY AREA

### General Description

The study was undertaken in a section of a Western Cape Province river, the Eerste River (Figure 2(a)). This river, 40 km in length, rises in the Dwarsberg, at the head of the Jonkershoek Valley (Petitjean, 1987), and flows through the Jonkershoek Forest Reserve, Assegaaibosch Nature Reserve, as well as several vineyards, after which it passes through the town of Stellenbosch (33°56'10"S; 18°51'34"E). For the rest of its course, the river is surrounded by agricultural land and a few settlements, until it reaches the sea at Macassar Beach on the False Bay coast.

In 1981 the Kleinplaas Dam was constructed in the Jonkershoek Forest Reserve, to regulate the flow of the upper Eerste River. This is a balancing and diversion structure and forms part of the Riviersonderend-Berg River Water Transfer Project (Petitjean, 1987). This dam also receives water from the Theewaterskloof Dam, near Villiersdorp (Department of Water Affairs, 1986).

Immediately downstream from Stellenbosch, the Eerste River receives its first main tributary, the Plankenburg River. This is a 10 km long river which runs past the townships of Cloetesville and Kaya Mandi, as well as past the Stellenbosch industrial area. The Plankenburg River joins the Eerste River at the Adam Tas bridge. Another stream, the Krom River, flows through forest reserves and grasslands before it joins the Plankenburg River at the George Blake road (Petitjean, 1987). The main abstraction point of the Lower Eerste River Irrigation Board is situated immediately below the Adam Tas bridge (Petitjean, 1987). The remaining three tributaries of the Eerste River are the small Veldwagters, Blouklip and Sanddrif Rivers. The Veldwagters River carries the treated effluent of the Stellenbosch Municipality sewage works. These three rivers were, however, not included in the specific study area.

### Climate and rainfall

Stellenbosch and the Eerste River are situated in the winter rainfall region, characterised by a Mediterranean-type climate of cool, wet winters in the south-west (as in the area chosen for the present study), grading eastwards along the south coast into spring and autumn rain peaks (Davies et al., 1993). According to the Rutherford and Westfall (1986) classification, Stellenbosch is situated in a part of the Fynbos Biome, which, apart from mainly winter rainfall, is also characterised by short-lived snowfalls on the higher mountain peaks in winter, whereas

hail is rare. Summer wind is, however, a common occurrence and is often very strong, persisting for several days.

The mean daily temperature and rainfall and total annual rainfall for Stellenbosch and Jonkershoek, respectively, during the period of study, 1993-1995, including absolute maximum and minimum temperatures for each year of study, are recorded in Tables 2.1 and 2.2. The marked difference in temperature and rainfall between Stellenbosch and Jonkershoek is an important consideration, since, of the two localities chosen for the study, one fell within the boundaries of Stellenbosch and the other within those of Jonkershoek.

All rainfall and temperature data were provided by the South African Weather Bureau, Agromet and Forestech, Jonkershoek.

Table 2.1: Rainfall data for the period 1993-1995, for Stellenbosch and Jonkershoek, including total annual and mean daily rainfall (mm).

| | Year | Stellenbosch | Jonkershoek |
|---|---|---|---|
| Total annual rainfall (mm) | 1993 | 823.0 | 1298.0 |
| | 1994 | 611.0 | 968.0 |
| | 1995 | 638.0 | 1217.0 |
| Mean daily rainfall (mm) | 1993 | 2.3 | 3.6 |
| | 1994 | 1.7 | 2.7 |
| | 1995 | 1.7 | 3.9 |

Table 2.2: Temperature data for the period 1993-1995, for Stellenbosch and Jonkershoek, including mean daily, absolute maximum and absolute minimum temperatures (°C).

| | Year | Stellenbosch | Jonkershoek |
|---|---|---|---|
| Mean daily temperatures (°C) | 1993 | 17.8 | 15.7 |
| | 1994 | 17.7 | 15.7 |
| | 1995 | 17.5 | 18.0 |
| Absolute maximum temp. (°C) | 1993 | 39.3 | 40.3 |
| | 1994 | 38.8 | 37.8 |
| | 1995 | 38.7 | 38.7 |

| | | | |
|---|---|---|---|
| Absolute | 1993 | 3.5 | 2.0 |
| minimum | 1994 | 3.0 | 2.2 |
| temp. (°C) | 1995 | 2.3 | 1.5 |

## Vegetation

The vegetation types which characterise the Fynbos Biome are the usually evergreen, sclerophyllous phanerophytes, chamaephytes and hemicryptophytes, which occur codominantly (Rutherford and Westfall, 1986). The plant species found along the banks of the Eerste River vary throughout its course and indigenous trees have, to a large extent, been replaced by exotic species. The specific species found at each locality will be discussed later.

## Fauna

Although the area is rich in endemic fauna, only a few species will be mentioned. Listed below are those species, endemic and exotic, which are considered significant to the study and which will be referred to in the final discussion.

1. Invertebrates:
Freshwater crab (*Potamonautes perlatus*)
2. Fishes (all exotics):
Smallmouth bass (*Micropterus dolomieu*)
Largemouth bass (*Micropterus salmoides*)
Rainbow trout (*Onchorhynchus mykiss*)
3. Birds:
Giant kingfisher (*Megaceryle maxima*)
4. Mammals:
Cape clawless otter (*Aonyx capensis*)
Water mongoose (*Atilax paludinosus*)

## Localities

In order to investigate the effects of the Stellenbosch industries on the Eerste River ecosystem, two localities were chosen for this study: one to represent a relatively uncontaminated environment, upstream from the town, and the other a section of the river downstream from Stellenbosch, where human intervention and pollution are clearly visible.

The first locality was situated in the Assegaaibosch Nature Reserve, at a small weir (33°58'21"S; 18°56'4"E). Here the river is approximately 10 m wide in summer, and forms a pool upstream from the weir, but flows fast thereafter. A narrow, fast flowing canal runs alongside the main stream at this point. The river substrate consists of boulders, large stones, bedrock (King, 1981), as well as pebbles and coarse sand. Algal growth is normally sparse, although *Spirogyra* is common in summer. The river is lined by tough-leaved, evergreen trees, e.g. *Metrosideros angustifolia* and *Brabejum stellatifolium*, but these are mostly replaced by the exotic oak, *Quercus robur* (King, 1981). The trees form a canopy over the stream and the fall of their leaves through late spring and early summer forms the major source of instream organic material, a fact which is of great importance to the freshwater crab, a detritus feeder. This coincidence of leaf fall and high temperatures is in contrast with the autumnal leaf fall at decreasing temperatures in the Northern Hemisphere (King et al., 1987; 1988).

Apart from crab-eating fish species in the river, a number of other animal species which rely on crabs in their diet have been recorded at this locality: a Giant kingfisher pair (*Megaceryle maxima*) was often observed here during the study period. Signs of Cape clawless otter (*Aonyx capensis*), in the form of scats and eaten crab, were frequently found at the weir. The water mongoose, *Atilax paludinosus*, which is a potential competitor of the Cape clawless otter for crabs, is also found in this area. Although no signs of water mongoose were recorded at this specific locality during the study, previous personal observations and local home range studies on these animals (Purves, 1995) have proved that they do utilize this area.

The water at this locality is clear and apparently free of organic pollution. Although some human activity occurs here, such as hiking and occasionally swimming, the area is kept clean and is free of litter.

The second locality, which will in future chapters be referred to as SFW, was situated downstream from the Adam Tas bridge, where the Eerste and Plankenburg Rivers meet, directly behind Stellenbosch Farmers' Winery (33°56'47"S; 18°50'32"E). This station is also located just below a small weir, where the river is approximately 5-8 m wide in summer. Similar to the first locality, the river at this point forms a pool upstream from the weir, but is fast-flowing thereafter. The river substrate consists of stones and pebbles on coarse sand (King, 1981) as well as finer, muddy sand. Some algal and fungal growth can be found on the rocky substrate and, compared to Locality 1, fewer water-dwelling invertebrates are found here (King, 1981). This is a well known sign of pollution. Evergreen as well as deciduous trees line the banks, with the two dominant species being the exotics, *Quercus robur* and *Populus canescens* (King, 1981). Similar to the first locality, the fall of the leaves also forms the main source of instream organic material during the warmer months.

The site includes an otter latrine, which was frequently used during the study period, indicating that these animals also often utilize this area for foraging.

Possibly as a result of human activities such as picnicking and swimming, the water is murky and often foul-smelling. The water quality is also greatly affected by runoff from the Plankenburg River and winery effluent from Stellenbosch Farmers' Winery.

At both the chosen localities the water level remains relatively low throughout the drier months as a result of draw-off for water supply to Greater Cape Town and excessive irrigation demand (Davies et al., 1993), but slight increases in level and flow rate do occur, coinciding with the occasional opening of the Kleinplaas Dam sluices in Jonkershoek. During the months of heaviest rainfall, however, the water level at Locality 2, especially, rises dramatically with the increase in water volume and flow rate. This results in a large amount of sludge and litter being washed downstream and settling at this site.

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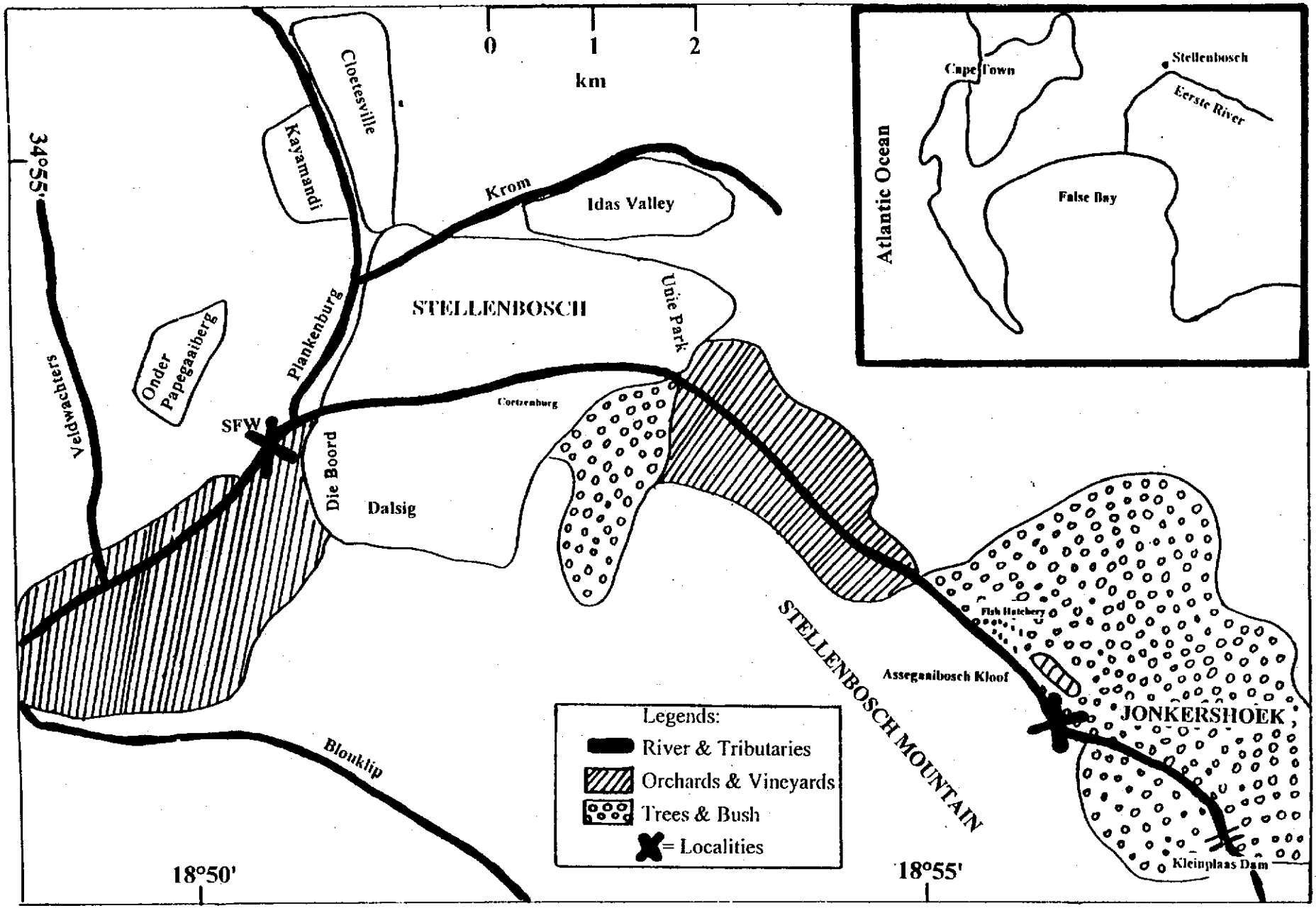


Figure 2(a): Course of the Eerste River through Jonkershoek and Stellenbosch.

# CHAPTER 3

# MATERIALS AND METHODS

## 1. Crab samples

Crabs, *Potamonautes perlatus* (Milne Edwards), were collected seasonally for a period of two years, at both localities. Collections were done during December/January, April/May, July/August and October/November, to represent summer, autumn, winter and spring respectively. Large (i.e. >40 mm carapace width) and medium sized individuals (i.e. 21-40 mm carapace width) were caught in baited funnel traps, set in the afternoons and left overnight, or until a large enough sample size (>10 individuals; >5 of each gender and size class) was obtained. Small-sized crabs (i.e. <20 mm carapace width) were infrequently caught in the traps and numbers often had to be supplemented with individuals collected by hand.

The crabs were immediately transported to the laboratory, where they were killed by freezing. After thawing and prior to dissection, carapace width, sex and wet mass were determined, upon which all individuals were grouped into three size classes, according to carapace width: Large (>40 mm carapace width), Medium (between 21 and 40 mm carapace width) and Small (<20 mm carapace width). These size classes were chosen to represent different stages of maturity. A number of representatives from each size class were used whole, for metal analysis, whereas others were dissected. Dissections were performed with stainless steel instruments on a metal free surface and the carapace as well as the following tissues were removed: the digestive gland, gills, gonads and claw muscle. The tissues and carapaces from different individuals of the same size class and gender were occasionally pooled, in order to obtain the correct sample size (1 g). All samples were stored at -10°C in acid-rinsed bottles.

The whole crabs and carapaces were then dried at 105°-110°C for 24 h, or until their weight stabilized. (These temperatures were chosen since Fourie & Peisach (1976) have shown that temperatures above and below these may cause losses of certain metals). After drying, the samples were ground, using a pestle and mortar. At this point some samples were once again pooled to obtain a large enough sample.

One gram of each whole crab, carapace and thawed tissue sample was weighed on a Mettler AE 200 and digested in a Labcon dual digester, using a 10:1 ratio of 55% nitric acid and 70% perchloric acid. (The choice of acids used was based on the method described by Van Eeden and Schoonbee, (1991)). The samples were firstly digested with 10 ml nitric acid at room temperature for 24 h, then at 40°-50°C for 2 h, after which the temperature was increased to 140 °C for one hour. The one hour digestion at 140°C was repeated after adding 1 ml perchloric acid

to each sample. A blank sample containing only a 10:1 ratio nitric:perchloric acid solution was also prepared, using the same method. The samples were allowed to cool only after obtaining a clear solution.

After cooling, each sample was filtered through Whatman 9.0 cm qualitative filterpaper, as well as a Sartorius Minisart 0.45 µm pore size filter, using a needle and syringe. The filtrate was then diluted to 100 ml with distilled water and stored at 6°C until needed for heavy metal analysis. To avoid contamination of samples, all glassware used was carefully washed and rinsed several times with a solution of 32% hydrochloric acid and distilled water.

The concentrations of zinc, manganese, copper, lead and cadmium in the various samples were determined by atomic absorption spectrophotometry on a Varian Spectr.AA 250 Plus model. Atomic Absorption Standard Solutions from Sigma, as well as deionised water, were used to prepare the analytical standards.

The metal concentrations in the whole crab and carapace samples, expressed as µg metal per gram dry mass, were recalculated, using the dry and wet mass of each sample, to be expressed as µg metal per gram wet mass. All tissue samples, expressed as µg metal per gram wet tissue mass, were in turn recalculated to µg metal per gram dry tissue mass, in order to use in the statistical analysis and to compare with results in the literature. This recalculation was done by dissecting a number of crabs and weighing and drying the carapace and tissues of each individual, as well as a few whole individuals, at 60 °C for 24 h, or until the mass stabilized. Using the wet and dry mass of each sample, the percentage water loss, thus also percentage dry matter, was ascertained. The mean dry matter percentage for whole crabs, each tissue and for the carapace were then used for the final recalculation.

A bioconcentration factor (BCF) was calculated for whole crabs, tissues and carapace, for each metal. The formula used to calculate the BCF (from Van Straalen & Verkleij, 1993), was:

BCF = [metal] in crab sample ÷ [metal] in water/sediment,

yielding a value of <1, 0 or >1 and giving an indication of the degree of heavy metal accumulation in the animal, from the water ($BCF_W$) and sediments ($BCF_S$).

Statistical analysis of the data was done with the use of the Quattro Pro and StatGraphics computer programs, as well as Student's t-test. In order to avoid confusion, the results for the five selected heavy metals will be discussed in separate chapters (Chapters 5-9).

2. **Water and sediment samples**

With every seasonal collection of crabs, water and sediment samples were also taken at each locality.
Water samples (50ml), were taken once every season, in fast flowing sections of the river, approximately 5-10 cm below the surface. Sediment samples, also taken once per season, were collected from the river bottom, in shallower sections, closer to the river banks. Only the top 10 cm was taken. In each instance a 100 ml container was filled. All samples were stored in pre-washed, acid-rinsed containers, at -10°C, until needed for analysis.

In order to determine the total metal concentration in each water sample, these were acidified with 55% nitric acid and filtered through Whatman 9.0 cm qualitative filterpaper, as well as a Sartorius Minisart 0.45 µm pore size filter, using a needle and syringe. Thereafter, each sample was diluted to 100 ml with distilled water and kept at 6°C until analysed.

Sediment samples were prepared for heavy metal analysis by firstly drying the samples at 100°C for 24 h, or until the weight stabilized, after which each sample was sieved to obtain a homogenous sample. These were then finely ground with a pestle and mortar and a 1 g subsample then weighed off on a Mettler AE 200.

The choice of acids used for digestion was based on the method described by Anderson (1974), in which a 1:1 ratio of hydrochloric and nitric acid was used. Ten ml 55% nitric acid was added to each sample and left at room temperature for 24 h, after which the temperature of the Labcon dual digester used was adjusted to 40°-50°C for 2 h. The temperature was then increased to 140°C for 1 h. This 1 h digestion at 140°C was repeated after adding 10 ml 32% hydrochloric acid to each sample. A blank sample containing only a 1:1 hydrochloric:nitric acid solution was also prepared, using the same method. The samples were allowed to cool only after a clear solution was obtained. Each sample was filtered through Whatman 9.0 cm qualitative filterpaper as well as a Sartorius Minisart 0.45µm pore size filter, using a needle and syringe. The filtrate was then diluted to 100 ml with distilled water and the samples stored at 6°C for further analysis. The residues were dried at 100°C until the weight stabilized, and then weighed accurately, to determine the actual amount digested. To avoid contamination of the samples, all glassware was carefully washed and rinsed several times with a 32% hydrochloric acid and distilled water solution, prior to use.

Heavy metal, i.e. zinc, manganese, copper, lead and cadmium concentrations, were determined by atomic absorption spectrophotometry on a Varian Spectr.AA 250 Plus model. Atomic Absorption Standard Solutions from Sigma, as well as deionised water, were used to prepare the analytical standards.

All other water analysis data were obtained from the Department of Water Affairs, Cape Town. Several sampling stations along the Eerste River were chosen and frequented by researchers from this Department. Of these stations, the following were selected for the present study: a site in the Jonkershoek Nature Reserve, one in the Assegaaibosch Nature Reserve (a few hundred meters downstream of Locality 1 of the present study), a site in the Plankenburg River (under Adam Tas bridge), a site behind Stellenbosch Farmers' Winery (at Locality 2 of the present study) and finally, one downstream of the point where the Veldwachters and Eerste Rivers meet. Of these sites, a few were only visited on one or two occasions, or selected recently, hence the lack of chemical data on some of them (see Table 4.3).

Chemical analyses of samples from these stations were done by the Department of Water Affairs, whereas the data from bacteriological analyses were provided by the Cape Town Municipality and Regional Services Council. Statistical analysis of the data was done with the use of the Quattro Pro and StatGraphics computer programs as well as Student's t-test.

## 3. **Sperm ultrastructure**

The testes and vasa deferentia from selected mature male crabs (±3 individuals from each locality, seasonally) were dissected and stored in 4% gluteraldehyde at 6°C for primary fixation, until needed for transmission electron microscopic preparation.

Samples were washed 3 times in Millonig phosphate buffer for 10 min each, after which they were post fixated for 1 h in 0.5% watery osmium tetroxide. The samples were then washed 3 times for 10 min each with distilled water. Staining was done with 2% uranyl acetate for 30 min, after which the samples were once more washed with distilled water: 3 times for 10 min each. Five different concentrations of acetone were used in the dehydration process for 10 min each, namely 25%, 50%, 75%, 95% and 100% acetone. The 100% acetone dehydration was repeated but left overnight and the acetone removed before commencing the embedding process.

The samples were infiltrated with 3 different ratios of acetone:Spurr resin solutions, for 1 h each. The ratios were 3:1, 2:1 and 1:1. Hereafter infiltrating was done for 1 h in 100% Spurr resin, which was repeated a second time but left overnight and the resin removed before embedding the samples in silicone rubber embedding moulds, and filling them with 100% Spurr resin. Polymerization was then done at 70°C for 24 h.

The resin blocks were prepared for transmission electron microscopy by firstly trimming and shaping them, using razor blades, after which thick sectioning and fine trimming with an ultramicrotome were done. Ultrathin sections (±70-90 nm) were finally made. These were stained as follows: the sections were evened out with xylene and mounted on 200 square size

copper grids. The staining procedure started with 5% uranyl acetate for 10 min, after which the sections were washed with distilled water and dried on filter paper. The last staining was done with Reynolds' lead citrate (Reynolds, 1963) for 2 min and the sections finally washed and dried once more. Sections were viewed in a Phillips 201 transmission electron microscope, operated at 60 kV.

Sections of the vasa deferentia of four animals from each locality were used for the sperm counts. These were done by counting the total number of sperm in 2-3 blocks of a grid, as well as the number of abnormal spermatozoa per block, and determining the average thereof.

Statistical comparisons of the two localities were done with the help of the StatGraphics computer program and Student's t-test.

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# CHAPTER 4

# PHYSICO-CHEMICAL CONDITIONS AT THE CHOSEN LOCALITIES IN THE EERSTE RIVER

## Introduction

An important objective of most water management programmes is the preservation of aquatic life. To meet this objective, it is necessary to maintain, within certain limits, variables such as water temperature, turbidity and other parameters which contribute to the chemical quality of the water (Abel, 1989). For this reason, management bodies such as the Department of Environment Affairs and Tourism have developed quality criteria for, among others, river water.

A number of hydrobiological studies have been undertaken in the Eerste River, at approximately the same sites as were chosen for the present study, of which the investigations of King (1981) and Petitjean (1987) are examples. These studies have, however, concentrated on the physical and selected chemical features of the river and have not addressed the occurrence of heavy metals. An assessment of the levels of heavy metals in river water is of great significance, since it can, if present in high concentrations in the water, hold serious implications for aquatic life (See introductions to Chapters 5 to 9).

The present study dealt with both the physical and chemical (including heavy metal) features of Eerste River water. All these features should be considered together since the physical features on their own not only affect the physiology and behaviour of organisms (Warburg et al., 1982 and Van Aardt, 1993) but may also affect heavy metal availability and toxicity. Kempster et al. (1980) reported that the toxicity of metals such as lead, zinc and copper to fish is higher in water with a low conductivity, whilst Campbell & Stokes (1985), Crowder (1991) and Wren & Stephenson (1991) discussed the effects of water acidification on the toxicity and availability of certain heavy metals.

Since many aquatic organisms, including freshwater crabs, are closely associated with river sediments the latter should be considered as an additional source of heavy metals together with the water and therefore analysed as well. Even heavy metals which are "immobilized" in the bottom sediments of rivers constitute a potential hazard since they may be released as a result of chemical changes in the aquatic environment. These changes may include increased salinity, a lowering of pH, the introduction of synthetic complexing agents, microbial activities and physical effects such as erosion (Förstner & Prosi, 1979).

## Materials and Methods

All samples for heavy metal analysis were prepared, tested and statistically analysed according to the method described in Chapter 3. All physical and chemical data for Eerste River water were gathered during the present study or obtained from the Department of Water Affairs, Cape Town.

## Results

### WATER ANALYSIS

#### Differences in heavy metal concentrations between Jonkershoek and SFW

Student's t-test, and the Mann-Whitney test in the case of the zinc concentration data, were used to determine whether statistically significant differences ($p<0.05$) in heavy metal concentrations existed between the two chosen localities. These results are shown in Tables 4.1 and 4.2.

Although all metals, except manganese, had their highest concentrations at SFW, no significant differences ($p>0.05$) were found between the two localities. Figure 4(a) shows the mean metal concentrations in the water at both localities.

#### Analysis of physical and other chemical features of Eerste River water

A list of the physical and chemical features tested for at each station during the study period, as well as their respective mean concentrations/values, is given in Table 4.3. A comparison of these features, with the water quality criteria as stipulated by the Department of Environmental Affairs and Tourism and other international bodies, is made in the discussion of this chapter.

The chemical and physical data for Assegaaibosch and SFW (stations 2 and 4 in Table 4.3) were compared by means of the nonparametric Mann-Whitney test, in order to investigate possible significant differences ($p<0.05$). Table 4.4 lists the results of these tests.

In the cases of all the chemical and physical factors tested for, except the dissolved oxygen, water temperature and nitrates, the data from SFW had the highest mean concentrations/values. Statistically significant differences in these factors between the two localities/stations were found for pH, conductivity, suspended solids, phosphates, faecal coliforms and total coliforms.

# SEDIMENT ANALYSIS

## Differences in heavy metal concentrations between Jonkershoek and SFW

When the mean metal concentrations in the sediments from Jonkershoek and SFW were compared statistically (Table 4.5), it was found that significant differences ($p<0.05$) existed between the manganese and between the zinc concentrations of the two localities.

The sediments from Jonkershoek exhibited the highest manganese concentration of the two sites, whereas the SFW sediments exhibited the highest concentrations for zinc and also, though not statistically significant, for the other three metals. Figure 4(b) shows the mean metal concentrations in the sediments from both localities.

Table 4.1: Results of Student's t-test for the differences in water heavy metal concentrations ($mg.l^{-1}$) between the two localities: Jonkershoek (J) and SFW (S).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Manganese(J) | 8 | 0.138 | 0.118 | 0-0.26 | 0.22 | >0.05 |
| vs | | | | | | |
| Manganese(S) | 8 | 0.125 | 0.133 | 0-0.27 | | |
| Copper(J) | 8 | 0.062 | 0.048 | 0.01-0.14 | -0.4 | >0.05 |
| vs | | | | | | |
| Copper(S) | 8 | 0.074 | 0.071 | 0.01-0.192 | | |
| Lead(J) | 8 | 0.027 | 0.019 | 0.01-0.06 | -0.78 | >0.05 |
| vs | | | | | | |
| Lead(S) | 8 | 0.035 | 0.025 | 0.015-0.08 | | |
| Cadmium(J) | 8 | 0.0055 | 0.0029 | 0.002-0.009 | -0.53 | >0.05 |
| vs | | | | | | |
| Cadmium(S) | 8 | 0.0063 | 0.0027 | 0.003-0.01 | | |

Table 4.2: Results of the Mann-Whitney test for the differences in water zinc concentrations ($mg.l^{-1}$) between the two localities: Jonkershoek (J) and SFW (S).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Zinc (J) | 8 | 0.219 | 0.325 | 0-0.874 | 0.563 | >0.05 |
| vs | | | | | | |
| Zinc (S) | 8 | 0.226 | 0.295 | 0-0.818 | | |

Table 4.3: Mean values of physical and chemical features of water, collected at selected stations in the Eerste River. Stations: 1. Jonkershoek Nature Reserve; 2. Assegaaibosch Nature Reserve; 3. Plankenburg River (Adam Tas bridge); 4. Stellenbosch Farmers' Winery; 5. Below confluence of Veldwachters and Eerste Rivers.

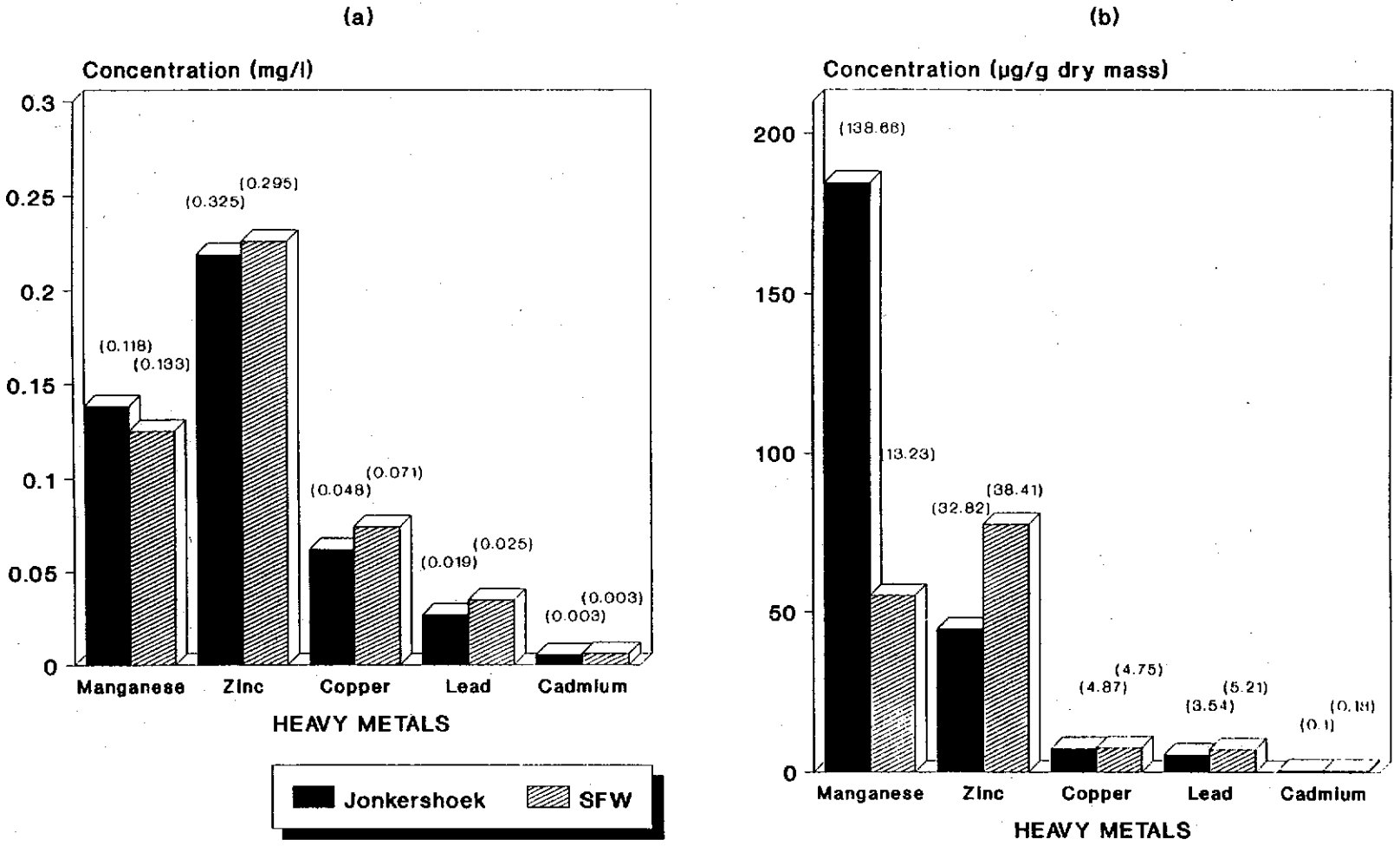
| **Determinand** | **Unit** | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|---|
| pH | pH | 6.19 | 6.74 | 7.27 | 7.1 | 7.17 |
| Conductivity | mS/m | 4.23 | 6.76 | 45.53 | 18.5 | 32.32 |
| Temperature | °C | 16.15 | 18.33 | -- | 16.3 | 17.7 |
| Dissolved $O_2$ | $mg.l^{-1}$ | 10.38 | 10.07 | -- | 9.03 | 7.45 |
| Suspended solids | $mg.l^{-1}$ | 5.0 | 7.38 | 14.13 | 9.07 | 14.69 |
| Nitrates | $mg.l^{-1}$ | 0.2 | 0.48 | 0.43 | 0.27 | 1.57 |
| Phosphates | $mg.l^{-1}$ | 0.03 | 0.05 | 0.05 | 0.06 | 2.2 |
| Hardness ($CaCO_3$) | $mg.l^{-1}$ | 4.0 | 14.5 | -- | 45.0 | -- |
| Total coliforms | 100ml | 13.29 | 498.83 | -- | 128227.3 | 53209 |
| Faecal coliforms | 100ml | 2.9 | 105.83 | -- | 15347.3 | 17664 |

Table 4.4: Results of the Mann-Whitney test for differences between chemical and physical data of water from stations 2 and 4: Assegaaibosch (J) and SFW (S) (Cond. = conductivity; Temp. = temperature; Diss.$O_2$ = dissolved oxygen; S.sol. = suspended solids; $NO_3$ = nitrates; $PO_4$ = phosphates; Hard = hardness; T.col. = total coliforms and F.col. = faecal coliforms).

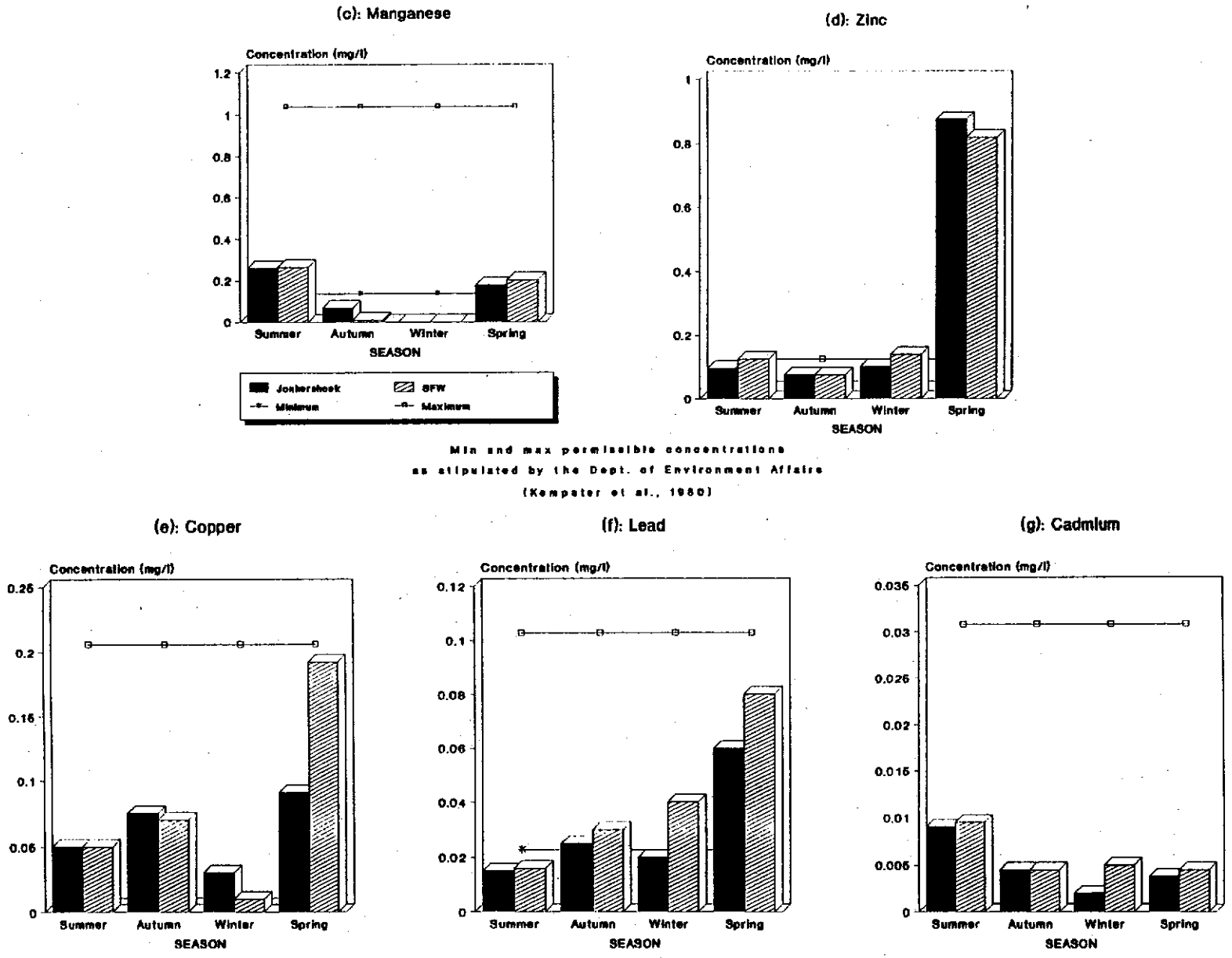
| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| pH (J) | 26 | 6.74 | 0.24 | 5.9-7.0 | 3.685 | <0.05 |
| vs | | | | | | |
| pH (S) | 43 | 7.1 | 0.38 | 6.3-7.6 | | |
| Cond. (J) | 24 | 6.76 | 1.31 | 3.44-8.88 | 6.558 | <0.05 |
| vs | | | | | | |
| Cond. (S) | 43 | 18.5 | 7.08 | 7.2-35.0 | | |
| Temp. (J) | 3 | 18.33 | 5.13 | 14.0-24.0 | -0.524 | >0.05 |
| vs | | | | | | |
| Temp. (S) | 21 | 16.3 | 3.59 | 10.1-25.7 | | |
| Diss.$O_2$ (J) | 3 | 10.07 | 0.64 | 9.47-10.75 | -1.484 | >0.05 |
| vs | | | | | | |
| Diss.$O_2$ (S) | 21 | 9.02 | 5.81 | 4.6-32.6 | | |
| S.sol. (J) | 24 | 7.38 | 11.64 | 5.0-62.0 | 2.198 | <0.05 |
| vs | | | | | | |
| S.sol. (S) | 43 | 9.07 | 9.58 | 5.0-57.0 | | |
| $NO_3$ (J) | 18 | 0.48 | 1.2 | 0.2-5.3 | 0.944 | >0.05 |
| vs | | | | | | |
| $NO_3$ (S) | 42 | 0.27 | 0.16 | 0.1-0.8 | | |
| $PO_4$ (J) | 18 | 0.05 | 0.07 | 0.03-0.33 | -2.504 | <0.05 |
| vs | | | | | | |
| $PO_4$ (S) | 42 | 0.06 | 0.17 | 0.03-1.1 | | |
| Hard (J) | 2 | 14.5 | 14.14 | 4.5-24.5 | 0.866 | >0.05 |
| vs | | | | | | |
| Hard (S) | 3 | 45.0 | 21.93 | 20.0-61.0 | | |
| T.col. (J) | 6 | 498.83 | 371.01 | 93-1100 | 3.69 | <0.05 |
| vs | | | | | | |
| T.col. (S) | 22 | 128227 | 255768 | 2400-1100000 | | |
| F. col. (J) | 6 | 105.83 | 71.44 | 23.0-240.0 | 3.573 | <0.05 |
| vs | | | | | | |
| F. col. (S) | 22 | 15347 | 32185 | 110-110000 | | |

Table 4.5: Results of Student's t-test for the differences between sediment heavy metal concentrations ($\mu g.g^{-1}$ dry mass) at the two localities: Jonkershoek (J) and SFW (S).

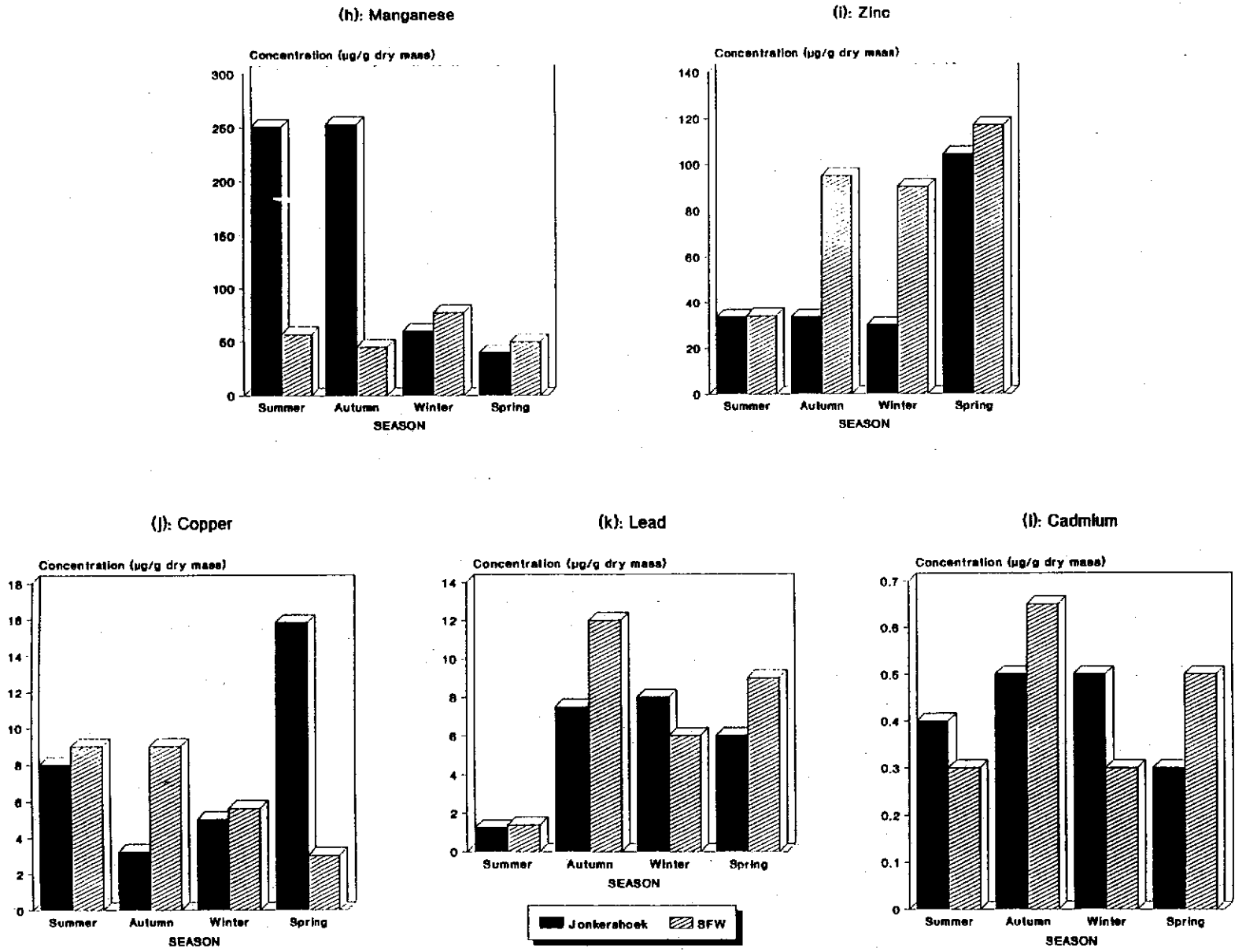
| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Manganese(J) | 8 | 184.68 | 138.66 | 39.6-386.5 | 2.63 | <0.05 |
| vs | | | | | | |
| Manganese(S) | 8 | 55.27 | 13.23 | 40-77.3 | | |
| Zinc(J) | 8 | 44.72 | 32.82 | 10-104.3 | -1.84 | <0.05 |
| vs | | | | | | |
| Zinc(S) | 8 | 77.53 | 38.41 | 32-120 | | |
| Copper(J) | 8 | 7.2 | 4.87 | 1.4-15.8 | -0.1 | >0.05 |
| vs | | | | | | |
| Copper(S) | 8 | 7.43 | 4.75 | 3-15 | | |
| Lead(J) | 8 | 5.25 | 3.54 | 1.2-10 | -0.77 | >0.05 |
| vs | | | | | | |
| Lead(S) | 8 | 6.97 | 5.21 | 1.4-15 | | |
| Cadmium (J) | 8 | 0.43 | 0.1 | 0.3-0.6 | -0.23 | >0.05 |
| vs | | | | | | |
| Cadmium (S) | 8 | 0.45 | 0.18 | 0.3-0.7 | | |




Figures 4(a)-(b): Mean heavy metal concentration in the water (a) and sediments (b) at Jonkershoek and SFW (SD in parenthesis).




Figures 4(c)-(g): Mean seasonal heavy metal concentration (mg/l) in the water at Jonkerhoek and SFW.




Figures 4(h)-(l): Mean seasonal heavy metal concentration (µg/g dry mass) in the sediments at Jonkershoek and SFW.

## Discussion

Kempster et al. (1980) presented lists of the South African water quality criteria for the various water uses, as stipulated by the Department of Environment Affairs. Among other criteria, the minimum and maximum permissible heavy metal concentrations, so as to protect wildlife in river and dam water, were provided. The corresponding mean water Mn, Cu, Pb and Cd concentrations for Jonkershoek and SFW (Figures 4 (c)-(g)) were found to be well under the maximum permissible concentrations (as also indicated in Figures 4 (c)-(g)), although the mean spring Cu and Pb concentrations were found to be relatively high. On a seasonal basis, zinc concentrations at Jonkershoek and SFW, especially during spring, were however very high, often exceeding the maximum allowed (Figure 4(d)).

Palmer & O'Keeffe (1990) cited the work of Ward & Stanford (1983), who developed the Serial Discontinuity Concept (SDC), which states that dams are physical barriers in river systems and that they and their associated impoundment water can result in changes in, among others, the chemistry of the receiving river. As the river progresses downstream from the dam, natural riverine conditions are restored as a result of natural processes and tributary inflows. Palmer & O'Keeffe (1990) tested this concept on the Buffalo River, Eastern Cape and found that, in agreement with this concept, impoundments which received water from a near-pristine upper catchment caused alterations of the water quality. Also, recovery to natural riverine conditions was within 2.6 and 18.4 km of the dam, depending on flow. The fact that the water from Jonkershoek contained high levels of zinc indicated that this locality is perhaps not as uncontaminated as would be believed and that the human activities in the Kleinplaas Dam, as well as the inflow of water from the Theewaterskloof Dam, may have a significant effect on the quality of the Eerste River water upstream from Stellenbosch.

