The ecology of free-living nematodes in nearshore marine and estuarine sediments of the microtidal lower west coast of Australia.

Submitted by

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Quotes of note

"We must not recoil with childish aversion from the examination of the humbler animals. In every realm of nature there is something marvellous."

-Aristotle-

"Those who truly love small life are deep lovers of mother nature."

-Takashi Amano-

"Sometimes a scream is better than a thesis."

-Ralph Waldo Emerson-

Declaration

I declare that this thesis is my own account of my research
and contains as its main content work which has
not previously been submitted for a degree
at any tertiary education institution.

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Mathew Hourston

Abstract

The overarching aim of this thesis was to describe the ecology of the assemblages of free-living nematodes in the sediments of nearshore marine and estuarine waters on the microtidal lower west coast of Australia. The thesis also provides descriptions of ten previously undescribed species as well as develops and tests a habitat classification scheme for the Swan River Estuary using these biotic assemblages.

The first section of my thesis has determined the ways in which the characteristics of the nematode assemblages in nearshore marine waters along the microtidal lower west coast of Australia are related to habitat type, time of year and shore-perpendicular zones. Three habitat types, which had previously been identified on the basis of a suite of enduring environmental characteristics, could be broadly described as highly sheltered from wave activity and containing dense seagrass (habitat type 1), moderately sheltered from wave activity and with sparse seagrass (habitat type 2) and relatively exposed to wave activity and with no seagrass (habitat type 6). Sampling in five consecutive seasons yielded > 15 000 nematodes, representing 75 species. The number of species and densities in habitat type 1, and particularly those in its subtidal zone, were far greater than those in the other two habitat types. Both of these biotic variables underwent marked seasonal changes, declining to low levels during winter. The compositions of the assemblages differed significantly among the three habitat types, with the differences between habitat types 1 and 6 being particularly marked. *Paracomesoma sipho*, *Dichromadora* sp., *Marylynnia annae* and *Pomponema* sp., which, on the basis of their buccal cavity morphology, are assumed to feed primarily on benthic diatoms, were particularly abundant at the most sheltered habitat type, whereas *Gonionchus australis*, *Theristus* sp. and *Bathylaimus australis*, which are assumed to be deposit feeders, were relatively abundant at the most highly exposed habitat type. The

compositions of the assemblages differed among seasons and were most discrete in spring, due to marked increases in the densities of certain species. However, differences in the compositions in the different zones of each habitat type were relatively small, presumably reflecting the influence of the small tidal regime of this region.

The second component of this thesis has determined the ways in which the density, number of species, species composition and trophic structure of free-living nematode assemblages in the subtidal waters of a large microtidal estuary change spatially and temporally, and has explored whether those four biotic characteristics are related to certain environmental factors. Based on data derived from samples collected seasonally at 12 sites throughout the estuary, the densities and number of species of nematodes decreased progressively with distance from estuary mouth, to reach a minimum at sites where salinities were most variable, and then increased slightly in the uppermost part of the estuary where salinities were least. Densities were also generally greatest in spring, due largely to increases in the abundance of epistrate-grazing species at the time when the amount of primary food (microphytobenthos) peaked. The spatial distribution of the composition of the nematode assemblages was closely correlated with salinity and, to a lesser extent, grain-size composition and amount of particulate organic material in the sediment (%POM). Although species composition changed sequentially along the estuary, the change was particularly pronounced between sites above and below the area where salinities started to decline markedly and become more variable and %POM increased markedly. This reflected, in particular, far greater abundances of *Spirinia parasitifera* at the six downstream sites, and of *Theristus* sp. 1 at the six sites further upstream. Species composition underwent pronounced seasonal cyclical changes at all sites, presumably reflecting interspecific differences in the timing of peak reproduction and thus of recruitment. The trophic structure of the nematode assemblages changed both spatially and temporally in relation to the relative abundance of different food

sources. Thus, for example, non-selective deposit feeders, such as *Theristus* sp. 1, dominated samples in the upper estuary, where %POM was by far the greatest, and was rare or absent at downstream sites. Conversely, epistrate grazers, such as species of the Chromadoridae, were most abundant at downstream sites in spring, when the density of the microphytobenthos reached its maximum.

The data for the nematode assemblages in nearshore subtidal marine sediments of the lower west coast of Australia were compared with those in nearshore subtidal sediments in the upper and lower regions of the Swan River Estuary. The densities and average species richness in cores from the marine environment were much lower than in cores from both estuarine regions. However, the total number of species found in the marine environment was much greater than in the estuary. The compositions of the nematode assemblages were more variable in marine than estuarine sediments. The assemblages from the two estuarine regions were far more similar to each other than to those from the marine region at a species level, and also, but to a lesser extent, at the generic and family levels.