Through comparisons of the water metal concentrations at Jonkershoek and SFW it was established that no significant differences existed between the two localities (Tables 4.1 and 4.2), indicating that the contribution of household, agricultural and industrial input to metal concentrations was not significant. This, however, does not mean that the town of Stellenbosch has no negative effect on the Eerste River water quality and therefore on the health of the aquatic fauna, since it is well known that the physico-chemistry of river water may greatly affect the bioavailability and toxicity of heavy metals to animals (e.g. Förstner & Prosi, 1979; Kempster, 1980; Moore & Ramamoorthy, 1984 and Lewis & McIntosh, 1986. See Chapters 5-9).

The influence of run-off from various sources in and around Stellenbosch on the physico-chemistry of the Eerste River was evident from the statistically significant differences between the two localities as far as pH, conductivity, suspended solids, phosphates, total coliforms and faecal coliforms are concerned, with SFW having had the highest values/concentrations for each of these features (Table 4.4).

The majority of physical and chemical factors listed in Table 4.3 for Jonkershoek (station 2) and SFW were compared with the quality criteria set for river and dam water by the Department of Environment Affairs (Kempster et al., 1980). The exception to this was the number of coliforms, which was compared with the criteria for drinking water since no values for river and dam water are available. According to these criteria (a maximum of 2000 faecal coliforms and a total maximum of 50 000 coliforms per 100 ml allowed), the numbers found at SFW and further downstream were extremely high. However, these criteria have been determined rather subjectively, as the criteria for drinking water set by the Canadian government (1989) stipulate that a maximum of 10 coliforms per 100 ml is allowable and may not include any faecals. Furthermore, their quality criteria for water for recreational use (1983) stipulate a maximum of 200 faecal coliforms per 100 ml. Since the number of coliforms in the water is correlated with the degree of pollution, Jonkershoek (station 2) can, according to these Canadian criteria, be considered polluted.

On the other hand, since the values/concentrations of the other physical and chemical factors listed in Table 4.3 for the two localities were well within the limits set by the Department of Environment Affairs (Kempster et al., 1980), the Eerste River, from Jonkershoek to SFW, can still be considered relatively unpolluted. Ironically, the very low pH and conductivity values found at Jonkershoek could be a disadvantage to aquatic life, rather than an advantage: it has been found that decreases in these values increase the toxicity of certain metals to animals (e.g. Kempster et al., 1980 and Lewis & McIntosh, 1986).

Two studies in which chemical and physical water data were collected have previously been done on the Eerste River, namely those by King (1981) and more recently Petitjean (1987). In both cases samples were taken at Jonkershoek and SFW, at sites in close proximity of the present study's localities. It was found that, compared to the results of King (1981), only the mean nitrate concentration in water from Jonkershoek has increased, whereas the mean values/concentrations of the other factors have remained relatively constant at both localities. Comparisons with the results of Petitjean (1987) showed that the mean temperature of, and mean suspended solid and nitrate concentrations in, water from Jonkershoek have increased, as well as the mean phosphate concentrations of Jonkershoek and SFW water. The mean values/concentrations of the other factors have all remained relatively constant or have decreased, as in the case of the conductivity. The observed increases in the concentrations of certain water features since 1987 and gradual increase in nitrate concentration at Jonkershoek since 1981, can possibly be ascribed to increased pollution from the Kleinplaas Dam, as well as from the industrial, agricultural and municipal areas in and around Stellenbosch. However, since these features are dependent on various other external factors, this can only remain a theory.

Studies of the metal concentrations in the sediments from the two localities (Figures 4(b), 4(h)-(l) and Table 4.5), showed that whilst the Zn concentrations in the sediments from SFW were

significantly higher than in the sediments from Jonkershoek, the opposite was true for the Mn concentrations. There were no differences in sediment Cu, Pb or Cd concentrations between the two localities. Overall, the metal concentrations were, however, still relatively low. Moore & Ramamoorthy (1984) listed the average Zn, Cu, Pb and Cd concentrations found in sediments associated with freshwaters. They gave the Zn concentrations in sediments of unpolluted and industrially/municipally polluted waters as <50 and 6 to 339 $mg.kg^{-1}$ dry mass respectively. The sediment Zn concentrations from SFW, listed in Table 4.5, compared better with the latter, whereas the mean Zn concentration in the sediments from Jonkershoek compared favourably with the former, although the maximum Zn concentration found in sediments from this locality (104.3 $\mu g.g^{-1}$ dry mass) was much higher. The Cu and Pb concentrations in sediments from Jonkershoek and SFW (Table 4.5) all compared well with the concentrations cited for unpolluted waters, i.e. $\leq$20 and 2 to 50 $mg.kg^{-1}$ dry mass for Cu and Pb respectively. The mean Cd concentrations given for unpolluted and industrially/municipally polluted freshwater sediments were $\pm$0.1 and $\pm$2.7 $mg.kg^{-1}$ dry mass respectively. The sediment Cd concentrations from both localities (Table 4.5) were found to be higher than the former but still much lower than the latter.

Watling & Watling (1983) studied heavy metal concentrations in the sediments of the relatively unpolluted Boesmans, Kowie, Kariega and Great Fish Rivers and found Mn concentrations to be between 36 and 920 $\mu g.g^{-1}$ dry mass. The mean Mn concentrations in Eerste River sediments (both localities) (Table 4.5) were relatively low to average in comparison, therefore comparing favourably with Mn concentrations in unpolluted freshwater sediments.

Finally, it is clear that overall, the Eerste River, from its origin to the SFW locality, can be classified as relatively unpolluted, in terms of the parameters surveyed during this study, although the Kleinplaas Dam and agricultural, municipal and industrial areas in and around Stellenbosch definitely influence the physico-chemistry and general quality of the water, up- and downstream from the town. This situation certainly needs to be monitored regularly.

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# CHAPTER 5

# CONCENTRATIONS OF MANGANESE IN THE FRESHWATER CRAB, *POTAMONAUTES PERLATUS*

## Introduction

Manganese is a heavy metal which occurs widely in nature. It is found in many minerals, e.g. pyrolusite, as the oxide, and rhodochronite, where it occurs as the carbonate. Manganese also occurs in nodules in the sea floor and in most iron ores. It is not, however, found in nature as the free metal (Piscator, 1979b and Richardson & Gangolli, 1994). In waters, manganese is in dissolved, colloidal and complex forms. It is known that Mn-organic matter complexes may form, which are stable and cause major problems in water treatment processes (Galvin, 1996).

Manganese is an essential trace element in animal nutrition. It is a constituent of the enzymes pyruvatecarboxylase and superoxidedismutase, as well as being an enzymatic catalyst (Piscator, 1979b). This metal is also involved in the synthesis of flavo proteins, cholesterol dynamics and haemoglobin production (Galvin, 1996). Although manganese deficiency has not been found in the general population, it has been shown experimentally that, in animals, a deficiency will cause heart disease, skeletal abnormalities and impaired growth (Piscator, 1979b and Galvin, 1996).

Aquatic ecosystems are in danger of receiving unnaturally high loads of manganese from especially industrial waste water, since this metal is mainly used in metallurgical processes of which ferromanganese is the main product. Other uses are as alloys with other metals in the electrical industry and as manganese dioxide for dry cell batteries (Piscator, 1979b).

High concentrations of manganese in industrial and agricultural runoff pose a great threat to aquatic animals exposed to resultant increased levels. Experiments with animals have shown that excessive exposure to manganese may cause a variety of symptoms, e.g. mononuclear reactions, changes in enzymatic activities and testicular morphology, fibrosis as well as aggressive reactions and irritation (Piscator, 1979b). It is well known that water pH is an important factor influencing the toxicity and bioavailability of manganese to aquatic fauna: a lowered pH increases the amounts of Mn accumulated by, as well as the toxicity of the metal to the animals (France, 1987 and Rouleau et al., 1996).

Very little research has, as yet, been done on manganese concentrations in freshwater decapod crustaceans. The crayfish *Orconectes virilis* has been studied by France (1987), whereas Van Eeden & Schoonbee (1991) and Steenkamp et al. (1994b) investigated the bioaccumulation of manganese in the crab *Potamonautes warreni*.

## Materials and Methods

All crab samples were prepared, tested and statistically analysed according to the method described in Chapter 3.

## Results

In all cases only the manganese concentrations per gram wet mass were used for statistical analysis. Crabs showed large individual variation in whole body, as well as tissue and carapace manganese concentrations. This was an important consideration in the interpretation of the statistical results.

### The relationship between crab size and whole crab manganese concentration

In order to investigate the possible relationship between whole crab manganese concentrations and the size of the crabs, the data from Jonkershoek and SFW were pooled. The carapace widths of the crabs, in decreasing order, were compared with the respective manganese concentrations and the correlation coefficient (r) thereof, calculated. With 53 degrees of freedom and an $r^2$-value of 27.64%, an r-value of -0.526 was obtained. This indicated a reasonably strong negative correlation between sizes of crabs and the concentrations of manganese in the body: as crab size decreased, so the manganese concentration increased (Figure 5(b)).

### Differences in manganese concentrations of the various size classes at each locality

Since the whole crab data for large, medium and small-sized crabs from each locality were not normally distributed, the data were logarithmically transformed, in order to test for statistically significant differences ($p<0.05$). The transformed data for medium-sized crabs from SFW, however, also failed to provide a normal distribution, thus a nonparametric test, the Mann-Whitney test, was used in this case. The results of both Student's t-test and the Mann-Whitney test, are shown in Tables 5.1 and 5.2.

Differences in concentrations were found between all three size classes from Jonkershoek. The small class showed the highest mean manganese concentration (1163.7±710.66 $\mu g.g^{-1}$ wet mass) and large sized crabs the lowest (152.98±111.36 $\mu g.g^{-1}$). No significant differences were, however, found between any of the size classes from SFW. Once more the small and large classes exhibited the highest and lowest mean manganese concentrations (743.15±667.43 and 313.5±107.82 $\mu g.g^{-1}$ wet mass respectively).

The relationship between crab size, and tissue and carapace manganese concentration

In order to investigate the possible relationship between crab size and manganese concentration in selected tissues, namely muscle tissue, digestive gland, gills and gonads, as well as the carapace, the data from Jonkershoek and SFW were pooled, after which the carapace widths of crabs, in decreasing order, were compared with the respective tissue or carapace manganese concentration. Table 5.3 shows the r-values calculated for each tissue and for the carapace.

Strong negative correlations were found between crab size and the manganese concentration in the muscles, digestive gland, gills and gonads. Only the manganese concentration in the carapace proved to be poorly negatively correlated with the size of the crabs.

Differences in manganese concentrations of the various selected tissues and the carapace

The mean manganese concentration found in the selected tissues and the carapace of crabs from Jonkershoek and SFW were pooled, in order to determine whether statistically significant differences ($p<0.05$) existed between the various tissues and the carapace. Since the data were not normally distributed, the mean logarithms of the tissue and carapace manganese concentrations were used in the t-test. The respective mean concentrations and standard deviations are tabulated in Table 5.4. Table 5.5 shows the results of the t-test performed on the tissue and carapace concentrations.

When comparing values obtained from the selected tissues and carapace, significant differences in mean manganese concentration were found between the muscles and digestive gland, muscles and carapace, digestive gland and carapace, gonads and carapace and gills and carapace. The carapace showed the highest mean concentration (229.73±213.33 $\mu g.g^{-1}$ wet mass), whereas the lowest mean was found in the gills (30.25±55.08 $\mu g.g^{-1}$ wet mass) (Figure 5(c)).

Differences in manganese concentrations of crabs from Jonkershoek and SFW

(a) Differences in concentration values for whole crabs

The results of Student's t-test performed on the mean logarithms of the whole crab manganese concentrations from Jonkershoek and SFW are shown in Table 5.6. The highest mean manganese concentration (596.21±1113.83 $\mu g.g^{-1}$ wet mass), was found in crabs from SFW (Figure 5(a)). A t-value of -3.9 indicated that there was a statistically significant difference ($p<0.05$) in mean whole crab manganese concentrations between the two localities.

(a) (i) Differences in mean whole crab concentrations of the various size classes

Through a comparison of the mean logarithms of whole crab manganese concentration values between large and small crabs from Jonkershoek and SFW, with the use of Student's t-test

(Table 5.7), and between medium crabs from Jonkershoek and SFW, with the use of the Mann-Whitney test (Table 5.8), it was established that statistically significant differences ($p<0.05$) for this parameter existed between large crabs as well as between medium sized crabs, from the two localities. In both cases the crabs from SFW had higher mean concentrations (313.49±107.82 and 617.1±1247.3 $\mu g.g^{-1}$ wet mass respectively) than those from Jonkershoek (Figure 5(b)).

(b) Differences in concentration values for the selected tissues and the carapace

The mean manganese concentration in the muscle, digestive gland, gills and gonads of crabs from Jonkershoek and SFW were compared with the use of Student's t-test and tabulated in Table 5.9. Since the data were not normally distributed, the mean logarithms of the manganese concentrations in the tissues and carapace were used to calculate the t-value. In the case of the carapace, however, a normal distribution could not be obtained after transformation of the concentration value data. A nonparametric test, namely the Mann-Whitney test, was performed on the original data, to investigate possible differences (Table 5.10).

No statistically significant differences ($p>0.05$) in tissue and carapace manganese concentrations were found between Jonkershoek and SFW crabs. Figure 5(d) exhibits the mean manganese concentrations found for the various tissues and the carapace.

Differences in manganese concentrations of males and females

In order to determine whether statistically significant differences ($p<0.05$) existed between the mean whole crab, tissue and carapace manganese concentrations of males and females, the data obtained for crabs from Jonkershoek and SFW were pooled for each sex. In all cases the original data failed the normal probability test, therefore each concentration value was logarithmically transformed.

(a) Differences in whole crab concentrations

Table 5.11 shows the results of Student's t-test, performed on the mean logarithms of the whole crab manganese concentrations in males and females of *Potamonautes perlatus*. The highest mean manganese concentration was found in females (599.4±1238.36 $\mu g.g^{-1}$ wet mass) but no statistically significant difference ($p>0.05$) existed between the two genders.

(b) Differences in the manganese concentrations of selected tissues and the carapace

Although females exhibited the highest mean manganese concentrations in two of the four tissue types, namely muscle and gonads, as well as in the carapace, no statistically significant differences ($p>0.05$) were found between the two genders (Table 5.12).

Seasonal variations in manganese concentration

The manganese concentrations in whole crabs, selected tissues and carapace from the different seasons were tested for statistically significant differences ($p<0.05$). In each instance, the data for crabs collected from Jonkershoek and SFW were pooled and these then tested for normality. None of the data sets were normally distributed and were thus logarithmically transformed. The transformed carapace data for spring also failed the normal probability test, thus the Mann-Whitney test was performed.

(a) Seasonal differences in mean whole crab manganese concentration

Student's t-test showed statistically significant differences in whole crab manganese concentrations, between summer and autumn, autumn and winter and winter and spring (Table 5.13). The highest mean whole crab manganese concentration (664.37±1170.08 $\mu g.g^{-1}$ wet mass), was observed for summer, whereas winter showed the lowest mean (149.7±120.02 $\mu g.g^{-1}$ wet mass) (Figure 5(e)).

(b) Seasonal differences in tissue and carapace manganese concentrations

1. Carapace

Statistically significant differences ($p<0.05$) in carapace manganese concentrations were found between summer and the other three seasons. Summer showed the highest mean manganese concentration (337.79±257.15 $\mu g.g^{-1}$ wet mass) and winter the lowest (117.24±126.9 $\mu g.g^{-1}$ wet mass) (Figure 5(f)). The results of Student's t-test, performed on the mean logarithms of the summer, autumn and winter manganese concentrations as well as the Mann-Whitney test, performed on the spring manganese concentrations, are tabulated in Tables 5.14 and 5.15.

2.Muscle tissue

The highest mean muscle manganese concentration (51.2±68.18 $\mu g.g^{-1}$ wet mass) occurred in summer, whereas in autumn the mean concentration was the lowest (1.69±1.97 $\mu g.g^{-1}$ wet mass) (Figure 5(g)). Through pairwise comparisons of the different seasons, it was found that statistically significant differences ($p<0.05$) existed between summer and autumn and between summer and spring (Table 5.16).

3. Gill tissue

Statistically significant differences ($p<0.05$) in gill manganese concentrations were found when comparing values for summer and autumn, and summer and spring. In Table 5.17 and Figure 5(h) it can be seen that the highest and lowest mean manganese concentrations occurred in summer (45.74±68.66 $\mu g.g^{-1}$ wet mass) and spring (7.25±9.62 $\mu g.g^{-1}$ wet mass) respectively.

4. Digestive gland

The results of Student's t-test on the mean logarithms of the digestive gland manganese concentrations, when comparing values for the different seasons, are given in Table 5.18. Comparisons of summer with the other seasons yielded statistical differences ($p<0.05$). The summer value for mean manganese concentration was the highest (174.99±198.06 $\mu g.g^{-1}$ wet mass), whilst the lowest mean concentration occurred in autumn (11.69±21.0 $\mu g.g^{-1}$ wet mass) (Figure 5(i)).

5. Gonads

When the data for the ovaries and testes were pooled, the highest and lowest mean manganese concentrations (88.55±143.32 and 15.54±24.2 $\mu g.g^{-1}$ wet mass) occurred in summer and autumn respectively (Figure 5(j)). A statistically significant difference was observed between summer and autumn (Table 5.19).

Differences in manganese concentrations of whole crabs, tissues, carapace, and Eerste River water

The original data from water samples failed to provide a normal distribution, therefore the logarithmically transformed concentrations in water were used to test for statistically significant differences ($p<0.05$).

(a) Differences between concentration values for whole crabs and water

Student's t-test (Table 5.20) yielded significant differences between the whole crab and water manganese concentrations in both localities, i.e. Jonkershoek as well as SFW.

(b) Differences between concentration values for the selected tissues, carapace and water

The manganese concentrations in all tissues and the carapace from both localities proved to be significantly different ($p<0.05$) from the concentrations in the water. The results of Student's t-test and the Mann-Whitney test in the case of comparisons with the carapace data, are recorded in Tables 5.21 and 5.22 respectively.

Differences in manganese concentrations of whole crabs, tissues, carapace, and sediments

(a) Differences between concentration values for whole crabs and sediments

Student's t-test, performed on the mean logarithms of whole crab and sediment manganese concentrations for Jonkershoek and SFW, yielded a statistically significant difference ($p<0.05$) in the whole crab and sediment concentrations from SFW. The results of the test are tabulated in Table 5.23.

(b) Differences between concentration values for the selected tissues, carapace and sediments

The mean logarithms of the tissue and carapace manganese concentrations were used to test for statistically significant differences ($p<0.05$) between tissue, carapace and sediment manganese concentrations within the two localities. The transformed carapace data, however, were not normally distributed, therefore the Mann-Whitney test was performed. Significant differences in manganese concentrations were found between the sediments and all four tissue types from Jonkershoek and all but two of the tissues (digestive gland and gonads) from SFW. Tables 5.24 and 5.25 list the results of Student's t- and the Mann-Whitney tests.

Table 5.1: Results of Student's t-test for differences in whole crab manganese concentrations ($\mu g.g^{-1}$ wet mass) of the various size classes at each locality.

| JONKERSHOEK | | | | | | |
|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t-value | z-value |
| Large | 22 | 152.98 | 111.36 | 52.04-373.38 | -2.16 | <0.05 |
| vs | | | | | | |
| Medium | 34 | 277.66 | 260.72 | 45.54-1148.33 | | |
| Large | 22 | 152.98 | 111.36 | 52.04-373.38 | -2.9 | <0.05 |
| vs | | | | | | |
| Small | 4 | 1163.7 | 710.66 | 106.4-1644.04 | | |
| Medium | 34 | 277.66 | 260.72 | 45.54-1148.33 | -4.34 | <0.05 |
| vs | | | | | | |
| Small | 4 | 1163.7 | 710.66 | 106.4-1644.04 | | |
| **SFW** | | | | | | |
| | n | Mean | SD | Range | t-value | p-value |
| Large | 6 | 313.49 | 107.82 | 162.73-480.99 | -1.56 | >0.05 |
| vs | | | | | | |
| Small | 6 | 743.15 | 667.43 | 228.17-1831.96 | | |

Table 5.2: Results of the Mann-Whitney test for differences in manganese concentrations ($\mu g.g^{-1}$ wet mass) of medium sized crabs and the other size classes, from SFW.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Large | 6 | 313.49 | 107.82 | 162.73-480.99 | -0.15 | >0.05 |
| vs | | | | | | |
| Medium | 39 | 617.1 | 1247.3 | 120.53-6138.67 | | |
| Medium | 39 | 617.1 | 1247.3 | 120.53-6138.67 | 1.619 | >0.05 |
| vs | | | | | | |
| Small | 6 | 743.15 | 667.43 | 228.17-1831.96 | | |

Table 5.3: $R^2$-values calculated for sizes of crabs and selected tissue and carapace manganese concentrations (DF = Degrees of freedom; Dig.gland = digestive gland).

| | DF | r-value | R-sq. (%) |
|---|---|---|---|
| Crab size and Muscle | 27 | -0.687 | 47.21 |
| Crab size and Dig.gland | 29 | -0.603 | 36.41 |
| Crab size and Gills | 24 | -0.602 | 36.18 |
| Crab size and Gonads | 23 | -0.603 | 36.32 |
| Crab size and Carapace | 28 | -0.267 | 7.10 |

Table 5.4: Mean manganese concentrations ($\mu g.g^{-1}$ wet mass) in the muscles, digestive gland, gonads, gills and carapace of crabs from the Eerste River (Jonkershoek and SFW data pooled).

| | n | Mean | SD | Range |
|---|---|---|---|---|
| Muscle | 29 | 30.68 | 53.48 | 0.0-193.52 |
| Digestive gland | 33 | 86.96 | 154.94 | 0.0-740.7 |
| Gonads | 25 | 54.5 | 108.34 | 0.0-500.0 |
| Gills | 26 | 30.25 | 55.08 | 0.0-235.97 |
| Carapace | 39 | 229.73 | 213.33 | 0.0-1082.73 |

Table 5.5: Results of Student's t-test for the differences in mean tissue and carapace manganese concentration ($\mu g.g^{-1}$ wet mass) of crabs (Jonkershoek and SFW data pooled; Mean logarithms of manganese concentrations used for the calculation of the t-value).

| | t-value | p-value |
|---|---|---|
| Muscle vs Digestive gland | -1.87 | <0.05 |
| Muscle vs Gonads | -1.44 | >0.05 |
| Muscle vs Gills | -0.25 | >0.05 |
| Muscle vs Carapace | -7.97 | <0.05 |
| Digestive gland vs Gonads | 0.46 | >0.05 |
| Digestive gland vs Gills | 1.59 | >0.05 |
| Digestive gland vs Carapace | -5.06 | <0.05 |
| Gonads vs Gills | 1.18 | >0.05 |
| Gonads vs Carapace | -5.92 | <0.05 |

| | | |
|---|---|---|
| Gills vs Carapace | -7.61 | <0.05 |

Table 5.6: Results of Student's t-test for the differences in mean whole crab manganese concentration ($\mu g.g^{-1}$ wet mass) from Jonkershoek and SFW (Mean logarithms of the whole crab manganese concentrations used in the t-test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Jonkershoek | 60 | 291.01 | 356.31 | 45.54-1644.04 | -3.9 | <0.05 |
| vs | | | | | | |
| SFW | 51 | 596.21 | 1113.83 | 120.53-6138.67 | | |

Table 5.7: Results of Student's t-test for the differences in mean whole crab manganese concentration ($\mu g.g^{-1}$ wet mass) of large and small size classes from Jonkershoek (J) and SFW (S) (Mean logarithms of manganese concentrations used in the t-test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Large (J) | 22 | 152.98 | 111.36 | 52.04-373.38 | -3.09 | <0.05 |
| vs | | | | | | |
| Large (S) | 6 | 313.49 | 107.82 | 162.73-480.99 | | |
| Small (J) | 4 | 1163.7 | 710.66 | 106.4-1644.04 | 0.54 | >0.05 |
| vs | | | | | | |
| Small (S) | 6 | 743.15 | 667.43 | 228.17-1831.96 | | |

Table 5.8: Results of the Mann-Whitney test for the differences in manganese concentrations ($\mu g.g^{-1}$ wet mass) of medium sized crabs from Jonkershoek (J) and SFW (S).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Medium (J) | 34 | 277.66 | 260.72 | 45.54-1148.33 | 2.217 | <0.05 |
| vs | | | | | | |
| Medium (S) | 39 | 617.1 | 1247.3 | 120.53-6138.67 | | |

Table 5.9: Results of Student's t-test for the differences in mean tissue manganese concentration ($\mu g.g^{-1}$ wet mass) of crabs from Jonkershoek (J) and SFW (S) (Mean logarithms of the tissue manganese concentrations used in the t-test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Muscle (J) | 16 | 23.93 | 46.92 | 0.0-187.12 | -0.62 | >0.05 |
| vs | | | | | | |
| Muscle (S) | 13 | 38.98 | 61.53 | 0.0-193.52 | | |
| Dig.gland (J) | 18 | 97.68 | 196.16 | 0.0-444.4 | -0.79 | >0.05 |
| vs | | | | | | |
| Dig.gland (S) | 15 | 74.09 | 88.47 | 0.0-275.0 | | |
| Gills (J) | 14 | 36.47 | 73.77 | 0.0-235.97 | -1.21 | >0.05 |
| vs | | | | | | |
| Gills (S) | 12 | 23.01 | 18.88 | 2.64-54.09 | | |
| Gonads (J) | 14 | 28.8 | 32.21 | 0.0-100.0 | -0.84 | >0.05 |
| vs | | | | | | |
| Gonads (S) | 11 | 87.21 | 157.22 | 0.0-500.0 | | |

Table 5.10: Results of the Mann-Whitney test for the differences in mean carapace manganese concentration ($\mu g.g^{-1}$ wet mass) of crabs from the two localities, Jonkershoek (J) and SFW (S).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (J) | 19 | 245.0 | 278.72 | 0.0-1082.73 | 0.815 | >0.05 |
| vs | | | | | | |
| Carapace (S) | 20 | 215.23 | 130.26 | 16.52-519.18 | | |

Table 5.11: Results of Student's t-test for the differences in mean whole crab manganese concentration ($\mu g.g^{-1}$ wet mass) of males and females (Mean logarithms of the whole crab manganese concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Females | 40 | 599.4 | 1238.36 | 55.82-6138.67 | 1.03 | >0.05 |
| vs | | | | | | |
| Males | 49 | 334.29 | 334.18 | 58.62-1831.96 | | |

Table 5.12: Results of Student's t-test for the differences in mean tissue and carapace manganese concentration ($\mu g.g^{-1}$ wet mass) of male (m) and female (f) crabs (Mean logarithms of the tissue and carapace manganese concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (f) | 15 | 271.17 | 240.64 | 93.17-1052.73 | 1.29 | >0.05 |
| vs | | | | | | |
| Carapace (m) | 18 | 226.74 | 207.0 | 16.52-858.09 | | |
| Muscle (f) | 11 | 30.99 | 57.04 | 3.67-193.52 | 0.02 | >0.05 |
| vs | | | | | | |
| Muscle (m) | 13 | 27.82 | 40.0 | 0.0-143.8 | | |
| Dig.gland (f) | 12 | 70.56 | 95.89 | 0.0-275.0 | -0.4 | >0.05 |
| vs | | | | | | |
| Dig.gland (m) | 15 | 83.69 | 120.37 | 0.0-444.42 | | |
| Gills (f) | 11 | 14.66 | 18.89 | 0.0-54.09 | -0.63 | >0.05 |
| vs | | | | | | |
| Gills (m) | 14 | 31.89 | 60.42 | 0.0-235.97 | | |
| Ovaries | 11 | 69.7 | 145.09 | 0.0-92.05 | -0.67 | >0.05 |
| vs | | | | | | |
| Testes | 10 | 57.8 | 80.57 | 5.97-272.71 | | |

Table 5.13: Results of Student's t-test for the seasonal differences in whole crab manganese concentrations ($\mu g.g^{-1}$ wet mass).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 49 | 664.37 | 1170.08 | 55.82-6138.67 | 0.34 | >0.05 |
| vs | | | | | | |
| Autumn | 16 | 304.48 | 104.71 | 64.64-451.2 | | |
| Summer | 49 | 664.37 | 1170.08 | 55.82-6138.67 | 3.59 | <0.05 |
| vs | | | | | | |
| Winter | 21 | 149.7 | 120.02 | 45.54-496.54 | | |
| Summer | 49 | 664.37 | 1170.08 | 55.82-6138.67 | 0.85 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 291.92 | 188.25 | 112.44-901.36 | | |
| Autumn | 16 | 304.48 | 104.71 | 64.64-451.2 | 4.31 | <0.05 |
| vs | | | | | | |
| Winter | 21 | 149.7 | 120.02 | 45.54-496.54 | | |
| Autumn | 16 | 304.48 | 104.71 | 64.64-451.2 | 0.65 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 291.92 | 188.25 | 112.44-901.36 | | |
| Winter | 21 | 149.7 | 120.02 | 45.54-496.54 | -4.28 | <0.05 |
| vs | | | | | | |
| Spring | 25 | 291.92 | 188.25 | 112.44-901.36 | | |

Table 5.14: Results of Student's t-test for the differences in mean crab carapace summer, autumn and winter manganese concentrations ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal manganese concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 18 | 337.79 | 257.15 | 100.34-1082.73 | 2.68 | <0.05 |
| vs | | | | | | |
| Autumn | 5 | 155.4 | 154.46 | 0.0-395.9 | | |
| Summer | 18 | 337.79 | 257.15 | 100.34-1082.73 | 3.78 | <0.05 |
| vs | | | | | | |
| Winter | 6 | 117.24 | 126.9 | 13.65-307.1 | | |
| Autumn | 5 | 155.4 | 154.46 | 0.0-395.9 | -0.09 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 117.24 | 126.9 | 13.65-307.1 | | |

Table 5.15: Results of the Mann-Whitney test for differences in crab carapace manganese concentration ($\mu g.g^{-1}$ wet mass) of spring and the other seasons.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 18 | 337.79 | 257.15 | 100.34-1082.73 | -2.9 | <0.05 |
| vs | | | | | | |
| Spring | 10 | 139.89 | 62.15 | 17.68-232.42 | | |
| Autumn | 5 | 155.4 | 154.46 | 0.0-395.9 | 0.306 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 139.89 | 62.15 | 17.68-232.42 | | |
| Winter | 6 | 117.24 | 126.9 | 13.65-307.1 | 0.813 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 139.89 | 62.15 | 17.68-232.42 | | |

Table 5.16: Results of Student's t-test for the seasonal differences in mean crab muscle manganese concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal manganese concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 51.2 | 68.18 | 3.67-193.52 | 3.1 | <0.05 |
| vs | | | | | | |
| Autumn | 4 | 1.69 | 1.97 | 0.0-3.77 | | |
| Summer | 15 | 51.2 | 68.18 | 3.67-193.52 | 1.43 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 11.62 | 14.96 | 0.0-33.53 | | |
| Summer | 15 | 51.2 | 68.18 | 3.67-193.52 | 1.74 | <0.05 |
| vs | | | | | | |
| Spring | 6 | 11.41 | 13.85 | 0.0-37.35 | | |
| Autumn | 4 | 1.69 | 1.97 | 0.0-3.77 | -1.49 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 11.62 | 14.96 | 0.0-33.53 | | |
| Autumn | 4 | 1.69 | 1.97 | 0.0-3.77 | -1.44 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 11.41 | 13.85 | 0.0-37.35 | | |
| Winter | 4 | 11.62 | 14.96 | 0.0-33.53 | 0.05 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 11.41 | 13.85 | 0.0-37.35 | | |

Table 5.17: Results of Student's t-test for seasonal differences in mean crab gill manganese concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal manganese concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 45.74 | 68.66 | 4.0-235.97 | 2.05 | <0.05 |
| vs | | | | | | |
| Autumn | 3 | 11.33 | 19.62 | 0.0-33.99 | | |
| Summer | 15 | 45.74 | 68.66 | 4.0-235.97 | 1.27 | >0.05 |
| vs | | | | | | |
| Winter | 3 | 10.12 | 9.28 | 2.64-20.51 | | |
| Summer | 15 | 45.74 | 68.66 | 4.0-235.97 | 2.52 | <0.05 |
| vs | | | | | | |
| Spring | 5 | 7.25 | 9.62 | 0.48-24.23 | | |
| Autumn | 3 | 11.33 | 19.62 | 0.0-33.99 | -0.62 | >0.05 |
| vs | | | | | | |
| Winter | 3 | 10.12 | 9.28 | 2.64-20.51 | | |
| Autumn | 3 | 11.33 | 19.62 | 0.0-33.99 | -0.1 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 7.25 | 9.62 | 0.48-24.23 | | |
| Winter | 3 | 10.12 | 9.28 | 2.64-20.51 | 0.75 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 7.25 | 9.62 | 0.48-24.23 | | |

Table 5.18: Results of Student's t-test for seasonal differences in mean crab digestive gland manganese concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal manganese concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 174.99 | 198.06 | 27.0-740.7 | 5.19 | <0.05 |
| vs | | | | | | |
| Autumn | 5 | 11.69 | 21.0 | 0.0-48.45 | | |
| Summer | 15 | 174.99 | 198.06 | 27.0-740.7 | 4.89 | <0.05 |
| vs | | | | | | |
| Winter | 6 | 14.67 | 22.9 | 0.0-59.43 | | |
| Summer | 15 | 174.99 | 198.06 | 27.0-740.7 | 5.08 | <0.05 |
| vs | | | | | | |
| Spring | 7 | 14.04 | 16.05 | 2.9-49.94 | | |
| Autumn | 5 | 11.69 | 21.0 | 0.0-48.45 | -0.41 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 14.67 | 22.9 | 0.0-59.43 | | |
| Autumn | 5 | 11.69 | 21.0 | 0.0-48.45 | -1.36 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 14.04 | 16.05 | 2.9-49.94 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Winter | 6 | 14.67 | 22.9 | 0.0-59.43 | -0.86 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 14.04 | 16.05 | 2.9-49.94 | | |

Table 5.19: Results of Student's t-test for seasonal differences in mean crab gonad manganese concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal manganese concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 13 | 88.55 | 143.32 | 5.97-500.0 | 2.31 | <0.05 |
| vs | | | | | | |
| Autumn | 4 | 15.54 | 24.2 | 0.0-50.97 | | |
| Summer | 13 | 88.55 | 143.32 | 5.97-500.0 | 1.53 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 18.98 | 18.62 | 0.0-44.32 | | |
| Summer | 13 | 88.55 | 143.32 | 5.97-500.0 | 1.52 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 18.32 | 18.16 | 2.9-41.74 | | |
| Autumn | 4 | 15.54 | 24.2 | 0.0-50.97 | -0.58 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 18.98 | 18.62 | 0.0-44.32 | | |
| Autumn | 4 | 15.54 | 24.2 | 0.0-50.97 | -0.7 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 18.32 | 18.16 | 2.9-41.74 | | |
| Winter | 4 | 18.98 | 18.62 | 0.0-44.32 | -0.07 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 18.32 | 18.16 | 2.9-41.74 | | |

Table 5.20: Results of Student's t-test for differences in whole crab ($\mu g.g^{-1}$ wet mass) and water manganese concentrations ($mg.l^{-1}$) at each locality: Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_W$), calculated for whole crabs (Mean logarithms of manganese concentrations used for the t-test; t = t-value, p = p-value).

| | n | Mean | SD | Range | t | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water (J) | 8 | 0.138 | 0.118 | 0.0-0.26 | -17.94 | <0.05 | 2108.77 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 291.01 | 356.31 | 45.54-1644.04 | | | |
| Water (S) | 8 | 0.125 | 0.133 | 0.0-0.27 | -20.22 | <0.05 | 4769.68 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 596.21 | 1113.83 | 120.53-6138.67 | | | |

Table 5.21: Results of Student's t-test for differences in crab tissue ($\mu g.g^{-1}$ wet mass) and water manganese concentrations ($mg.l^{-1}$) at each locality, as well as the bioconcentration factor ($BCF_W$) calculated for all tissues (Dig.gland = digestive gland; t = t-value, p = p-value).

| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_W$ |
| **Water** | 8 | 0.138 | 0.118 | 0.0-0.26 | | | |
| vs Muscle | 16 | 23.93 | 46.92 | 0.0-187.12 | -5.42 | <0.05 | 173.41 |
| vs Dig.gland | 18 | 97.68 | 196.16 | 0.0-444.4 | 4.67 | <0.05 | 707.83 |
| vs Gills | 14 | 36.47 | 73.77 | 0.0-235.97 | -4.47 | <0.05 | 264.28 |
| vs Gonads | 14 | 28.8 | 32.21 | 0.0-100.0 | -6.42 | <0.05 | 208.7 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_W$ |
| **Water** | 8 | 0.125 | 0.133 | 0.0-0.27 | | | |
| vs Muscle | 13 | 38.98 | 61.53 | 0.0-193.52 | -5.19 | <0.05 | 311.84 |
| vs Dig.gland | 15 | 74.09 | 88.47 | 0.0-275.0 | -3.78 | <0.05 | 592.72 |
| vs Gills | 12 | 23.0 | 18.88 | 2.64-54.09 | -7.32 | <0.05 | 184.0 |
| vs Gonads | 11 | 87.21 | 157.22 | 0.0-500.0 | -5.92 | <0.05 | 697.68 |

Table 5.22: Results of the Mann-Whitney test for differences in crab carapace ($\mu g.g^{-1}$ wet mass) and water manganese concentrations at each locality, Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_W$) calculated for the carapace (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water (J) | 8 | 0.138 | 0.118 | 0.0-0.26 | 3.281 | <0.05 | 1775.36 |
| vs | | | | | | | |
| Carapace (J) | 19 | 245.0 | 278.72 | 0.0-1082.73 | | | |
| Water (S) | 8 | 0.125 | 0.133 | 0.0-0.27 | 3.622 | <0.05 | 1721.84 |
| vs | | | | | | | |
| Carapace (S) | 20 | 215.23 | 130.26 | 16.52-519.18 | | | |

Table 5.23: Results of Student's t-test for the differences in manganese concentrations ($\mu g.g^{-1}$ wet mass) in whole crabs and sediments from Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for whole crabs (Mean logarithms of manganese concentrations used to calculate the t-value; t = t-value, p = p-value).

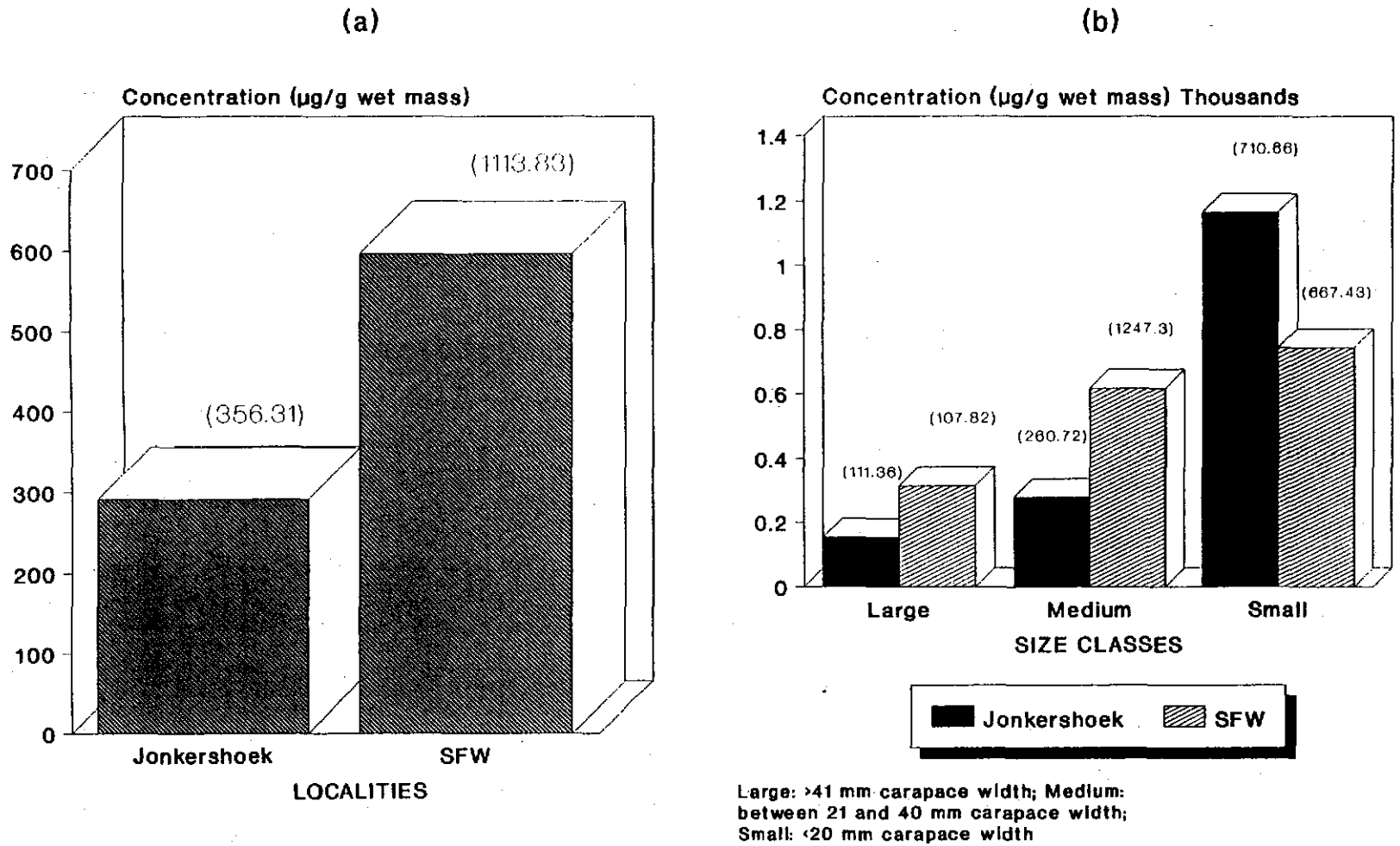
| | n | Mean | SD | Range | t | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 157.81 | 118.49 | 33.84-330.26 | -1.21 | >0.05 | 1.84 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 291.01 | 356.31 | 45.54-1644.04 | | | |
| Sediment (S) | 8 | 47.23 | 11.3 | 34.18-66.05 | -6.8 | <0.05 | 12.62 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 596.21 | 1113.83 | 120.53-6138.67 | | | |

Table 5.24: Results of Student's t-test for the differences in mean manganese concentration ($\mu g.g^{-1}$ wet mass) in selected crab tissues and sediments at each locality, as well as the bioconcentration factor ($BCF_S$) calculated for all tissues (Dig.gland = digestive gland; Mean logarithms of manganese concentrations used to calculate the t-value; t = t-value, p = p-value).

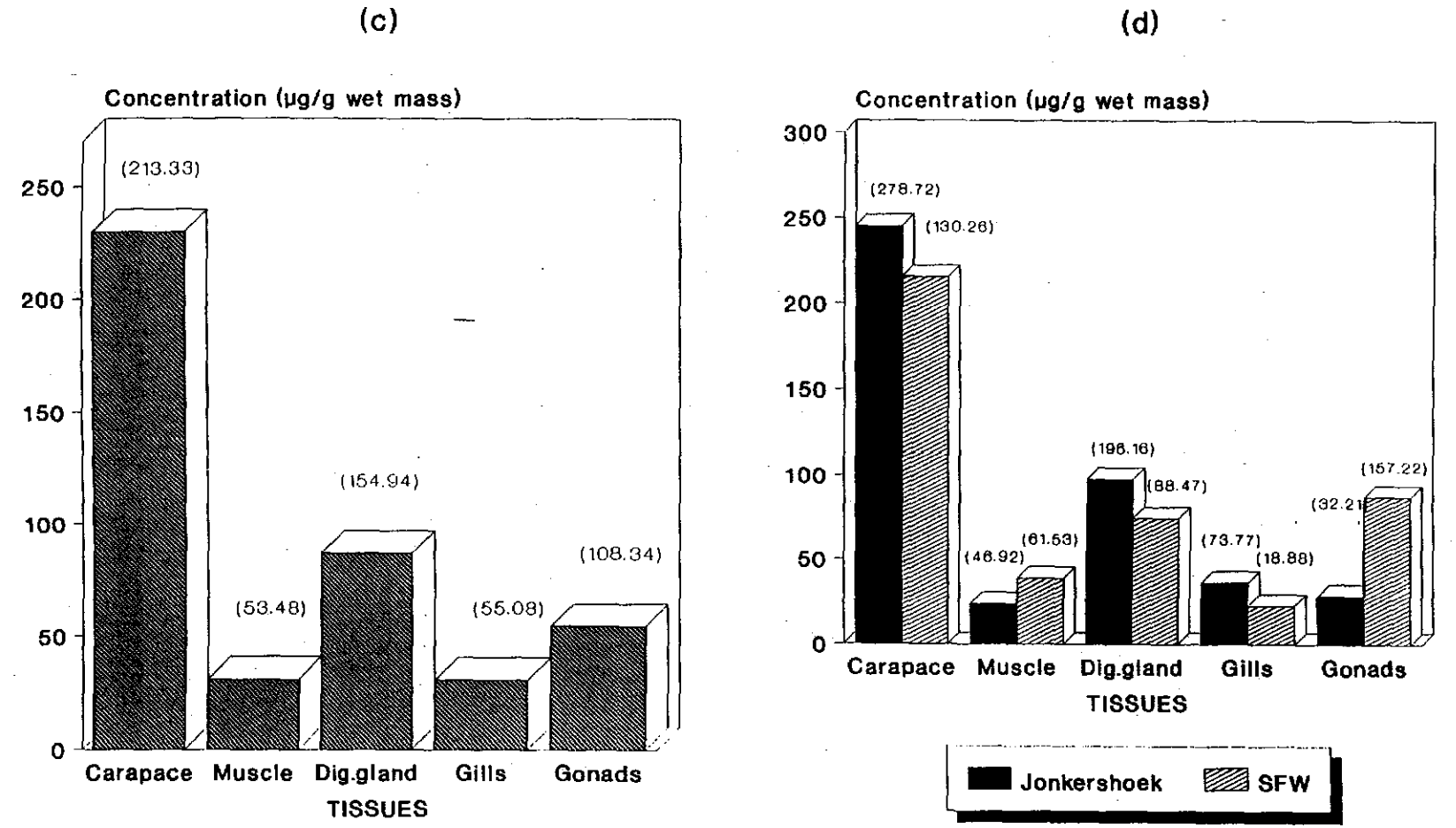
| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 157.81 | 118.49 | 33.84-330.26 | | | |
| vs Muscle | 16 | 23.93 | 46.92 | 0.0-187.12 | 4.66 | <0.05 | 0.15 |
| vs Dig.gland | 18 | 97.68 | 196.16 | 0.0-444.4 | 2.3 | <0.05 | 0.62 |
| vs Gills | 14 | 36.47 | 73.77 | 0.0-235.97 | 3.97 | <0.05 | 0.23 |
| vs Gonads | 14 | 28.8 | 32.21 | 0.0-100.0 | 3.71 | <0.05 | 0.18 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 47.23 | 11.3 | 34.18-66.05 | | | |
| vs Muscle | 13 | 38.98 | 61.53 | 0.0-193.52 | 2.24 | <0.05 | 0.83 |
| vs Dig.gland | 15 | 74.09 | 88.47 | 0.0-275.0 | 0.74 | >0.05 | 1.57 |
| vs Gills | 12 | 23.01 | 18.88 | 2.64-54.09 | 2.95 | <0.05 | 0.49 |
| vs Gonads | 11 | 87.21 | 157.22 | 0.0-500.0 | 1.04 | >0.05 | 1.85 |

Table 5.25: Results of the Mann-Whitney test for the differences in manganese concentrations ($\mu g.g^{-1}$ wet mass) of the crab carapace and sediments at each locality, Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for the carapace (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 157.81 | 118.49 | 33.84-330.26 | 0.415 | >0.05 | 1.55 |
| vs | | | | | | | |
| Carapace (J) | 19 | 245.0 | 278.72 | 0.0-1082.73 | | | |
| Sediment (S) | 8 | 47.23 | 11.3 | 34.18-66.05 | 2.769 | <0.05 | 4.56 |
| vs | | | | | | | |
| Carapace (S) | 20 | 215.23 | 130.26 | 16.52-519.18 | | | |

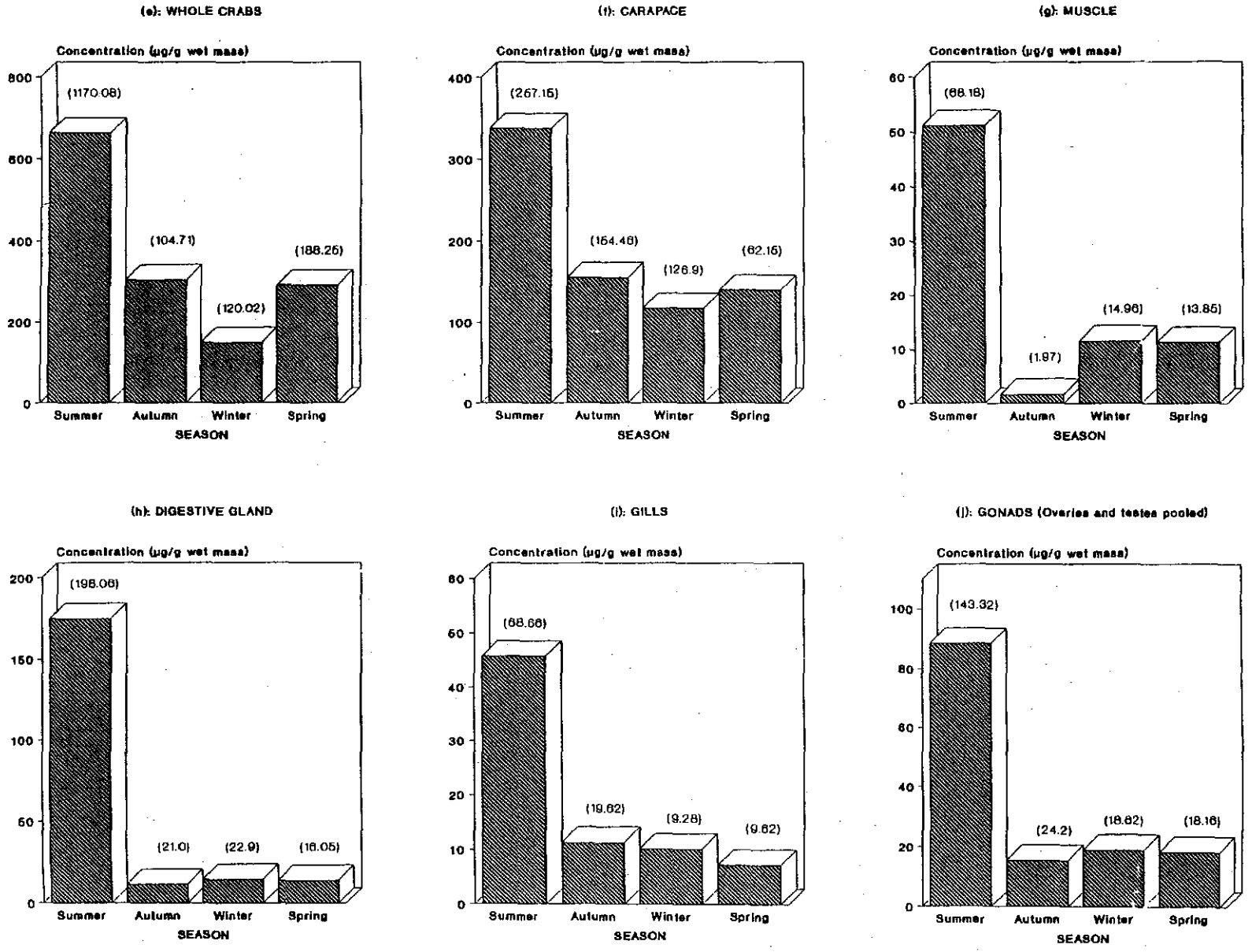



Figures 5(a)-(b): Mean manganese concentration in whole crabs (a) and various size classes (b) from both localities (SD in parenthesis).




Jonkershoek and SFW data pooled

Figures 5(c)-(d): Mean manganese concentration in carapace and tissues of *P. perlatus* from the Eerste River (SD in parenthesis).




Figures 5(e)-(j): Mean seasonal manganese concentration in whole crabs, carapace and tissues of *P. perlatus* (Jonkershoek and SFW data pooled; SD in parenthesis).

## Discussion

Comparisons of the mean manganese concentration found in the various selected tissues and the carapace of *Potamonautes perlatus* (Table 5.4; Figure 5(c)), with the results obtained by Bryan & Ward (1965) for the lobster *Homarus vulgaris* and Steenkamp et al. (1994b) for the freshwater crab *Potamonautes warreni*, were made. Steenkamp et al. (1994b) found mean concentrations of 11±9 to 39±46 $\mu g.g^{-1}$ wet muscle mass, 24±19 to 67±41 $\mu g.g^{-1}$ wet gonad mass, 66±64 to 252±253 $\mu g.g^{-1}$ wet digestive gland mass, 52±57 to 117±277 $\mu g.g^{-1}$ wet gill mass and 275±130 to 973±513 $\mu g.g^{-1}$ wet carapace mass for *P. warreni*, from polluted freshwater ecosystems. It was found that, in comparison, the manganese concentrations in the gills and carapace of *P. perlatus* were relatively low. The mean muscle, gonad and digestive gland concentrations compared well. However, compared to the findings of Bryan & Ward (1965) for unexposed individuals of *Homarus vulgaris*, namely mean manganese concentrations of 0.8, 4.8, 1.6, 20.8 and 225 $\mu g.g^{-1}$ wet mass for the muscles, digestive gland, gonads, gills and carapace respectively, the manganese concentrations in the muscles, digestive gland and gonads of *P. perlatus* were extremely high and the gill and carapace manganese somewhat high.

Despite the fact that fairly high manganese concentrations were found in certain tissues of *P. perlatus*, the whole body manganese concentrations in crabs from Jonkershoek and SFW, i.e. 119.0±152.19 and 161.76±221.31 $\mu g.g^{-1}$ dry mass respectively, when compared to the dry mass Mn concentrations found in *P. warreni* by Van Eeden & Schoonbee (1991) (568.0±0.03 to 1675.0±0.02 $\mu g.g^{-1}$), were shown to be very low.

It is clear that large intra- and interspecific variations in manganese concentrations in whole body, tissue and carapace exist, complicating efforts to determine the degree of accumulation in the species. The low whole crab dry mass concentrations in *P. perlatus* indicate that Mn is relatively well regulated in this species in the Eerste River. However, the statistically significant differences in whole body Mn, observed between Jonkershoek and SFW, as well as the differences in Mn between large-sized and between medium-sized crabs from the two localities (Tables 5.6 to 5.8), indicate Mn accumulation from the environment in the SFW population. This is supported by the significantly higher concentrations in whole crabs than in the sediments from SFW (Table 5.23), as well as the higher $BCF_W$'s and $BCF_S$'s for whole crabs, tissues (except gills) and carapace from SFW (Tables 5.20-5.25). The reasons for the observed differences between the two localities are not clear, but it is probable that the availability of Mn from the water and sediments of SFW were increased by certain physico-chemical factors at this site.

The determination of the specific sites of manganese storage in the crab body revealed that the carapace contained the most Mn, and can therefore be considered the most important storage site, with the digestive gland the second most important site (Tables 5.4 and 5.5). The $BCF_W$ and

the $BCF_S$ for the carapace (Tables 5.21, 5.22, 5.24 and 5.25), from Jonkershoek and SFW, were also clearly higher than BCF's of any of the selected tissues and therefore support these findings. The results of the present study agree with those of Bryan & Ward (1965) and Steenkamp et al. (1994b) for the marine lobster *Homarus vulgaris* and the freshwater crab *Potamonautes warreni* respectively. Bryan & Ward (1965) stated that most of the manganese in the lobster is incorporated into calcified regions such as the "shell" and ossicles and teeth of the gastric mill. It was also suggested that the "shell" could possibly act as a reservoir for Mn. It is therefore possible that the storage of Mn in the carapace is a mechanism by which the animal protects itself against long-term Mn accumulation, since the carapace is shed during moulting. Similarly, collembola store metals in the intestinal epithelium and rid themselves of these metals through excretion of a type of intestinal "plug" with every moult (Van Straalen & Verkleij, 1993).

Food was found to be an important source of Mn for the lobster, *Homarus vulgaris*. When Bryan & Ward (1965) pipetted a Mn solution into the stomach fluid of individuals of this species, the Mn content of the "hepatopancreas" reached a maximum after 2 h, suggesting that much of the absorption takes place via this organ. It is therefore expected that the digestive gland would show high but variable concentrations of manganese, as was indeed the case with *P. perlatus* (Table 5.4). The importance of food as source of manganese can also possibly explain the relatively high and variable concentrations of manganese in the muscles, gills and gonads (Table 5.4): Bryan & Ward (1965) found corresponding increases in Mn in abdominal muscle and gills with increases in blood Mn, indicating contamination of these tissues from the blood. They also showed that Mn might be absorbed across the gills, but that this may be a passive rather than an active process.

Whilst the carapace, and to a lesser extent the digestive gland, are clearly the most important sites for Mn storage, the remaining three tissue types do not differ significantly in their concentrations of manganese and therefore in their contribution to Mn storage (Table 5.5).

The relationship between crab size and whole body, tissue and carapace manganese concentrations were investigated. The relatively strong negative correlation ($r = -0.526$) between sizes of crabs and whole crab Mn concentrations, as well as the statistically significant differences in whole crab Mn found between large and small, and between medium and small crabs from Jonkershoek (Table 5.1), prove that smaller (therefore younger) individuals of *P. perlatus* take up more manganese per unit body mass than larger (older) individuals. These results are supported by the strong negative correlations found between crab size and tissue manganese concentrations (Table 5.3). Steenkamp et al. (1994b) reported that *P. warreni* showed a significant decrease in muscle manganese with an increase in crab size at two of the sampling localities. Hill & O'Keeffe (1992) studied the feeding ecology of *P. perlatus* and found that smaller individuals preyed significantly more on aquatic invertebrates than larger individuals, whilst the latter preferred vegetable material. It is known that aquatic invertebrates

(e.g. insects) are able to accumulate heavy metals in their bodies (Albers & Camardese, 1993 and Kiffney & Clements, 1993). It is therefore possible that smaller individuals of *P. perlatus* bioaccumulate more Mn from their prey and that upon reaching an unspecified size/age, a change in food preference from invertebrates to vegetable material results in a decrease in Mn bioaccumulation, which would explain the observed lower Mn concentrations in larger crabs. Steenkamp et al. (1994b) suggested that the higher manganese concentrations in smaller individuals are due to the fact that the regulation mechanisms are not yet fully developed and that younger crabs have a relatively higher metabolic rate. Gherardi et al. (1987) and Gherardi & Micheli (1989) found that younger individuals of *Potamon fluviatile* and *P. potamios palestinensis* respectively, tend to hide more under stones and rocks, especially during the night. Since this type of microhabitat offers a relatively constant environment, it is possible that the younger crabs are exposed to specific manganese concentrations for longer periods, which might also contribute to these elevated manganese concentrations.

Despite the difficulty in explaining the observed higher manganese concentrations in small crabs, it is clear that body size is an important factor influencing the uptake of manganese by *Potamonautes perlatus*.

As far as the relationships between factors such as gender and seasonality and whole body, tissue and carapace manganese concentrations are concerned, it was found that no statistically significant differences in whole body, tissue or carapace manganese concentrations existed between males and females (Tables 5.11 and 5.12). The results of Steenkamp et al. (1994b) varied between their three sampling locations. Whilst at one of the locations no differences occurred between the sexes, at the remaining two locations significant differences indeed occurred between the two sexes. Despite the fact that gender seems to be of some importance in the accumulation of Mn in species such as *P. warreni*, it is concluded from this study that gender does not seem to be an influencing factor in *P. perlatus*.

There were, however, clear relationships between seasonality and whole crab, tissue and carapace manganese concentrations. All selected tissues and the carapace, as well as whole crabs, exhibited a number of statistical differences between the seasons (Tables 5.13 to 5.19). In all cases a summer peak in manganese concentration occurred, corresponding with a slight summer increase in water manganese concentration (Figures 4(c) and 5(e)-(j)). Although the water is a probable source of Mn to the crabs, it is also likely that a change in diet and activity patterns during summer could be responsible for the summer Mn peaks in the crabs. As food is such an important source of Mn, and as all four selected tissues and the carapace are affected by raised blood manganese concentrations (Bryan & Ward, 1965), a possible manganese-rich diet in summer could increase the Mn levels in the various tissues and the carapace, as well as in the entire body.

Finally, it is concluded from the preliminary results, that *P. perlatus* in the Eerste River, similar to *P. warreni* (Steenkamp et al., 1994b), qualifies as a potentially useful monitor of Mn pollution. This is based entirely on the observed high concentrations of Mn in certain tissues of *P. perlatus* and the summer water Mn peak, reflected in whole crabs, tissues and carapace. On the other hand, it must be noted that the degree of regulation of Mn in the body, as shown in the present study, as well as the large intraspecific variations in Mn concentrations, may greatly influence the accuracy to which Mn pollution in the riverine ecosystem is reflected in the crab. Final conclusions can only be made after further intensive research has been undertaken on, especially, the uptake of Mn in *P. perlatus* and the factors affecting the availability of Mn to the species.

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# CHAPTER 6

## CONCENTRATIONS OF ZINC IN THE FRESHWATER CRAB, *POTAMONAUTES PERLATUS*

### Introduction

Zinc is a heavy metal which occurs frequently in nature and is generally associated with Fe, Cu, Cd and Pb sulphides (Galvin, 1996). It is found in several minerals such as zinc blends, smithsonite, $ZnCO_3$, willemite, $Zn_2SiO_4$, zincite, ZnO etc. (Moore & Ramamoorthy, 1984), but particularly in ZnS (Galvin, 1996). The ability of zinc to form complexes with particularly ammonia, amines, halide ions and cyanide is well known (Moore & Ramamoorthy, 1984).

Zinc is an essential element in animal nutrition and is required in several processes e.g. the biosynthesis of nucleic acids, namely RNA and DNA polymerases, the healing of tissues, hormone metabolism, immune response and the stabilization of ribosomes and membranes (Moore & Ramamoorthy, 1984).

According to Moore and Ramamoorthy (1984), zinc ranks fourth among metals next to steel, aluminium and copper in annual global consumption. It has several industrial uses, of which the galvinizing of iron and steel products, the use in brass products and the use as zinc-base alloys for mechanical components in automobiles, are the major and most well known uses (Elinder & Piscator, 1979 and Moore & Ramamoorthy, 1984). Other uses include as rolled zinc for e.g. dry cell battery production and as zinc oxide for paints and agricultural products, among others (Moore & Ramamoorthy, 1984).

An excess of zinc in aquatic ecosystems, originating from industrial, household and agricultural runoff, can occur and this holds serious implications for those aquatic animals which cannot regulate the levels of zinc in their bodies but accumulate the metal to high concentrations. The sublethal effects of zinc on animals include, among others, retarded growth, poor appetite, delayed wound healing (Richardson & Gangolli, 1994), leg paralysis, non-viable newborn, cartilage erosion, changes in tissue minerals and decreased retention of calcium and phosphorus (Mills, 1989). Abel (1989) reported that zinc is a fairly potent bactericide and fungicide and could therefore, in an aquatic ecosystem, interfere with the processing of detritus by decomposer organisms, thus depriving other aquatic animals of a major food source.

There are several factors influencing the bioavailability and toxicity of zinc in freshwater ecosystems: the toxicity of zinc, for instance, decreases with an increase in water hardness and pH (Förstner & Prosi, 1979 and Moore & Ramamoorthy, 1984) as well as a decrease in age of the animal: immature stages are, in general, more sensitive to zinc than adults (Moore &

Ramamoorthy, 1984). The interactions between Zn and Cd, and Zn and Cu, also play important rôles (Elinder & Piscator, 1979). Certain organic substances in solution can remove zinc ions from the water by the formation of organic compounds, which subsequently lower the availability of zinc to the aquatic organisms (Förstner & Prosi, 1979). Zinc toxicity, in the animal, may also be modified by sequestration in metallothionein, a small protein synthesized when zinc is administered to experimental animals (Ottaway, 1980). The synthesis of metallothioneins has also been reported for decapod crustaceans: Rainbow & Scott (1979) and Engel & Brouwer (1987) discussed the production of a heavy metal- (notably zinc) binding protein in the midgut-/digestive gland of the crabs, *Carcinus maenas* and *Callinectes sapidus*, respectively.

The accumulation of zinc by freshwater decapod crustaceans has been studied in several species, e.g. the crayfishes *Orconectes virilis* (Anderson & Brower, 1978 and France, 1987) and *Austopotamobius pallipes* (Bryan, 1968) as well as the crabs *Oziotelphusa senex senex* (Radhakrishnaiah, 1987) and *Potamonautes warreni* (du Preez et al., 1993; Steenkamp, 1992 and Van Eeden & Schoonbee, 1991).

## Materials and Methods

All crab samples were prepared, tested and statistically analysed according the method described in Chapter 3.

## Results

In all cases only the zinc concentrations per gram wet mass were used for statistical analysis. Crabs showed large individual variation in whole body, as well as tissue and carapace zinc concentrations. This was an important consideration in the interpretation of the statistical results.

### The relationship between crab size and whole crab zinc concentration

In order to investigate the possible relationship between whole crab zinc concentrations and the size of the crabs, the data from Jonkershoek and SFW were pooled. The carapace widths of the crabs, in decreasing order, were compared with the respective zinc concentrations and the correlation coefficient (r) thereof, calculated. With 53 degrees of freedom and an $r^2$-value of 23.77%, an r-value of -0.4875 was obtained. This indicated a reasonably strong negative

correlation between sizes of crabs and the concentrations of zinc in the body: as crab size decreased, so did the zinc concentration increase (Figure 6(b)).

Differences in zinc concentrations of the various size classes at each locality

Since the whole crab data for large, medium and small-sized crabs from each locality were not normally distributed, the mean logarithm of the zinc concentrations in each class was used to test for statistically significant differences ($p<0.05$) (Table 6.1).

Differences in concentrations were found between large and small crabs from Jonkershoek. The small size class showed the highest mean zinc concentration (131.22±61.23 $\mu g.g^{-1}$ wet mass) and large sized crabs the lowest (74.64±25.85 $\mu g.g^{-1}$ wet mass).

Significant differences existed between large and medium and between medium and small size classes from SFW, with small and large crabs also exhibiting highest and lowest mean concentrations respectively (345.66±260.36 and 48.7±24.9 $\mu g.g^{-1}$ wet mass).

The relationship between crab size, and tissue and carapace zinc concentration

In order to investigate the possible relationship between crab size and zinc concentration in selected tissues, namely muscle tissue, digestive gland, gills and gonads, as well as the carapace, the data from Jonkershoek and SFW were pooled, after which the carapace widths of crabs, in decreasing order, were compared with the respective tissue and carapace zinc concentration. Table 6.2 shows the r-values calculated for each tissue and for the carapace.

Strong negative correlations were found between crab size and the zinc concentration in the muscles and carapace. Reasonably strong negative correlations existed between crab size and zinc concentration in the digestive gland and gonads, whereas the zinc concentration in the gills proved to be poorly negatively correlated with the size of the crabs.

Differences in zinc concentrations of the various selected tissues and the carapace

The mean zinc concentration values for the selected tissues and the carapace of crabs from Jonkershoek and SFW, were pooled in order to determine whether statistically significant differences ($p<0.05$) existed between the various tissues and the carapace. Since the data were not normally distributed, the mean logarithms of the tissue and carapace zinc concentrations were used in the t-test. The respective mean concentrations and standard deviations are tabulated in Table 6.3. Table 6.4 shows the results of the t-test performed on the tissue and carapace concentrations.

When comparing values obtained for the selected tissues and the carapace, significant differences were found between the mean zinc concentration in all the tissues, except between muscle and gonads, digestive gland and gonads, and digestive gland and gill tissue. The carapace showed the highest mean concentration (78.55±91.926 $\mu g.g^{-1}$ wet mass), whereas the lowest mean was found in the digestive gland (26.968±31.423 $\mu g.g^{-1}$ wet mass) (Figure 6(c)).

Differences in zinc concentrations of crabs from Jonkershoek and SFW

(a) Differences in concentration values for whole crabs

The results of Student's t-test performed on the mean logarithms of the whole crab zinc concentrations from Jonkershoek and SFW are shown in Table 6.5.

The highest mean zinc concentration (112.69±155.88 $\mu g.g^{-1}$ wet mass), was found in crabs from SFW (Figure 6(a)). A t-value of 1.33 indicated that there was no statistically significant difference ($p>0.05$) in mean whole crab zinc concentrations between the two localities.

(a)(i) Differences in mean whole crab zinc concentration of the various size classes

Through a comparison of the mean logarithms of whole crab zinc concentration values between large, medium and small crabs, from Jonkershoek and SFW, it was established that statistically significant differences ($p<0.05$) existed between large crabs as well as between medium sized crabs from the two localities. In both cases the crabs from Jonkershoek had higher mean concentrations (74.64±25.85 and 87.25±80.08 $\mu g.g^{-1}$ wet mass respectively) than those from SFW (Table 6.6; Figure 6(b)).

(b) Differences in concentration values for the selected tissues and the carapace

The mean zinc concentration in the carapace, muscle, digestive gland, gills and gonads of crabs from Jonkershoek and SFW were compared with the use of Student's t-test and tabulated in Table.6.7. Since the data were not normally distributed, the mean logarithm of the zinc concentrations in the tissues and carapace were used to calculate the t-value. In the case of the carapace, however, a normal distribution could not be obtained after transformation of the concentration value data. A nonparametric test, namely the Mann-Whitney test, was therefore performed on the original data, to investigate possible differences (Table 6.8).

Statistically significant differences ($p<0.05$) were found to exist between the zinc concentration values for the muscles, digestive gland, gills and gonads, from crabs originating from Jonkershoek and SFW respectively. In the case of all four tissues, the highest mean zinc concentrations were found in crabs collected from SFW. The respective means were 63.87±72.22 $\mu g.g^{-1}$ wet mass for muscle tissue, 31.843±38.98 $\mu g.g^{-1}$ wet mass for the digestive gland, 56.54±73.93 $\mu g.g^{-1}$ wet mass for the gills and 113.93±193.48 $\mu g.g^{-1}$ wet mass for the gonads (Figure 6(d)).

Differences between zinc concentrations of males and females

In order to determine whether statistically significant differences ($p<0.05$) existed in the mean whole crab, tissue and carapace zinc concentrations between males and females, the data obtained for crabs from Jonkershoek and SFW, were pooled for each sex. In all cases the original data failed the normal probability test, therefore each concentration value was logarithmically transformed. In the case of the muscle and gill tissues, the transformed data also failed to provide a normal distribution, therefore the Mann-Whitney nonparametric test was performed.

(a) Differences in the zinc concentrations of whole crabs

Table 6.9 shows the results of Student's t-test, performed on the mean logarithm of the whole crab zinc concentrations in males and females of *Potamonautes perlatus*. The highest mean zinc concentration was found in males ($116.89\pm152.4$ $\mu g.g^{-1}$ wet mass) but no statistically significant difference ($p>0.05$) existed between the two genders.

(b) Differences in zinc concentrations of selected tissues and the carapace

Although males exhibited the highest mean zinc concentrations in the carapace and all but one of the four tissue types (gonads), no statistically significant differences ($p>0.05$) were found between the two genders (Tables 6.10 and 6.11).

Seasonal variations in zinc concentration

The zinc concentrations in whole crabs, selected tissues and carapace from the different seasons were tested for statistically significant differences ($p<0.05$). In each instance, the data for crabs collected from Jonkershoek and SFW were pooled and these then tested for normality. None of the data sets were normally distributed and were thus logarithmically transformed. The transformed whole crab data also failed the normal probability test, thus the Mann-Whitney test was performed.

(a) Seasonal differences in whole crab zinc concentrations

The Mann-Whitney nonparametric test produced statistically significant differences ($p<0.05$) in whole crab zinc concentrations for spring and the other three seasons (Table 6.12). The highest mean whole crab zinc concentration ($177.6\pm213.89$ $\mu g.g^{-1}$ wet mass), was observed for winter, whereas spring showed the lowest mean ($50.36\pm67.3$ $\mu g.g^{-1}$ wet mass) (Figure 6(e)).

(b) Seasonal differences in tissue and carapace zinc concentrations

1. Carapace

Statistically significant differences ($p<0.05$) in carapace zinc concentrations were found between all seasonal comparisons, except between autumn and spring. The highest mean zinc concentration occurred in winter (176.95±180.25 $\mu g.g^{-1}$ wet mass) and the lowest in autumn (40.43±32.94 $\mu g.g^{-1}$ wet mass) (Figure 6(f)). The results of the t-test, performed on the mean logarithms of the seasonal zinc concentrations, are tabulated in Table 6.13.

2. Muscle tissue

The highest mean muscle zinc concentration (71.95±72.19 $\mu g.g^{-1}$ wet mass) occurred in winter, whereas in autumn the mean concentration was the lowest (9.65±3.93 $\mu g.g^{-1}$ wet mass) (Figure 6(g)). Through pairwise comparisons of the different seasons, it was found that statistically significant differences ($p>0.05$) existed between summer and autumn and between autumn and winter (Table 6.14).

3. Gill tissue

Statistically significant differences ($p<0.05$) in gill zinc concentrations were found when comparing values for summer and winter, and winter and spring. In Table 6.15 it can be seen that the highest and lowest mean zinc concentrations occurred in autumn (127.96±98.65 $\mu g.g^{-1}$ wet mass) and summer (10.23±14.03 $\mu g.g^{-1}$ wet mass) respectively (Figure 6(h)).

4. Digestive gland

The results of Student's t-test on the mean logarithms of the digestive gland zinc concentrations, when comparing values from the different seasons, are tabulated in Table 6.16. Only the comparisons of summer and winter and of winter and spring showed significant differences ($p<0.05$). The mean zinc concentration was the highest in winter (37.02±25.62 $\mu g.g^{-1}$ wet mass), whilst the lowest mean concentration occurred in summer (17.533±23.617 $\mu g.g^{-1}$ wet mass) (Figure 6(i)).

5. Gonads

When the data for the ovaries and testes were pooled, the highest and lowest mean gonad zinc concentrations (120.93±118.97 and 22.704±22.5 $\mu g.g^{-1}$ wet mass) occurred in winter and spring respectively (Figure 6(j)). No statistically significant differences ($p>0.05$) were however found to exist between the values for any of the seasons (Table 6.17).

<u>Differences in zinc concentrations of whole crabs, tissues, carapace, and Eerste River water</u>

The original as well as the transformed data from water samples failed to provide a normal distribution, therefore the Mann-Whitney test was performed in each case, to test for statistically significant differences ($p<0.05$).

(a) Differences in concentration values for whole crabs and water

The Mann-Whitney test (Table 6.18) indicated significant differences between the whole crab and water zinc concentrations in both localities, i.e. Jonkershoek as well as SFW.

(b) Differences in concentration values for the selected tissues, carapace and water
The zinc concentrations in all the tissues as well as the carapace from both localities proved to be significantly different ($p<0.05$) from the concentrations in the water. The results of the Mann-Whitney test are listed in Table 6.19.

Differences in zinc concentrations of whole crabs, tissues, carapace, and sediments

(a) Differences in concentration values for whole crabs and sediments
Student's t-test, performed on the mean logarithms of whole crab and sediment zinc concentrations, for Jonkershoek and SFW, yielded a statistically significant difference ($p<0.05$) in the whole crab and sediment concentrations from Jonkershoek. The results of the t-test are tabulated in Table 6.20.

(b) Differences in concentration values for the selected tissues, carapace and sediments
The mean logarithms of the tissue and carapace zinc concentrations were used to test for statistically significant differences ($p<0.05$) between tissue, carapace and sediment zinc concentrations within the two localities. The transformed carapace concentrations, however, were not normally distributed, therefore the Mann-Whitney test was performed.

Significant differences in zinc concentrations were found between the sediment and digestive gland and gills from Jonkershoek and between the sediment and muscles from SFW.
Table 6.21 lists the results of Student's t-test, performed on the mean logarithms of the tissue zinc concentrations from the two localities. The results of the Mann-Whitney test, performed on the original carapace data, are shown in Table 6.22.

Table 6.1: Results of Student's t-test for differences in whole crab zinc concentrations ($\mu g.g^{-1}$ wet mass) of the various size classes at each locality.

| JONKERSHOEK | | | | | | |
|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t-value | p-value |
| Large vs Medium | 22<br>34 | 74.64<br>87.25 | 25.85<br>80.08 | 40.47-133.94<br>30.72-432.64 | 0.04 | >0.05 |
| Large vs Small | 22<br>4 | 74.64<br>131.22 | 25.85<br>61.23 | 40.47-133.94<br>41.65-171.65 | -2.24 | <0.05 |
| Medium vs Small | 34<br>4 | 87.25<br>131.22 | 80.08<br>61.23 | 30.72-432.64<br>41.65-171.65 | -1.55 | >0.05 |
| **SFW** | | | | | | |
| | n | Mean | SD | Range | t-value | p-value |
| Large vs Medium | 6<br>39 | 48.7<br>86.69 | 24.9<br>114.28 | 17.48-71.78<br>10.39-624.87 | -0.3 | >0.05 |
| Large vs Small | 6<br>6 | 48.7<br>345.68 | 24.9<br>260.36 | 17.48-71.78<br>98.03-690.63 | -4.58 | <0.05 |
| Medium vs Small | 39<br>6 | 86.69<br>345.68 | 114.28<br>260.36 | 10.39-624.87<br>98.03-690.63 | -3.73 | <0.05 |

Table 6.2: $R^2$-values calculated for sizes of crabs and selected tissue and carapace zinc concentrations (DF = Degrees of freedom; Dig.gland = digestive gland).

| | DF | r-value | R-sq.(%) |
|---|---|---|---|
| Crab size and Muscle | 27 | -0.7061 | 49.85 |
| Crab size and Dig.gland | 29 | -0.5683 | 32.3 |
| Crab size and Gills | 24 | -0.2076 | 4.31 |
| Crab size and Gonads | 23 | -0.466 | 21.68 |
| Crab size and Carapace | 28 | -0.612 | 37.45 |

Table 6.3: Mean zinc concentration ($\mu g.g^{-1}$ wet mass) in the muscles, digestive gland (dig. gland), gonads, gills and carapace of crabs from the Eerste River (Jonkershoek and SFW data pooled).

| | n | Mean | SD | Range |
|---|---|---|---|---|
| Muscle | 29 | 47.14 | 63.82 | 5.90-236.11 |
| Dig. gland | 32 | 26.97 | 31.42 | 2.0-112.35 |
| Gonads | 25 | 70.42 | 135.35 | 1.75-639.68 |
| Gills | 26 | 30.72 | 55.81 | 2.04-235.65 |
| Carapace | 39 | 78.55 | 91.93 | 0.0-517.88 |

Table 6.4: Results of Student's t-test for the differences in mean tissue and carapace zinc concentration ($\mu g.g^{-1}$ wet mass) of crabs (Jonkershoek and SFW data pooled; Mean logarithms of zinc concentrations used for the calculation of the t-value).

| | t-value | p-value |
|---|---|---|
| Muscle vs Digestive gland | 2.08 | <0.05 |
| Muscle vs Gonads | 0.17 | >0.05 |
| Muscle vs Gills | 2.66 | <0.05 |
| Muscle vs Carapace | -2.03 | <0.05 |
| Digestive gland vs Gonads | -1.54 | >0.05 |
| Digestive gland vs Gills | 0.8 | >0.05 |
| Digestive gland vs Carapace | -4.06 | <0.05 |
| Gonads vs Gills | 2.04 | <0.05 |
| Gonads vs Carapace | -1.86 | <0.05 |
| Gills vs Carapace | -4.43 | <0.05 |

Table 6.5: Results of Student's t-test for the differences in mean whole crab zinc concentration ($\mu g.g^{-1}$ wet mass) from Jonkershoek and SFW (Mean logarithms of the whole crab zinc concentrations used in the t-test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Jonkershoek | 60 | 85.56 | 64.83 | 30.7-432.6 | 1.333 | >0.05 |
| vs | | | | | | |
| SFW | 51 | 112.69 | 155.88 | 10.4-690.6 | | |

Table 6.6: Results of Student's t-test for the differences in mean whole crab zinc concentration ($\mu g.g^{-1}$ wet mass) for the various size classes, small, medium and large, from Jonkershoek (J) and SFW (S) (Mean logarithms of zinc concentrations used in the t-test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Large (J) | 22 | 74.64 | 25.85 | 40.47-133.94 | 2.74 | <0.05 |
| vs | | | | | | |
| Large (S) | 6 | 48.7 | 24.9 | 17.48-71.18 | | |
| Medium (J) | 34 | 87.25 | 80.08 | 30.72-432.64 | 1.78 | <0.05 |
| vs | | | | | | |
| Medium (S) | 39 | 86.69 | 114.28 | 10.39-624.87 | | |
| Small (J) | 4 | 131.22 | 61.23 | 41.65-171.65 | -1.78 | >0.05 |
| vs | | | | | | |
| Small (S) | 6 | 345.68 | 260.36 | 98.03-690.63 | | |

Table 6.7: Results of Student's t-test for the differences in mean tissue zinc concentration ($\mu g.g^{-1}$ wet mass) of crabs from Jonkershoek (J) and SFW (Mean logarithms of the tissue zinc concentrations used in the test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Muscle (J) | 16 | 33.55 | 54.69 | 5.9-236.1 | -5.7 | <0.05 |
| vs | | | | | | |
| Muscle (SFW) | 13 | 63.87 | 72.22 | 6.0-196.8 | | |
| Dig.gland (J) | 17 | 22.67 | 31.84 | 3.5-79.5 | -3.52 | <0.05 |
| vs | | | | | | |
| Dig.gland (SFW) | 15 | 23.28 | 38.98 | 1.2-112.3 | | |
| Gills (J) | 14 | 8.52 | 9.36 | 2.0-38.5 | -3.09 | <0.05 |
| vs | | | | | | |
| Gills (SFW) | 12 | 56.54 | 73.93 | 3.5-235.6 | | |
| Gonads (J) | 14 | 36.24 | 46.49 | 4.8-184.2 | -3.61 | <0.05 |
| vs | | | | | | |
| Gonads (SFW) | 11 | 113.93 | 193.48 | 1.8-639.7 | | |

Table 6.8: Results of the Mann-Whitney test for the differences in mean crab carapace zinc concentration ($\mu g.g^{-1}$ wet mass) from the two localities, Jonkershoek (J) and SFW.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (J) | 19 | 64.63 | 52.81 | 0-222.7 | 0.084 | >0.05 |
| vs | | | | | | |
| Carapace (SFW) | 20 | 91.78 | 117.82 | 5.7-577.9 | | |

Table 6.9: Results of Student's t-test for the differences in mean whole crab zinc concentration ($\mu g.g^{-1}$ wet mass) of males and females (Mean logarithms of the whole crab zinc concentrations used to calculate the t-value).

| | n | Mean | SD | t-value | p-value |
|---|---|---|---|---|---|
| Females | 40 | 88.73 | 85.97 | -0.71 | >0.05 |
| vs | | | | | |
| Males | 49 | 116.89 | 152.4 | | |

Table 6.10: Results of Student's t-test for the differences in mean tissue and carapace zinc concentration ($\mu g.g^{-1}$ wet mass) of male (m) and female (f) crabs (Mean logarithms of the tissue and carapace zinc concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (f) | 15 | 72.93 | 65.99 | 8.1-517.9 | -0.3 | >0.05 |
| vs | | | | | | |
| Carapace (m) | 18 | 95.8 | 118.73 | 11.9-222.7 | | |
| Dig.gland (f) | 11 | 19.065 | 25.9 | 2.4-112.3 | -0.66 | >0.05 |
| vs | | | | | | |
| Dig.gland (m) | 15 | 27.78 | 36.6 | 1.2-93.7 | | |
| Ovaries | 11 | 94.87 | 189.6 | 7.4-241.6 | -0.47 | >0.05 |
| vs | | | | | | |
| Testes | 10 | 67.5 | 80.8 | 4.8-639.7 | | |

Table 6.11: Results of the Mann-Whitney test for the differences in muscle and gill zinc concentrations ($\mu g.g^{-1}$ wet mass) of male (m) and female (f) crabs.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Muscle (f) | 10 | 26.72 | 23.4 | 7.8-180.1 | -0.589 | >0.05 |
| vs | | | | | | |
| Muscle (m) | 13 | 47.23 | 59.0 | 14.1-89.7 | | |
| Gills (f) | 10 | 15.9 | 18.7 | 3.3-235.6 | -0.674 | >0.05 |
| vs | | | | | | |
| Gills (m) | 14 | 42.96 | 72.5 | 2.0-56.3 | | |

Table 6.12: Results of the Mann-Whitney test for the seasonal differences in whole crab zinc concentrations ($\mu g.g^{-1}$ wet mass).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 49 | 83.44 | 72.66 | 35.0-432.6 | 0.38 | >0.05 |
| vs | | | | | | |
| Autumn | 16 | 86.29 | 56.6 | 35.1-253.9 | | |
| Summer | 49 | 83.44 | 72.66 | 35.0-432.6 | 1.69 | >0.05 |
| vs | | | | | | |
| Winter | 21 | 177.6 | 213.89 | 30.7-690.6 | | |
| Summer | 49 | 83.44 | 72.66 | 35.0-432.6 | -3.38 | <0.05 |
| vs | | | | | | |
| Spring | 25 | 50.36 | 67.3 | 10.4-304.6 | | |
| Autumn | 16 | 86.29 | 56.6 | 35.1-253.9 | 1.16 | >0.05 |
| vs | | | | | | |
| Winter | 21 | 177.6 | 213.89 | 30.7-690.6 | | |
| Autumn | 16 | 86.29 | 56.6 | 35.1-253.9 | -3.01 | <0.05 |
| vs | | | | | | |
| Spring | 25 | 50.36 | 67.3 | 10.4-304.6 | | |
| Winter | 21 | 177.6 | 213.89 | 30.7-690.6 | -3.85 | <0.05 |
| vs | | | | | | |
| Spring | 25 | 50.36 | 67.3 | 10.4-304.6 | | |

Table 6.13: Results of Student's t-test for seasonal differences in mean crab carapace zinc concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal zinc concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 18 | 67.02 | 44.09 | 19.0-185.8 | 2.02 | <0.05 |
| vs | | | | | | |
| Autumn | 5 | 40.43 | 32.94 | 0-75.6 | | |
| Summer | 18 | 67.02 | 44.09 | 19.0-185.8 | -2.06 | <0.05 |
| vs | | | | | | |
| Winter | 6 | 176.95 | 180.25 | 29.3-517.9 | | |
| Summer | 18 | 67.02 | 44.09 | 19.0-185.8 | 1.93 | <0.05 |
| vs | | | | | | |
| Spring | 10 | 59.32 | 74.41 | 5.7-222.7 | | |
| Autumn | 5 | 40.43 | 32.94 | 0-75.6 | -1.99 | <0.05 |
| vs | | | | | | |
| Winter | 6 | 176.95 | 180.25 | 29.3-517.9 | | |
| Autumn | 5 | 40.43 | 32.94 | 0-75.6 | -0.33 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 59.32 | 74.41 | 5.7-222.7 | | |
| Winter | 6 | 176.95 | 180.25 | 29.3-517.9 | 2.25 | <0.05 |
| vs | | | | | | |
| Spring | 10 | 59.32 | 74.41 | 5.7-222.7 | | |

Table 6.14: Results of Student's t-test for seasonal differences in mean crab muscle zinc concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal zinc concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 50.73 | 69.82 | 13.2-236.1 | 2.29 | <0.05 |
| vs | | | | | | |
| Autumn | 4 | 9.65 | 3.93 | 5.9-15.0 | | |
| Summer | 15 | 50.73 | 69.82 | 13.2-236.1 | -1.17 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 71.95 | 72.19 | 31.3-180.1 | | |
| Summer | 15 | 50.73 | 69.82 | 13.2-236.1 | 0.67 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 22.28 | 11.22 | 6.0-37.1 | | |
| Autumn | 4 | 9.65 | 3.93 | 5.9-15.0 | -3.91 | <0.05 |
| vs | | | | | | |
| Winter | 4 | 71.95 | 72.19 | 31.3-180.1 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Autumn | 4 | 9.65 | 3.93 | 5.9-15.0 | -0.81 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 22.28 | 11.22 | 6.0-37.1 | | |
| Winter | 4 | 71.95 | 72.19 | 31.3-180.1 | 1.05 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 22.28 | 11.22 | 6.0-37.1 | | |

Table 6.15: Results of Student's t-test for seasonal differences in mean crab gill zinc concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal zinc concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 10.23 | 14.03 | 2.0-56.3 | -1.07 | >0.05 |
| vs | | | | | | |
| Autumn | 3 | 127.96 | 98.65 | 3.6-159.3 | | |
| Summer | 15 | 10.23 | 14.03 | 2.0-56.3 | -4.88 | <0.05 |
| vs | | | | | | |
| Winter | 3 | 55.84 | 89.57 | 42.0-235.6 | | |
| Summer | 15 | 10.23 | 14.03 | 2.0-56.3 | -1.34 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 15.21 | 13.49 | 5.2-38.5 | | |
| Autumn | 3 | 127.96 | 98.65 | 3.6-159.3 | -1.51 | >0.05 |
| vs | | | | | | |
| Winter | 3 | 55.84 | 89.57 | 42.0-235.6 | | |
| Autumn | 3 | 127.96 | 98.65 | 3.6-159.3 | 0.17 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 15.21 | 13.49 | 5.2-38.5 | | |
| Winter | 3 | 55.84 | 89.57 | 42.0-235.6 | 3.67 | <0.05 |
| vs | | | | | | |
| Spring | 5 | 15.21 | 13.49 | 5.2-38.5 | | |

Table 6.16: Results of Student's t-test for the seasonal differences in mean crab digestive gland zinc concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of digestive gland zinc concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 17.53 | 23.62 | 3.5-93.7 | -0.51 | >0.05 |
| vs | | | | | | |
| Autumn | 5 | 30.47 | 46.1 | 3.7-112.3 | | |
| Summer | 15 | 17.53 | 23.62 | 3.5-93.7 | -2.57 | <0.05 |
| vs | | | | | | |
| Winter | 6 | 37.02 | 25.62 | 17.8-79.5 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Summer | 15 | 17.53 | 23.62 | 3.5-93.7 | 0.27 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 18.27 | 21.73 | 1.2-64.8 | | |
| Autumn | 5 | 30.47 | 46.1 | 3.7-112.3 | -1.31 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 37.02 | 25.62 | 17.8-79.5 | | |
| Autumn | 5 | 30.47 | 46.1 | 3.7-112.3 | 0.51 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 18.27 | 21.73 | 1.2-64.8 | | |
| Winter | 6 | 37.02 | 25.62 | 17.8-79.8 | 1.95 | <0.05 |
| vs | | | | | | |
| Spring | 7 | 18.27 | 21.73 | 1.2-64.8 | | |

Table 6.17: Results of Student's t-test for the seasonal differences in mean crab gonad zinc concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal gonad zinc concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 13 | 73.8 | 171.62 | 4.8-639.7 | 0.14 | >0.05 |
| vs | | | | | | |
| Autumn | 4 | 56.66 | 85.67 | 5.8-184.2 | | |
| Summer | 13 | 73.8 | 171.62 | 4.8-639.7 | -1.2 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 120.93 | 118.97 | 10.9-241.6 | | |
| Summer | 13 | 73.8 | 171.62 | 4.8-639.7 | 0.97 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 22.7 | 22.5 | 1.8-49.3 | | |
| Autumn | 4 | 56.66 | 85.67 | 5.8-184.2 | -0.94 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 120.93 | 118.97 | 10.9-241.6 | | |
| Autumn | 4 | 56.66 | 85.67 | 5.8-184.2 | 0.57 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 22.7 | 22.5 | 1.8-49.3 | | |
| Winter | 4 | 120.93 | 118.97 | 10.9-241.6 | 1.54 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 22.7 | 22.5 | 1.8-49.3 | | |

Table 6.18: Results of the Mann-Whitney test for differences in whole crab ($\mu g.g^{-1}$ wet mass) and water zinc concentrations ($mg.l^{-1}$) at each locality: Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_W$) calculated for whole crabs (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water (J) | 8 | 0.23 | 0.33 | 0-0.88 | 4.004 | <0.05 | 390.68 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 85.56 | 64.83 | 30.7-432.6 | | | |
| Water (S) | 8 | 0.23 | 0.3 | 0-0.82 | 3.965 | <0.05 | 498.63 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 112.69 | 155.88 | 10.4-690.6 | | | |

Table 6.19: Results of the Mann-Whitney test for differences in crab tissue, carapace ($\mu g.g^{-1}$ wet mass) and water zinc concentrations ($mg.l^{-1}$) at each locality, as well as the bioconcentration factor ($BCF_W$) calculated for all tissues and the carapace (Dig.gland = digestive gland; z = z-value, p = p-value).

| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | z | p | $BCF_W$ |
| **Water** | 8 | 0.219 | 0.325 | 0-0.875 | | | |
| vs Carapace | 19 | 64.63 | 52.81 | 0-222.68 | 3.503 | <0.05 | 295.11 |
| vs Muscle | 16 | 33.55 | 54.69 | 5.9-236.1 | 3.503 | <0.05 | 153.2 |
| vs Dig.gland | 17 | 22.67 | 23.28 | 3.5-79.5 | 3.537 | <0.05 | 103.52 |
| vs Gills | 14 | 8.52 | 9.36 | 2.0-38.5 | 3.424 | <0.05 | 38.9 |
| vs Gonads | 14 | 36.24 | 46.49 | 4.8-184.2 | 3.424 | <0.05 | 165.48 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | z | p | $BCF_W$ |
| **Water** | 8 | 0.226 | 0.295 | 0-0.818 | | | |
| vs Carapace | 20 | 91.76 | 117.82 | 5.7-517.9 | 3.621 | <0.05 | 406.02 |
| vs Muscle | 13 | 63.87 | 72.22 | 6.0-196.8 | 3.377 | <0.05 | 282.61 |
| vs Dig.gland | 15 | 31.84 | 38.98 | 1.2-112.3 | 3.464 | <0.05 | 140.88 |
| vs Gills | 12 | 56.54 | 73.93 | 3.5-235.6 | 3.325 | <0.05 | 250.18 |
| vs Gonads | 11 | 113.93 | 193.48 | 1.8-639.7 | 3.266 | <0.05 | 504.12 |

Table 6.20: Results of Student's t-test for the differences in zinc concentrations ($\mu g.g^{-1}$ wet mass) of whole crabs and sediments from the two localities, Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for whole crabs (Mean logarithms of zinc concentrations used to calculate the t-value; t = t-value, p = p-value).

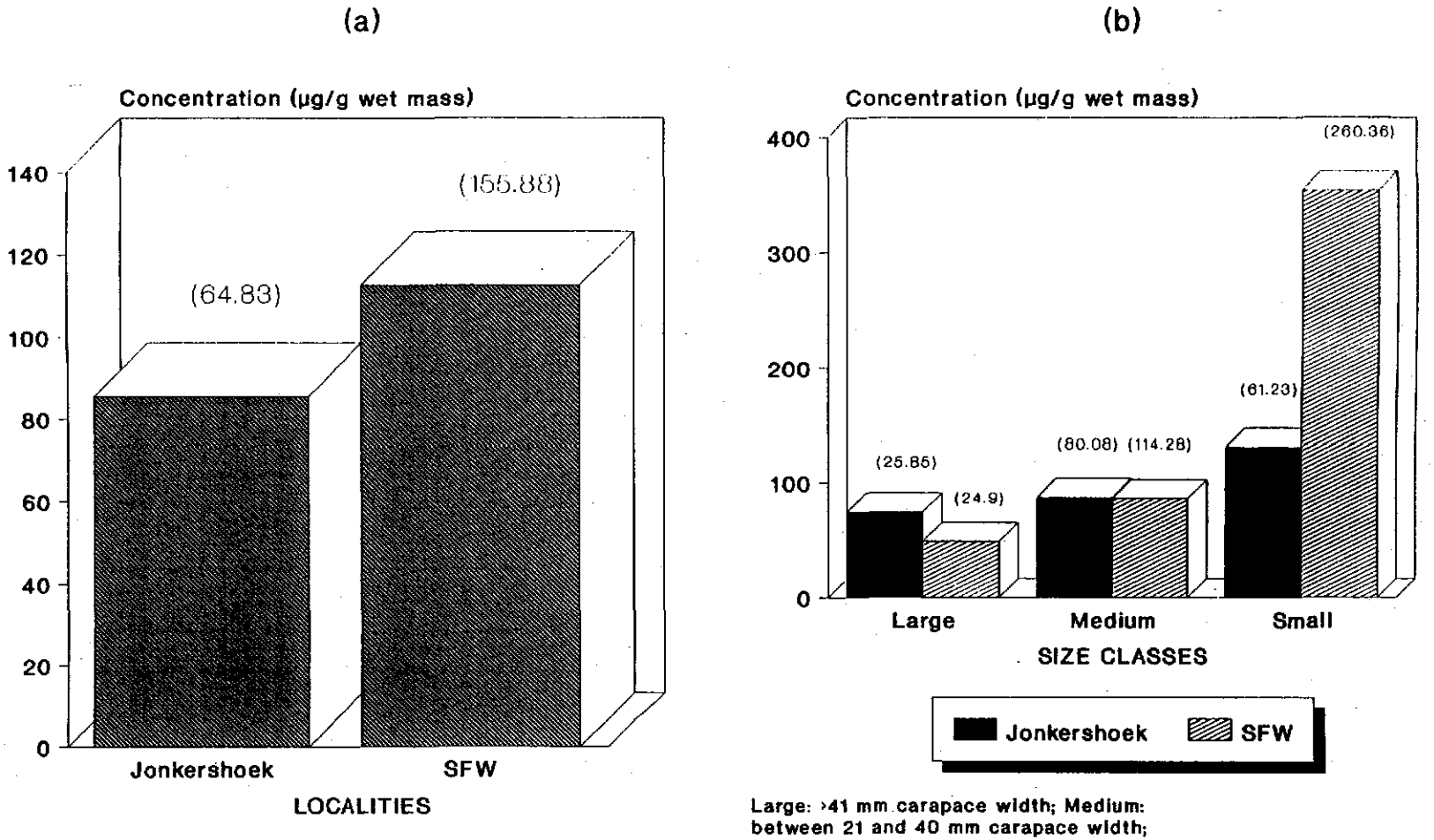
| | n | Mean | SD | Range | t | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 38.21 | 28.05 | 8.55-89.12 | -4.2 | <0.05 | 2.24 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 85.56 | 64.83 | 30.7-432.6 | | | |
| Sediment (S) | 8 | 66.25 | 32.82 | 27.34-102.54 | 0.01 | >0.05 | 1.7 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 112.69 | 155.88 | 104-690.6 | | | |

Table 6.21: Results of Student's t-test for the differences in zinc concentrations ($\mu g.g^{-1}$ wet mass) of the sediments and crab tissues at each locality: Jonkershoek and SFW, as well as the bioconcentration factor ($BCF_S$) calculated for all tissues (Dig.gland = digestive gland; mean logarithms of manganese concentrations used to calculate the t-value; t = t-value, p = p-value).

| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 38.21 | 28.05 | 8.55-89.12 | | | |
| vs Muscle | 16 | 33.55 | 54.69 | 5.9-236.1 | 1.02 | >0.05 | 0.89 |
| vs Dig.gland | 17 | 22.67 | 31.84 | 3.5-79.5 | 1.86 | <0.05 | 0.59 |
| vs Gills | 14 | 8.52 | 9.36 | 2.0-38.5 | 4.59 | <0.05 | 0.22 |
| vs Gonads | 14 | 36.24 | 46.49 | 4.8-184.2 | 0.86 | >0.05 | 0.95 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 66.25 | 32.82 | 27.34-102.54 | | | |
| vs Muscle | 13 | 63.87 | 72.22 | 6.0-196.8 | -2.41 | <0.05 | 0.96 |
| vs Dig.gland | 15 | 23.28 | 38.98 | 1.2-112.3 | -0.08 | >0.05 | 0.35 |
| vs Gills | 12 | 56.54 | 73.93 | 3.5-235.6 | 1.66 | >0.05 | 0.85 |
| vs Gonads | 11 | 113.93 | 193.48 | 1.8-639.7 | -1.57 | >0.05 | 1.72 |

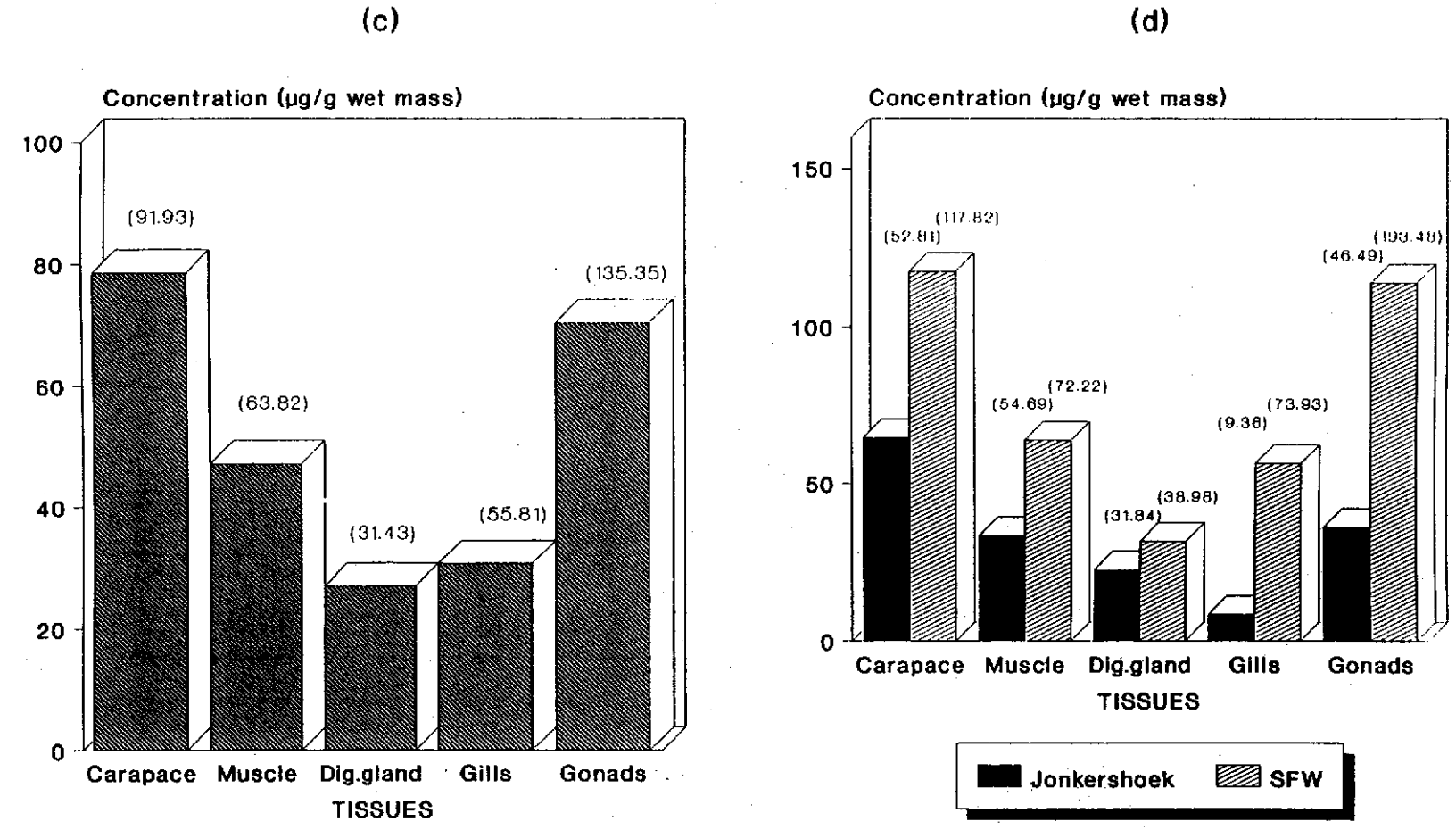
Table 6.22: Results of the Mann-Whitney test for differences in zinc concentrations ($\mu g.g^{-1}$ wet mass) of the crab carapace and sediment from Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for the carapace (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 38.21 | 28.05 | 8.55-89.12 | 0.923 | >0.05 | 1.69 |
| vs | | | | | | | |
| Carapace (J) | 19 | 64.63 | 52.81 | 0-222.7 | | | |
| Sediment (S) | 8 | 66.25 | 32.82 | 27.34-102.54 | -0.152 | >0.05 | 1.39 |
| vs | | | | | | | |
| Carapace (S) | 20 | 91.78 | 117.82 | 5.7-577.9 | | | |



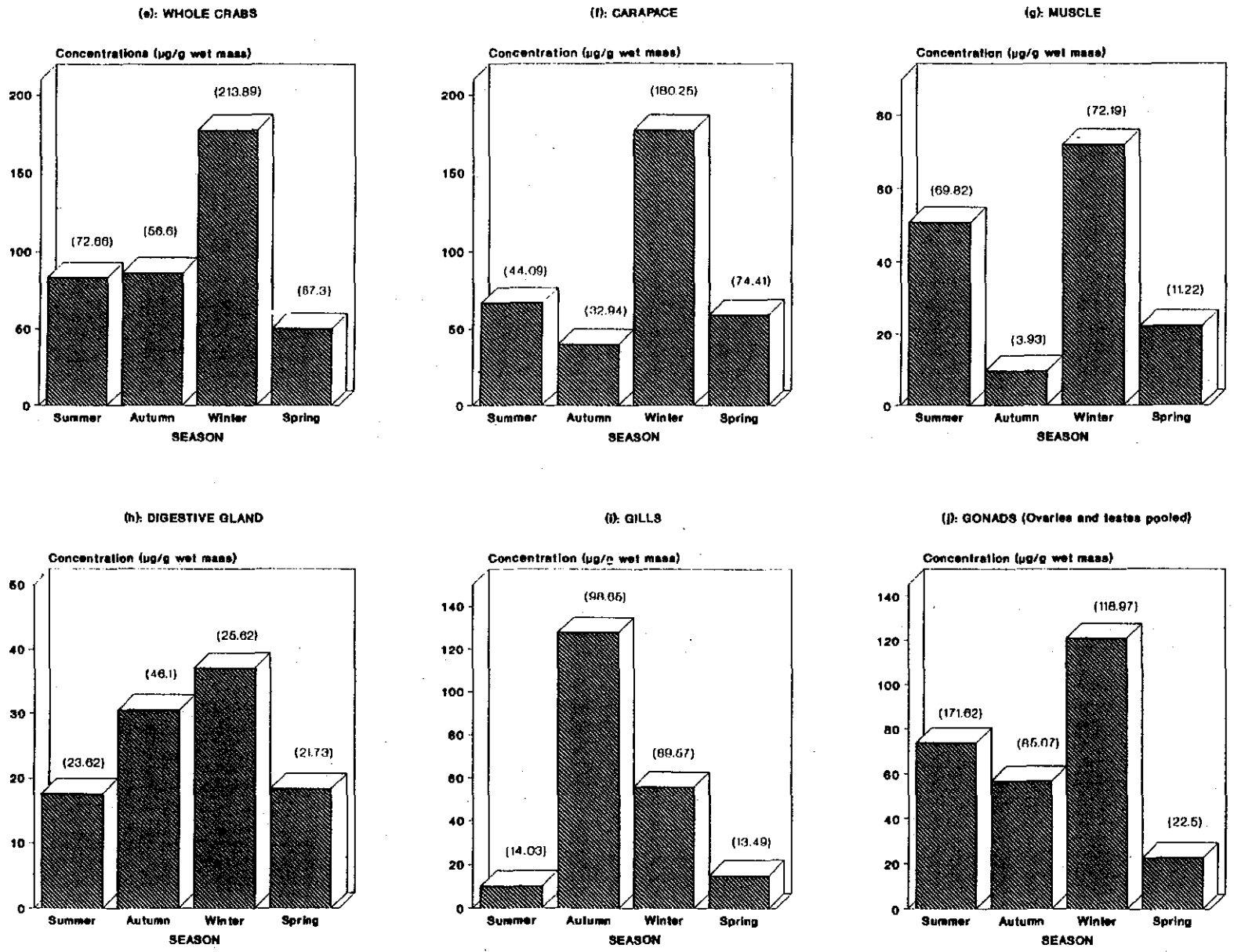

Large: >41 mm carapace width; Medium: between 21 and 40 mm carapace width; Small: <20 mm carapace width

Figures 6(a)-(b): Mean zinc concentration in whole crabs (a) and various size classes (b) from both localities (SD in parenthesis).




Jonkershoek and SFW data pooled

Figures 6(c)-(d): Mean zinc concentration in carapace and tissues of P. *perlatus* from the Eerste River (SD in parenthesis).




Figures 6(e)-(j): Mean seasonal zinc concentration in whole crabs, carapace and tissues of *P. perlatus* (Jonkershoek and SFW data pooled; SD in parenthesis).

## Discussion

The mean wet mass zinc concentration found in each of the selected tissues and the carapace of *P. perlatus* (Table 6.3; Figure 6(c)) was compared with the results of Steenkamp (1992) and du Preez et al. (1993) for *P. warreni* from polluted freshwater ecosystems, as well as with those of Bryan (1968) for various uncontaminated marine and freshwater decapods. Steenkamp (1992) found mean Zn concentrations of 31.2±15.5 to 80.5±263.1 $\mu g.g^{-1}$ wet digestive gland mass, 23.8±12.6 to 67.5±275.6 $\mu g.g^{-1}$ wet gill mass, 63.1±17.8 to 101.7±256.7 $\mu g.g^{-1}$ wet muscle mass, 30.2±10.4 to 120.8±366.5 $\mu g.g^{-1}$ wet gonad mass and 15.2±9.3 to 51.7±276.3 $\mu g.g^{-1}$ wet carapace mass. The mean zinc concentrations in the gonads and carapace of *P. warreni*, found by du Preez et al. (1993), were very similar, namely 112.9±413.9 and 16.0±5.1 $\mu g.g^{-1}$ wet mass respectively. While the mean zinc concentrations in the digestive gland and muscles of *P. perlatus*, in comparison with the above mentioned results, were relatively low and those in the carapace relatively high, the concentrations in the gills and gonads compared well. In comparison with the results of Bryan (1968), namely mean wet mass Zn concentrations of 15 to 66 $\mu g.g^{-1}$ for muscle tissue, 24 to 169 $\mu g.g^{-1}$ for the digestive gland, 8 to 69 $\mu g.g^{-1}$ for gill tissue, 3 to 28 $\mu g.g^{-1}$ for the "shell" and 13 to 87 $\mu g.g^{-1}$ for the gonads, the mean Zn concentration in the carapace of *P. perlatus* was found to be very high. The concentrations in the digestive gland, muscles, gills and gonads, however, compared favourably.

When comparing the mean dry mass zinc concentration of whole individuals of *Potamonautes perlatus* from Jonkershoek (28.93±25.04 $\mu g.g^{-1}$) and SFW (27.69±37.68 $\mu g.g^{-1}$) with the results of Van Eeden & Schoonbee (1991) for *P. warreni* (73.3±0.03 to 138.3±0.01 $\mu g.g^{-1}$), it was clear that the zinc concentrations in *P. perlatus* were comparatively low.

Despite the fairly high concentrations observed in some of the tissues of *P. perlatus*, it seems that Zn is generally well regulated in this species in the Eerste River.

Comparisons of the two localities, Jonkershoek and SFW, showed that, although crabs from SFW had higher tissue zinc concentrations than those from Jonkershoek (Tables 6.7-6.8), and although large- and medium-sized crabs from Jonkershoek in turn had higher whole body zinc concentrations than those from SFW (Table 6.6), no statistically significant differences in whole crab zinc concentrations existed overall between the two populations (Table 6.5). The water at both localities can certainly be considered as a source of zinc, as indicated by the $BCF_W$'s for whole crabs, tissues and carapace (Tables 6.18 and 6.19), but since it was found that there was no difference in water zinc concentrations between Jonkershoek and SFW (Table 4.5), the reason for the observed differences between the Jonkershoek and SFW populations need to be searched for elsewhere: bioaccumulation from the sediments also fail to fully explain the observed tissue and size class population differences, since, though the sediment from SFW was found to contain significantly more zinc than sediment from Jonkershoek (Table 4.11), the crabs

from the latter had significantly higher zinc concentrations than the sediments and a larger $BCF_S$ for whole crabs (Table 6.20). Another possible source of zinc is of course food, but the most likely reason for these differences can possibly be found in the differences in water physico-chemistry between the two localities (Table 4.8), affecting the bioavailability of the metal to the animals.

It was established that, in *P. perlatus*, most zinc was stored in the carapace, with the gonads being the second most important storage site (Tables 6.3 and 6.4). The higher $BCF_W$'s and $BCF_S$'s for the carapace from Jonkershoek and the gonads from SFW (Tables 6.19 and 6.21) confirm these findings. These results are, however, in contrast to those of Steenkamp (1992) and du Preez et al. (1993), who found that the carapace of *P. warreni* contained the lowest zinc concentration of the sites and tissue types they tested. Bryan (1968) also found relatively low zinc concentrations (3 to 28 $\mu g.g^{-1}$ wet mass) in the "shells" of various decapods, compared to their tissues. Rainbow (1985), however, contradicted Bryan's findings by showing that *Carcinus maenas* had a mean "cuticle" Zn concentration of 76.1±10.8 $\mu g.g^{-1}$ dry mass, which is far greater than the concentrations found by Bryan.

Bryan (1968) reported that much of the Zn in the "shell" appears to be absorbed from the external environment. The large variations in individual carapace zinc concentrations may be a result of the stage in the moult cycle (Nugegoda & Rainbow, 1987) or may be residual zinc which remains when the "shell" is renewed during moulting (Bryan, 1968). White & Rainbow (1984) exposed the shrimp *Palaemon elegans* to zinc and found that the animals that moulted during the experiment took up greater amounts of labelled zinc than those that were not moulting. All these factors, if applicable, could have contributed to the high mean carapace concentration found in *P. perlatus*, as well as the great individual variations observed.

The digestive gland and gills are the store for excess zinc absorbed from food or from the surrounding water, and the main site of absorption and loss of zinc across the body surface, respectively (Bryan, 1968). The zinc concentrations in these tissues are therefore subject to environmental fluctuations and feeding status and can thus be expected to vary greatly between individuals, as indicated in Table 6.3. Bryan (1968) also reported that internal organs such as the muscles and gonads are provided with a controlled environment, since the concentrations of zinc in the blood is regulated, but that when changes in the blood zinc concentrations do occur, these organs may themselves be able to control internal homeostasis by regulating against these changes. Although the mean zinc concentrations in the muscles and gonads of *P. perlatus* were observed to be the second highest and highest of the four tissues respectively (Table 6.3), the present results still seem to support the conclusions of Bryan (1968), since, as mentioned before, the concentrations in these two tissues were found to be relatively low to average, compared to the results of other authors.

Individual variation in zinc concentrations is clearly large in most species of decapod crustaceans, as illustrated by Bryan (1968) and this complicates comparisons between species and individuals. However, despite these variations, the preliminary results from the present study show that the carapace seems to be of greater importance in the storage of zinc in *P. perlatus* than the four tissue types studied, and that these do not differ significantly in their contribution to zinc storage.

The present study also investigated the possible relationship between body size and zinc concentrations. A reasonably strong negative correlation between crab size and whole crab zinc concentrations ($r = -0.488$), as well as the strong and reasonably strong negative correlations between crab size, tissue (except gill) and carapace concentrations (Table 6.2), confirmed that smaller (immature) individuals are subjected to greater Zn loads. This is also confirmed by the statistically significant differences in zinc concentrations between large and small crabs within each locality (Table 6.1). Steenkamp (1992) found that smaller individuals of *P. warreni* accumulated zinc to a higher degree in two tissues, the carapace and the gills. Upon exposing a number of ephemeropteran and one plecopteran species to zinc, Kiffney & Clements (1996) also observed an inverse relationship between body size and survivorship. These results are in contrast to those of Hilmy et al. (1988), who exposed *Portunus pelagicus* to zinc and found that the accumulation of the toxicant in mature (therefore larger) individuals, was significantly greater than in immature animals.

Hill & O'Keeffe (1992) studied the feeding ecology of *P. perlatus* and found a statistically significant difference in food preferences between large and small crabs. While smaller individuals preyed significantly more on aquatic invertebrates, larger individuals preferred vegetable matter. This is a significant finding and could explain the present results when considering the fact that heavy metals are accumulated in aquatic invertebrates (e.g. insects). Albers & Camardese (1993) and Kiffney & Clements (1993) showed that aquatic invertebrates accumulate heavy metals to high levels. The heavy metals can possibly, in turn, be accumulated in the small individuals of *P. perlatus* who feed on these invertebrates. Also, Gherardi et al. (1987) and Gherardi & Micheli (1989) found that younger individuals of *Potamon fluviatile* and *P. potamios palestinensis*, respectively, tend to hide more under stones and rocks, especially during the night. This type of behaviour could prolong smaller individuals' exposure time to a certain metal concentration, in this particular microhabitat, resulting in the observed elevated zinc concentrations.

The possible influence of factors such as seasonality and gender on the levels of Zn in *P. perlatus* was also investigated. Bryan (1964), Hilmy et al. (1988) and Nugegoda & Rainbow (1989) found no significant difference between zinc concentrations in the tissues and carapace of male and female decapods. The only exception was the significant difference between the mature gonads, found by Bryan (1964) and Hilmy et al. (1988). This pattern was also observed

by Bryan (1968) in most of the decapods studied. The results of the present study are in agreement with these findings, except that the difference in zinc concentrations in the ovaries and testes of *P. perlatus* was not significant (Tables 6.9-6.11). Steenkamp (1992) also found no such significant difference between the ovaries and testes of *P. warreni* but did, however, detect differences between the zinc concentrations of the digestive gland, gills and muscles of males and females from one of the sampling localities. She concluded that this could not be attributed to variation between males and females alone but rather to possible sampling variation.

In general, there seems to be no clear relationship between gender and body zinc concentrations in *P. perlatus*, and gender can therefore not be considered an influencing factor in Zn uptake and distribution in this species.

When the possible relationship between seasonality and zinc concentrations in *P. perlatus* was investigated (Tables 6.13-6.17), it was established that there were no significant seasonal differences for gonad concentrations, whereas the muscle tissue, gills and digestive gland showed a number of significant seasonal differences. Only the carapace exhibited significant differences between almost all seasons. It is important to note that even though certain tissues and the carapace exhibited significant seasonal differences in zinc concentrations, there were no seasonal differences in whole body zinc concentrations (Table 6.12). Peaks in whole crab, tissue and carapace zinc concentrations in winter, and in gill concentrations in autumn, although not significant in all cases, were observed (Figures 6(e)-(j)). The marked seasonal variations in carapace zinc agrees with the findings of Steenkamp (1992), although the peaks differ. Whilst *Potamonautes perlatus* showed peaks in winter, *P. warreni* exhibited a summer peak.

The observed peaks in whole crab, tissue and carapace concentrations in the colder months can not be explained by seasonal variations in the water and sediment zinc concentrations, since no corresponding peaks were observed (Figures 4(c)-(l)). However, Warburg et al. (1982) showed that the crab *Potamon potamios* preferred the thermal zone between 24° and 27°C and that its activity dropped below and above these temperatures. Similarly, *Potamonautes perlatus*' activity is also affected by environmental temperatures. This species has been observed to be relatively inactive in winter (personal observations and marked decrease in capture rate) and tend to retreat into burrows or under rocks and stones, in order to avoid the force of the water flow. The exposure time to a specific zinc concentration is therefore possibly lengthened. The decreased activity implies a lowered metabolic rate which would mean that metal loads are not disposed of as effectively as during normal activity.

Engel & Brouwer (1987) found that in the blue crab, *Callinectes sapidus*, zinc metallothioneins in the digestive gland is at its highest during the pre-moult period, when the new epidermis is being synthesized beneath the existing exoskeleton, but is possibly mobilized via the haemolymph at moult. The carapace and digestive gland zinc concentrations are therefore clearly

coupled with the moult cycle. Steenkamp (1992) reported for *Potamonautes warreni* and Gherardi et al. (1987) for *Potamon fluviatile* that moulting takes place in the warmer months and in summer/autumn respectively, while Raübenheimer (1986) reported gender-related moulting peaks for *Potamonautes sidneyii*: males moulted in autumn and winter, and females in autumn, summer and spring, depending on their age and therefore reproductive status. Although it is not certain when *P. perlatus* moults in the Eerste River, and though it seems certain that moulting is not synchronized in all individuals (clearly shown by the great individual variations), it is possible that a number of individuals in the two populations (Jonkershoek and SFW) moulted during winter, with a corresponding peak in mean carapace zinc concentration. The peak in digestive gland zinc concentration during winter supports this theory.

Apart from its relationship with the moulting cycle, the seasonal variations in concentration values for the digestive gland (Table 6.16) can also be ascribed to the relative inactivity of the animals during winter, as a result of the high rainfall (Table 2.1) and therefore fast flow of the river, which would certainly necessitate changes in the diets of the animals. Similarly, the seasonal variations in gill zinc concentrations, with peaks in the colder months (Table 6.15), may possibly also be explained by the fact that *P. perlatus* is relatively inactive during winter and may possibly be exposed to specific zinc concentrations for longer periods.

Also, Bryan (1968) found large differences in zinc concentrations between samples of muscle from different species. These differences seemed to correlate with the degree of activity of the species. He found the lowest and highest concentrations of zinc in leg muscles of the swimming crab *Portunus depurator* and the slow-moving *Maia squinado* and *Cancer pagurus* respectively, and therefore concluded that the results suggested that zinc concentrations in muscle tissue may be related to the speed with which it can contract. Since *P. perlatus* is physically less active in winter, Bryan's results give a possible explanation for the observed winter peak in muscle zinc concentration. The fact that no seasonal differences in gonad zinc concentrations were observed, and that these concentrations remained relatively constant throughout all seasons, supports the conclusions of Bryan (1968) on the concentrations of zinc in the internal organs such as muscles and gonads, as mentioned previously.

Although a few seasonal variations in tissue and carapace zinc concentrations were observed, the whole body concentrations remained relatively constant throughout the seasons, implying an ability of *P. perlatus* to regulate the body zinc concentrations and suggesting that seasonality did not have a major influence on the concentrations of zinc in this species.

Finally, the fact that *P. perlatus* in the Eerste River was shown to regulate Zn in its body, as well as the fact that seasonal fluctuations in environmental Zn concentrations were not reflected in the crab body, imply that this species in the Eerste River does not qualify as a potential monitor of environmental Zn pollution. Also, the fact that the carapace was shown to contain the highest Zn

concentrations and that periodic moulting is probably a mechanism in which the animal rids itself of excess Zn and therefore protects itself against long term accumulation, as well as the observed large intraspecific variations in Zn concentrations, provide further proof that this species would probably not be a suitable monitor of environmental Zn pollution. Steenkamp (1992) also concluded that there is no certainty that Zn concentrations in the tissues of *P. warreni* would accurately reflect environmental Zn contamination and that of all the tissues tested, only the digestive gland proved to be useful as indicator of the degree of Zn pollution. However, more frequent observations on *P. perlatus* are needed before final conclusions can be made.

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# CHAPTER 7

# CONCENTRATIONS OF COPPER IN THE FRESHWATER CRAB, *POTAMONAUTES PERLATUS*

## Introduction

Copper is widely distributed in nature and is found either in the element form or in several compounds, together with hard bases such as carbonate, nitrate, sulfate, chloride, ammonia and hydroxide (Moore & Ramamoorthy, 1984 and Galvin, 1996). In aquatic environments copper can exist in three broad categories: particulate, colloidal and soluble. Speciation of Cu in natural waters is determined by the physico-chemical, hydro-dynamic characteristics and the biological state of the water (Moore & Ramamoorthy, 1984).

Copper is an essential trace element in animals, since it is contained in many proteins, such as oxygen-binding haemocyanin, cytochrome oxidase, tyrosinase and laccase (Piscator, 1979a; Moore & Ramamoorthy, 1984 and Galvin, 1996). Piscator (1979) reported that copper deficiency may occur when the intake of molybdenum is excessive.

This metal poses a threat to aquatic ecosystems, as it can reach water bodies via industrial and agricultural runoff: the largest single user of copper is the electrical industry, accounting for ≥50% of the total consumption (Piscator, 1979a; Moore & Ramamoorthy, 1984 and Richardson & Gangolli, 1993). Use under this category includes power transmission, electronics and electrical equipment. Construction and plumbing is the second largest user (Moore & Ramamoorthy, 1984). Other uses include contribution to alloys, together with other metals such as silver, cadmium, tin and zinc (Piscator, 1979a) and in agriculture as fungicides and insecticides (Richardson & Gangolli, 1993).

High levels of copper is highly toxic to aquatic animals, especially freshwater invertebrates. Through animal experiments, it has been shown that exposure to copper may cause a wide variety of symptoms, including e.g. a loss of cellular adhesion in the gills, cell necrosis, retarded growth and a lowered rate of reproduction and egg survival. Behavioural changes such as decreased degree of concealment and ability to orientate may also occur (Moore & Ramamoorthy, 1984).

Several factors are known to influence copper availability and toxicity to aquatic animals: toxicity of copper is generally greater in freshwater than in marine waters, reflecting the relative proportion of the toxic free Cu ion in solution. A lowered pH (Stouthart et al., 1996), soft water and salinity can often increase copper toxicity, especially to invertebrates. Also, sensitivity to copper generally increases with decreasing age/size (Moore & Ramamoorthy, 1984).

Owen (1982) states that copper concentration and toxicity in animals may be affected by various other metals: cadmium, lead, manganese and zinc, e.g., are all, generally speaking, antagonistic to copper. The toxicity of copper to decapods is also decreased by Cu binding proteins (metallothioneins) in the digestive glands of these animals (Rainbow & Scott, 1979 and Engel & Brouwer, 1987).

Several authors have reported on the accumulation of copper in freshwater decapod crustaceans: the crayfish species *Austropotamobius pallipes* and *Orconectes virilis* have been studied by Bryan (1968), Anderson & Brower (1978) and France (1987). Two crab species have also been thoroughly researched, namely *Barytelphusa guerini* (Tulasi & Ramana Rao, 1988) and *Potamonautes warreni* (Van Eeden & Schoonbee, 1991 and Steenkamp et al., 1994a).

## Materials and Methods

All crab samples were prepared, tested and statistically analysed according to the method described in Chapter 3.

## Results

In all cases only the copper concentrations per gram wet mass were used for statistical analysis. Crabs showed large individual variation in whole body, tissue as well as carapace copper concentrations. This was an important consideration in the interpretation of the statistical results.

### The relationship between crab size and whole crab copper concentration

In order to investigate the possible relationship between whole crab copper concentrations and the sizes of the crabs, the data from Jonkershoek and SFW were pooled. The carapace widths of the crabs, in decreasing order, were compared with the respective copper concentrations and the correlation coefficient (r) thereof, calculated. With 53 degrees of freedom and an $r^2$ of 33.61%, an r-value of -0.58 was obtained. This indicates a reasonably strong negative correlation between size of crab and the concentrations of copper in the body: as size decreased, so did the copper concentrations increase (Figure 7(b)).

Differences in copper concentrations of the various size classes at each locality

Since the logarithmically transformed whole crab data for large, medium and small-sized crabs from each locality also failed to provide normal distributions, the nonparametric Mann-Whitney test was performed to test for statistically significant differences ($p<0.05$) (Table 7.1).

Differences in concentrations were found between all size classes from Jonkershoek. The small size class crabs showed the highest mean copper concentration (35.61±17.38 $\mu g.g^{-1}$ wet mass) and large sized crabs the lowest (11.68±10.34 $\mu g.g^{-1}$ wet mass).

Significant differences were found between the copper concentrations of large and small and between medium and small size classes from SFW, with the small and large-sized crabs also exhibiting highest and lowest mean concentrations respectively (73.36±63.26 and 13.54±4.23 $\mu g.g^{-1}$ wet mass).

The relationship between crab size, and tissue and carapace copper concentration

In order to investigate the possible relationship between crab size and copper concentration in the carapace and selected tissues, namely muscle tissue, digestive gland, gills and gonads, the data from Jonkershoek and SFW were pooled, after which the carapace widths, in decreasing order, were compared with the respective tissue and carapace copper concentration. Table 7.2 shows the r-values calculated for each tissue and for the carapace.

Reasonably strong negative correlations were found to exist between crab sizes and the copper concentrations in the muscles, digestive gland and carapace, whereas the copper concentrations in the gills and gonads proved to be poorly negatively correlated with the sizes of the crabs.

Differences in copper concentrations between the various selected tissues and the carapace

The mean copper concentration values from the selected tissues and carapace of crabs from Jonkershoek and SFW were pooled, in order to determine whether statistically significant differences ($p<0.05$) in copper concentrations existed between the various tissues and the carapace. Since the data were not normally distributed, the mean logarithms of the tissue and carapace copper concentrations were used in the t-test. The respective mean concentrations and standard deviations are tabulated in Table 7.3. Table 7.4 shows the results of the t-test performed on the tissue and carapace concentrations.

When comparing values obtained for the selected tissues and the carapace, significant differences were found between the mean copper concentration of the carapace and all tissues, except between muscle and gonads, muscle and gills, muscle and gonads, and gonads and gills.

The digestive gland showed the highest mean concentration (29.95±21.42 $\mu g.g^{-1}$ wet mass), whereas the lowest mean was found for the carapace (6.78±9.12 $\mu g.g^{-1}$ wet mass) (Figure 7(c)).

Differences in copper concentrations of crabs from Jonkershoek and SFW

(a) Differences in whole crab values

The results of the Mann-Whitney test performed on the whole crab copper concentrations from Jonkershoek and SFW are shown in Table 7.5.

The highest mean copper concentration (27.6±31.19 $\mu g.g^{-1}$ wet mass) was found in crabs from SFW (Figure 7(a)). A z-value of 3.056 indicated that there was a statistically significant difference in whole crab copper concentrations between crabs from the two localities.

(a) (i) Differences in whole crab concentrations between the various size classes

Through a comparison of the whole crab copper concentration values between large, medium and small crabs from Jonkershoek and SFW, with the use of the Mann-Whitney test, it was established that no significant differences ($p>0.05$) existed between copper concentrations in any of the size classes from the two localities.

(b) Differences in copper concentrations of the selected tissues and the carapace

The mean copper concentration in the carapace, muscle, digestive gland, gills and gonads of crabs from Jonkershoek and SFW were compared with the use of Student's t-test and tabulated in Table 7.7. Since the data were not normally distributed, the mean logarithms of the copper concentrations in the tissues and carapace were used to calculate the t-value. In the case of the gills, however, a normal distribution could not be obtained even after transformation of the concentration value data, therefore the Mann-Whitney test was performed on the original data, in order to investigate possible differences (Table 7.8).

Although the mean copper concentrations observed in all four tissues were highest in crabs collected from SFW (Figure 7(d)), no statistically significant differences ($p>0.05$) could be found between the two localities.

Differences in copper concentrations of males and females

In order to determine whether statistically significant differences ($p<0.05$) existed in the mean whole crab, tissue and carapace copper concentrations between males and females, the data obtained for crabs from Jonkershoek and SFW, were pooled for each sex. In all cases the original data failed the normal probability test, therefore each concentration value was logarithmically transformed. In the case of the whole crab concentrations the transformed data

also failed to provide a normal distribution, therefore the Mann-Whitney nonparametric test was performed.

(a) Differences in whole crab concentrations

Table 7.9 shows the results of the Mann-Whitney test, performed on the whole crab copper concentrations of males and females of *Potamonautes perlatus*. A statistically significant difference ($p<0.05$) was found between the two genders; males had the highest mean whole body copper concentration (26.89±30.5b $\mu g.g^{-1}$ wet mass).

(b) Differences in the copper concentrations of the selected tissues and the carapace

Although males exhibited the highest mean copper concentrations for the carapace and all but one of the four tissue types (gonads), no statistically significant differences ($p>0.05$) were found to exist between the two genders (Table 7.10).

Seasonal variations in copper concentrations

The copper concentrations in whole crabs, selected tissues and carapace from the different seasons were tested for statistically significant differences ($P<0.05$). In each instance, the data from crabs collected at Jonkershoek and SFW were pooled and these then tested for normality. None of the data sets were normally distributed and were thus logarithmically transformed. The transformed data for whole crabs, as well as the digestive gland summer and spring data and the gill summer data, also failed the normal probability test, therefore the Mann-Whitney test was performed.

(a) Seasonal differences in whole crab copper concentrations

The Mann-Whitney test produced statistically significant differences ($p<0.05$) in whole crab copper concentrations, between summer and autumn and between autumn and spring (Table 7.11). The highest mean whole crab copper concentration (30.79±23.38 $\mu g.g^{-1}$ wet mass) occurred in autumn, whereas summer showed the lowest mean (16.52±14.02 $\mu g.g^{-1}$ wet mass) (Figure 7(e)).

(b) Seasonal differences in tissue and carapace copper concentrations

1. Carapace

Statistically significant differences ($p<0.05$) in seasonal carapace copper concentrations were found to exist only between summer and spring values. Data for autumn had the highest mean copper concentration (12.45±12.97 $\mu g.g^{-1}$ wet mass) and summer the lowest (4.65±3.45 $\mu g.g^{-1}$ wet mass) (Figure 7(f)). The results of Student's t-test, performed on the mean logarithms of the seasonal copper concentrations, are given in Table 7.12.

2. Muscle tissue

The highest mean muscle copper concentration (54.73±75.63 $\mu g.g^{-1}$ wet mass) was observed in winter, whereas autumn showed the lowest mean concentration (2.59±1.48 $\mu g.g^{-1}$ wet mass) (Figure 7(g)). However, through pairwise comparisons of the different seasons, it was found that no significant differences ($p>0.05$) existed between any of the seasons (Table 7.13).

3. Gill tissue

Statistically significant differences ($p<0.05$) in seasonal gill copper concentrations were found between almost all seasonal comparisons, except between autumn and winter and between autumn and spring. Tables 7.14 and 7.15 show the results of Student's t-test and the Mann-Whitney test respectively, performed on the original data. It can be seen in Figure 7(h) that the highest and lowest mean copper concentrations were found in autumn (50.25±71.11 $\mu g.g^{-1}$ wet mass) and summer (6.16±4.48 $\mu g.g^{-1}$ wet mass) respectively.

4. Digestive gland

The results of the Mann-Whitney test, performed on the original data from samples collected in summer and spring, and Student's t-test, performed on the logarithmically transformed data from autumn and winter, are given in Tables 7.16 and 7.17 respectively. Summer showed the highest mean copper concentration (39.07±23.2 $\mu g.g^{-1}$ wet mass), with the lowest mean concentration found in winter (15.51±9.35 $\mu g.g^{-1}$ wet mass) (Figure 7(i)). However, none of the comparisons between the different seasons yielded statistical differences ($p>0.05$).

5. Gonads

When the data for the ovaries and testes were pooled, the highest and lowest mean copper concentrations (23.3±60.12 and 7.93±9.2 $\mu g.g^{-1}$ wet mass) occurred in summer and autumn respectively (Figure 7(j)). No statistically significant differences ($p>0.05$) were however found to exist between values from any of the seasons (Table 7.18).

Differences in copper concentrations of whole crabs, tissues, carapace, and Eerste River water

(a) Differences between concentration values for whole crabs and water

Since the logarithmically transformed whole crab data from Jonkershoek and SFW were not normally distributed, the Mann-Whitney test was performed on the original data (Table 7.19). It was ascertained that statistically significant differences ($p<0.05$) existed between the whole crab and water copper concentrations from both localities.

(b) Differences between concentration values for the selected tissues, carapace and water

The copper concentrations in all the tissues and the carapace from both localities proved to be significantly different ($p<0.05$) from the concentrations in the water. This was ascertained with the use of the Mann-Whitney test (Table 7.21), performed on the gill and water copper

concentrations from Jonkershoek, and Student's t-test (Table 7.20) performed on all the other data.

Differences in copper concentrations of whole crabs, tissues, carapace, and sediments

(a) Differences between concentration values for whole crabs and sediments
The Mann-Whitney test, performed on the whole crab and sediment copper concentrations, from Jonkershoek and SFW, showed statistically significant differences ($p<0.05$) to exist at both localities. The results of the test are tabulated in Table 7.22.

(b) Differences between concentration values for the selected tissues, carapace and sediments
The mean logarithms of the tissue and carapace copper concentrations were used to test for statistically significant differences ($p<0.05$) between tissue, carapace and sediment copper concentrations at the two localities. The transformed gill concentration values from Jonkershoek and SFW, however, were both not normally distributed, therefore the Mann-Whitney test was performed.

From both the Jonkershoek and SFW data, significant differences in copper concentrations were found only between concentrations for the sediments and the digestive gland. Table 7.23 lists the results of Student's t-test, performed on the mean logarithms of the tissue and carapace copper concentrations from the two localities. The results of the Mann-Whitney test, performed on the original Jonkershoek and SFW gill data, are shown in Table 7.24.

Table 7.1: Results of the Mann-Whitney test for differences in whole crab copper concentrations ($\mu g.g^{-1}$ wet mass) of the various size classes at each locality.

| **JONKERSHOEK** | | | | | | |
|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | z-value | p-value |
| Large | 22 | 11.07 | 10.34 | 5.1-41.8 | 2.491 | <0.05 |
| vs | | | | | | |
| Medium | 34 | 15.26 | 7.88 | 4.64-35.39 | | |
| Large | 22 | 11.07 | 10.34 | 5.1-41.8 | 2.594 | <0.05 |
| vs | | | | | | |
| Small | 4 | 35.61 | 17.38 | 9.59-45.18 | | |
| Medium | 34 | 15.26 | 7.88 | 4.64-35.39 | 1.926 | =0.05 |
| vs | | | | | | |
| Small | 4 | 35.61 | 17.38 | 9.59-45.18 | | |
| **SFW** | | | | | | |
| | n | Mean | SD | Range | z-value | p-value |
| Large | 6 | 13.54 | 4.23 | 8.8-20.71 | 0.718 | >0.05 |
| vs | | | | | | |
| Medium | 39 | 22.72 | 19.1 | 7.48-92.3 | | |
| Large | 6 | 13.54 | 4.23 | 8.8-20.71 | 2.802 | <0.05 |
| vs | | | | | | |
| Small | 6 | 73.36 | 63.26 | 32.69-200.32 | | |
| Medium | 39 | 22.72 | 19.1 | 7.48-92.3 | 3.222 | <0.05 |
| vs | | | | | | |
| Small | 6 | 73.36 | 63.26 | 32.69-200.32 | | |

Table 7.2: $R^2$-values calculated for sizes of crabs and selected tissue and carapace copper concentrations (DF = Degrees of freedom; Dig.gland = digestive gland).

| | DF | r-value | R-sq. (%) |
|---|---|---|---|
| Crab size and Muscle | 27 | -0.571 | 32.59 |
| Crab size and Dig.gland | 29 | -0.466 | 21.69 |
| Crab size and Gills | 24 | -0.257 | 6.61 |
| Crab size and Gonads | 23 | -0.361 | 13.02 |
| Crab size and Carapace | 28 | -0.415 | 17.23 |

Table 7.3: Mean copper concentration ($\mu g.g^{-1}$ wet mass) in the muscles, digestive gland (dig. gland), gills, gonads and carapace of crabs from the Eerste River (Jonkershoek and SFW data pooled).

| | n | Mean | SD | Range |
|---|---|---|---|---|
| Muscle | 29 | 18.1 | 34.26 | 0.0-166.32 |
| Dig. gland | 33 | 29.95 | 21.42 | 1.9-74.1 |
| Gonads | 25 | 18.43 | 43.91 | 1.0-222.2 |
| Gills | 26 | 16.99 | 25.93 | 2.56-132.35 |
| Carapace | 39 | 6.78 | 9.12 | 0.0-44.54 |

Table 7.4: Results of Student's t-test for the differences in mean crab tissue and carapace copper concentration ($\mu g.g^{-1}$ wet mass) (Jonkershoek and SFW data pooled; Mean logarithms of copper concentrations used for the calculation of the t-value).

| | t-value | p-value |
|---|---|---|
| Muscle vs Digestive gland | -4.28 | <0.05 |
| Muscle vs Gonads | -0.46 | >0.05 |
| Muscle vs Gills | -1.56 | >0.05 |
| Muscle vs Carapace | 1.05 | >0.05 |
| Digestive gland vs Gonads | 4.06 | <0.05 |
| Digestive gland vs Gills | 3.21 | <0.05 |
| Digestive gland vs Carapace | 7.61 | <0.05 |
| Gonads vs Gills | -1.16 | >0.05 |
| Gonads vs Carapace | 1.79 | <0.05 |
| Gills vs Carapace | 3.58 | <0.05 |

Table 7.5: Results of the Mann-Whitney test for the differences in whole crab copper concentrations ($\mu g.g^{-1}$ wet mass) from Jonkershoek and SFW.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Jonkershoek | 60 | 15.31 | 11.0 | 6.64-45.18 | 3.056 | <0.05 |
| vs | | | | | | |
| SFW | 51 | 27.6 | 31.19 | 7.48-200.32 | | |

Table 7.6: Results of the Mann-Whitney test for the differences in whole crab copper concentrations ($\mu g.g^{-1}$ wet mass) of the various size classes, small, medium and large, from Jonkershoek (J) and SFW (S).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Large (J) | 22 | 11.68 | 10.34 | 5.1-41.8 | 1.595 | >0.05 |
| vs | | | | | | |
| Large (S) | 6 | 13.54 | 4.23 | 8.8-20.71 | | |
| Medium (J) | 34 | 15.26 | 7.88 | 4.64-35.39 | 1.288 | >0.05 |
| vs | | | | | | |
| Medium (S) | 39 | 22.72 | 19.1 | 4.48-92.3 | | |
| Small (J) | 4 | 35.61 | 17.38 | 9.59-45.18 | 0.533 | >0.05 |
| vs | | | | | | |
| Small (S) | 6 | 73.36 | 63.26 | 32.69-200.32 | | |

Table 7.7: Results of Student's t-test for the differences in mean tissue and carapace copper concentration ($\mu g.g^{-1}$ wet mass) of crabs from Jonkershoek (J) and SFW (S) (Mean logarithms of the tissue and carapace copper concentrations used in the test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (J) | 19 | 6.82 | 8.9 | 0.0-34.57 | -0.33 | >0.05 |
| vs | | | | | | |
| Carapace (S) | 20 | 6.75 | 9.55 | 2.02-44.54 | | |
| Muscle (J) | 16 | 10.24 | 17.26 | 0.0-66.6 | -1.42 | >0.05 |
| vs | | | | | | |
| Muscle (S) | 13 | 27.78 | 46.72 | 0.35-166.32 | | |
| Dig.gland (J) | 18 | 24.62 | 20.95 | 2.3-74.1 | -1.28 | >0.05 |
| vs | | | | | | |
| Dig.gland (S) | 15 | 36.33 | 20.87 | 1.9-68.0 | | |
| Gonads (J) | 11 | 11.53 | 13.61 | 1.0-50.7 | -0.38 | >0.05 |
| vs | | | | | | |
| Gonads (S) | 14 | 27.22 | 65.07 | 1.64-222.2 | | |

Table 7.8: Results of the Mann-Whitney test for the differences in crab gill copper concentrations ($\mu g.g^{-1}$ wet mass) from the two localities, Jonkershoek (J) and SFW.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Gills (J) | 14 | 10.93 | 6.91 | 3.5-19.71 | 0.386 | >0.05 |
| vs | | | | | | |
| Gills (SFW) | 12 | 24.07 | 37.02 | 2.56-132.35 | | |

Table 7.9: Results of the Mann-Whitney test for the differences in whole crab copper concentrations ($\mu g.g^{-1}$ wet mass) of males and females.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Females | 40 | 17.17 | 15.08 | 4.64-60.87 | -2.738 | <0.05 |
| vs | | | | | | |
| Males | 49 | 26.89 | 30.5 | 5.37-200.32 | | |

Table 7.10: Results of Student's t-test for the differences in mean tissue and carapace copper concentration ($\mu g.g^{-1}$ wet mass) of male (m) and female (f) crabs (Mean logarithms of the tissue and carapace copper concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (f) | 15 | 5.61 | 8.26 | 0.0-34.57 | -0.96 | >0.05 |
| vs | | | | | | |
| Carapace (m) | 18 | 8.22 | 10.89 | 1.61-44.54 | | |
| Muscle (f) | 11 | 11.51 | 14.31 | 1.9-42.86 | 0.02 | >0.05 |
| vs | | | | | | |
| Muscle (m) | 13 | 24.76 | 46.93 | 0.0-166.32 | | |
| Dig.gland (f) | 12 | 22.77 | 16.26 | 2.3-50.0 | -0.72 | >0.05 |
| vs | | | | | | |
| Dig.gland (m) | 15 | 33.29 | 24.57 | 1.9-74.1 | | |
| Gills (f) | 11 | 11.58 | 10.31 | 3.5-37.52 | -0.46 | >0.05 |
| vs | | | | | | |
| Gills (m) | 14 | 21.09 | 34.18 | 2.56-132.35 | | |
| Ovaries | 11 | 29.59 | 65.44 | 1.0-50.7 | 0.12 | >0.05 |
| vs | | | | | | |
| Testes | 10 | 11.61 | 9.66 | 1.64-22.9 | | |

Table 7.11: Results of the Mann-Whitney test for the seasonal differences in whole crab copper concentrations ($\mu g.g^{-1}$ wet mass).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 49 | 16.52 | 14.02 | 4.64-60.87 | 2.825 | <0.05 |
| vs | | | | | | |
| Autumn | 16 | 30.79 | 23.38 | 10.05-92.3 | | |
| Summer | 49 | 16.52 | 14.02 | 4.64-60.87 | 0.564 | >0.05 |
| vs | | | | | | |
| Winter | 21 | 28.78 | 42.97 | 5.37-300.32 | | |
| Summer | 49 | 16.52 | 14.02 | 4.64-60.87 | 1.829 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 16.77 | 6.87 | 7.48-31.15 | | |
| Autumn | 16 | 30.79 | 23.38 | 10.05-92.3 | -1.855 | >0.05 |
| vs | | | | | | |
| Winter | 21 | 28.78 | 42.97 | 5.37-200.32 | | |
| Autumn | 16 | 30.79 | 23.38 | 10.05-92.3 | -1.991 | <0.05 |
| vs | | | | | | |
| Spring | 25 | 16.77 | 6.87 | 7.48-31.15 | | |
| Winter | 21 | 28.78 | 42.97 | 5.37-200.32 | 0.132 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 16.77 | 6.87 | 7.48-31.15 | | |

Table 7.12: Results of Student's t-test for the seasonal differences in mean crab carapace copper concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal copper concentration used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 18 | 4.65 | 3.45 | 1.61-14.44 | -2.27 | <0.05 |
| vs | | | | | | |
| Autumn | 5 | 12.45 | 12.97 | 2.93-34.57 | | |
| Summer | 18 | 4.65 | 3.45 | 1.61-14.44 | -0.17 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 10.16 | 17.01 | 1.03-44.54 | | |
| Summer | 18 | 4.65 | 3.45 | 1.61-14.44 | -0.12 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 5.76 | 7.57 | 0.0-26.66 | | |
| Autumn | 5 | 12.45 | 12.97 | 2.93-34.57 | 1.0 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 10.16 | 17.01 | 1.03-44.54 | | |
| Autumn | 5 | 12.45 | 12.97 | 2.93-34.57 | 1.59 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 5.76 | 7.57 | 0.0-26.66 | | |
| Winter | 6 | 10.16 | 17.01 | 1.03-44.54 | 0.07 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 5.76 | 7.57 | 0.0-26.66 | | |

Table 7.13: Results of Student's t-test for the seasonal differences in mean crab muscle copper concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of seasonal copper concentration used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 14.83 | 24.32 | 1.3-71.43 | 1.28 | >0.05 |
| vs | | | | | | |
| Autumn | 4 | 2.59 | 1.48 | 0.58-4.1 | | |
| Summer | 15 | 14.83 | 24.32 | 1.3-71.43 | -1.37 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 54.73 | 75.63 | 0.9-166.32 | | |
| Summer | 15 | 14.83 | 24.32 | 1.3-71.43 | -0.34 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 12.21 | 12.05 | 0.0-31.09 | | |
| Autumn | 4 | 2.59 | 1.48 | 0.58-4.1 | -1.8 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 54.73 | 75.63 | 0.9-166.32 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Autumn | 4 | 2.59 | 1.48 | 0.58-4.1 | -1.46 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 12.21 | 12.05 | 0.0-31.09 | | |
| Winter | 4 | 54.73 | 75.63 | 0.9-166.32 | 0.86 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 12.21 | 12.05 | 0.0-31.09 | | |

Table 7.14: Results of Student's t-test for the differences in mean crab gill copper concentration ($\mu g.g^{-1}$ wet mass) of autumn, winter and spring (Mean logarithms of seasonal copper concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Autumn | 3 | 50.25 | 71.11 | 8.1-132.35 | 0.34 | >0.05 |
| vs | | | | | | |
| Winter | 3 | 36.01 | 11.04 | 24.3-46.22 | | |
| Autumn | 3 | 50.25 | 71.11 | 8.1-132.35 | 1.07 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 18.14 | 1.04 | 17.22-19.71 | | |
| Winter | 3 | 36.01 | 11.04 | 24.3-46.22 | 3.81 | <0.05 |
| vs | | | | | | |
| Spring | 5 | 18.14 | 1.04 | 17.22-19.71 | | |

Table 7.15: Results of the Mann-Whitney test for the differences in crab gill copper concentrations ($\mu g.g^{-1}$ wet mass) of summer and the other seasons.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 6.16 | 4.48 | 2.56-19.23 | 2.132 | <0.05 |
| vs | | | | | | |
| Autumn | 3 | 50.25 | 71.11 | 8.1-132.35 | | |
| Summer | 15 | 6.16 | 4.48 | 2.56-19.23 | 2.606 | <0.05 |
| vs | | | | | | |
| Winter | 3 | 36.01 | 11.04 | 24.3-46.22 | | |
| Summer | 15 | 6.16 | 4.48 | 2.56-19.23 | 2.88 | <0.05 |
| vs | | | | | | |
| Spring | 5 | 18.14 | 1.04 | 17.22-19.71 | | |

Table 7.16: Results of the Mann-Whitney test for the differences in crab digestive gland copper concentrations ($\mu g.g^{-1}$ wet mass) of summer, spring and the other seasons.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 39.07 | 23.2 | 7.0-74.1 | -1.267 | >0.05 |
| vs | | | | | | |
| Autumn | 5 | 25.75 | 19.82 | 2.3-49.0 | | |
| Summer | 15 | 39.07 | 23.2 | 7.0-74.1 | -1.752 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 15.51 | 9.35 | 1.9-27.0 | | |
| Summer | 15 | 39.07 | 23.2 | 7.0-74.1 | -0.846 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 25.78 | 20.13 | 7.32-68.0 | | |
| Autumn | 5 | 25.75 | 19.82 | 2.3-49.0 | 0.0 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 25.78 | 20.13 | 7.32-68.0 | | |
| Winter | 6 | 15.51 | 9.35 | 1.9-27.0 | 0.643 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 25.78 | 20.13 | 7.32-68.0 | | |

Table 7.17: Results of Student's t-test for the differences in mean crab digestive gland copper concentration ($\mu g.g^{-1}$ wet mass) of autumn and winter.

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Autumn | 5 | 25.75 | 19.82 | 2.3-49.0 | 1.13 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 15.51 | 9.35 | 1.9-27.0 | | |

Table 7.18: Results of Student's t-test for the seasonal differences in mean crab gonad copper concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of gonad copper concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 13 | 23.3 | 60.12 | 1.0-222.2 | 0.16 | >0.05 |
| vs | | | | | | |
| Autumn | 4 | 7.93 | 9.2 | 2.4-21.67 | | |
| Summer | 13 | 23.3 | 60.12 | 1.0-222.2 | -0.23 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 10.28 | 8.91 | 1.6-22.69 | | |
| Summer | 13 | 23.3 | 60.12 | 1.0-222.2 | -1.07 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 21.24 | 21.29 | 4.14-50.7 | | |
| Autumn | 4 | 7.93 | 9.2 | 2.4-21.67 | -0.42 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 10.28 | 8.91 | 1.6-22.69 | | |
| Autumn | 4 | 7.93 | 9.2 | 2.4-21.67 | -1.29 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 21.24 | 21.29 | 4.14-50.7 | | |
| Winter | 4 | 10.28 | 8.91 | 1.6-22.69 | -0.83 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 21.24 | 21.29 | 4.14-50.7 | | |

Table 7.19: Results of the Mann-Whitney test for the differences in whole crab ($\mu g.g^{-1}$ wet mass) and water copper concentrations ($mg.l^{-1}$) at each locality: Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_W$) calculated for whole crabs (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water (J) | 8 | 0.06 | 0.05 | 0.01-0.14 | 4.0 | <0.05 | 255.17 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 15.31 | 11.0 | 4.64-45.18 | | | |
| Water (S) | 8 | 0.07 | 0.07 | 0.01-0.192 | 3.965 | <0.05 | 394.29 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 27.6 | 31.19 | 7.48-200.32 | | | |

Table 7.20: Results of Student's t-test for differences in mean crab tissue and carapace ($\mu g.g^{-1}$ wet mass), and water copper concentration ($mg.l^{-1}$) at each locality, as well as the bioconcentration factor ($BCF_W$) calculated for the tissues and carapace (Dig.gland = digestive gland; Mean logarithms of copper concentrations used to calculate the t-value; t = t-value, p = p-value).

| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_W$ |
| **Water** | 8 | 0.06 | 0.05 | 0.01-0.14 | | | |
| vs Carapace | 19 | 6.82 | 8.9 | 0.0-34.57 | -10.72 | <0.05 | 113.67 |
| vs Muscle | 16 | 10.24 | 17.26 | 0.0-66.6 | -8.43 | <0.05 | 170.67 |
| vs Dig.gland | 18 | 24.62 | 20.95 | 2.3-74.1 | -16.0 | <0.05 | 410.33 |
| vs Gonads | 11 | 11.53 | 13.61 | 1.0-50.7 | -10.10 | <0.05 | 192.17 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_W$ |
| **Water** | 8 | 0.07 | 0.07 | 0.01-0.192 | | | |
| vs Carapace | 20 | 6.75 | 9.55 | 2.02-44.54 | -12.20 | <0.05 | 96.43 |
| vs Muscle | 13 | 27.78 | 46.72 | 1.35-166.32 | -8.15 | <0.05 | 396.86 |
| vs Dig.gland | 15 | 36.33 | 20.87 | 1.9-68.0 | -14.10 | <0.05 | 519.0 |
| vs Gills | 12 | 24.07 | 37.02 | 2.56-132.35 | -10.20 | <0.05 | 343.86 |
| vs Gonads | 14 | 27.22 | 65.07 | 1.64-222.2 | -8.61 | <0.05 | 388.86 |

Table 7.21: Results of the Mann-Whitney test for differences in crab gill ($\mu g.g^{-1}$ wet mass) and water copper concentrations ($mg.l^{-1}$) in Jonkershoek, as well as the bioconcentration factor ($BCF_W$) calculated for the gills (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water | 8 | 0.06 | 0.05 | 0.01-0.14 | 3.424 | <0.05 | 182.17 |
| vs Gills | 14 | 10.93 | 6.91 | 3.5-19.71 | | | |

Table 7.22: Results of the Mann-Whitney test for differences in copper concentrations ($\mu g.g^{-1}$ wet mass) of whole crabs and sediments from the two localities, Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for whole crabs (z = z-value, p = p-value).

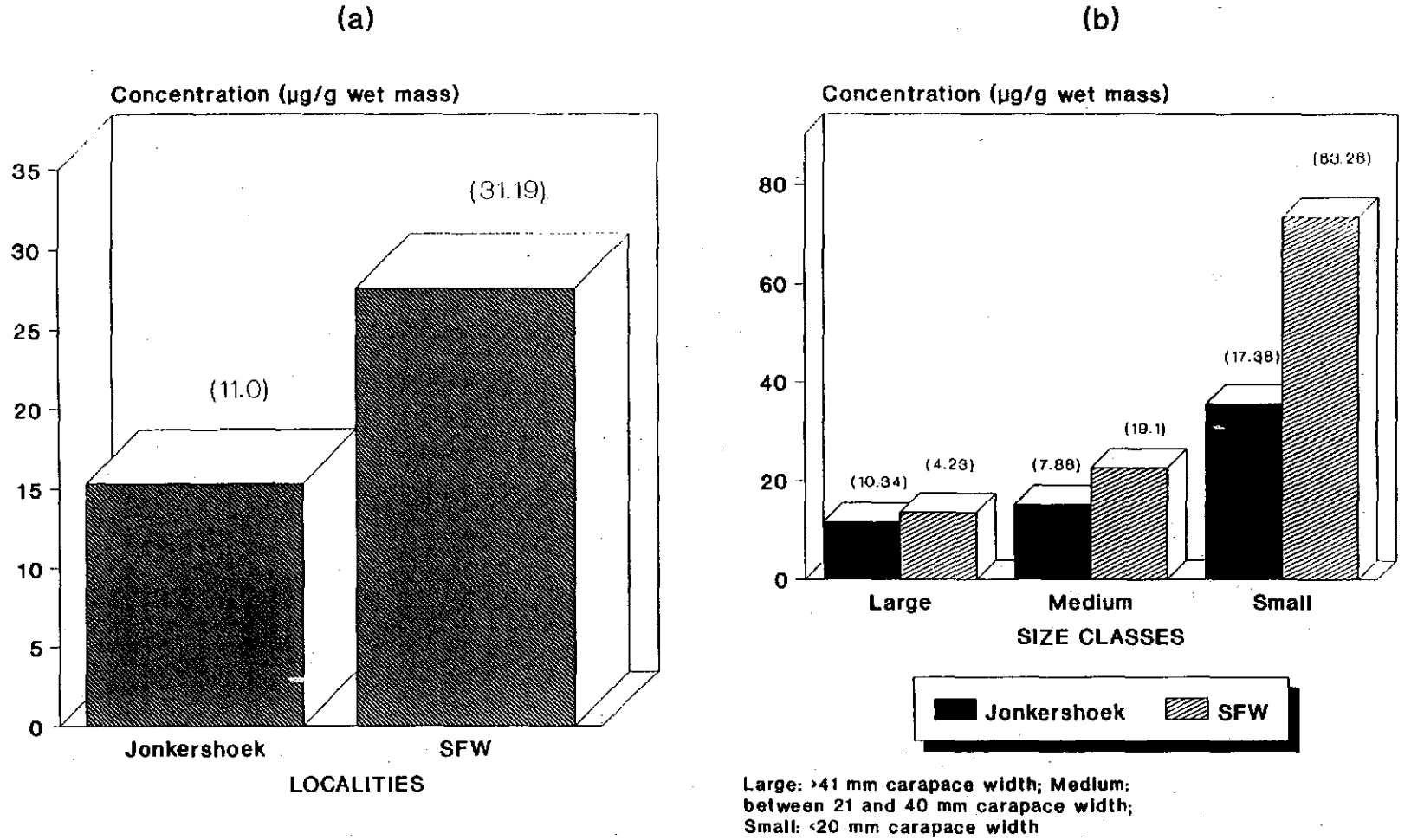
| | n | Mean | SD | Range | z | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 6.15 | 4.16 | 1.2-13.5 | 2.331 | <0.05 | 2.49 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 15.31 | 11.0 | 6.64-45.18 | | | |
| Sediment (S) | 8 | 6.35 | 4.06 | 2.56-12.82 | 3.29 | <0.05 | 4.35 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 27.6 | 31.19 | 7.48-200.32 | | | |

Table 7.23: Results of Student's t-test for differences in mean copper concentration ($\mu g.g^{-1}$ wet mass) of the sediments, crab tissues and carapace at each locality: Jonkershoek and SFW, as well as the bioconcentration factor ($BCF_S$) calculated for the tissues and carapace (Dig.gland = digestive gland; Mean logarithms of copper concentrations used to calculate the t-value; t = t-value, p = p-value).

| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 6.15 | 4.16 | 1.2-13.5 | | | |
| vs Carapace | 19 | 6.82 | 8.9 | 0.0-34.57 | 0.48 | >0.05 | 1.11 |
| vs Muscle | 16 | 10.24 | 17.26 | 0.0-66.6 | 0.36 | >0.05 | 1.67 |
| vs Dig.gland | 15 | 24.62 | 20.95 | 2.3-74.1 | -3.66 | <0.05 | 4.0 |
| vs Gonads | 11 | 11.53 | 13.61 | 1.0-50.7 | -0.53 | >0.05 | 1.87 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 6.35 | 4.06 | 2.56-12.82 | | | |
| vs Carapace | 20 | 6.75 | 9.55 | 2.02-44.54 | 0.6 | >0.05 | 1.06 |
| vs Muscle | 13 | 27.78 | 46.72 | 0.35-166.32 | -0.83 | >0.05 | 4.37 |
| vs Dig.gland | 15 | 36.33 | 20.87 | 1.9-68.0 | -4.15 | <0.05 | 5.72 |
| vs Gonads | 14 | 27.22 | 65.07 | 1.64-222.2 | -0.66 | >0.05 | 4.29 |

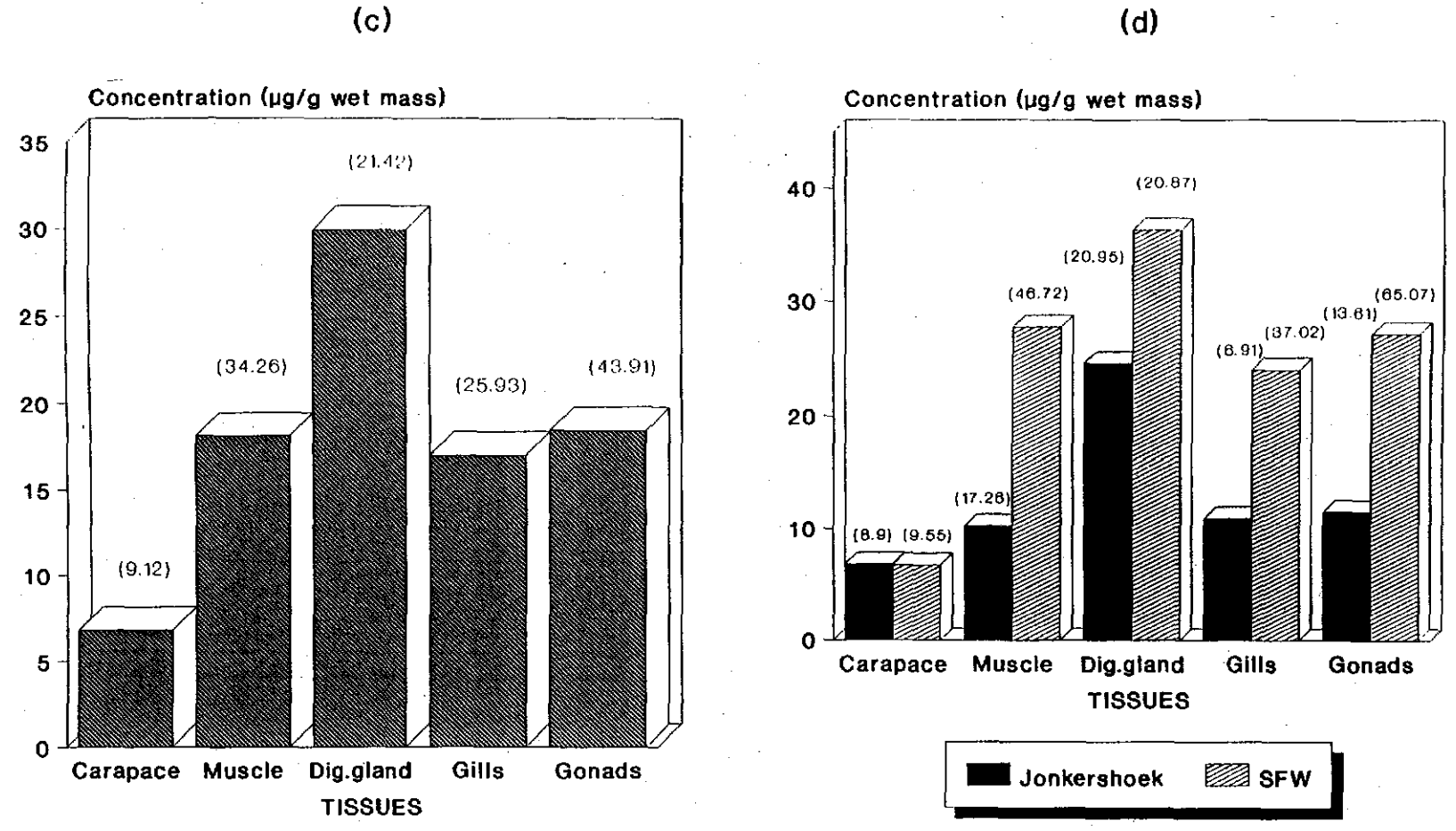
Table 7.24: Results of the Mann-Whitney test for differences in copper concentrations ($\mu g.g^{-1}$ wet mass) of sediment and crab gills from Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for the gills (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 6.15 | 4.16 | 1.2-13.5 | 1.279 | >0.05 | 1.78 |
| vs | | | | | | | |
| Gills (J) | 14 | 10.93 | 6.91 | 3.5-19.71 | | | |
| Sediment (S) | 8 | 6.35 | 4.06 | 2.56-12.82 | 1.078 | >0.05 | 3.79 |
| vs | | | | | | | |
| Gills (S) | 12 | 24.07 | 37.02 | 2.56-132.35 | | | |



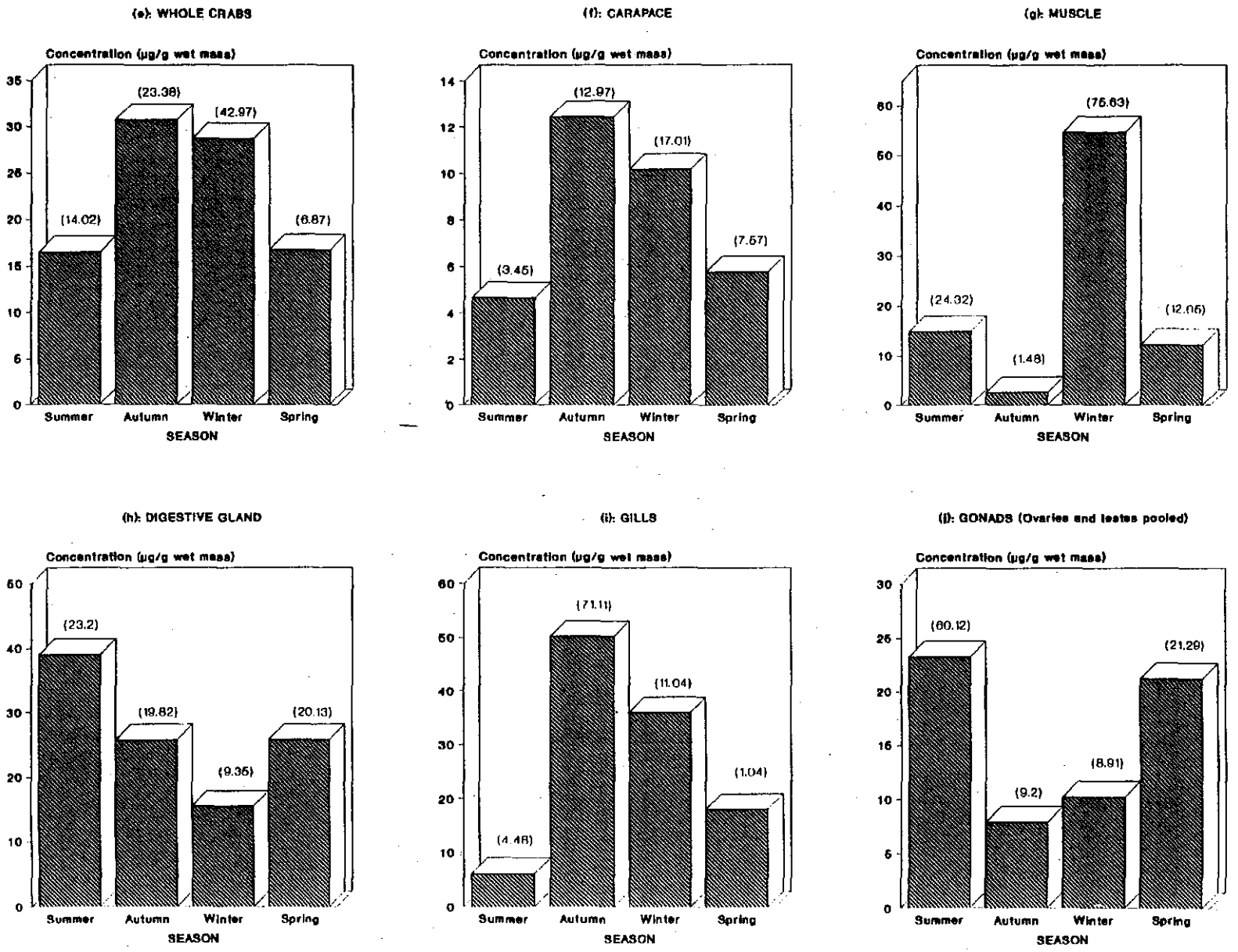

Large: >41 mm carapace width; Medium: between 21 and 40 mm carapace width; Small: <20 mm carapace width

Figures 7(a)-(b): Mean copper concentration in whole crabs (a) and various size classes (b) from both localities (SD in parenthesis).




Jonkershoek and SFW data pooled

Figures 7(c)-(d): Mean copper concentration in carapace and tissues of *P. perlatus* from the Eerste River (SD in parenthesis).




Figures 7(e)-(j): Mean seasonal copper concentration in whole crabs, carapace and tissues of *P. perlatus* (Jonkershoek and SFW data pooled; SD in parenthesis).

## Discussion

Comparisons of whole body, tissue, and carapace copper concentrations in *Potamonautes perlatus* (Table 7.3; Figure 7(c)), with the corresponding concentrations found by Bryan (1968) for several uncontaminated marine and freshwater decapods, Arumugam & Ravindranath (1983) for the green lagoon crab *Scylla serrata* and Steenkamp et al. (1994a) for *Potamonautes warreni*, were made.

Bryan (1968) illustrated mean wet mass Cu concentrations of 3.2 to 11.0 $\mu g.g^{-1}$ for muscle tissue, 34.0 to 603.0 $\mu g.g^{-1}$ for the digestive gland, 7.0 to 57.0 $\mu g.g^{-1}$ for the gills, 3.0 to 50.0 $\mu g.g^{-1}$ for the gonads and trace concentrations to 9.5 $\mu g.g^{-1}$ for the "shell". Unexposed individuals of the green lagoon crab, *Scylla serrata*, contained mean wet mass Cu concentrations of 39.5±4.3, 152.3±24.3, 58.2±9.2, 30.2±2.2 and 14.8±1.3 $\mu g.g^{-1}$ in the muscles, digestive gland, gills, gonads and "cuticle" respectively (Arumugam & Ravindranath, 1983). It was found that, when compared with the results of Bryan (1968), the mean Cu concentrations in the tissues and carapace of *P. perlatus* were all relatively low to very low, whereas, compared to the results of Arumugam & Ravindranath (1983), the Cu concentrations in the digestive gland of *P. perlatus* were relatively low and the concentrations in the muscles relatively high. The mean Cu concentrations in the gills, gonads and carapace of *P. perlatus*, however, compared well. Steenkamp et al. (1994a) observed mean wet mass Cu concentrations of 13.2±4.7 to 28.9±14.8 $\mu g.g^{-1}$ in the muscles of *P. warreni* from polluted waters, 20.6±18.0 to 67.0±205.5 $\mu g.g^{-1}$ in the digestive gland, 35.3±25.1 to 61.2±56.3 $\mu g.g^{-1}$ in the gills, 15.9±9.7 to 23.7±15.2 $\mu g.g^{-1}$ in the gonads and 8.8±4.6 to 14.9±6.8 $\mu g.g^{-1}$ in the carapace. In comparison, the mean concentrations in the carapace and gills of *P. perlatus* were found to be relatively low, whilst the concentrations in the remaining tissues compared favourably. Also, when the whole crab copper concentrations in several uncontaminated marine and freshwater decapods (10 to 33 $\mu g.g^{-1}$ wet mass) (Bryan, 1968) were compared with the whole body Cu concentrations in *Potamonautes perlatus* (Table 7.5), the concentrations in the latter were found to compare favourably.

Despite the fact that the mean Cu concentrations in the muscles, digestive gland and gonads of *P. perlatus* were observed to be similar to the concentrations in *P. warreni* collected from polluted freshwater ecosystems (Steenkamp et al., 1994a), the whole body Cu concentrations in *P. perlatus* indicate that this metal is generally well regulated in this species in the Eerste River. However, comparisons of the concentration values from the two localities produced results to the contrary: whilst no significant differences between either the various size classes or the selected tissues and carapace (Tables 7.6-7.8) were found, the statistically significant differences in Cu concentrations observed between whole crabs (Table 7.5), as well the higher $BCF_W$ and $BCF_S$ for whole crabs from SFW (Tables 7.19 and 7.22), indicated a Cu accumulation in crabs from this locality.

Since no differences in copper concentrations existed between either the water or the sediments from the two localities (Tables 4.5 and 4.11), the reason for the observed whole crab differences between the localities must lie within differences in the physico-chemistry of the water (Table 4.8), possibly resulting in, among others, an increase in the bioavailability of Cu to the animals. Also, as mentioned, food is an important source of Cu, therefore differences in the diets of the two populations may also have been an influencing factor.

Upon researching the distribution of Cu in *P. perlatus*, it was found that the tissue into which the most copper was concentrated, was the digestive gland (Tables 7.3 and 7.4), supported by the higher $BCF_W$'s and $BCF_S$'s observed for this tissue from both localities (Tables 7.20, 7.21, 7.23 and 7.24), as well as the fact that this was the only tissue in which the Cu concentrations were significantly higher than in the sediments, at both localities (Table 7.23). The carapace, on the other hand, contained the lowest Cu concentrations (Table 7.3). Hilmy et al. (1988) also found the highest Cu concentrations in the digestive gland of *Portunus pelagicus* and a relatively low Cu concentration in the carapace. These results also agree with those of Bryan (1968) and Arumugam & Ravindranath (1983). Although Steenkamp et al. (1994a) also found low Cu concentrations in the carapace of *Potamonautes warreni*, they detected the highest concentrations in the gills, followed by the digestive gland.

Since food is the major source of copper in decapod crustaceans (Wieser, 1968), the copper concentrations in the digestive gland are expected to be fairly high but to vary with feeding status. Authors such as Rainbow & Scott (1979) and Engel & Brouwer (1987) have reported on specific heavy metal-binding proteins (metallothioneins) in the digestive glands of the marine crabs *Carcinus maenas* and *Callinectes sapidus* respectively. Arumugam & Ravindranath (1983) stated that the digestive gland is a unique tissue in storing Cu associated with lipids, and that this may be a mechanism to detoxify Cu.

In the haemolymph Cu is present in the TCA-insoluble fraction, suggesting that all the copper may be very tightly bound to the TCA-protein precipitate which is probably mainly haemocyanin (Arumugam & Ravindranath, 1983). Bryan (1968) suggested that the Cu found in the muscles and gills of decapod crustaceans are related to the blood concentration and are normally probably due to contamination by the blood. One can assume that this is also applicable in the case of other internal organs such as the gonads.

It is not known to what extent copper is absorbed by the exoskeleton or whether copper is incorporated in the exoskeleton, but the consistently low Cu concentrations found in the carapaces of *P. perlatus* and various other species suggest that the exoskeleton (specifically the carapace) does not play a significant rôle in the uptake and storage of Cu.

It is concluded that the digestive gland is the most important tissue for Cu storage in *P. perlatus* and that the carapace is of relatively little importance. The remaining three selected tissue types do not seem to vary significantly in their contribution to Cu storage.

Investigations into the relationship between crab body size and whole body, tissue and carapace copper concentrations, brought to light that reasonably strong negative correlations existed between body size and whole body ($r = -0.58$), muscle, gonad and carapace Cu concentrations (Table 7.2). The statistically significant differences between Cu concentrations of the various size classes from the two localities (except between large and medium crabs from SFW) (Table 7.1), provide more proof that smaller individuals of *P. perlatus* take up more copper than larger individuals. Although this contradicts the findings of Hilmy et al. (1988), who found that mature crabs (*Portunus pelagicus*) accumulated more Cu than immature crabs, it supports the findings of Steenkamp et al. (1994a), who found that immature individuals of *Potamonautes warreni* were able to bioaccumulate more Cu per unit weight than mature crabs. Also, Kiffney & Clements (1996) demonstrated an inverse relationship between body size and survivorship, upon exposing three ephemeropteran and one plecopteran species to copper.

It is therefore concluded that body size can be considered to be a factor which influences the uptake of copper in *Potamonautes perlatus*. The reasons for this phenomenon however, are not clear. Hill & O'Keeffe (1992) who studied the feeding ecology of *P. perlatus*, found that smaller individuals preyed significantly more on aquatic invertebrates than larger individuals, while the latter preferred vegetable material. It is known that aquatic invertebrates (e.g. insects) are able to accumulate heavy metals in their bodies (Albers & Camardese, 1993 and Kiffney & Clements, 1993). It is therefore possible that smaller individuals of *P. perlatus* bioaccumulate more Cu from their prey. Steenkamp et al. (1994a) suggested that the higher Cu concentrations in smaller individuals are due to the fact that the regulation mechanisms of these animals are not yet fully developed and that younger crabs have relatively higher metabolic and growth rates. Also, Gherardi et al. (1987) and Gherardi & Micheli (1989) found that younger individuals of *Potamon fluviatile* and *P. potamios palestinensis* respectively tend to hide more under stones and rocks, especially during the night. Since these microhabitats create relatively constant environments, it is possible that younger (therefore smaller) individuals of *P. perlatus* are exposed to specific Cu concentrations for longer periods, resulting in the observed higher Cu concentrations.

An investigation into the relationship between gender and whole body, tissue and carapace Cu concentrations showed that gender also seems to be a factor influencing the uptake of Cu in *P. perlatus*. Although no significant differences were found in tissue or carapace Cu concentrations in the two genders, the copper concentrations of whole male individuals of *P. perlatus* were found to be significantly higher than the concentrations of whole females (Table 7.9). Hilmy et al. (1988) also found no significant differences in tissue or carapace Cu concentrations between

males and females of *Portunus pelagicus* (Table 7.10). Bryan (1968) and Steenkamp et al. (1994a), on the other hand, found a singular difference between the concentrations in the mature gonads. Since the differences in whole body Cu between males and females of *P. perlatus* cannot be attributed to the copper concentrations in any of the tissues or the carapace, it must be assumed that males had higher Cu concentrations in the haemolymph or other internal organs but, as mentioned previously, the concentrations in the latter are influenced by the concentrations in the former. Gherardi et al. (1989) and Gherardi & Vannini (1989) studied the foraging strategies and spatial behaviour of *Potamon fluviatile* and determined that, in relation to the reproductive phases, females moved faster and occupied a larger area during summer and autumn. In contrast, males moved more slowly and occupied a smaller area, displaying a sedentary behaviour. These seasonal "nomadic" movements of females have also been observed for *Potamonautes perlatus* during the present study and could explain the higher Cu concentrations found in whole males: the less active males are possibly exposed to specific Cu concentrations for longer periods, suggesting that more Cu is absorbed per time unit.

Seasonality was also found to influence Cu uptake to some extent: a number of seasonal variations were found in whole crab, carapace and gill copper concentrations (Tables 7.11, 7.12, 7.14 and 7.15) In each case, peaks in Cu were observed during autumn/winter (Figures 7(e),(f) and (i)). Authors Steenkamp et al. (1994a) and Devescovi & Lucu (1995) however, found summer Cu peaks in tissues of *P. warreni* and whole individuals of *Carcinus mediterraneus* respectively. The observed peaks for *P. perlatus* do not correspond with the slight seasonal peaks in water and sediment Cu (Figures 4(c)-(l)), therefore another explanation for these peaks in this species need to be considered. It is known that *P. perlatus* is less active in winter and retreats into burrows or under rocks and stones (personal observations and lowered catch rate), in order to escape the increased water flow rate. The constant environment created by this type of microhabitat and the close contact with the sediments could increase the amount of Cu absorbed by the animals, through lengthened exposure to a specific copper concentration. The copper is possibly absorbed via the gills, since Arumugam & Ravindranath (1983; 1987) found that the gills of *Scylla serrata* were capable of sequestering Cu from the environment.

The seasonal carapace copper concentrations are probably not directly related to environmental copper and absorption via the gills but more likely a result of the stage of the moult cycle, as suggested by Arumugam & Ravindranath (1983). Steenkamp et al. (1994a) speculated that since the carapace is shed periodically, this could represent a route of excretion of Cu in decapods. Since the exact time of moulting in *P. perlatus* is not yet known, it will be impossible to substantiate this theory in the case of this species.

Although no significant seasonal differences were observed for the remaining tissues, namely the muscles, digestive gland and gonads, these tissues exhibited peaks during autumn/winter (muscles), in summer (digestive gland) and during spring/summer (gonads) (Figures 7(g), (h)

and (j)). Devescovi & Lucu (1995) also observed a summer peak in digestive gland Cu for *Carcinus mediterraneus*. Bryan (1968) reported that the Cu in the digestive gland increases following a moult and reaches a maximum at the start of the intermoult period, after which it decreases. It is likely that the Cu is mobilized into the haemolymph and partly incorporated into the exoskeleton, especially the carapace, to be excreted during the moult. It is possible that the intermoult period, for a number of individuals of *P. perlatus*, may have commenced in summer, explaining the summer peak in digestive gland Cu. However, again, owing to the limited information on the moulting cycle of this species, this theory cannot be substantiated. Gherardi et al. (1989) showed that during early summer, when second vitellogenesis takes place in females of *Potamon fluviatile*, a corresponding deposition of fats in the gonads occurred. The lipid content of the digestive gland was also significantly higher. Considering the opinion of Arumugam & Ravindranath (1983), that the digestive gland stores Cu associated with lipids, this could be a plausible explanation for the observed summer peaks in digestive gland Cu in *Potamonautes perlatus*.

Finally, in agreement with the findings of Steenkamp et al. (1994a) for *P. warreni*, it is concluded from the preliminary results, that *P. perlatus* in the Eerste River does not seem to qualify as a monitor of environmental Cu pollution. This is based on the fact that seasonal variations in whole body, carapace and gill Cu were observed, indicating that long term accumulation would not occur, as well as the fact that fluctuations in environmental Cu were not reflected in any body parts analysed. The observed large intraspecific variations in Cu concentrations also support these findings. However, more intensive research on Cu uptake and distribution in *P. perlatus* is needed before final conclusions can be made.

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# CHAPTER 8

## CONCENTRATIONS OF LEAD IN THE FRESHWATER CRAB, *POTAMONAUTES PERLATUS*

### Introduction

Lead has played an important rôle in human societies over many thousands of years: the decline of the Roman Empire has been ascribed to the deleterious effect of lead poisoning. It occurs in several ores, namely galena, galenite, lead sulphide, cerussite, anglesite, lancarksite, massicot and matlockite. The main Pb minerals are the sulphides and carbonates. The $Pb^{+2}$ forms complexes which are slightly stable with nitrate, chloride and cyanide, and other fairly stable complexes with acetic, organic hydroxyl and thiosulphuric acids. The salts of lead (II), lead oxides and lead sulphide are poorly soluble in water with the exception of lead acetate, lead chlorate and, to some extent, lead chloride (Galvin, 1996; Harrison & Laxen, 1984; Richardson & Gangolli, 1994 and Tsuchiya, 1979).

The world consumption of lead was about 4.1 million tons in 1975, more than 50% of which was used by the automobile industry, in the form of batteries and alkyllead. The largest consumer of lead in 1974 was the storage battery industry, followed by alkyllead production and cable sheathing. Other uses are e.g. as an element of alloys, in paint pigments, in petrol to give anti-knock properties, for x-ray and atomic radiation protection, as well as a means of sweetening wine (Harrison & Laxen, 1984; Newland & Daum, 1982; Richardson & Gangolli, 1994 and Tsuchiya, 1979).

Lead can reach riverine ecosystems via industrial runoff and atmospheric fallout; the latter is known to be the main source of Pb in marine and freshwaters (Moore & Ramamoorthy, 1984). This metal poses a threat to riverine fauna since it is not a physiologically essential element, but has a toxic character (Galvin, 1996). It is known to be less toxic to invertebrates than copper, cadmium, zinc and mercury but generally more toxic than nickel, cobalt and manganese (Moore & Ramamoorthy, 1984).

Lead, accumulated to sublethal levels in animals, can cause a number of symptoms of which the following are examples: anemia, mental deterioration, aggressive behaviour, loss of appetite, insomnia, vomiting, decreased male fertility, stillbirths and miscarriages. Acute and chronic lead poisoning may lead to gastrointestinal colic and encephalopathy (Harrison & Laxen, 1984; Richardson & Gangolli, 1994 and Tsuchiya, 1979).

There are several factors which influence the toxicity and bioavailability of lead in freshwaters, such as the presence of other salts in the water, which reduce the bioavailability of lead because

it precipitates. An increase in water pH lowers the concentration of $Pb^{+2}$, therefore lowering its toxicity to invertebrates. Chelators and complexing agents bind $Pb^{+2}$, thus decreasing Pb toxicity. It is also known that the acute toxicity of lead to freshwater organisms is greater in soft water than in hard water. Age may also be an important factor: it has been shown that young people absorb lead faster and are more susceptible to its toxic effects than adults. This may also be true for immature aquatic animals (Galvin, 1996; Newland & Daum; 1982 and Richardson & Gangolli, 1994). Lastly, Roldan & Shivers (1987) reported that the toxicity of Pb to decapods (e.g. the crayfish *Orconectes propinquus*), may be decreased by the formation of Pb-containing vacuoles in the digestive gland.

The accumulation of lead by freshwater decapods have been studied in a number of crayfish and crab species. Lead concentrations in the two crayfish species *Orconectes virilis* and *O. propinquus* were investigated by Anderson & Brower (1978), France (1987) and Roldan & Shivers (1987). Tulasi et al. (1987) and Tulasi & Ramana Rao (1988) studied lead accumulation in the crab *Barytelphusa guerini*, whereas for the South African crab species *Potamonautes warreni* intensive research was undertaken by Van Eeden & Schoonbee (1991), Steenkamp et al. (1992) and du Preez et al. (1993).

## Materials and Methods

All samples were prepared, tested and statistically analysed according to the method described in Chapter 3.

## Results

In all cases only the lead concentrations per gram wet mass were used for statistical analysis. Crabs showed large individual variation in whole body, tissue and carapace lead concentrations. This was an important consideration in the interpretation of the statistical results.

### The relationship between crab size and whole crab lead concentration

In order to investigate the possible relationship between whole crab lead concentrations and the sizes of the crabs, the data from Jonkershoek and SFW were pooled. The carapace widths of the crabs, in decreasing order, were compared with the respective lead concentrations and the correlation coefficient (r) thereof, calculated. With 53 degrees of freedom and an $r^2$-value of 21.69%, an r-value of -0.466 was obtained. This indicated a reasonably strong negative

correlation between sizes of crabs and the concentration of lead in the body: as size decreased, so did the concentrations increase (Figure 8(b)).

Differences in lead concentrations of the various size classes at each locality

Since both the original and logarithmically transformed whole crab data for large, medium and small-sized crabs from each locality were not normally distributed, a nonparametric test, the Mann-Whitney test, was performed on the original data, in order to test for statistically significant differences ($p<0.05$) (Table 8.1).

Differences in lead concentrations between medium and small crabs from SFW were found. The small size class showed the highest mean lead concentration ($72.62\pm58.78$ $\mu g.g^{-1}$ wet mass), and large sized crabs the lowest ($3.62\pm1.25$ $\mu g.g^{-1}$ wet mass).

The relationship between crab size, and tissue and carapace lead concentration

In order to investigate the possible relationship between crab size and lead concentrations in selected tissues, namely muscle tissue, digestive gland, gills and gonads, as well as the carapace, the data from Jonkershoek and SFW were pooled, after which the carapace widths of the crabs, in decreasing order, were compared with the respective tissue and carapace lead concentrations. Table 8.2 shows the r-values calculated for each tissue and for the carapace.

Reasonably strong negative correlations were found between crab size and the lead concentration in three of the four tissue types, namely muscle tissue, gills and gonads, as well as the carapace. Only the lead concentration of the digestive gland proved to be poorly correlated with crab size.

Differences in lead concentrations of the various selected tissues and the carapace

The mean lead concentration values of the selected tissues and the carapace of crabs from Jonkershoek and SFW were pooled, in order to determine whether statistically significant differences ($p<0.05$) existed between the concentrations of the various tissues and the carapace. Since the data were not normally distributed, the mean logarithms of the tissue and carapace lead concentrations were used in Student's t-test. The respective mean concentrations and standard deviations are given in Table 8.3. Table 8.4 shows the results of the t-test performed on the tissue and carapace concentrations.

Through comparison of the selected tissues and the carapace, significant differences in mean lead concentrations were found between the digestive gland and gonads, digestive gland and carapace, and gills and carapace. The gonads showed the highest mean lead concentration

(23.44±32.45 $\mu g.g^{-1}$ wet mass), and the digestive gland the lowest (7.43±12.45 $\mu g.g^{-1}$ wet mass) (Figure 8(c)).

Differences in lead concentrations of crabs from Jonkershoek and SFW

(a) Differences in whole crab lead concentration

The logarithmically transformed whole crab data for Jonkershoek and SFW failed the normal probability test, thus the Mann-Whitney test was performed (Table 8.5).

The highest mean lead concentration (19.49±32.36 $\mu g.g^{-1}$ wet mass) was found in crabs from SFW (Figure 8(a)) but a z-value of 0.145 indicated that there were no statistically significant differences ($p>0.05$) between whole crab lead concentrations in the two localities.

(a)(i) Differences in whole crab lead concentrations of the various size classes

Through a comparison of the whole crab lead concentration values between large, medium and small crabs from Jonkershoek and SFW, it was established with the Mann-Whitney test that no statistically significant differences existed in lead concentration between any of the size classes (Table 8.6).

(b) Differences between concentration values of selected tissues and the carapace

The mean lead concentrations in the carapace, muscle, digestive gland, gills and gonads of crabs from Jonkershoek and SFW were compared with the use of Student's t-test and the results tabulated in Table 8.7. Since the data were not normally distributed, the mean logarithms of the lead concentrations in the tissues and carapace were used to calculate the t-value. In the case of the carapace data from SFW, however, a normal distribution could not be obtained after transformation of the concentration data. The Mann-Whitney test was therefore performed on the original data, to investigate possible differences (Table 8.8).

Although the tissue and carapace samples from SFW showed the highest mean lead concentration in each instance (Figure 8(d)), no significant differences could be found between the two localities.

Differences in lead concentrations of males and females

In order to determine whether statistically significant differences ($p<0.05$) existed between the mean whole crab, tissue and carapace lead concentration of males and females, the data for crabs collected from Jonkershoek and SFW, were pooled for each sex. In all cases the original data failed the normal probability test, therefore each concentration was logarithmically transformed. In all cases except the digestive gland and gonads, the transformed data also failed to provide a normal distribution, thus the Mann-Whitney nonparametric test was performed.

(a) Differences in whole crab concentrations

Table 8.9 shows the results of the Mann-Whitney test, performed on the original whole crab lead concentrations of males and females of *Potamonautes perlatus*. The highest mean lead concentration was found in males (24.39±34.24 $\mu g.g^{-1}$ wet mass) but no statistically significant difference ($p>0.05$) existed between the two genders.

(b) Differences in lead concentration of the selected tissues and the carapace

Although males exhibited the highest mean lead concentrations in the carapace and all but one of the four tissue types (gonads), the digestive gland was the only tissue type in which a significant difference between the two genders was found (Tables 8.10 and 8.11).

Seasonal variations in lead concentrations

The lead concentrations of whole crabs, selected tissues and carapace from the different seasons were tested for statistically significant differences ($p<0.05$). In each instance, the data for crabs collected at Jonkershoek and SFW were pooled and these then tested for normality. None of the data sets were normally distributed and were therefore logarithmically transformed. The transformed whole crab, carapace and summer muscle and gill data also failed the normal probability test, thus the Mann-Whitney test was performed.

(a) Seasonal differences in whole crab lead concentrations

The Mann-Whitney nonparametric test produced statistically significant differences between whole crab lead concentrations of all the seasons, except between summer and spring and between winter and spring (Table 8.12). The highest mean whole crab lead concentration (39.25±35.89 $\mu g.g^{-1}$ wet mass), was found in autumn, whereas summer showed the lowest mean (10.78±13.97 $\mu g.g^{-1}$ wet mass) (Figure 8(e)).

(b) Seasonal differences in tissue and carapace lead concentrations

1. Carapace

No statistically significant differences ($p>0.05$) between carapace lead concentrations of any of the four seasons were found. The results of the Mann-Whitney test, performed on the seasonal lead concentrations, are tabulated in Table 8.13. Figure 8(f) shows the mean seasonal carapace lead concentrations.

2. Muscle tissue

Through pairwise comparisons of the different seasons, it was found that no statistically significant differences in muscle lead concentrations existed between any of the seasons (Tables 8.14 and 8.15). Figure 8(g) exhibits the mean seasonal muscle lead concentration.

3. Gill tissue

A statistically significant difference ($p<0.05$) in gill lead concentrations of summer and winter was observed. In Tables 8.16 and 8.17 it can be seen that the highest and lowest mean lead concentrations were found in autumn (39.65±54.81 $\mu g.g^{-1}$ wet mass) and summer (5.89±7.65 $\mu g.g^{-1}$ wet mass) respectively (Figure 8(h)).

4. Digestive gland

The results of Student's t-test on the mean logarithms of the digestive gland lead concentrations between the different seasons, are tabulated in Table 8.18. Only the comparison of summer and autumn yielded a significant difference ($p<0.05$). In autumn the highest mean lead concentration (17.24±26.01 $\mu g.g^{-1}$ wet mass) was observed, whilst the lowest mean concentration was found in summer (4.07±4.26 $\mu g.g^{-1}$ wet mass) (Figure 8(i)).

5. Gonads

When the data for the ovaries and testes were pooled, the highest and lowest mean gonad lead concentrations (34.17±36.5 and 16.39±18.04 $\mu g.g^{-1}$ wet mass) were found in spring and autumn respectively. No statistically significant differences ($p>0.05$), however, existed between any of the seasons (Table 8.19). Figure 8(j) exhibits the mean seasonal gonad lead concentration.

Differences in lead concentrations of whole crabs, tissues, carapace, and Eerste River water

(a) Differences between concentration values for whole crabs and water

The Mann-Whitney test (Table 8.20) showed significant differences between the whole crabs and water lead concentrations in both two localities, Jonkershoek as well as SFW.

(b) Differences between concentration values for the selected tissues, carapace and water

The lead concentrations in all the tissues and the carapace from both localities proved to be significantly different ($p<0.05$) from that in the water. The results of Student's t-test and the Mann-Whitney test (the latter having been performed on the lead concentrations in the water and carapace from SFW), are listed in Tables 8.21 and 8.22 respectively.

Differences in lead concentrations of whole crabs, tissues, carapace, and sediments

(a) Differences between concentration values for whole crabs and sediments

The Mann-Whitney test, performed on the whole crab and sediment lead concentrations from Jonkershoek and SFW, showed no statistically significant differences ($p>0.05$) (Table 8.23).

(b) Differences between concentration values for the selected tissues, carapace and sediments

The mean logarithms of the tissue and carapace lead concentrations were used to test for statistically significant differences ($p<0.05$) between tissue, carapace and sediment lead

concentrations at the two localities. The transformed carapace concentrations, however, were not normally distributed, therefore the Mann-Whitney test was performed.

The data from both localities showed no significant differences in lead concentrations between the sediments, tissues and carapace. Table 8.24 lists the results of Student's t-test, performed on the mean logarithms of the tissue lead concentrations from the two localities. The results of the Mann-Whitney test, performed on the original carapace data, are shown in Table 8.25.

Table 8.1: Results of the Mann-Whitney test for differences in whole crab lead concentrations ($\mu g.g^{-1}$ wet mass) of the various size classes at each locality.

| **JONKERSHOEK** | | | | | | |
|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | z-value | p-value |
| Large | 22 | 9.27 | 11.89 | 0.78-33.29 | 1.334 | >0.05 |
| vs | | | | | | |
| Medium | 34 | 19.45 | 23.39 | 0.97-75.62 | | |
| Large | 22 | 9.27 | 11.89 | 0.78-33.29 | 1.173 | >0.05 |
| vs | | | | | | |
| Small | 4 | 21.4 | 14.03 | 1.31-33.85 | | |
| Medium | 34 | 19.45 | 23.39 | 0.97-75.62 | 0.499 | >0.05 |
| vs | | | | | | |
| Small | 4 | 21.4 | 14.03 | 1.31-33.85 | | |
| **SFW** | | | | | | |
| | n | Mean | SD | Range | z-value | p-value |
| Large | 6 | 3.62 | 1.25 | 2.34-5.22 | -0.083 | >0.05 |
| vs | | | | | | |
| Medium | 39 | 13.76 | 20.1 | 1.16-74.68 | | |
| Large | 6 | 3.62 | 1.25 | 2.34-5.22 | 1.841 | >0.05 |
| vs | | | | | | |
| Small | 6 | 72.62 | 58.78 | 1.95-164.7 | | |
| Medium | 39 | 13.76 | 20.1 | 1.16-74.68 | 2.655 | <0.05 |
| vs | | | | | | |
| Small | 6 | 72.62 | 58.78 | 1.95-164.7 | | |

Table 8.2: $R^2$-values calculated for sizes of crabs and selected tissue and carapace lead concentrations (DF = Degrees of freedom; Dig.gland = digestive gland)

| | DF | r-value | R-sq (%) |
|---|---|---|---|
| Crab size and Muscle | 27 | -0.549 | 30.15 |
| Crab size and Dig.gland | 29 | -0.343 | 11.79 |
| Crab size and Gills | 24 | -0.498 | 24.79 |
| Crab size and Gonads | 23 | -0.463 | 21.44 |
| Crab size and Carapace | 28 | -0.467 | 21.81 |

Table 8.3: Mean lead concentration ($\mu g.g^{-1}$ wet mass) in the muscles, digestive gland (dig. gland), gonads, gills and carapace of crabs from the Eerste River (Jonkershoek and SFW data pooled).

| | n | Mean | SD | Range |
|---|---|---|---|---|
| Muscle | 29 | 17.84 | 24.96 | 0.3-85.7 |
| Dig. gland | 33 | 7.43 | 12.45 | 0.0-63.2 |
| Gonads | 25 | 23.44 | 32.45 | 0.1-125.0 |
| Gills | 26 | 14.11 | 23.43 | 0.0-102.94 |
| Carapace | 39 | 23.41 | 53.28 | 0.73-329.63 |

Table 8.4: Results of Student's t-test for the differences in mean crab tissue and carapace lead concentration ($\mu g.g^{-1}$ wet mass) (Jonkershoek and SFW pooled; Mean logarithms of lead concentrations used for the calculation of the t-value).

| | t-value | p-value |
|---|---|---|
| Muscle vs Digestive gland | 1.65 | >0.05 |
| Muscle vs Gonads | -0.23 | >0.05 |
| Muscle vs Gills | 0.27 | >0.05 |
| Muscle vs Carapace | -1.31 | >0.05 |
| Digestive gland vs Gonads | -1.74 | <0.05 |
| Digestive gland vs Gills | -1.39 | >0.05 |
| Digestive gland vs Carapace | -3.53 | <0.05 |
| Gonads vs Gills | 0.48 | >0.05 |
| Gonads vs Carapace | -1.69 | <0.05 |
| Gills vs Carapace | -0.89 | >0.05 |

Table 8.5: Results of the Mann-Whitney test for the differences in whole crab lead concentrations ($\mu g.g^{-1}$ wet mass) from Jonkershoek and SFW.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Jonkershoek | 60 | 15.84 | 19.8 | 0.78-75.62 | 0.145 | >0.05 |
| vs | | | | | | |
| SFW | 51 | 19.49 | 32.36 | 1.16-164.7 | | |

Table 8.6: Results of the Mann-Whitney test for the differences in whole crab lead concentrations ($\mu g.g^{-1}$ wet mass) for the various size classes, small, medium and large, from Jonkershoek (J) and SFW (S).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Large (J) | 22 | 9.27 | 11.89 | 0.78-33.29 | 0.364 | >0.05 |
| vs | | | | | | |
| Large (S) | 6 | 3.62 | 1.25 | 2.34-5.22 | | |
| Medium (J) | 34 | 19.45 | 23.39 | 0.97-75.62 | -0.824 | >0.05 |
| vs | | | | | | |
| Medium (S) | 39 | 13.76 | 20.1 | 1.61-74.68 | | |
| Small (J) | 4 | 21.4 | 41.03 | 1.31-33.85 | 1.173 | >0.05 |
| vs | | | | | | |
| Small (S) | 6 | 72.62 | 58.78 | 1.95-164.7 | | |

Table 8.7: Results of Student's t-test for the differences in mean tissue lead concentration ($\mu g.g^{-1}$ wet mass) of crabs from Jonkershoek (J) and SFW (SF) (Mean logarithms of the tissue lead concentrations used in the test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Muscle (J) | 16 | 14.15 | 18.98 | 0.4-63.63 | -0.14 | >0.05 |
| vs | | | | | | |
| Muscle (SF) | 13 | 22.38 | 31.04 | 0.3-85.7 | | |
| Dig.gland (J) | 18 | 5.72 | 8.52 | 0.0-31.96 | -0.86 | >0.05 |
| vs | | | | | | |
| Dig.gland (SF) | 15 | 9.49 | 16.12 | 0.2-63.2 | | |
| Gills (J) | 14 | 9.52 | 16.51 | 0.0-61.4 | -1.24 | >0.05 |
| vs | | | | | | |
| Gills (SF) | 12 | 19.46 | 29.45 | 0.9-102.94 | | |
| Gonads (J) | 14 | 19.46 | 23.6 | 0.2-69.4 | 0.04 | >0.05 |
| vs | | | | | | |
| Gonads (SF) | 11 | 28.5 | 41.86 | 0.1-125.0 | | |

Table 8.8: Results of the Mann-Whitney test for the differences in carapace lead concentrations ($\mu g.g^{-1}$ wet mass) of crabs from the two localities, Jonkershoek (J) and SFW (SF).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (J) | 19 | 17.5 | 17.23 | 0.73-55.08 | -0.829 | >0.05 |
| vs | | | | | | |
| Carapace (SF) | 20 | 29.03 | 73.0 | 1.95-329.63 | | |

Table 8.9: Results of the Mann-Whitney test for the differences in whole crab lead concentrations ($\mu g.g^{-1}$ wet mass) of males and females.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Females | 40 | 15.74 | 17.6 | 1.16-66.44 | -0.037 | >0.05 |
| vs | | | | | | |
| Males | 49 | 24.39 | 34.24 | 1.23-164.7 | | |

Table 8.10: Results of Student's t-test for the differences in mean digestive gland and gonad lead concentration ($\mu g.g^{-1}$ wet mass) of male (m) and female (f) crabs (Mean logarithms of the tissue lead concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Dig.gland (f) | 12 | 2.63 | 2.78 | 0.0-7.8 | -2.28 | <0.05 |
| vs | | | | | | |
| Dig.gland (m) | 15 | 12.29 | 16.87 | 0.4-63.2 | | |
| Ovaries | 11 | 32.47 | 41.85 | 0.1-125.0 | -0.31 | >0.05 |
| vs | | | | | | |
| Testes | 10 | 22.51 | 23.15 | 0.9-69.4 | | |

Table 8.11: Results of the Mann-Whitney test for the differences in carapace, muscle and gill lead concentrations ($\mu g.g^{-1}$ wet mass) of male (m) and female (f) crabs.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (f) | 15 | 16.26 | 16.42 | 2.64-51.28 | -0.289 | >0.05 |
| vs | | | | | | |
| Carapace (m) | 18 | 35.67 | 76.07 | 1.95-329.63 | | |
| Muscle (f) | 11 | 15.89 | 25.17 | 0.9-85.7 | -0.261 | >0.05 |
| vs | | | | | | |
| Muscle (m) | 13 | 22.3 | 26.76 | 0.8-71.43 | | |
| Gills (f) | 11 | 6.27 | 8.39 | 0.0-25.02 | -1.425 | >0.05 |
| vs | | | | | | |
| Gills (m) | 14 | 19.36 | 30.12 | 0.9-102.94 | | |

Table 8.12: Results of the Mann-Whitney test for the seasonal differences in whole crab lead concentrations ($\mu g.g^{-1}$ wet mass).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 49 | 10.78 | 13.97 | 1.62-66.44 | 2.597 | <0.05 |
| vs | | | | | | |
| Autumn | 16 | 39.25 | 35.89 | 1.25-99.61 | | |
| Summer | 49 | 10.78 | 13.97 | 1.62-66.44 | -1.935 | =0.05 |
| vs | | | | | | |
| Winter | 21 | 21.1 | 37.95 | 0.78-164.7 | | |
| Summer | 49 | 10.78 | 13.97 | 1.62-66.44 | -1.309 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 13.82 | 18.23 | 1.16-64.83 | | |
| Autumn | 16 | 39.25 | 35.89 | 1.25-99.61 | -2.652 | <0.05 |
| vs | | | | | | |
| Winter | 21 | 21.1 | 37.95 | 0.78-164.7 | | |
| Autumn | 16 | 39.25 | 35.89 | 1.25-99.61 | -2.713 | <0.05 |
| vs | | | | | | |
| Spring | 25 | 13.82 | 18.23 | 1.16-64.83 | | |
| Winter | 21 | 21.1 | 37.95 | 0.78-164.7 | 1.434 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 13.82 | 18.23 | 1.16-64.83 | | |

Table 8.13: Results of the Mann-Whitney test for the seasonal differences in crab carapace lead concentrations ($\mu g.g^{-1}$ wet mass).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 18 | 8.82 | 6.24 | 3.08-21.83 | 0.112 | >0.05 |
| vs | | | | | | |
| Autumn | 5 | 15.0 | 15.97 | 1.03-35.16 | | |
| Summer | 18 | 8.82 | 6.24 | 3.08-21.83 | 1.168 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 81.02 | 124.64 | 0.73-329.63 | | |
| Summer | 18 | 8.82 | 6.24 | 3.08-21.83 | -0.672 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 19.32 | 21.77 | 1.95-55.08 | | |
| Autumn | 5 | 15.0 | 15.97 | 1.03-35.16 | 0.822 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 81.02 | 124.64 | 0.73-329.63 | | |
| Autumn | 5 | 15.0 | 15.97 | 1.03-35.16 | 0.184 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 19.32 | 21.77 | 1.95-55.08 | | |
| Winter | 6 | 81.02 | 124.64 | 0.73-329.63 | -0.706 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 19.32 | 21.77 | 1.95-55.08 | | |

Table 8.14: Results of the Mann-Whitney test for differences in crab muscle lead concentrations ($\mu g.g^{-1}$ wet mass) of summer and the other seasons.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 16.15 | 27.23 | 0.8-85.7 | 0.0 | >0.05 |
| vs | | | | | | |
| Autumn | 4 | 6.55 | 6.55 | 0.6-12.89 | | |
| Summer | 15 | 16.15 | 27.23 | 0.8-85.7 | 0.751 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 29.43 | 31.42 | 0.4-71.43 | | |
| Summer | 15 | 16.15 | 27.23 | 0.8-85.7 | 0.974 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 21.86 | 23.64 | 0.3-63.63 | | |

Table 8.15: Results of Student's t-test for differences in mean crab muscle lead concentration ($\mu g.g^{-1}$ wet mass) of autumn, winter and spring (Mean logarithms of seasonal lead concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Autumn | 4 | 6.55 | 6.55 | 0.6-12.89 | -0.83 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 29.43 | 31.42 | 0.4-71.43 | | |
| Autumn | 4 | 6.55 | 6.55 | 0.6-12.89 | -0.93 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 21.86 | 23.64 | 0.3-63.63 | | |
| Winter | 4 | 29.43 | 31.42 | 0.4-71.43 | 0.07 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 21.86 | 23.64 | 0.3-63.63 | | |

Table 8.16: Results of the Mann-Whitney test for differences in crab gill lead concentrations ($\mu g.g^{-1}$ wet mass) of summer and the other seasons.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 5.89 | 7.65 | 0.9-26.9 | 1.665 | >0.05 |
| vs | | | | | | |
| Autumn | 3 | 39.65 | 54.81 | 8.0-102.94 | | |
| Summer | 15 | 5.89 | 7.65 | 0.9-26.9 | 2.02 | <0.05 |
| vs | | | | | | |
| Winter | 3 | 26.87 | 19.3 | 8.56-47.02 | | |
| Summer | 15 | 5.89 | 7.65 | 0.9-26.9 | 0.0 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 15.78 | 25.89 | 0.0-61.4 | | |

Table 8.17: Results of Student's t-test for differences in mean crab gill lead concentration ($\mu g.g^{-1}$ wet mass) of autumn, winter and spring (Mean logarithms of the seasonal lead concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Autumn | 3 | 39.65 | 54.81 | 8.0-102.94 | -0.14 | >0.05 |
| vs | | | | | | |
| Winter | 3 | 26.87 | 19.3 | 8.56-47.02 | | |
| Autumn | 3 | 39.65 | 54.81 | 8.0-102.94 | 1.08 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 15.78 | 25.89 | 0.0-61.4 | | |
| Winter | 3 | 26.87 | 19.3 | 8.56-47.02 | 1.25 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 15.78 | 25.89 | 0.0-61.4 | | |

Table 8.18: Results of Student's t-test for the seasonal differences in mean crab digestive gland lead concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of lead concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 4.07 | 4.26 | 0.7-14.8 | -1.77 | <0.05 |
| vs | | | | | | |
| Autumn | 5 | 17.24 | 26.01 | 1.0-63.2 | | |
| Summer | 15 | 4.07 | 4.26 | 0.7-14.8 | 0.3 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 6.34 | 9.33 | 0.3-24.56 | | |
| Summer | 15 | 4.07 | 4.26 | 0.7-14.8 | 0.25 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 8.57 | 12.23 | 0.0-31.96 | | |
| Autumn | 5 | 17.24 | 26.01 | 1.0-63.2 | 1.22 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 6.34 | 9.33 | 0.3-24.56 | | |
| Autumn | 5 | 17.24 | 26.01 | 1.0-63.2 | 1.12 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 8.57 | 12.23 | 0.0-31.96 | | |
| Winter | 6 | 6.34 | 9.33 | 0.3-24.56 | -0.02 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 8.57 | 12.23 | 0.0-31.96 | | |

Table 8.19: Results of Student's t-test for the seasonal differences in mean crab gonad lead concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of the lead concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 13 | 20.59 | 34.64 | 0.1-125.0 | 0.05 | >0.05 |
| vs | | | | | | |
| Autumn | 4 | 16.39 | 18.04 | 0.8-34.8 | | |
| Summer | 13 | 20.59 | 34.64 | 0.1-125.0 | 0.09 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 29.01 | 40.83 | 0.2-87.39 | | |
| Summer | 13 | 20.59 | 34.64 | 0.1-125.0 | -0.47 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 34.17 | 36.5 | 0.43-69.4 | | |
| Autumn | 4 | 16.39 | 18.04 | 0.8-34.8 | 0.03 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 29.01 | 40.83 | 0.2-87.39 | | |
| Autumn | 4 | 16.39 | 18.04 | 0.8-34.8 | -0.38 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 34.17 | 36.5 | 0.43-69.4 | | |
| Winter | 4 | 29.01 | 40.83 | 0.2-87.39 | -0.36 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 34.17 | 36.5 | 0.43-69.4 | | |

Table 8.20: Results of the Mann-Whitney test for differences in whole crab ($\mu g.g^{-1}$ wet mass) and water lead concentrations ($mg.l^{-1}$) at each locality: Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_W$) calculated for whole crabs (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water (J) | 8 | 0.03 | 0.02 | 0.01-0.06 | 4.004 | <0.05 | 528.0 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 15.84 | 19.8 | 0.78-75.62 | | | |
| Water (S) | 8 | 0.04 | 0.03 | 0.02-0.08 | 3.966 | <0.05 | 487.25 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 19.49 | 32.36 | 1.16-164.7 | | | |

Table 8.21: Results of Student's t-test for differences in mean crab tissue and carapace ($\mu g.g^{-1}$ wet mass), and water lead concentration ($mg.l^{-1}$) at each locality, as well as the bioconcentration factor ($BCF_W$) calculated for all tissues and the carapace (Dig.gland = digestive gland; t = t-value, p = p-value).

| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_W$ |
| **Water** | 8 | 0.03 | 0.02 | 0.01-0.06 | | | |
| vs Carapace | 19 | 17.5 | 17.23 | 0.73-55.08 | -12.73 | <0.05 | 583.33 |
| vs Muscle | 16 | 14.15 | 18.98 | 0.4-63.63 | -8.91 | <0.05 | 471.67 |
| vs Dig.gland | 18 | 5.72 | 8.42 | 0.0-31.96 | -8.44 | <0.05 | 190.67 |
| vs Gills | 14 | 9.52 | 16.51 | 0.0-61.4 | -8.62 | <0.05 | 317.33 |
| vs Gonads | 14 | 19.46 | 23.6 | 0.2-69.4 | -8.17 | <0.05 | 648.67 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_W$ |
| **Water** | 8 | 0.04 | 0.03 | 0.02-0.08 | | | |
| vs Muscle | 13 | 22.38 | 31.04 | 0.3-85.7 | -7.27 | <0.05 | 559.5 |
| vs Dig.gland | 15 | 9.49 | 16.12 | 0.2-63.2 | -8.18 | <0.05 | 237.25 |
| vs Gills | 12 | 19.46 | 29.45 | 0.9-102.94 | -9.19 | <0.05 | 486.5 |
| vs Gonads | 11 | 28.5 | 41.86 | 0.1-125.0 | -6.29 | <0.05 | 712.5 |

Table 8.22: Results of the Mann-Whitney test for differences in crab carapace ($\mu g.g^{-1}$ wet mass) and water lead concentrations ($mg.l^{-1}$) from SFW, as well as the bioconcentration factor ($BCF_W$) calculated for the carapace (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water | 8 | 0.04 | 0.03 | 0.02-0.08 | 3.625 | <0.05 | 725.75 |
| vs Carapace | 20 | 29.03 | 73.0 | 1.95-329.63 | | | |

Table 8.23: Results of the Mann-Whitney test for the differences in lead concentrations ($\mu g.g^{-1}$ wet mass) of whole crabs and sediments from the two localities, Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for whole crabs (z = z-value, p = p-value).

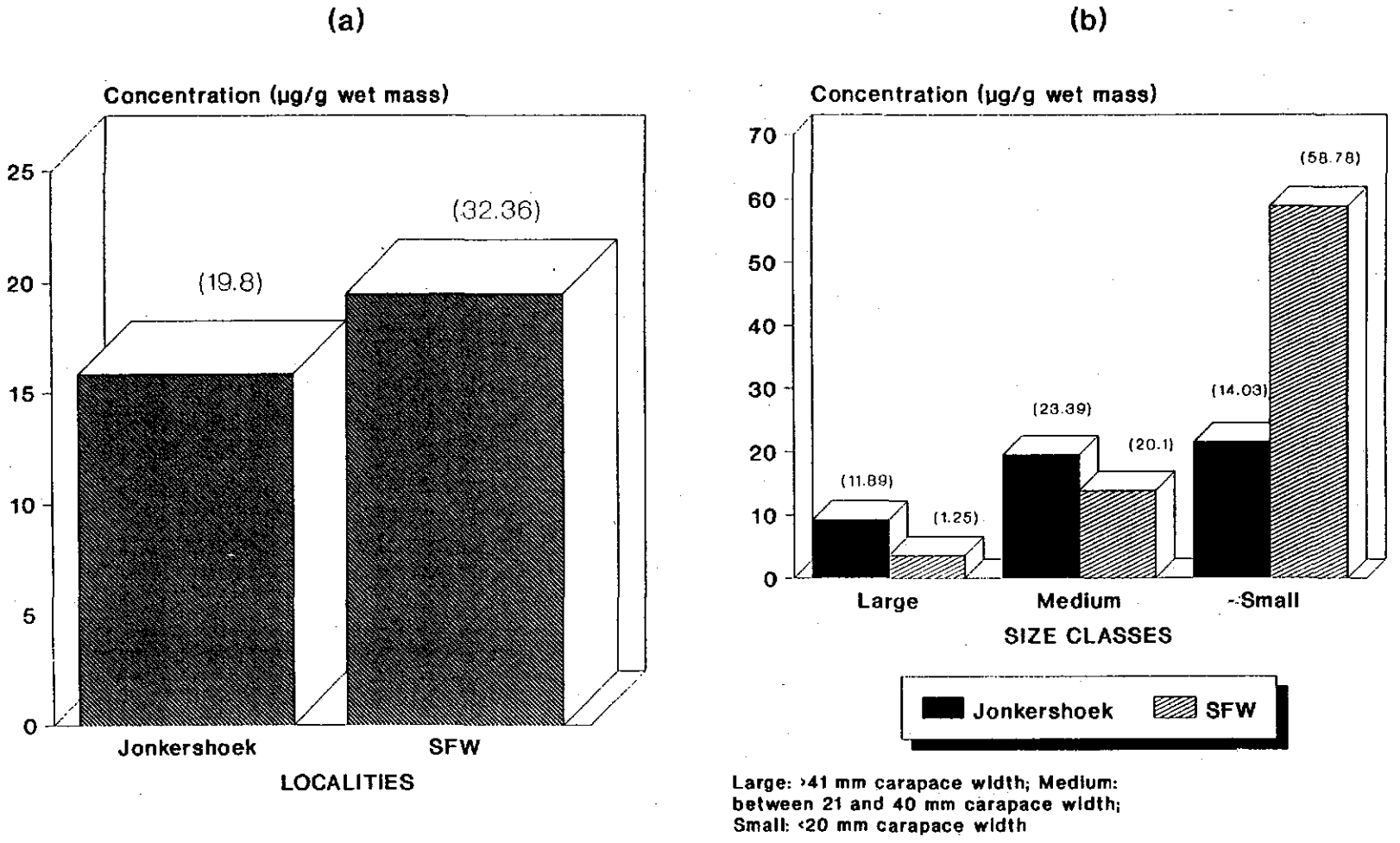
| | n | Mean | SD | Range | z | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 4.38 | 2.96 | 1.0-8.35 | 1.149 | >0.05 | 3.62 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 15.84 | 19.8 | 0.78-75.62 | | | |
| Sediment (S) | 8 | 5.81 | 4.35 | 1.17-12.52 | 0.481 | >0.05 | 3.35 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 19.49 | 32.36 | 1.16-164.7 | | | |

Table 8.24: Results of Student's t-test for differences in mean lead concentration ($\mu g.g^{-1}$ wet mass) of the sediments and crab tissues at each locality: Jonkershoek and SFW, as well as the bioconcentration factor ($BCF_S$) calculated for all tissues (Dig.gland = digestive gland; t = t-value, p = p-value).

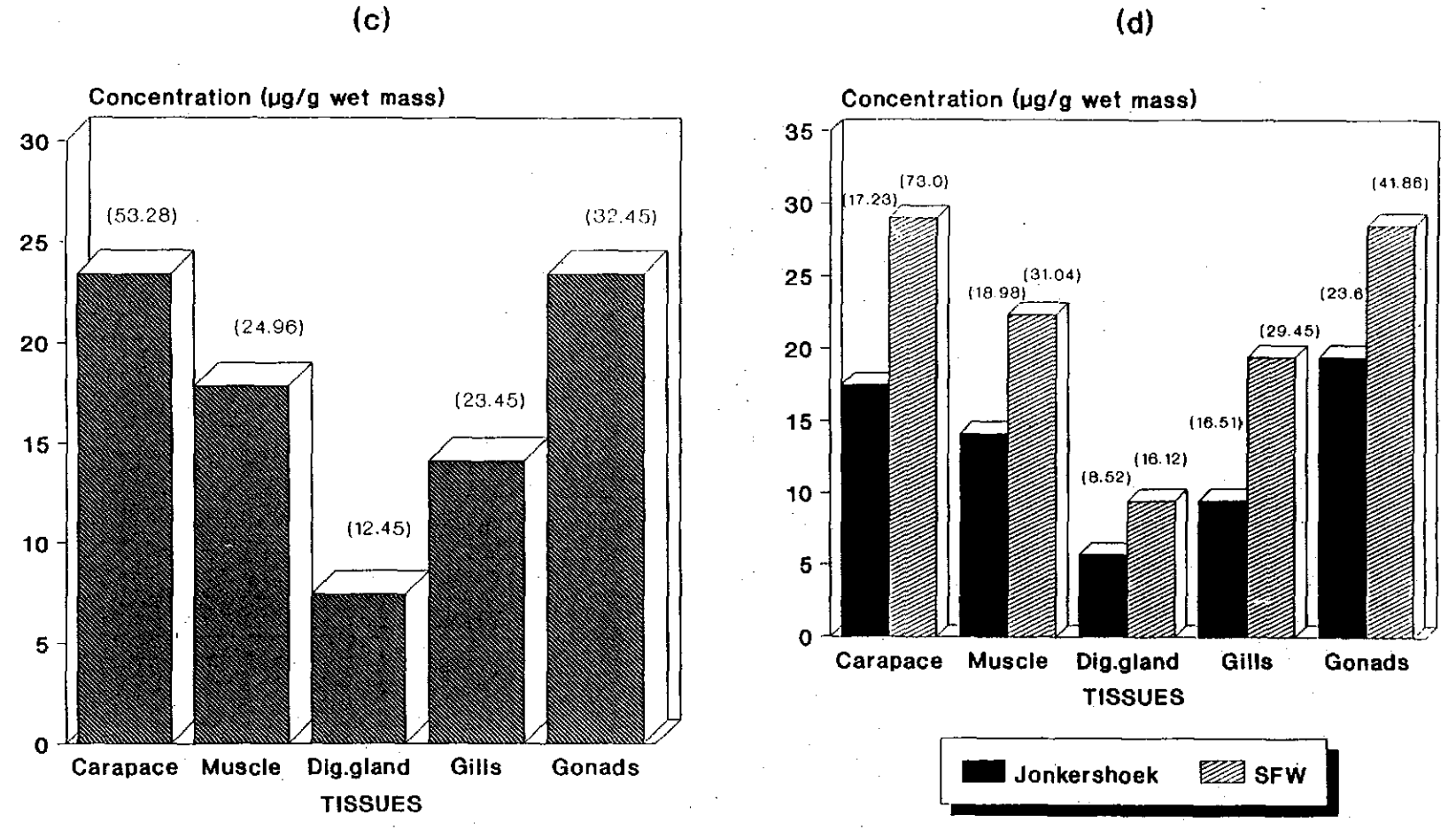
| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 4.38 | 2.96 | 1.0-8.35 | | | |
| vs Muscle | 16 | 14.15 | 18.98 | 0.4-63.63 | -0.69 | >0.05 | 3.23 |
| vs Dig.gland | 18 | 5.72 | 8.52 | 0.0-31.96 | 0.73 | >0.05 | 1.31 |
| vs Gills | 14 | 9.52 | 16.51 | 0.0-61.4 | -0.00 | >0.05 | 2.17 |
| vs Gonads | 14 | 19.46 | 23.6 | 0.2-69.4 | -0.87 | >0.05 | 4.44 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 5.81 | 4.35 | 1.17-12.52 | | | |
| vs Muscle | 13 | 22.38 | 31.04 | 0.3-85.7 | -0.43 | >0.05 | 3.85 |
| vs Dig.gland | 15 | 9.49 | 16.12 | 0.2-63.2 | 0.27 | >0.05 | 1.63 |
| vs Gills | 12 | 19.46 | 29.45 | 0.9-102.94 | -0.88 | >0.05 | 3.35 |
| vs Gonads | 11 | 28.5 | 41.86 | 0.1-125.0 | -0.42 | >0.05 | 4.91 |

Table 8.25: Results of the Mann-Whitney test for differences in lead concentrations ($\mu g.g^{-1}$ wet mass) of crab carapace and sediment from Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for the carapace (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 4.38 | 2.96 | 1.0-8.35 | 1.495 | >0.05 | 4.0 |
| vs | | | | | | | |
| Carapace (J) | 20 | 17.5 | 17.23 | 0.73-55.08 | | | |
| Sediment (S) | 8 | 5.81 | 4.35 | 1.17-12.52 | 0.518 | >0.05 | 5.0 |
| vs | | | | | | | |
| Carapace (S) | 20 | 29.03 | 73.0 | 1.95-329.63 | | | |

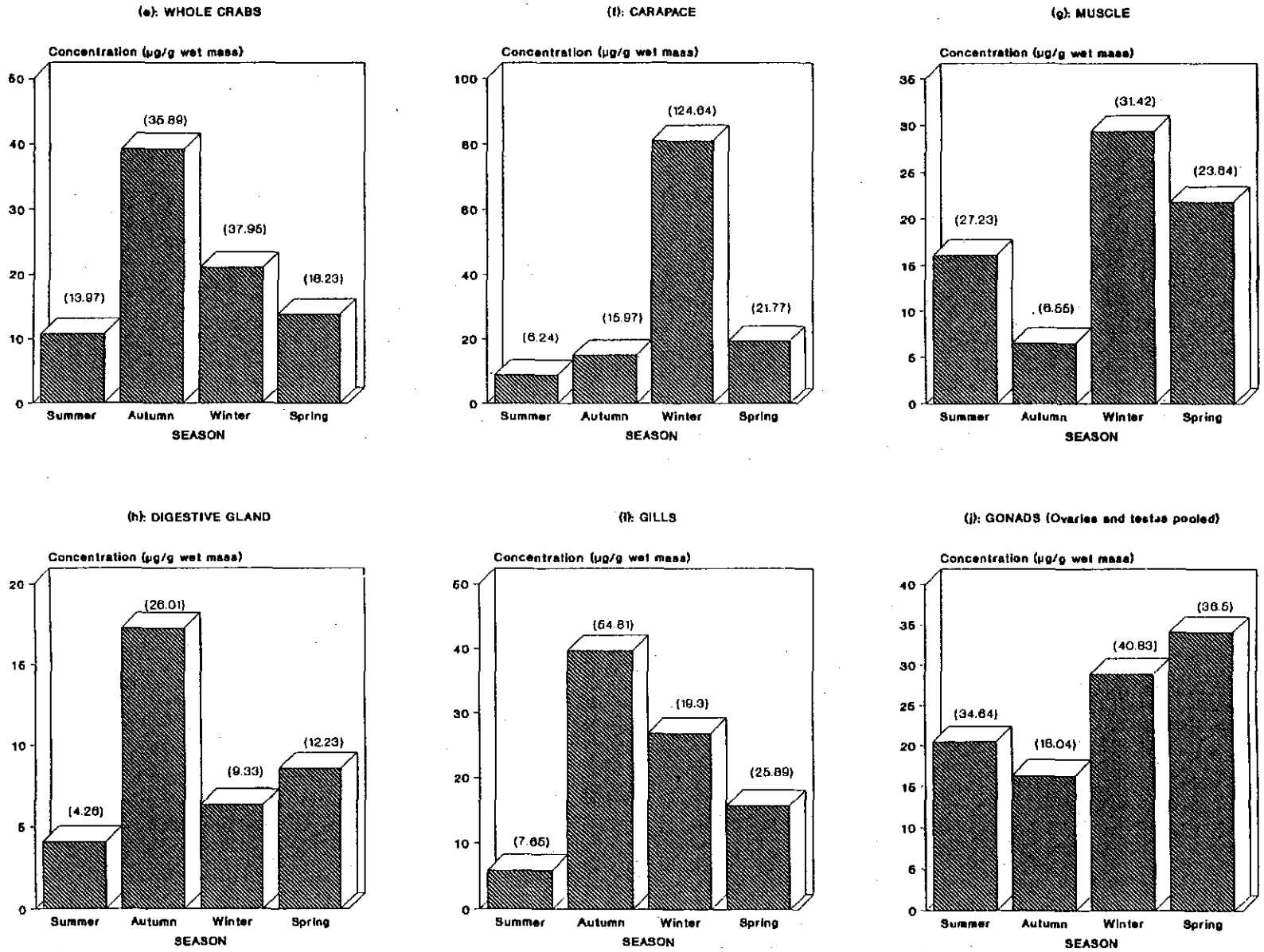



Figures 8(a)-(b): Mean lead concentration in whole crabs (a) and various size classes (b) from both localities (SD in parenthesis).




Jonkershoek and SFW data pooled

Figures 8(c)-(d): Mean lead concentration in carapace and tissues of *P. perlatus* from the Eerste River (SD in parenthesis).




Figures 8(e)-(j): Mean seasonal lead concentration in whole crabs, carapace and tissues of *P. perlatus* (Jonkershoek and SFW data pooled; SD in parenthesis).

**Discussion**

Comparisons of the mean wet mass lead concentrations determined for *Potamonautes perlatus* (Table 8.3; Figure 8(c)), with those found by Steenkamp (1992) and du Preez et al. (1993) for *P. warreni* from polluted freshwater ecosystems, were made. Steenkamp (1992) found mean Pb concentrations of 8.3±7.3 to 30.0±44.1 $\mu g.g^{-1}$, 7.0±7.0 to 21.0±28.2 $\mu g.g^{-1}$, 6.4±6.3 to 29.8±161.2 $\mu g.g^{-1}$, 6.7±7.9 to 38.9±58.5 $\mu g.g^{-1}$ and 8.0±16.3 to 50.5±67.7 $\mu g.g^{-1}$ in the digestive gland, muscles, gills, gonads and carapace respectively. The results of du Preez et al. (1993) for the carapace and muscle tissue, differed from those of Steenkamp (1992). The former found mean Pb concentrations of 86.6±73.4 $\mu g.g^{-1}$ wet mass in the carapace and 3.0±1.4 $\mu g.g^{-1}$ wet mass in the muscles. In comparison with Steenkamp's results, the mean Pb concentration in the digestive gland of *P. perlatus* was found to be relatively low, whilst the concentrations in the carapace and remaining tissues compared well. Comparisons with the results of du Preez et al. showed that the mean Pb concentrations in the carapace and muscles of *P. perlatus* were comparatively very low and high respectively.

When the dry mass whole crab lead concentrations of *P. perlatus* (4.3±5.65 $\mu g.g^{-1}$ for Jonkershoek and 5.49±9.27 $\mu g.g^{-1}$ for SFW), were compared with the findings of Van Eeden & Schoonbee (1991) (2.0±0.06 to 13.9±0.06 $\mu g.g^{-1}$), who studied *P. warreni* from a mine-polluted wetland, it was found that the mean concentrations in *P. perlatus* compared favourably, but that the individual variations in Pb concentrations were greater and the highest whole crab concentration from each population (22.83 and 52.5 $\mu g.g^{-1}$ dry mass for Jonkershoek and SFW respectively) was higher than in *P. warreni* (13.96 $\mu g.g^{-1}$ dry mass). This in itself is a cause for concern and indicates an accumulation of lead by *P. perlatus*. The theory of Pb accumulation in this species is supported by the high $BCF_W$'s and relatively high $BFC_S$'s found for whole crabs (Tables 8.20 and 8.23), tissues and carapace (Tables 8.21, 8.22, 8.24 and 8.25) at both localities. Furthermore, comparisons of data from the two localities yielded no statistically significant differences between whole crab, tissue, carapace, water or sediment Pb concentrations (Tables 8.20-8.25), indicating that accumulation of Pb took place at both localities.

It is therefore concluded that *P. perlatus* is not able to regulate the concentrations of Pb in its body and that accumulation takes place, even in aquatic environments where the Pb concentrations in the water and sediments are low and not considered a problem (see Chapter 4).

An investigation into the distribution of Pb in *P. perlatus* showed that the carapace and gonads contained the highest lead concentrations, whilst the digestive gland contained the lowest concentrations (Tables 8.3 and 8.4). These results were also substantiated by the higher $BCF_W$'s and $BCF_S$'s determined for the carapace in SFW and the gonads in Jonkershoek (Tables 8.21, 8.22. 8.24 and 8.25). Similarly, Steenkamp (1992) and du Preez et al. (1993) found the highest

Pb concentrations in the carapace of *P. warreni*, whilst Tulasi et al. (1987) observed the highest concentrations in the haemolymph and gills of *Barytelphusa guerini*.

Anderson & Brower (1978) suggested that Pb may be incorporated in the exoskeleton, so that this becomes a potential sink for Pb. This theory could explain the higher Pb concentrations in the carapace of *P. perlatus*. It is important to note that, due to periodic moulting, soft tissue burdens of this metal would be eliminated and long term accumulation would not occur (Anderson & Brower, 1978). Tulasi et al. (1987) reported that Pb is mostly absorbed through the gills and distributed via the haemolymph to the internal organs (and exoskeleton). Contamination of the internal organs by the haemolymph might explain the relatively high mean concentrations observed in the gonads, compared to the other tissues. Though the Pb concentrations in the digestive gland of *P. perlatus* were found to be relatively low, Pb concentrations in the digestive glands of decapods in general may be expected to increase dramatically upon exposure to this metal: Roldan & Shivers (1987) exposed crayfish, *Orconectes propinquus*, to low doses of lead acetate, which resulted in the accumulation of fine electron-dense particles, possibly lead, in the R-cells of the digestive gland. They stated that this mechanism prevents contact of excess metal with vital cell constituents and effectively detoxifies the metal until it is eliminated or passed on to other tissues.

It is concluded that in *P. perlatus*, the carapace is the most important Pb storage site, with the gonads being the second most important site. The remaining three tissue types do not seem to vary greatly in their contributions to Pb storage.

The possible relationship between crab body size and whole crab, tissue and carapace lead concentrations was investigated. The reasonably strong negative correlations between whole crab ($r = -0.466$), muscle, carapace, gonad and gill lead concentrations and size of crab (Table 8.2), as well as the statistically significant difference in lead concentration between medium and small crabs from SFW (Tables 8.1), showed that size (therefore age), can be considered to have some influence on the uptake and distribution of lead in *P. perlatus*. These findings are in agreement with those of Steenkamp (1992) and du Preez et al. (1993) for *P. warreni*. The latter found that the Pb concentrations in the digestive gland and muscles were significantly higher in immature crabs than in mature crabs. Steenkamp (1992) concluded that younger individuals seemed to be able to accumulate more Pb per unit weight than mature crabs. She ascribed this to the higher metabolic rates of the smaller/younger individuals. Hill & O'Keeffe (1991) found significant differences in the diets of large and small individuals of *P. perlatus*. Smaller crabs were shown to prey significantly more on aquatic invertebrates, whilst the larger and more mature crabs preferred vegetable material. Since it has been shown by several authors, e.g. Albers & Camardese (1993) and Kiffney & Clements (1993) that aquatic invertebrates (e.g. insects) accumulate heavy metals, bioaccumulation from their prey might be a plausible explanation for the observed higher Pb concentrations in the smaller individuals.

Investigations into the influence of gender on the whole crab, tissue and carapace Pb showed only one significant difference, namely between male and female digestive gland concentrations (Tables 8.9-8.11). This observed general lack of difference between the two genders agrees with the findings of Anderson & Brower (1978) for the crayfish *Orconectes virilis*. Steenkamp (1992) also found only one difference in Pb concentrations between male and female *Potamonautes warreni*, namely a significantly higher concentration in the mature ovaries than in the testes. It is therefore concluded that although gender may affect the concentrations of Pb in the digestive gland, it does not have a significant influence, generally, on lead uptake and distribution in *P. perlatus*.

It was ascertained, through the observed statistically significant differences in seasonal whole crab Pb concentrations, as well as the few seasonal variations in gill and digestive gland Pb concentrations (Tables 8.12-8.19), that seasonality can be considered an influencing factor in Pb uptake and distribution. Whereas *P. perlatus* exhibited autumn peaks in the Pb content of whole crabs, gills and digestive gland, winter peaks in carapace and muscle Pb content and a spring peak in gonad Pb content (Figures 8(e)-(j)), Steenkamp (1992) reported that *P. warreni* exhibited Pb peaks in tissues, only in the warmer months. Although the peaks in tissue and carapace Pb in *P. perlatus* did not follow the Pb peaks in water and sediment Pb closely (Figures 4(c)-(l)), the lowest Pb concentrations were found in summer for most of the tissues as well as for the water and sediments. This may indicate a relationship between environmental Pb concentrations and the concentrations in *P. perlatus*. The clear seasonal variations in whole crab Pb concentrations are also indications that lead is accumulated periodically, to some extent, in this species. However, it also implies that long term Pb accumulation does not occur and that excesses of the metal are excreted periodically, probably through moulting (Anderson & Brower, 1978).

Finally, although large intraspecific variation in Pb concentrations was observed for *P. perlatus* in the Eerste River, and although the seasonal fluctuations in environmental Pb were not accurately reflected in the crab, it is concluded from the preliminary results, that this species in the Eerste River can be considered as a potential monitor of environmental Pb pollution. This is based entirely on the fact that Pb accumulation was shown to occur at both localities. It must, however, be remembered that long term Pb accumulation probably does not occur, due to the carapace being an important store of Pb and periodic moulting taking place. This in itself suggests that *P. perlatus* might in fact not be a suitable monitor. Steenkamp (1992) also concluded that *P. warreni* may be useful as a potential indicator of Pb pollution but that there is no guarantee that environmental Pb concentrations will be accurately reflected by the tissue concentrations. However, final conclusions can only be made after further intensive research on especially the accumulation and distribution of Pb in *P.perlatus*, has been done.

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# CHAPTER 9

## CONCENTRATIONS OF CADMIUM IN THE FRESHWATER CRAB, *POTAMONAUTES PERLATUS*

### Introduction

Cadmium displays chemical similarity to and occurs together with zinc. It is contained in many inorganic compounds, of which several are quite soluble in water, e.g. the acetates, chlorides and sulfates. The common cadmium minerals are greenockite (hexagonal CdS), hawleyite (cubic CdS), otavite ($CdCO_3$), monteponite (CdO) and cadmoselite (hexagonal CdSe).

Cadmium is obtained as a by-product from the refining of zinc and other metals, particularly copper and lead. The world production of cadmium in 1970 was 16 000 tons. It had increased yearly by 14% during the preceding five years (Förstner, 1980 and Friberg et al., 1979). This metal is used in a number of industrial processes, of which electroplating is the most important. Other uses include in copper-cadmium alloys, for the production of fungicides, in nickel-cadmium batteries and as a stabilizer in PVC. Of these major industries employing cadmium, electroplating shops, pigment plants and producers of alloys and batteries can be expected to be major sources of cadmium pollution (Förstner, 1980; Friberg et al., 1979; Moore & Ramamoorthy, 1984 and Richardson & Gangolli, 1993).

Cadmium can reach riverine ecosystems via industrial and, to a lesser extent, agricultural runoff and pose a great threat to aquatic fauna, since it is known to be bioaccumulated and concentrated in the food chain (Richardson & Gangolli, 1993). Cadmium is regarded as one of the most toxic metals and may cause various symptoms and illnesses when taken in, either via the respiratory or the digestive system, e.g. nausea, vomiting, osteomalacia, anemia and disturbed liver function. It has been shown that injections of single doses of soluble cadmium salts (1-3 mg/kg body weight) give rise to dramatic effects in the testicles of several experimental animal species. Effects on non-ovulating ovaries of females as well as on sensory ganglia have also been reported. All these organs undergo complete destruction within hours after injection, without evident damage to other organs. It is also known that ingestion of highly contaminated food results in acute gastrointestinal effects (Förstner, 1980 and Friberg et al., 1979).

There are several factors which may influence the toxicity and bioavailability of cadmium in aquatic environments: crustaceans appear to be the most sensitive group to cadmium levels in the water, followed by molluscs and polychaetes. It also seems that, depending on the species, cadmium concentrations in specific organs may either increase or decrease with temperature and reproductive activity.

Cd-Zn interactions are thought to be of special importance in cadmium toxicity. A biochemical explanation is offered by the replacement of zinc by cadmium in zinc-dependent enzymes. The toxicity of cadmium is also affected by calcium levels: the latter has an antagonistic effect on the toxicity of the former (Friberg et al., 1979 and Moore & Ramamoorthy, 1984). Other influencing factors include water pH, hardness, salinity, temperature and the presence of ligands and co-existing metal cations: a lower pH increases the solubility of the metal, thus increasing its availability, whereas high concentrations of calcium and magnesium salts reduce cadmium uptake. It has also been shown that an increased salinity decreases cadmium toxicity to many estuarine species, and low temperature enhances the survival of most organisms (Rosenberg & Costlow, 1976). Waters rich in humic compounds contain the lowest Cd levels (Galvin, 1996; Moore & Ramamoorthy, 1984 and Richardson & Gangolli, 1993). Rainbow & Scott (1979) and Lyon (1984) illustrated a possible Cd detoxification mechanism, through Cd-binding proteins in the digestive glands of the crab *Carcinus maenas* and the crayfish *Austropotamobius pallipes* respectively.

Studies on the accumulation of cadmium by freshwater decapod crustaceans has as yet been neglected. The only known studies to date are those undertaken by Anderson & Brower (1978) and France (1987), on the crayfish *Orconectes virilis* and Lyon (1984) on the crayfish *Austropotamobius pallipes*.

## Materials and Methods

All samples were prepared, tested and statistically analysed according to the method described in Chapter 3.

## Results

In all cases only the cadmium concentrations per gram wet mass were used for statistical analysis. Crabs showed large individual variation in whole body, tissue as well as carapace cadmium concentrations. This was an important consideration in the interpretation of the statistical results.

### The relationship between crab size and whole crab cadmium concentration

In order to investigate the possible relationship between whole crab cadmium concentrations and the sizes of the crabs, the data from Jonkershoek and SFW were pooled. The carapace widths of

the crabs, in decreasing order, were compared with the respective cadmium concentrations and the correlation coefficient (r) thereof, calculated. With 53 degrees of freedom and an $r^2$-value of 27.23%, an r-value of -0.522 was obtained. This indicated a reasonably strong negative correlation between size of crab and the concentration of cadmium in the body: as size decreased, so did the concentrations increase (Figure 9(b)).

Differences in cadmium concentrations of the various size classes at each locality

Since the whole crab concentration data for large, medium and small-sized crabs from each locality were not normally distributed, the cadmium concentrations for each size class were logarithmically transformed. Apart from the large and small size classes from SFW, all transformed data sets also failed the normal probability test, therefore the Mann-Whitney nonparametric test was used to test for statistically significant differences ($p<0.05$) (Table 9.1).

Differences were found between cadmium concentrations in large and small, and in medium and small crabs from SFW. The small size class had the highest mean cadmium concentration ($16.65\pm9.97$ $\mu g.g^{-1}$ wet mass) and large sized crabs the lowest ($2.08\pm0.82$ $\mu g.g^{-1}$ wet mass).

The relationship between crab size, and tissue and carapace cadmium concentration

In order to investigate the possible relationship between crab size and cadmium concentration in the selected tissues, namely muscle tissue, digestive gland, gills and gonads, as well as the carapace, the data from Jonkershoek and SFW were pooled, after which the carapace widths of crabs, in decreasing order, were compared with the respective tissue or carapace cadmium concentration. Table 9.2 shows the r-values calculated for each tissue and for the carapace.

Reasonably strong negative correlations were found between crab size and the cadmium concentration in the carapace and all but one of the four tissue types (muscle). The cadmium concentration in the latter proved to be poorly negatively correlated with the size of the crabs.

Differences in cadmium concentrations of the various selected tissues and the carapace

The cadmium concentration values for the selected tissues and the carapace of crabs from Jonkershoek and SFW were pooled, in order to determine whether statistically significant differences ($p<0.05$) existed between the various tissues and the carapace. Since the data were not normally distributed, the mean logarithms of the tissue and carapace cadmium concentrations were used in Student's t-test. The logarithmically transformed data sets for the carapace and muscle tissue, however, also failed to provide a normal distribution, thus the Mann-Whitney test was performed in order to compare these the carapace and muscles with the other three tissue types. The respective mean concentrations and standard deviations are tabulated in Table 9.3.

Tables 9.4 and 9.5 show the results of the t-test and Mann-Whitney test performed on the tissue and carapace concentrations.

When comparing values obtained for the selected tissues and the carapace, significant differences in cadmium concentrations were found for muscle and carapace, digestive gland and gonads, digestive gland and carapace, and gills and carapace. The gonads showed the highest mean concentration (5.29±6.46 $\mu g.g^{-1}$ wet mass), and the digestive gland the lowest (2.21±3.37 $\mu g.g^{-1}$ wet mass) (Figure 9(c)).

Differences in cadmium concentrations of whole crabs from Jonkershoek and SFW

(a) Differences in whole crab concentrations
The result of the Mann-Whitney test performed on the whole crab cadmium concentrations from Jonkershoek and SFW are shown in Table 9.6.

The highest mean cadmium concentration (5.04±6.26 $\mu g.g^{-1}$ wet mass), occurred in crabs from SFW (Figure 9(a)), but a z-value of 0.896 indicated that there was no statistically significant difference ($p>0.05$) between whole crab cadmium concentrations of the two localities.

(a)(i) Differences in whole crab concentrations of the various size classes
Through comparisons of the whole crab cadmium concentration values between large-sized crabs and between medium-sized crabs, as well as a comparison of the mean logarithms of whole crab cadmium concentrations between small-sized crabs from Jonkershoek and SFW, it was established that a statistically significant difference ($p<0.05$) existed between the small size class crabs from the two localities. The small crabs from SFW had the highest mean cadmium concentration of the two localities (16.65±9.97 $\mu g.g^{-1}$ wet mass) (Tables 9.7 and 9.8).

(b) Differences in concentration values for the selected tissues and the carapace
The mean cadmium concentration of the digestive gland, gills and gonads of crabs from Jonkershoek and SFW were compared using of Student's t-test, and tabulated in Table 9.9, while the cadmium concentrations of the carapace and muscle tissue of crabs from the two localities were compared by means of the Mann-Whitney test, and recorded in Table 9.10.

Although the mean Cd concentrations in all the tissues and the carapace were higher in crabs collected from SFW (Figure 9(d)), no statistically significant differences in the cadmium concentrations of any of the tissues or the carapace were found between the two localities.

Differences in cadmium concentrations of males and females

In order to determine whether statistically significant differences ($p<0.05$) existed between the mean whole crab, tissue and carapace cadmium concentrations of males and females, the data obtained from Jonkershoek and SFW were pooled for each sex. In the case of the muscle tissue, the data for both sexes failed the normal probability test, therefore the Mann-Whitney test was performed.

(a) Differences in whole crab concentrations

Table 9.11 shows the result of Student's t-test, performed on the mean logarithms of the whole crab cadmium concentrations of males and females of *Potamonautes perlatus*. The highest mean cadmium concentration was found in males (5.48±6.13 μg.g-1 wet mass) but no statistically significant difference ($p>0.05$) existed between the two genders.

(b) Differences in concentration values for the selected tissues and the carapace

Although males exhibited the highest mean cadmium concentration in the carapace and all but one of the four tissue types (gonads), a significant difference was found only in one case, namely between the digestive gland cadmium concentrations of males and females (Tables 9.12 and 9.13).

Seasonal variations in cadmium concentrations

The cadmium concentrations in whole crabs, selected tissues and carapace from the different seasons were tested for statistically significant differences ($p<0.05$). In each instance, the data for crabs collected at Jonkershoek and SFW were pooled and these then tested for normality. None of the data sets were normally distributed and were thus logarithmically transformed. The transformed whole crab data also failed the normal probability test, therefore the Mann-Whitney test was performed.

(a) Seasonal differences in whole crab cadmium concentrations

The Mann-Whitney nonparametric test produced no statistically significant differences ($p>0.05$) between whole crab cadmium concentrations in the four seasons (Table 9.14). The highest mean whole crab cadmium concentration (5.77±5.92 $\mu g.g^{-1}$ wet mass), was observed for autumn, whereas summer showed the lowest mean (3.45±3.09 $\mu g.g^{-1}$ wet mass) (Figure 9(e)).

(b) Seasonal differences in tissue and carapace cadmium concentrations

1. Carapace

A statistically significant difference ($p<0.05$) between carapace cadmium concentrations in summer and autumn was found. Data for winter showed the highest mean cadmium

concentration (7.08±6.7 $\mu g.g^{-1}$ wet mass) and autumn the lowest (2.72±1.42 $\mu g.g^{-1}$ wet mass) (Figure 9(f)). The results of the t-test, performed on the mean logarithms of the seasonal cadmium concentrations, are tabulated in Table 9.15.

2. Muscle tissue

The highest mean cadmium concentration (4.31±3.4 $\mu g.g^{-1}$ wet mass) occurred in winter, whereas in summer the mean concentration was the lowest (1.8±2.08 $\mu g.g^{-1}$ wet mass) (Figure 9(g)). Through pairwise comparisons of the different seasons, it was found that a statistically significant difference in muscle tissue cadmium concentration existed between summer and spring (Table 9.16).

3. Gill tissue

Statistically significant differences ($p<0.05$) between mean gill cadmium concentrations were found when comparing values for summer and the other three seasons. In Table 9.17 it can be seen that the highest and lowest mean cadmium concentrations occurred in autumn (8.13±11.06 $\mu g.g^{-1}$ wet mass) and summer (1.74±2.25 $\mu g.g^{-1}$ wet mass) respectively (Figure 9(h)).

4. Digestive gland

The results of Student's t-test on the mean logarithms of the digestive gland cadmium concentrations, when comparing the different seasons, are tabulated in Table 9.18. No statistically significant differences ($p>0.05$) were found between any of the four seasons. The mean seasonal digestive gland cadmium concentration are shown in Figure 9(i).

5. Gonads

When the data for the ovaries and testes were pooled and tested by means of Student's t-test, no statistically significant differences were found to occur between any of the four seasons (Table 9.19). Figure 9(j) shows the mean seasonal gonad cadmium concentration.

<u>Differences in cadmium concentrations of whole crabs, tissues, carapace, and Eerste River water</u>

(a) Differences in concentration values of whole crabs and water

The Mann-Whitney nonparametric test (Table 9.20) showed significant differences between the whole crab and water cadmium concentrations in both localities, Jonkershoek and SFW.

(b) Differences between concentration values of the selected tissues, carapace and water

Student's t-test was used to investigate possible significant differences between tissue and water cadmium concentrations from the two localities (Table 9.21). In the case of the carapace and muscle tissue from Jonkershoek and the carapace from SFW, the logarithmically transformed data sets were not normally distributed, therefore the Mann-Whitney test was performed (Table 9.22).

The cadmium concentrations in all the tissues and the carapace from both localities proved to be significantly different ($p<0.05$) from the concentrations in the water.

Differences in cadmium concentrations of whole crabs, tissues, carapace, and sediments

(a) Differences between concentration values of whole crabs and sediments
The Mann-Whitney test, performed on the whole crab and sediment cadmium concentrations from Jonkershoek and SFW, yielded statistically significant differences ($p<0.05$) between these concentrations at both localities (Table 9.23).

(b) Differences between concentration values of the selected tissues, carapace and sediments
The mean logarithms of the tissue and carapace cadmium concentrations were used to test for statistically significant differences ($p<0.05$) between tissue, carapace and sediment cadmium concentrations at the two localities. The transformed carapace and muscle tissue data, however, were not normally distributed, therefore the Mann-Whitney test was performed.

The data from both localities showed significant differences between the cadmium concentrations of the sediment, the carapace and all tissues of crabs from Jonkershoek, and between the concentrations in the sediment, the carapace and all but one of the tissues (muscle) of crabs from SFW. Tables 9.24 and 9.25 list the results of Student's and the Mann-Whitney tests respectively.

Table 9.1: Results of the Mann-Whitney test for differences in whole crab cadmium concentrations ($\mu g.g^{-1}$ wet mass) of the various size classes at each locality.

| JONKERSHOEK | | | | | | |
|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | z-value | p-value |
| Large | 22 | 2.73 | 2.09 | 0.62-6.99 | 1.242 | >0.05 |
| vs | | | | | | |
| Medium | 34 | 3.61 | 2.93 | 0.77-11.2 | | |
| Large | 22 | 2.73 | 2.09 | 0.62-6.99 | 1.528 | >0.05 |
| vs | | | | | | |
| Small | 4 | 5.8 | 4.02 | 1.02-10.81 | | |
| Medium | 34 | 3.61 | 2.93 | 0.77-11.2 | 0.88 | >0.05 |
| vs | | | | | | |
| Small | 4 | 5.8 | 4.02 | 1.02-10.81 | | |
| **SFW** | | | | | | |
| | n | Mean | SD | Range | z-value | p-value |
| Large | 6 | 2.08 | 0.82 | 1.01-2.99 | 0.134 | >0.05 |
| vs | | | | | | |
| Medium | 39 | 3.72 | 3.73 | 0.49-14.6 | | |
| Large | 6 | 2.08 | 0.82 | 1.01-2.99 | 2.802 | <0.05 |
| vs | | | | | | |
| Small | 6 | 16.65 | 9.97 | 4.83-33.67 | | |
| Medium | 39 | 3.72 | 3.73 | 0.49-14.6 | 3.456 | <0.05 |
| vs | | | | | | |
| Small | 6 | 16.65 | 9.97 | 4.83-33.67 | | |

Table 9.2: $R^2$-values calculated for sizes of crabs and selected tissue and carapace cadmium concentrations (DF = degrees of freedom; Dig.gland = digestive gland).

| | DF | r-value | R-sq.(%) |
|---|---|---|---|
| Crab size and Muscle | 27 | -0.332 | 11.01 |
| Crab size and Dig.gland | 29 | -0.406 | 16.52 |
| Crab size and Gills | 24 | -0.454 | 20.62 |
| Crab size and Gonads | 23 | -0.511 | 26.11 |
| Crab size and Carapace | 28 | -0.542 | 29.38 |

Table 9.3: Mean cadmium concentration ($\mu g.g^{-1}$ wet mass) in the muscles, digestive gland (dig.gland), gonads, gills and carapace of crabs from the Eerste River (Jonkershoek and SFW data pooled).

| | n | Mean | SD | Range |
|---|---|---|---|---|
| Muscle | 29 | 2.65 | 2.44 | 0.1-7.65 |
| Dig.gland | 33 | 2.21 | 3.37 | 0.1-15.72 |
| Gonads | 25 | 5.29 | 6.46 | 0.1-22.15 |
| Gills | 26 | 3.29 | 4.52 | 0.2-20.9 |
| Carapace | 39 | 4.03 | 3.04 | 0.44-18.46 |

Table 9.4: Results of Student's t-test for the differences in mean crab tissue cadmium concentration ($\mu g.g^{-1}$ wet mass) (Jonkershoek and SFW data pooled; Mean logarithms of tissue cadmium concentrations used for the calculation of the t-value).

| | t-value | p-value |
|---|---|---|
| Digestive gland vs Gonads | -2.25 | <0.05 |
| Digestive gland vs Gills | -1.61 | >0.05 |
| Gonads vs Gills | 0.75 | >0.05 |

Table 9.5: Results of the Mann-Whitney test for differences in crab tissue and carapace cadmium concentrations ($\mu g.g^{-1}$ wet mass) (Jonkershoek and SFW data pooled).

| | z-value | p-value |
|---|---|---|
| Muscle vs Digestive gland | -1.026 | >0.05 |
| Muscle vs Gonads | 1.302 | >0.05 |
| Muscle vs Gills | 0.22 | >0.05 |
| Muscle vs Carapace | 2.084 | <0.05 |
| Digestive gland vs Carapace | 4.059 | <0.05 |
| Gonads vs Carapace | 0.826 | >0.05 |
| Gills vs Carapace | 2.512 | <0.05 |

Table 9.6: Results of the Mann-Whitney test for the differences in whole crab cadmium concentrations ($\mu g.g^{-1}$ wet mass) at Jonkershoek and SFW.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Jonkershoek | 60 | 3.43 | 2.79 | 0.62-11.2 | 0.896 | >0.05 |
| vs | | | | | | |
| SFW | 51 | 5.04 | 6.26 | 0.49-33.67 | | |

Table 9.7: Results of the Mann-Whitney test for the differences in whole crab cadmium concentrations ($\mu g.g^{-1}$ wet mass) of large and medium sized crabs from Jonkershoek (J) and SFW (S).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Large (J) | 22 | 2.73 | 2.09 | 0.62-6.99 | -0.028 | >0.05 |
| vs | | | | | | |
| Large (S) | 6 | 2.08 | 0.82 | 1.01-2.99 | | |
| Medium (J) | 34 | 3.61 | 2.93 | 0.77-11.2 | -0.194 | >0.05 |
| vs | | | | | | |
| Medium (S) | 39 | 3.72 | 3.73 | 0.49-14.6 | | |

Table 9.8: Results of Student's t-test for the differences in mean whole crab cadmium concentration ($\mu g.g^{-1}$ wet mass) of small sized crabs from Jonkershoek (J) and SFW (S) (Mean logarithms of cadmium concentrations used in the t-test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Small (J) | 4 | 5.8 | 4.02 | 1.02-10.81 | -2.23 | <0.05 |
| vs | | | | | | |
| Small (S) | 6 | 16.65 | 9.97 | 4.83-33.67 | | |

Table 9.9: Results of Student's t-test for the differences in mean digestive gland, gill and gonad cadmium concentration ($\mu g.g^{-1}$ wet mass) of crabs from Jonkershoek (J) and SFW (S) (Mean logarithms of the tissue cadmium concentrations used in the test).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Dig.gland (J) | 18 | 1.78 | 2.53 | 0.2-9.59 | -0.34 | >0.05 |
| vs | | | | | | |
| Dig.gland (S) | 15 | 2.72 | 4.21 | 0.1-15.72 | | |
| Gills (J) | 14 | 2.81 | 3.43 | 0.3-12.28 | -0.29 | >0.05 |
| vs | | | | | | |
| Gills (S) | 12 | 3.85 | 5.65 | 0.2-20.9 | | |
| Gonads (J) | 14 | 3.95 | 3.78 | 0.3-12.5 | -0.01 | >0.05 |
| vs | | | | | | |
| Gonads (S) | 11 | 7.0 | 8.71 | 0.1-22.15 | | |

Table 9.10: Results of the Mann-Whitney test for the differences in muscle and carapace cadmium concentrations ($\mu g.g^{-1}$ wet mass) of crabs from Jonkershoek (J) and SFW (S).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Muscle (J) | 16 | 2.56 | 2.19 | 0.2-7.05 | -0.088 | >0.05 |
| vs | | | | | | |
| Muscle (S) | 13 | 2.77 | 2.8 | 0.1-7.65 | | |
| Carapace (J) | 19 | 3.32 | 1.41 | 0.44-5.57 | 0.113 | >0.05 |
| vs | | | | | | |
| Carapace (S) | 20 | 4.71 | 3.95 | 1.9-18.46 | | |

Table 9.11: Results of Student's t-test for the differences in mean whole crab cadmium concentration ($\mu g.g^{-1}$ wet mass) of males and females (Mean logarithms of the whole crab cadmium concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Females | 40 | 3.79 | 3.04 | 0.86-14.6 | -0.94 | >0.05 |
| vs | | | | | | |
| Males | 49 | 5.48 | 6.13 | 0.49-33.67 | | |

Table 9.12: Results of Student's t-test for the differences in mean tissue and carapace cadmium concentration ($\mu g.g^{-1}$ wet mass) of male (m) and female (f) crabs (Mean logarithms of the tissue and carapace cadmium concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Carapace (f) | 15 | 3.99 | 1.79 | 2.2-8.2 | -0.29 | >0.05 |
| vs | | | | | | |
| Carapace (m) | 18 | 4.71 | 3.95 | 1.9-18.46 | | |
| Dig.gland (f) | 12 | 1.02 | 1.08 | 0.1-3.5 | -1.77 | <0.05 |
| vs | | | | | | |
| Dig.gland (m) | 15 | 3.47 | 4.52 | 0.2-15.72 | | |
| Gills (f) | 11 | 1.99 | 1.69 | 0.4-4.8 | -1.42 | >0.05 |
| vs | | | | | | |
| Gills (m) | 14 | 4.0 | 5.79 | 0.2-20.9 | | |
| Ovaries | 11 | 6.23 | 7.04 | 0.3-20.81 | -0.04 | >0.05 |
| vs | | | | | | |
| Testes | 10 | 6.13 | 6.67 | 0.3-22.15 | | |

Table 9.13: Results of the Mann-Whitney test for the differences in muscle cadmium concentrations ($\mu g.g^{-1}$ wet mass) of male (m) and female (f) crabs.

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Muscle (f) | 11 | 2.94 | 2.56 | 0.3-7.05 | -0.261 | >0.05 |
| vs | | | | | | |
| Muscle (m) | 13 | 2.97 | 2.55 | 0.1-7.65 | | |

Table 9.14: Results of the Mann-Whitney test for the seasonal differences in whole crab cadmium concentrations ($\mu g.g^{-1}$ wet mass).

| | n | Mean | SD | Range | z-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 49 | 3.45 | 3.09 | 0.49-14.6 | 1.728 | >0.05 |
| vs | | | | | | |
| Autumn | 16 | 5.77 | 5.92 | 1.1-21.34 | | |
| Summer | 49 | 3.45 | 3.09 | 0.49-14.6 | -1.0 | >0.05 |
| vs | | | | | | |
| Winter | 21 | 5.24 | 7.72 | 0.62-33.67 | | |
| Summer | 49 | 3.45 | 3.09 | 0.49-14.6 | 0.731 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 3.67 | 2.98 | 1.0-11.2 | | |
| Autumn | 16 | 5.77 | 5.92 | 1.1-21.34 | -1.794 | >0.05 |
| vs | | | | | | |
| Winter | 21 | 5.24 | 7.72 | 0.62-33.67 | | |
| Autumn | 16 | 5.77 | 5.92 | 1.1-21.34 | -0.842 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 3.67 | 2.98 | 1.0-11.2 | | |
| Winter | 21 | 5.24 | 7.72 | 0.62-33.67 | 1.5 | >0.05 |
| vs | | | | | | |
| Spring | 25 | 3.67 | 2.98 | 1.0-11.2 | | |

Table 9.15: Results of Student's t-test for the seasonal differences in mean crab carapace cadmium concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of the cadmium concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 18 | 3.86 | 1.48 | 2.34-7.47 | 1.97 | <0.05 |
| vs | | | | | | |
| Autumn | 5 | 2.72 | 1.42 | 0.44-4.1 | | |
| Summer | 18 | 3.86 | 1.48 | 2.34-7.47 | -0.44 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 7.08 | 6.7 | 0.59-18.46 | | |
| Summer | 18 | 3.86 | 1.48 | 2.34-7.47 | 1.35 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 3.16 | 1.04 | 1.9-5.13 | | |
| Autumn | 5 | 2.72 | 1.42 | 0.44-4.1 | -0.96 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 7.08 | 6.7 | 0.59-18.46 | | |
| Autumn | 5 | 2.72 | 1.42 | 0.44-4.1 | -1.06 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 3.16 | 1.04 | 1.9-5.13 | | |
| Winter | 6 | 7.08 | 6.7 | 0.59-18.46 | 0.79 | >0.05 |
| vs | | | | | | |
| Spring | 10 | 3.16 | 1.04 | 1.9-5.13 | | |

Table 9.16: Results of Student's t-test for the seasonal differences in mean crab muscle cadmium concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of cadmium concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 1.8 | 2.08 | 0.1-7.15 | -0.6 | >0.05 |
| vs | | | | | | |
| Autumn | 4 | 1.93 | 1.69 | 0.4-4.0 | | |
| Summer | 15 | 1.8 | 2.08 | 0.1-7.15 | -1.52 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 4.31 | 3.4 | 0.4-7.65 | | |
| Summer | 15 | 1.8 | 2.08 | 0.1-7.15 | -2.13 | <0.05 |
| vs | | | | | | |
| Spring | 6 | 4.18 | 2.24 | 0.4-7.05 | | |
| Autumn | 4 | 1.93 | 1.69 | 0.4-4.0 | -0.83 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 4.31 | 3.4 | 0.4-7.65 | | |
| Autumn | 4 | 1.93 | 1.69 | 0.4-4.0 | -1.29 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 4.18 | 2.24 | 0.4-7.05 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Winter | 4 | 4.31 | 3.4 | 0.4-7.65 | -0.21 | >0.05 |
| vs | | | | | | |
| Spring | 6 | 4.18 | 2.24 | 0.4-7.05 | | |

Table 9.17: Results of Student's t-test for the seasonal differences in mean crab gill cadmium concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of cadmium concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 1.74 | 2.25 | 0.2-7.7 | -1.96 | <0.05 |
| vs | | | | | | |
| Autumn | 3 | 8.13 | 11.06 | 1.4-20.9 | | |
| Summer | 15 | 1.74 | 2.25 | 0.2-7.7 | -1.96 | <0.05 |
| vs | | | | | | |
| Winter | 3 | 3.48 | 0.66 | 2.81-4.12 | | |
| Summer | 15 | 1.74 | 2.25 | 0.2-7.7 | -2.6 | <0.05 |
| vs | | | | | | |
| Spring | 5 | 4.91 | 4.3 | 1.9-12.28 | | |
| Autumn | 3 | 8.13 | 11.06 | 1.4-20.9 | 0.16 | >0.05 |
| vs | | | | | | |
| Winter | 3 | 3.48 | 0.66 | 2.81-4.12 | | |
| Autumn | 3 | 8.13 | 11.06 | 1.4-20.9 | 0.05 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 4.91 | 4.3 | 1.9-12.28 | | |
| Winter | 3 | 3.48 | 0.66 | 2.81-4.12 | -0.22 | >0.05 |
| vs | | | | | | |
| Spring | 5 | 4.91 | 4.3 | 1.9-12.28 | | |

Table 9.18: Results of Student's t-test for the seasonal differences in mean crab digestive gland cadmium concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of cadmium concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 15 | 1.29 | 1.58 | 0.1-5.6 | -0.61 | >0.05 |
| vs | | | | | | |
| Autumn | 5 | 2.17 | 3.51 | 0.4-8.43 | | |
| Summer | 15 | 1.29 | 1.58 | 0.1-5.6 | -1.56 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 4.1 | 5.89 | 0.4-15.72 | | |
| Summer | 15 | 1.29 | 1.58 | 0.1-5.6 | -0.94 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 2.57 | 3.47 | 0.2-9.59 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Autumn | 5 | 2.17 | 3.51 | 0.4-8.43 | -0.7 | >0.05 |
| vs | | | | | | |
| Winter | 6 | 4.1 | 5.89 | 0.4-15.72 | | |
| Autumn | 5 | 2.17 | 3.51 | 0.4-8.43 | -0.21 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 2.57 | 3.47 | 0.2-9.59 | | |
| Winter | 6 | 4.1 | 5.89 | 0.4-15.72 | 0.52 | >0.05 |
| vs | | | | | | |
| Spring | 7 | 2.57 | 3.47 | 0.2-9.59 | | |

Table 9.19: Results of Student's t-test for the seasonal differences in mean crab gonad cadmium concentration ($\mu g.g^{-1}$ wet mass) (Mean logarithms of cadmium concentrations used to calculate the t-value).

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Summer | 13 | 4.11 | 5.84 | 0.3-20.81 | 0.52 | >0.05 |
| vs | | | | | | |
| Autumn | 4 | 3.6 | 3.85 | 0.1-7.6 | | |
| Summer | 13 | 4.11 | 5.84 | 0.3-20.81 | -0.72 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 9.87 | 10.74 | 0.3-22.15 | | |
| Summer | 13 | 4.11 | 5.84 | 0.3-20.81 | -0.83 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 6.24 | 5.28 | 0.43-12.5 | | |
| Autumn | 4 | 3.6 | 3.85 | 0.1-7.6 | -0.71 | >0.05 |
| vs | | | | | | |
| Winter | 4 | 9.87 | 10.74 | 0.3-22.15 | | |
| Autumn | 4 | 3.6 | 3.85 | 0.1-7.6 | -0.82 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 6.24 | 5.28 | 0.43-12.5 | | |
| Winter | 4 | 9.87 | 10.74 | 0.3-22.15 | -0.02 | >0.05 |
| vs | | | | | | |
| Spring | 4 | 6.24 | 5.28 | 0.43-12.5 | | |

Table 9.20: Results of the Mann-Whitney test for differences in whole crab ($\mu g.g^{-1}$ wet mass) and water cadmium concentrations ($mg.l^{-1}$) at each locality: Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_W$) calculated for whole crabs (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water (J) | 8 | 0.005 | 0.003 | 0.002-0.009 | 4.004 | <0.05 | 686.0 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 3.43 | 2.79 | 0.62-11.2 | | | |
| Water (S) | 8 | 0.006 | 0.003 | 0.003-0.01 | 3.966 | <0.05 | 840.0 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 5.04 | 6.26 | 0.49-33.67 | | | |

Table 9.21: Results of Student's t-test for differences in mean crab tissue ($\mu g.g^{-1}$ wet mass) and water cadmium concentration ($mg.l^{-1}$) at each locality, as well as the bioconcentration factor ($BCF_W$) calculated for the tissues (Dig.gland = digestive gland; t = t-value, p = p-value).

| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_W$ |
| **Water** | 8 | 0.005 | 0.003 | 0.002-0.009 | | | |
| vs Dig.gland | 18 | 1.78 | 2.53 | 0.2-9.59 | -12.45 | <0.05 | 356.0 |
| vs Gills | 14 | 2.81 | 3.43 | 0.3-12.28 | -13.04 | <0.05 | 562.0 |
| vs Gonads | 14 | 3.95 | 3.78 | 0.3-12.5 | -12.76 | <0.05 | 790.0 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_W$ |
| **Water** | 8 | 0.006 | 0.003 | 0.03-0.01 | | | |
| vs Muscle | 13 | 2.77 | 2.8 | 0.1-7.65 | -9.9 | <0.05 | 461.67 |
| vs Dig.gland | 15 | 2.72 | 4.21 | 0.1-15.72 | -9.53 | <0.05 | 453.33 |
| vs Gills | 12 | 3.85 | 5.65 | 0.2-20.9 | -11.24 | <0.05 | 641.67 |
| vs Gonads | 11 | 7.0 | 8.71 | 0.1-22.15 | -8.89 | <0.05 | 1166.67 |

Table 9.22: Results of the Mann-Whitney test for differences in crab muscle and carapace ($\mu g.g^{-1}$ wet mass), and water cadmium concentrations ($mg.l^{-1}$) at each locality: Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_W$) calculated for the muscles and carapace (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_W$ |
|---|---|---|---|---|---|---|---|
| Water (J) | 8 | 0.005 | 0.003 | 0.002-0.009 | 3.6 | <0.05 | 664.0 |
| vs | | | | | | | |
| Carapace (J) | 19 | 3.32 | 1.41 | 0.44-5.57 | | | |
| Water (J) | 8 | 0.005 | 0.003 | 0.002-0.009 | 3.513 | <0.05 | 512.0 |
| vs | | | | | | | |
| Muscle (J) | 16 | 2.56 | 2.19 | 0.2-7.05 | | | |
| Water (S) | 8 | 0.006 | 0.003 | 0.003-0.01 | 3.624 | <0.05 | 785.0 |
| vs | | | | | | | |
| Carapace (S) | 20 | 4.71 | 3.95 | 1.9-18.46 | | | |

Table 9.23: Results of the Mann-Whitney test for differences in cadmium concentrations ($\mu g.g^{-1}$ wet mass) of whole crabs and sediments from the two localities: Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for whole crabs (z = z-value, p = p-value).

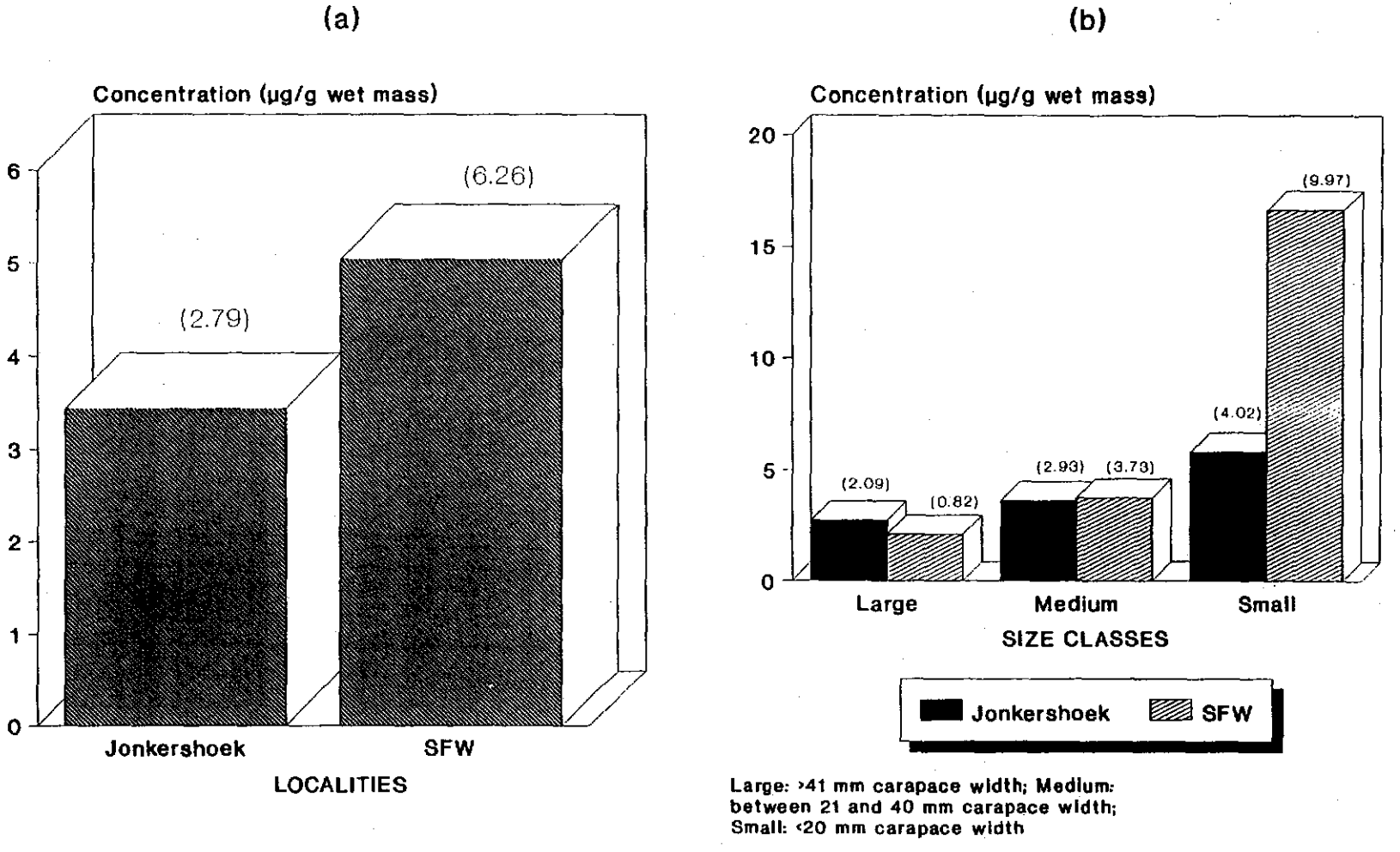
| | n | Mean | SD | Range | z | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 0.36 | 0.09 | 0.25-0.5 | 4.004 | <0.05 | 9.53 |
| vs | | | | | | | |
| Whole crab (J) | 60 | 3.43 | 2.79 | 0.62-11.2 | | | |
| Sediment (S) | 8 | 0.38 | 0.15 | 0.25-0.58 | 3.914 | <0.05 | 13.26 |
| vs | | | | | | | |
| Whole crab (S) | 51 | 5.04 | 6.26 | 0.49-33.67 | | | |

Table 9.24: Results of Student's t-test for differences in mean cadmium concentration ($\mu g.g^{-1}$ wet mass) of the sediments and crab tissues at each locality: Jonkershoek and SFW, as well as the bioconcentration factor ($BCF_S$) calculated for the tissues (Dig.gland = digestive gland; Mean logarithms of cadmium concentrations used to calculate the t-value; t = t-value, p = p-value).

| **JONKERSHOEK** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 0.36 | 0.09 | 0.25-0.5 | | | |
| vs Dig.gland | 18 | 1.78 | 2.53 | 0.2-9.59 | -2.3 | <0.05 | 4.94 |
| vs Gills | 14 | 2.81 | 3.43 | 0.3-12.28 | -3.48 | <0.05 | 7.81 |
| vs Gonads | 14 | 3.95 | 3.78 | 0.3-12.5 | -3.96 | <0.05 | 10.97 |
| **SFW** | | | | | | | |
| | n | Mean | SD | Range | t | p | $BCF_S$ |
| **Sediment** | 8 | 0.38 | 0.15 | 0.25-0.58 | | | |
| vs Dig.gland | 15 | 2.72 | 4.21 | 0.1-15.72 | -2.0 | <0.05 | 7.16 |
| vs Gills | 12 | 3.85 | 5.65 | 0.2-20.9 | -3.18 | <0.05 | 10.13 |
| vs Gonads | 11 | 7.0 | 8.71 | 0.1-22.15 | -2.75 | <0.05 | 18.42 |

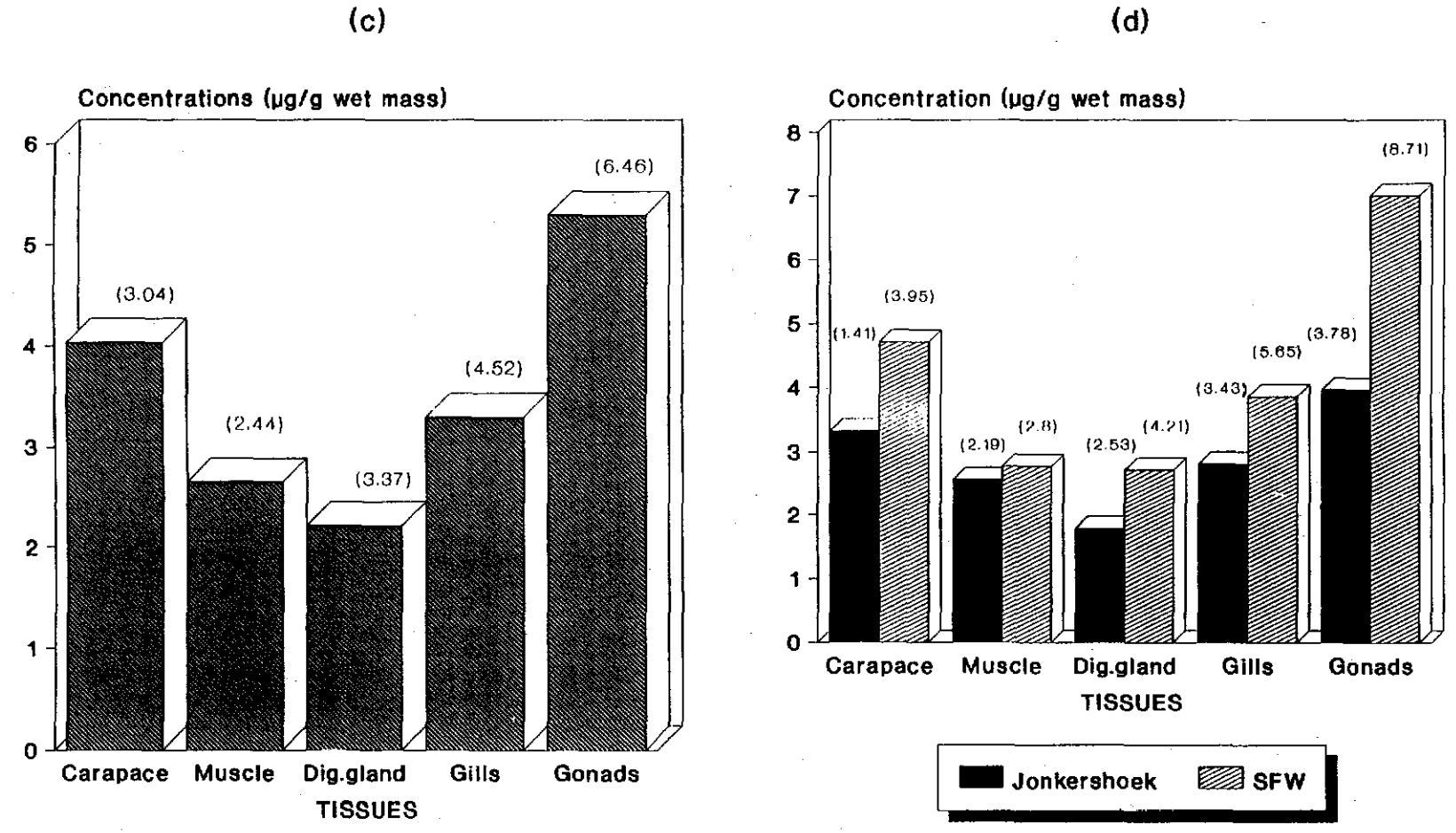
Table 9.25: Results of the Mann-Whitney test for differences in cadmium concentrations ($\mu g.g^{-1}$ wet mass) of sediments, crab muscles and carapace at each locality: Jonkershoek (J) and SFW (S), as well as the bioconcentration factor ($BCF_S$) calculated for the muscles and carapace (z = z-value, p = p-value).

| | n | Mean | SD | Range | z | p | $BCF_S$ |
|---|---|---|---|---|---|---|---|
| Sediment (J) | 8 | 0.36 | 0.09 | 0.25-0.5 | 3.538 | <0.05 | 9.22 |
| vs | | | | | | | |
| Carapace (J) | 19 | 3.32 | 1.41 | 0.44-5.57 | | | |
| Sediment (J) | 8 | 0.36 | 0.09 | 0.25-0.5 | 2.109 | <0.05 | 7.11 |
| vs | | | | | | | |
| Muscle (J) | 16 | 2.56 | 2.19 | 0.2-7.05 | | | |
| Sediment (S) | 8 | 0.38 | 0.15 | 0.25-0.58 | 3.627 | <0.05 | 12.39 |
| vs | | | | | | | |
| Carapace (S) | 20 | 4.71 | 3.95 | 1.9-18.46 | | | |
| Sediment (S) | 8 | 0.38 | 0.15 | 0.25-0.58 | 1.625 | >0.05 | 7.29 |
| vs | | | | | | | |
| Muscle (S) | 13 | 2.77 | 2.8 | 0.1-7.65 | | | |



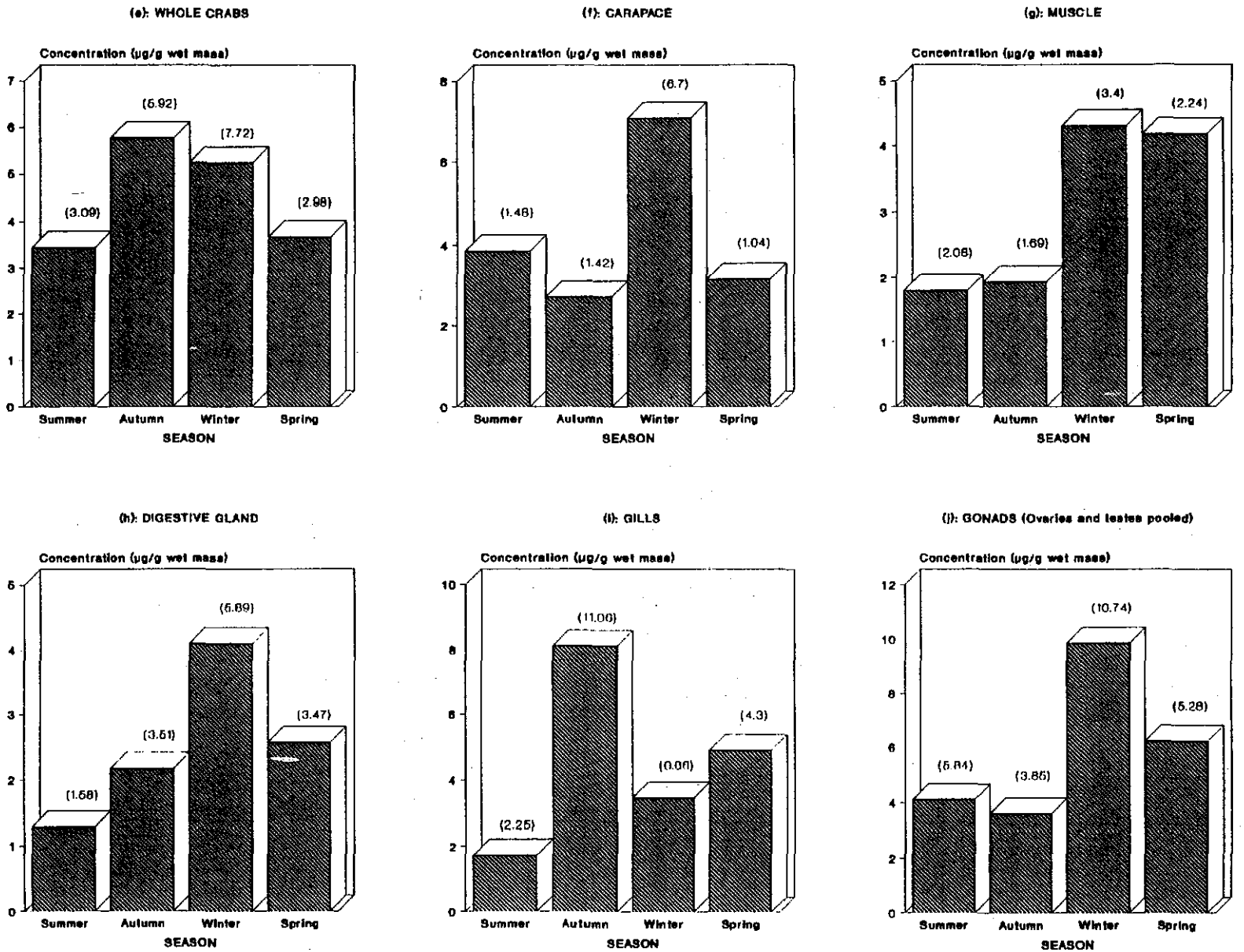

Large: >41 mm carapace width; Medium: between 21 and 40 mm carapace width; Small: <20 mm carapace width

Figures 9(a)-(b): Mean cadmium concentration in whole crabs (a) and various size classes (b) from both localities (SD in parenthesis).




Jonkershoek and SFW data pooled

Figures 9(c)-(d): Mean cadmium concentration in carapace and tissues of *P. perlatus* from the Eerste River (SD in parenthesis).




Figures 9(e)-(j): Mean seasonal cadmium concentration in whole crabs, carapace and tissues of P. *perlatus* (Jonkershoek and SFW data pooled; SD in parenthesis).

**Discussion**

The mean cadmium concentration in whole bodies, tissues and carapace of *Potamonautes perlatus* (Table 9.3; Figure 9(c)), were compared with the concentrations found by several other researchers in two decapod species, the crayfish *Orconectes virilis* and the shore crab *Carcinus maenas*. In comparison with the "Criteria for Cadmium", cited by Förstner (1980), ranging from 5 to 33.1 $mg.kg^{-1}$ dry mass for whole crabs from the UK and 22 $mg.kg^{-1}$ dry mass for whole crabs from Europe, the cadmium concentrations in whole crabs *P. perlatus* (0.99±0.9 and 1.57±2.71 $\mu g.g^{-1}$ dry mass for Jonkershoek and SFW respectively) were found to be very low. The whole crab Cd concentrations in *P. perlatus* did, however, compare favourably with the mean whole body Cd concentrations in the crayfish *Orconectes virilis*, collected from a relatively unpolluted site on the Fox River, Illinois, namely 0.83±0.13 to 2.38±0.74 $\mu g.g^{-1}$ dry mass (Anderson & Brower, 1978).

The mean dry mass Cd concentrations in the digestive gland and carapace of *P. perlatus*, i.e. 0.44±0.63 and 0.68±1.05 $\mu g.g^{-1}$ for the digestive gland from Jonkershoek and SFW respectively and 2.26±0.96 and 3.22±2.7 $\mu g.g^{-1}$ for the carapace from Jonkershoek and SFW respectively, were found to be low and high respectively, compared to the cadmium concentrations in unexposed *Carcinus maenas* (7.75±8.78 $\mu g.g^{-1}$ dry mass (digestive gland) and 0.55±0.3 $\mu g.g^{-1}$ dry mass (carapace)), as found by Rainbow (1985). The mean dry mass Cd concentration in the muscles of *P. perlatus* (0.52±0.19 and 0.55±0.55 $\mu g.g^{-1}$ for Jonkershoek and SFW respectively) were found to be relatively high, in comparison with the mean Cd concentrations in muscles of the crayfish *Orconectes virilis*, (0.1 to 0.22 $\mu g.g^{-1}$ dry mass) collected by France (1987) from lakes with low metal burdens, as well as from acid lakes with slightly higher metal levels than the non-acidified lakes. Anderson & Brower (1978) observed mean Cd concentrations of 0.78±0.13, <0.5 and 1.47±0.78 $\mu g.g^{-1}$ dry mass, respectively, in the exoskeleton, muscles and gills of *Orconectes virilis*. The carapace and muscle concentrations in *Potamonautes perlatus* (see above), in comparison with Anderson & Brower's results, were found to be relatively high and the gill Cd (0.41±0.5 and 0.56±0.82 $\mu g.g^{-1}$ for Jonkershoek and SFW respectively) relatively low.

It is clear that the Cd concentrations in *P. perlatus* compare well with the concentrations found in decapod species from relatively unpolluted fresh and marine waters elsewhere. However, the high dry mass concentrations in the carapace of crabs from Jonkershoek and SFW ranging from 0.3 to 3.8 and from 1.3 to 12.6 $\mu g.g^{-1}$ respectively, as well as the very high maximum whole crab dry mass concentration (18.67 $\mu g.g^{-1}$) found in the SFW population, are of some concern and indicate possible Cd accumulation in *P. perlatus* from the Eerste River. Also, the significantly higher Cd concentrations in whole crab, tissue and carapace cadmium concentrations than in the sediments from both localities, as well as the high $BCF_W$'s and $BCF_S$'s obtained (Tables 9.23-9.25), indicated that Cd was accumulated in *P. perlatus* from both

Jonkershoek and SFW, but to a higher degree in individuals from SFW, as indicated by the significantly higher Cd concentrations in small crabs from this locality, than in the small crabs from Jonkershoek (Table 9.8), as well as the higher $BCF_W$ and $BCF_S$ determined for whole crabs from SFW (Tables 9.20 and 9.23).

Since the Cd concentrations in the water and sediments at both localities were relatively low and no statistically significant differences in these concentrations were observed between the two localities (Tables 4.5 and 4.11), the observed difference in small crab Cd concentrations between the Jonkershoek and SFW populations was probably due to differences in the physico-chemistry of the water, between the two localities (Table 4.8), resulting in increases in the bioavailability and uptake rate of Cd at SFW.

Upon researching the distribution of Cd in *P. perlatus* it was established that the gonads had the highest mean Cd concentration, but this only differed significantly from the mean concentration in the digestive gland (Tables 9.3-9.5). The carapace, with the second highest mean concentration, exhibited significantly higher concentrations than all but one of the selected tissues (gonads), indicating perhaps that most of the body Cd was incorporated into the gonads and carapace. This theory is supported by the higher $BCF_W$'s and $BCF_S$'s observed for the gonads and carapace at both localities (Tables 9.21, 9.22, 9.24 and 9.25). The remaining tissues did not vary greatly in their contributions to Cd uptake and/or storage. As opposed to the results for *P. perlatus*, individuals of the shore crab *Carcinus maenas*, not exposed to Cd in the laboratory, had higher Cd concentrations in the digestive gland (Rainbow, 1985), while those exposed to cadmium contained most of this metal in the gills, followed by the carapace and digestive gland, with the muscles having the lowest Cd concentrations (Pedersen & Bjerregaard, 1995). Anderson & Brower (1978) found similar results for the crayfish *Orconectes virilis*.

It is concluded that although all four tissues as well as the carapace of *P. perlatus* seem to contain fairly similar Cd concentrations, the carapace and gonads are of more importance in Cd storage.

There is evidence that Cd is absorbed from the environment through the gills (Pedersen & Bjerregaard, 1995), after which it is distributed via the haemolymph to the various internal organs, resulting in the observed high concentrations in e.g. the gonads. There are indications that Cd also enters decapods via the alimentary canal, and that the digestive gland plays an important rôle in protecting the animal against high Cd concentrations by binding the metal to specific proteins (Rainbow & Scott, 1979 and Lyon, 1984).

The carapace may possibly act as a sink for Cd, offering some protection to the animal, since the exoskeleton is shed at each periodic moult. Van Straalen & Verkleij (1993) reported that collembola store heavy metals in the intestinal epithelium, the latter which is excreted as a type

of intestinal "plug" with each moult. Periodic moulting therefore clearly offers protection against accumulated metals to these animals.

Investigations into the relationship between crab size and Cd uptake in *P. perlatus* showed significantly higher concentrations in small crabs from SFW (Table 9.1). Furthermore, the reasonably strong negative correlations between size and whole crab (r = -0.522), tissue (except muscle) and carapace Cd (Table 9.2), showed that body size (therefore age) has some influence in the uptake of Cd from the environment, in this species. Kiffney & Clements (1996) exposed three ephemeropteran and one plecopteran species to cadmium and also found an inverse relationship between body size and survivorship. All the above mentioned results differ from those of Anderson & Brower (1978) who found no differences in Cd concentrations between the various size classes of *Orconectes virilis*.

One can speculate on the reason for these observed size/age differences. Hill & O'Keeffe (1991) demonstrated differences in food preference between different sized individuals of *P. perlatus*. Smaller crabs were shown to prefer aquatic invertebrates (e.g. insects), which are known to accumulate heavy metals (e.g. Albers & Camardese, 1993 and Kiffney & Clements, 1993). Small crabs may therefore bioaccumulate higher Cd concentrations from their food. Also, Gherardi et al. (1987) and Gherardi & Micheli (1989) showed that younger (smaller) individuals of *Potamon fluviatile* and *P. potamios palestinensis* respectively, tend to hide more under rocks and stones, especially at night. This type of microhabitat offers a relatively constant environment for the crabs, which might result in lengthened exposure times to specific Cd concentrations, possibly explaining the observed higher concentrations in the smaller individuals of *P. perlatus*.

As in the case of *Orconectes virilis* (Anderson & Brower, 1978), gender also proved to have little influence on Cd uptake and distribution. The only significant difference between cadmium concentrations of males and females of *P. perlatus* was found for the digestive gland, with males exhibiting the highest concentrations (Tables 9.12-9.13). Since metal levels in this tissue are mostly affected by levels in the diet, one may speculate that factors such as possible differences in diet between the genders could have resulted in the observed differences in digestive gland Cd. Comparisons between whole crab Cd concentrations of males and females did not, however, show a statistically significant difference, therefore the difference in digestive gland Cd is not considered of great importance.

Seasonality proved to be of some influence in cadmium uptake in *P. perlatus*. Seasonal differences in Cd levels were observed for the carapace, muscle tissue and gills (Tables 9.15-9.17). However, the latter two tissue types exhibited only very slight seasonal variations. Peaks in Cd concentrations in carapace, muscles and gill tissue were observed for autumn, winter/spring and winter respectively (Figures 9(e)-(j)). These peaks did not follow the peaks in

water and sediment Cd closely (Figures 4(c)-(l)), although the sediments did exhibit a slight autumn peak.

It has been shown that the crab *Potamon potamios* prefers the thermal zone between 24° and 27°C and that its activity drops below and above these temperatures (Warburg et al., 1982). Similarly, the activity of *Potamonautes perlatus* is affected by environmental temperatures: this species is relatively inactive during the colder, wetter months and tend to retreat into burrows or under rocks and stones (personal observations and marked decrease in capture rate), in order to avoid the full force of the water flow. The relatively constant environment produced by this type of microhabitat may cause the animals to be exposed to specific Cd concentrations for longer periods. This theory might explain the observed tissue and carapace Cd peaks in the colder months.

Finally, the fact that *P. perlatus* in the Eerste River at both localities was shown to accumulate Cd in its body, indicates that this species may be considered as a potential monitor of environmental Cd pollution in the Eerste River. On the other hand, it must be noted that since long term accumulation probably does not occur, due to loss of excesses of this metal through moulting, and since environmental fluctuations in Cd were not accurately reflected in the crab body, this species might in fact not be a suitable monitor. More frequent observations and a better understanding of the factors affecting bioavailability of Cd to, and uptake and distribution thereof in, *P. perlatus* are needed, in order to make final conclusions.

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# CHAPTER 10

# HEAVY METALS AND THE SPERMATOZOA OF *POTAMONAUTES PERLATUS*

## Introduction

The sperm ultrastructure and spermiogenesis of some decapod crustaceans, especially crabs, have been studied intensively, e.g. for the genus *Cancer* (Langreth, 1960), the species *Eriocheir japonicus* (Yasuzumi, 1960), *Ranina ranina*, *Portunus pelagicus*, *Potamonautes perlatus sidneyii* (Jamieson, 1989; 1993) and for *Uca tangeri* (Medina & Rodriguez, 1992).

A transmission electron microscopic study of the spermatozoon of the freshwater crab *Potamonautes perlatus sidneyii* (Jamieson, 1993) was published shortly after the present study had commenced. It revealed that each spermatozoon, contained in its own spermatophore, is spheroidal in shape, slightly depressed antero-posteriorly and has broadly based lateral projections. Furthermore, like all decapod sperm, it lacks a flagellum and like all brachyuran sperm, the nucleus, which surrounds the acrosome, consists of diffuse, fibrous chromatin. The dorsal tip of the sperm cell is covered by a dense caplike structure, the operculum, and two elongate centrioles are present at the base of the acrosomal perforatorium.

It has been shown by several researchers that accumulated pesticides and heavy metals may have adverse effects on the morphology, physiology and/or behaviour of animals (see Chapter 1). Of these, changes in sperm morphology and physiology are well known: Young & Nelson (1974) and Castagna et al. (1981) studied the effects of heavy metals on the sperm motility of the sea urchin species *Arbacia punctulata* and *A. lixula* respectively. Ackerman (1995) investigated the effects of copper on impala (*Aepyceros melampus*) sperm, whereas Reinecke et al. (1995) discussed the effects of dieldrin on the sperm structure of the earthworm *Eudrilus eugeniae*. The sperm structure of another earthworm species, *Eisenia fetida*, was investigated by Reinecke & Reinecke (1996 in press) after exposure to lead and manganese.

Studies on the effects of pollutants on the sperm ultrastructure of crabs are lacking. The possibility of sperm abnormalities occurring in freshwater crabs such as *Potamonautes perlatus*, caused by exposure to heavy metals also exists. It was therefore decided to study the sperm ultrastructure of the test animal, *Potamonautes perlatus* and the possible adverse effects of heavy metals. The aim was to establish whether the occurrence of sperm abnormalities and sperm counts could be related to exposure to heavy metals.

## Materials and Methods

Samples and sections were prepared for transmission electron microscopy according to the method described in Chapter 3.

## Results

Sperm were found in the testes and vasa deferentia of males of approximately 45 mm carapace width and larger. Also, sperm were only detected in these organs during April/May and late October to January, therefore during autumn, late spring and summer.

Figures 10(a)-(c) show the typical structure of normal spermatozoa of *Potamonautes perlatus*. These were found to be similar in structure to the spermatozoa of *P. perlatus sidneyii*, described by Jamieson (1993). Note the operculum, subacrosomal chamber, peripheral cytoplasm, dense membrane, elongate centrioles and nucleus, as indicated in Figures 10(a) and (b).
Abnormal spermatozoa were found in males from both localities, i.e. Jonkershoek and SFW. Figures 10(d)-(g) illustrate the various types of abnormalities observed, such as the deformed dense membrane and peripheral cytoplasm and the thickened operculum.

1. Differences between the two localities

(a) Differences in the total number of spermatozoa per grid block

Student's t-test was performed in order to establish whether statistically significant differences ($p<0.05$) between the total number of spermatozoa of males from the two localities existed (Table 10.1). It was found that the individuals from SFW had significantly higher sperm counts per grid block than those from Jonkershoek.

Table 10.1: Results of Student's t-test for the differences between the total number of spermatozoa of *P. perlatus* per grid block, of males from Jonkershoek and SFW.

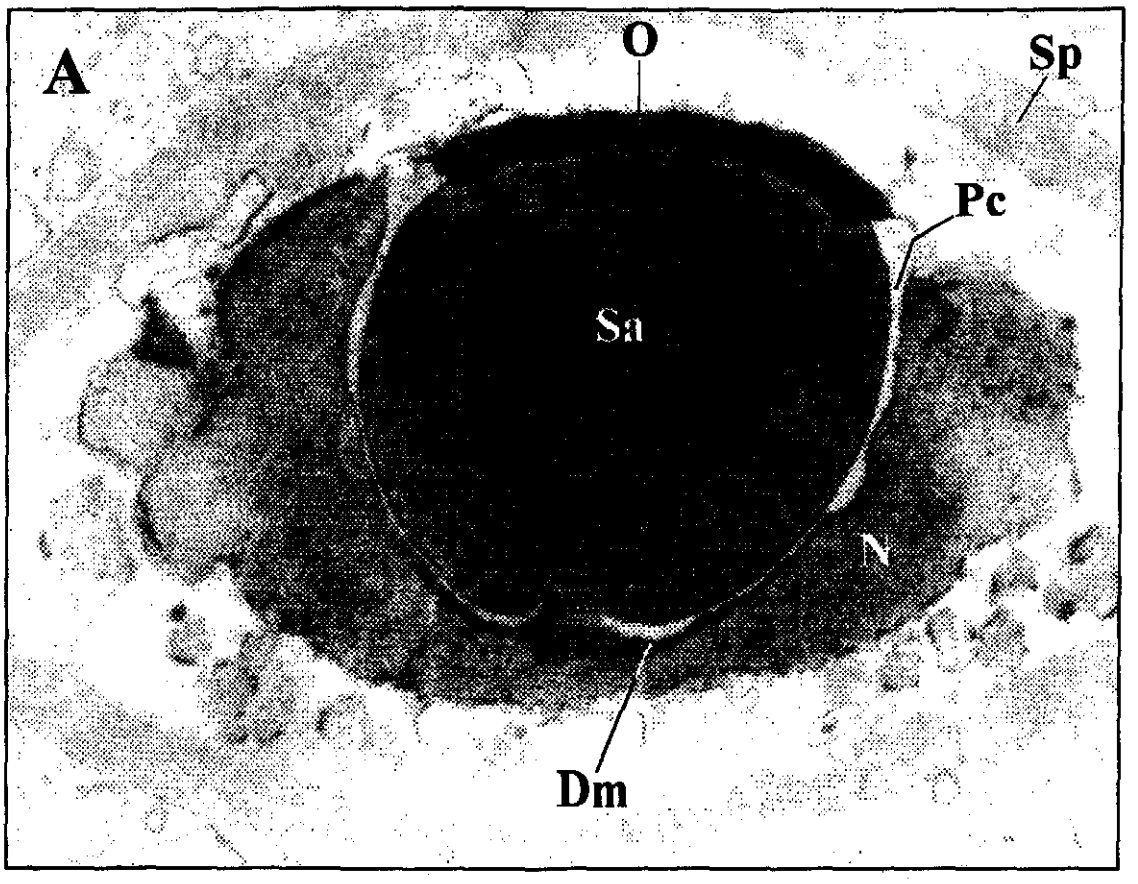
| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Jonkershoek | 4 | 37.75 | 22.16 | 16-65 | -3.3 | <0.05 |
| vs | | | | | | |
| SFW | 4 | 94.25 | 26.08 | 62-124 | | |

(b) Differences in the number of abnormal spermatozoa per grid block

The result of Student's t-test, performed on the number of abnormal spermatozoa of males from Jonkershoek and SFW counted per grid block, is shown in Table 10.2. A statistically significant difference ($p<0.05$) was found between individuals from the two localities, with individuals from SFW having the highest mean number of abnormal spermatozoa per grid block. Figure 10(h) shows the percentage of abnormal spermatozoa per grid block in males from Jonkershoek and SFW.

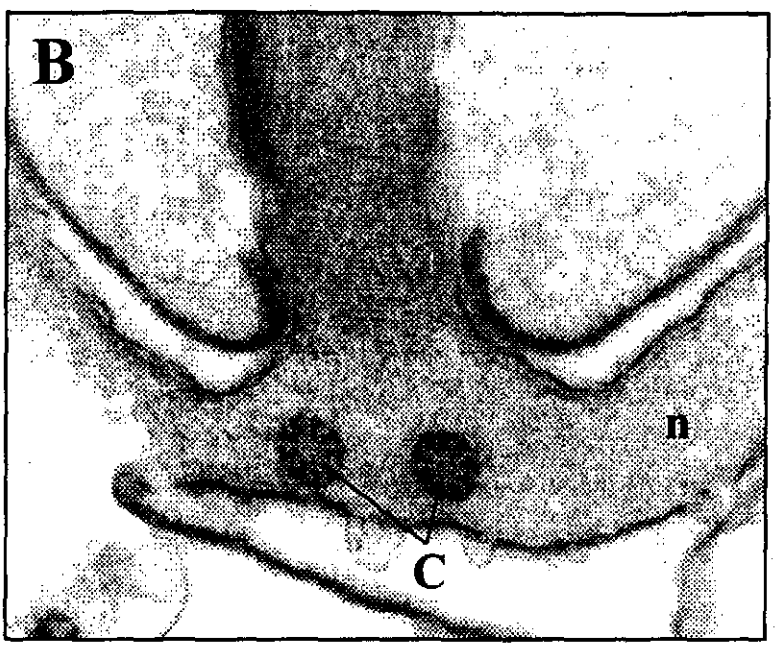
Table 10.2: Results of Student's t-test for the differences between the number of abnormal spermatozoa of *P. perlatus* per grid block, of males from Jonkershoek and SFW.

| | n | Mean | SD | Range | t-value | p-value |
|---|---|---|---|---|---|---|
| Jonkershoek | 4 | 4.0 | 3.56 | 1-8 | -8.38 | <0.05 |
| vs | | | | | | |
| SFW | 4 | 25.5 | 3.7 | 22-30 | | |

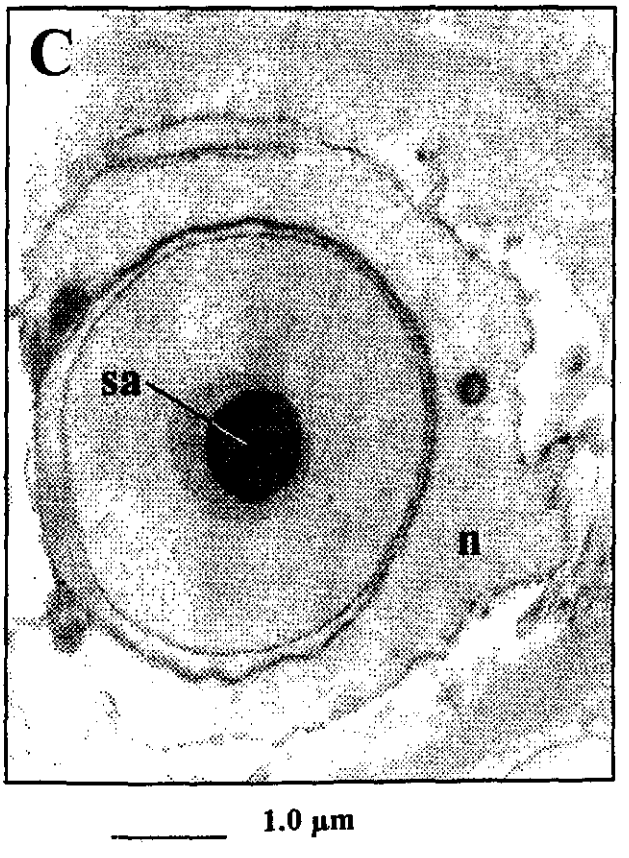


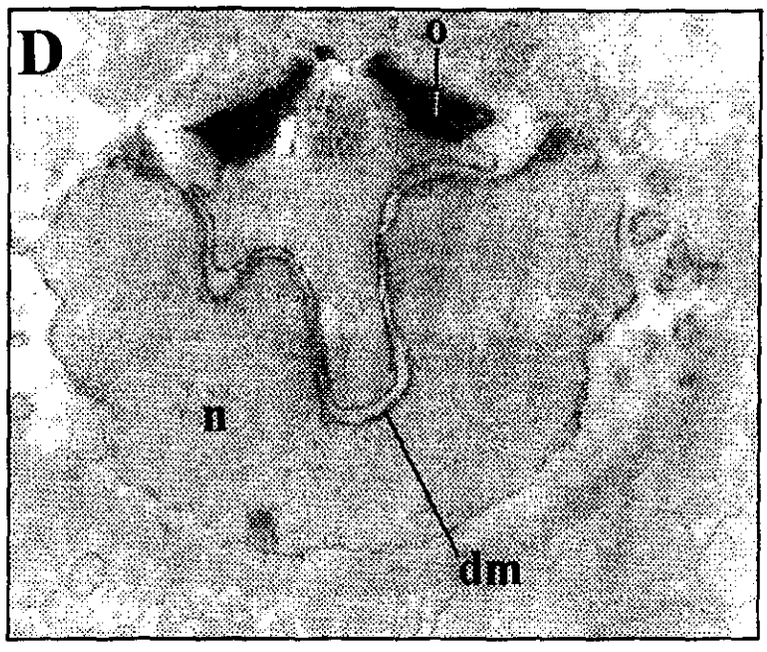

Figures 10(a):

Transmission electron micrograph of a longitudinal sections through the spermatozoon of *Potamonautes perlatus*. Abbreviations: Dm = dense membrane; N = nucleus; O = operculum; Pc = peripheral cytoplasm; Sa = subacrosomal chamber; Sp = spermatophore.

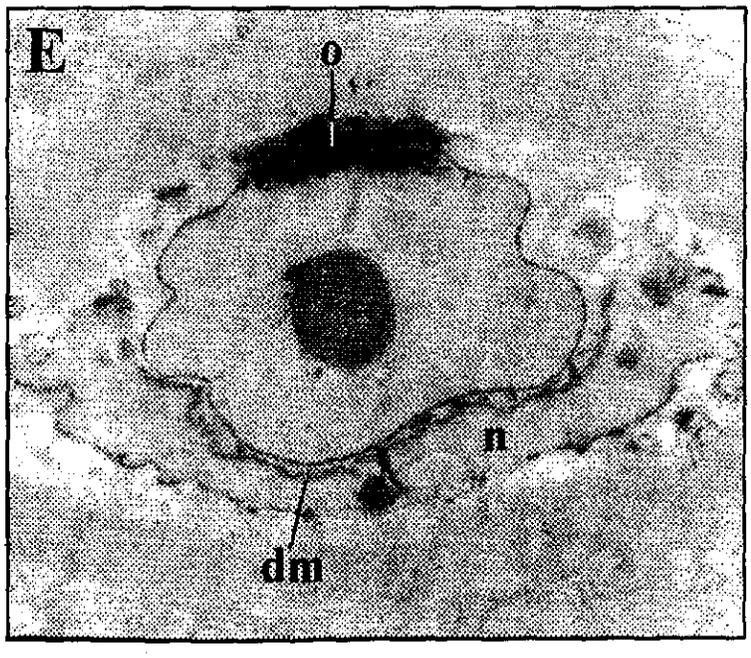


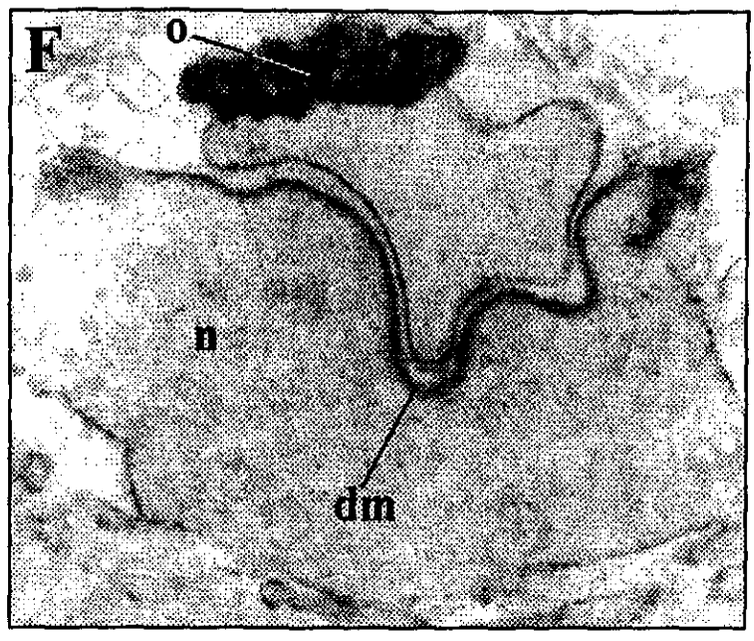

Figures 10(b)-(c): Transmission electron micrographs of a longitudinal (b) and a cross section (c) through the spermatozoon of *P. perlatus*. Abbreviations: C = centrioles; dm = dense membrane; n = nucleus; sa = subacrosomal chamber.



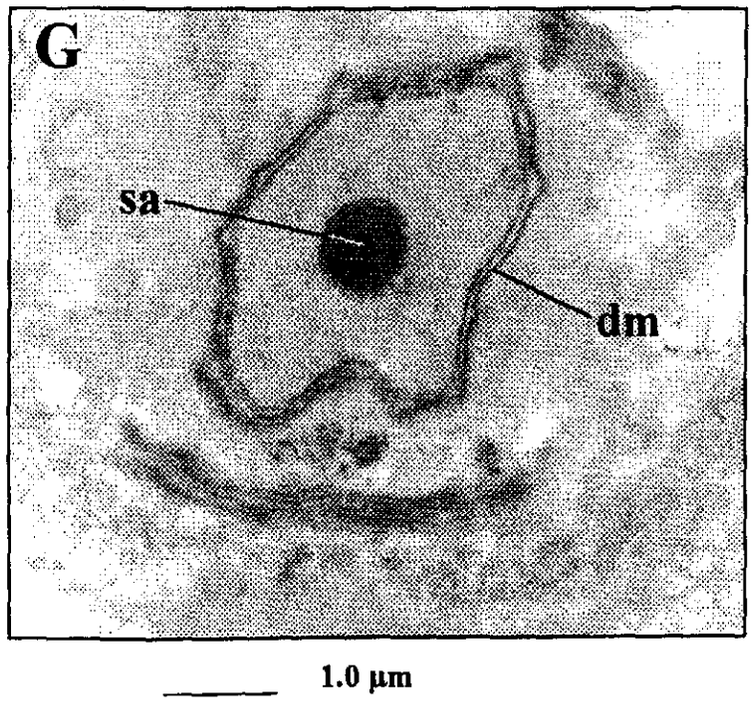


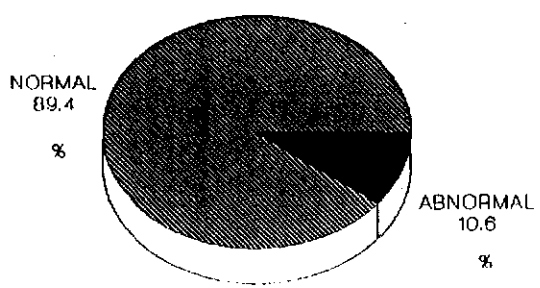

Figures 10(d)-(e): Transmission electron micrographs of longitudinal sections through abnormal spermatozoa of *P. perlatus*. Abbreviations: dm = dense membrane; n = nucleus; o = operculum and sa = subacrosomal chamber.



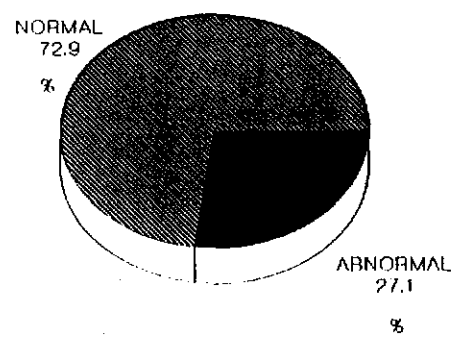



Figures 10(f)-(g): Transmission electron micrographs of a longitudinal (f) and a cross (g) section through abnormal spermatozoa of *P. perlatus*. Abbreviations: dm = dense membrane; n = nucleus; o = operculum and sa = subacrosomal chamber.






JONKERSHOEK




SFW

Figure 10(h): Percentage of abnormal spermatozoa per grid block, of *P. perlatus* from Jonkershoek and SFW.

## Discussion

Reproduction in *Potamonautes perlatus* is clearly periodical, probably seasonal but does not seem to be synchronized between individuals. It is also evident that only the largest and most mature males (≥45 mm carapace width) produce sperm and can reproduce (personal observations during dissections and transmission electron microscopy). Micheli et al. (1990) also found no sperm in subadult males (≤35 mm carapace width) of *Potamon fluviatile*, an Italian freshwater crab species.

A comparison of the total number of sperm cells counted for males of *P. perlatus*, collected at Jonkershoek and SFW, showed that individuals from SFW had a significantly higher number of spermatozoa per grid block than males from Jonkershoek (Table 10.1). Cikutovic et al. (1993) reported that exposure to heavy metals such as cadmium resulted in lowered sperm counts of earthworms, but the results of the present study differ from this finding, since it was shown that individuals of the SFW population had accumulated, and therefore been exposed to a greater variety of heavy metals (Mn, Cu, Pb and Cd) than those of the Jonkershoek population (Pb and Cd) (See discussions of Chapters 5-9). It must, however, be noted that it has not as yet been established whether the reproductive cycles of the two populations are synchronized, and that the possibility therefore exists that spermiogenesis commenced earlier in the season in the SFW population, resulting in higher sperm counts at the time of capture. The observed differences in total sperm counts of crabs from the two localities might therefore not be related to heavy metal exposure at all.

A number of abnormal spermatozoa were observed in all males investigated, from Jonkershoek as well as from SFW. These cells all differed from the normal form, which is illustrated in Figures 10(a)-(c), as they all had highly deformed dense membranes and also appeared to have thickened opercula (Figures 10(d)-(g)). One explanation for these abnormalities is the possible negative effects of chemical fixation techniques, as described by Reger et al. (1984). They found that cryofixed, as opposed to chemically fixed spermatozoa of the crab *Carcinus maenas*, exhibited greater cytonucleoplasmic density, the filament and membrane fine structure was more detailed, membranes appeared smoother and membrane particle distribution was more regular. Another explanation could be the technique used to kill the animals, namely by freezing.

However, this fails to explain the significantly higher number of abnormal spermatozoa found in individuals from SFW (Table 10.2). One would indeed expect a proportionally higher number of abnormal sperm cells in individuals from SFW, coinciding with the significantly higher total number of sperm cells but, upon investigation, it was found that the percentage of abnormal spermatozoa per grid block in SFW individuals were in fact more than twice as great as the percentage of abnormal spermatozoa per grid block in Jonkershoek individuals (Figure 10(h)).

There must, therefore, be alternative explanations for these observed differences, one of which might be the significantly higher Mn and Cu concentrations found in whole crabs from SFW (Tables 5.6 and 7.5). Ackerman (1995) and Reinecke & Reinecke (1996 in press) have clearly illustrated the negative influence of heavy metals, specifically copper (Ackerman, 1995) and manganese (Reinecke & Reinecke, 1996 in press), on the sperm ultrastructure of the impala, *Aepyceros melampus* and the earthworm, *Eisenia fetida*, respectively.

It is therefore concluded that the greater number of abnormal spermatozoa per grid block of males from SFW could possibly be related to heavy metal exposure. To test this hypothesis, it will of course be necessary to execute sublethal exposure experiments on *P. perlatus*, in a controlled laboratory environment.

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# CHAPTER 11

# CONCLUSIONS

Results of the present study indicated that heavy metal (except Zn) concentrations in the water and sediments at Jonkershoek and SFW were either well within the limits set by the Department of Environment Affairs and Tourism, or compared well with the results of other researchers on relatively unpolluted freshwater ecosystems. It was therefore concluded that the Eerste River, from its origin to the SFW locality, can still be considered relatively unpolluted in terms of heavy metals, but that runoff from the Stellenbosch municipal, industrial and agricultural areas definitely influence the physico-chemistry and general quality of the water.

Despite the relatively low heavy metal concentrations in the water and sediments at Jonkershoek and SFW, some of the heavy metals tested for, when compared with the results of various other researchers on several decapod crustaceans, were found to be relatively high in *Potamonautes perlatus*, such as Zn, Cd and Mn in the carapace, Mn, Cu, Pb and Cd in muscle tissue and Mn in the remaining tissues. Dry mass Pb concentrations in whole crabs from both localities, as well as dry mass Cd concentrations in whole crabs from SFW, were also relatively high. Heavy metal concentrations in the remaining tissues and dry mass Zn, Mn and Cd concentrations in whole crabs all compared favourably or were relatively low.

Comparisons between the metal concentrations of crabs from Jonkershoek and SFW showed significantly higher ($p<0.05$) whole body Mn and Cu concentrations of crabs collected at SFW. These results especially, as well as the bioconcentration factors ($BCF_W$ and $BCF_S$) calculated for whole crabs, tissues and carapace, aided in the decision whether the metals were accumulated in the crabs or generally well regulated. It was concluded that while Zn was well regulated in crabs from both localities, Mn and Cu were accumulated in *P. perlatus* from SFW, and Pb and Cd in individuals from both localities.

All five selected heavy metals were shown to be compartmentalized in the body of *P. perlatus*, i.e. concentrated more in certain body parts and/or tissues than in others. The carapace especially, as well as the digestive gland and gonads were indicated as the most important storage sites for these heavy metals: Mn was shown to concentrate in the carapace and, to a lesser degree in the digestive gland, Cd and Pb in both the carapace and gonads, and Zn and Cu mainly in the carapace and digestive gland respectively.

Investigations were made into the possible relationship between factors such as body size, gender and seasonality, and heavy metal concentrations in *P. perlatus* in the Eerste River.
Crab body size was shown to be an important factor influencing heavy metal uptake in this species: for all five selected metals, reasonably strong negative correlations were found between

body size and whole crab, carapace and gonad metal concentrations, indicating higher metal concentrations in smaller crabs.

Gender, on the other hand, generally seemed to be of little importance in heavy metal uptake in the crab. Only in the cases of Cu in whole crabs, and Pb and Cd in the digestive gland, were gender shown to be of some importance. In all these instances metal concentrations were found to be highest in male crabs.

Seasonal variations in all five selected heavy metals in this species indicated that seasonality can be considered of some influence in metal uptake in *P. perlatus*. Peaks in Zn, Cu, Pb and Cd in those body parts/tissues which showed significant seasonal variations, were found in the colder months, i.e. autumn and/or winter. These peaks did not follow the seasonal peaks in water and sediment metal concentrations. The Mn concentrations in whole crabs, tissues and carapace, however, showed summer peaks which corresponded with peaks in water and sediment Mn concentrations.

Factors such as the degree of regulation or accumulation of each metal in *P. perlatus*, the large intraspecific variations in metal concentrations in the crabs, as well as the degree to which metal concentrations in the riverine environment is reflected in the crabs, were all taken into account when the species was evaluated as monitor of environmental heavy metal pollution. It was concluded that the species in the Eerste River can be considered as a potential biomonitor of Mn, Pb and Cd pollution, but that there is no certainty that environmental metal concentrations would be accurately reflected in the crab.

The sperm ultrastructure of *P. perlatus*, on the other hand, might prove to be of greater value in environmental heavy metal pollution monitoring, since it was found that mature male crabs (>45 mm carapace width) from SFW had a significantly larger amount ($p<0.05$) of abnormal spermatozoa than those from Jonkershoek. Whether these observed abnormalities can definitely be related to the effects of heavy metal exposure, and if so, precisely which metals are responsible, still need to be investigated. It is also necessary that further investigations be made into possible differences between the two populations of *P. perlatus*, i.e. from Jonkershoek and SFW, in terms of biomass and population density, since the higher number of abnormal spermatozoa observed in the SFW population may hold serious long term implications for the population's reproduction. Also, an intensive study on the reproductive cycle of the species is crucial, since this may help to explain several of the trends observed in the present study, as well as results of future studies.

One of the major questions which arises from this study, is whether the heavy metal concentrations in *P. perlatus* influence animals further up in the food chain. Such predators along and in the Eerste River include the Cape clawless otter *Aonyx capensis*, the water

mongoose *Atilax paludinosus* and the Giant kingfisher *Megaceryle maxima*, which have all been shown to prey on crabs in the Eerste River and elsewhere (Purves et al., 1994; Purves, 1995 and Arkel, 1979), as well as a number of fish species such as the Small- and Largemouth basses (*Micropterus dolomieu* and *M. salmoides*) and the Rainbow trout *Onchorhynchus mykiss* (Skelton, 1993).

Mason et al. (1986) reported heavy metal, particularly lead, concentrations in tissues of the British otter *Lutra lutra*, approaching concentrations known to produce sublethal effects in mammals. This implies possible metal accumulation from the prey. These findings may be taken a step further, in light of the results of the present study on the distribution of heavy metals in the crab body, and the influence of crab body size on heavy metal concentrations: Cape clawless otters and water mongooses may possibly be exposed to higher metal concentrations than for instance the Giant kingfisher, since the former two have been shown to consume the entire crab, carapace included (although the water mongoose discards the carapaces of larger crabs) (Rowe-Rowe, 1977b; Baker, 1989 and Purves et al., 1994), whereas the kingfisher always discards the carapace (Arkel, 1979). Also, the studies of Arkel (1979) and Purves (1995) have shown that the Giant kingfisher takes larger crabs than otters and water mongooses which hunt along the Eerste River at Jonkershoek, and that otters, in turn, generally take larger crabs than the mongoose. This implies that otters at Jonkershoek are possibly exposed to higher concentrations of heavy metals than any other predators which feed on *P. perlatus* in this area, since they not only take large crabs which, although shown to contain lower concentrations of heavy metals per gram body mass than smaller crabs, would contain higher total body heavy metal concentrations, but also always consume the carapace, which was shown to contain the highest concentrations of most of the selected heavy metals. However, the opposite might possibly be true for the above mentioned animals found along the Bushmans River, since Somers & Purves (1996) showed that water mongooses along this river take significantly larger crabs than the Cape clawless otter.

In conclusion, the results of the present study have clearly raised many new questions on various issues surrounding the Eerste River ecosystem. With this study the grounds have been lain for several divergent and more intensive studies.

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