While the trophic compositions of the nematode assemblages in the upper estuarine region was dominated by non-selective deposit feeding species and those of the lower estuarine region were dominated by epistrate grazing species during spring and non-selective deposit feeding species in other seasons, the dominant functional feeding groups varied among the sites representing the marine region. That variability presumably reflects differences in the relative contributions of the different potential food sources. Surprisingly, the trophic composition in the upper estuarine region, *i.e.* comprising predominantly non-selective deposit feeders, was similar to that at the very different environment of the most exposed marine site. The dominance of this feeding group at the marine site is assumed to be attributable to the fact that the only food source of any note is POM and, even then, it occurs in only small amounts.

Taxonomic descriptions have been produced for ten new species of nematodes found during the ecological studies of the free-living aquatic nematofaunas of south-western Australia. These species were chosen because they were members of families for which the other species had been described and, in a number of cases, were important for distinguishing between the compositions of *a priori* groups. They comprised four species of Axonolaimidae, representing the genera *Ascolaimus*, *Parascolaimus*, *Odontophora* and *Parodontophora,* and six species of Desmodoridae, representing single species of *Bolbonema*, *Eubostrichus*, *Catanema* and *Leptonemella* and two species of *Onyx*.

As a complement to the nematological study of the Swan River Estuary, a novel habitat classification system was developed and then applied in this environment. This system was based on enduring environmental characteristics and employed the relatively new multivariate statistical routines SIMPROF and LINKTREE. The applicability of habitat types produced by this classification system to biotic assemblages was tested using the data for the estuarine nematode assemblages described above. The results demonstrate that the compositions of the assemblages differed significantly among each of the habitat types defined by the classification system. While there were also significant differences between the compositions of the nematofaunas at sites belonging to the same habitat type, the extent of these differences were generally less than those between habitat types. A significant and strong correlation was also found between the spatial pattern exhibited by the environmental characteristics used to define habitat types, and that of the nematofauna.

Publiction list

The contents of Chapters 2 and 3 are published as peer-reviewed journal articles, the references for which are listed below. Additionally, publications detailing the habitat classification procedure and the taxonomic descriptions contained in Chapters 4 and 5, respectively, are currently in press. Chapter 6 is currently being prepared for publication.

Chapter 2

Hourston, M., Warwick, R. M., Valesini, F. J. & Potter, I. C. (2005) To what extent are the characteristics of nematode assemblages in nearshore sediments on the west Australian coast related to habitat type, season and zone? *Estuarine, Coastal and Shelf Science.* **64:**601-612.

Chapter 3

Hourston, M., Potter, I. C., Warwick, R. M., Valesini, F. J. & Clarke, K. R. (2009) Spatial and seasonal variations in the ecological characteristics of the free-living nematode assemblages in a large microtidal estuary. *Estuarine, Coastal and Shelf Science.* **82:**309-322.

Chapter 4

Valesini, F.J., Hourston, M. Wildsmith, M.J., Coen N.J. & Potter, I.C. (in press) New quantitative approaches for classifying and predicting local-scale habitats in estuaries. *Estuarine, Coastal and Shelf Science.*

Chapter 5

Hourston, M. & Warwick, R.M. (in press) New species of free-living aquatic nematodes from south-western Australia (Nematoda: Axonolaimidae and Desmodoridae) *Records of the Western Australian Museum.*

Chapter 6

Hourston, M., Warwick, R.M., Potter, I.C.P., & Valesini, F.J. (in prep) Comparisons between nematode assemblages of nearshore marine and estuarine environments of south-western Australia: The importance of taxonomic resolution

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Chapter 1 General introduction

The initial aims of my thesis were to study the ecology of the nematode faunas found in nearshore waters along the lower west coast of Australia and in the large Swan River Estuary, which is likewise located on that microtidal coast. As many of the nematode species collected during this part of the study were undescribed, the scope of my thesis was extended to include descriptions of the species in two of the more dominant families. During the course of my PhD, I also participated in developing a classification scheme for the main habitat types in the Swan River Estuary, which, in the future, could be used to predict the faunal composition that would likely be found at any particular site in that system.

1.1 Meiofauna

1.1.1 Definition and components

Meiofauna are defined by Coull (1999) as those benthic metazoans that pass through a 500 µm sieve, but are retained on meshes of 40-63 µm. Such organisms are often found in very large numbers in the sediments of almost all aquatic environments, with densities as great as 10^6 individuals m^{-2} being common (Giere, 1993; Coull, 1999). Meiofauna play an important role in aquatic ecosystems as they form a substantial part of the diet of many aquatic animals (Gee, 1989; Coull, 1990) and facilitate the remineralisation of organic matter (Coull, 1988; Riera & Hubas, 2003).

The numerous taxa that represent the meiofaunal community comprise two functional groups, *i.e.* the meromeiobenthos and holomeiobenthos (Giere, 1993). The meromeiobenthos contains those taxa that do not constitute part of the meiofauna for all of their life cycle, through either having a larval stage that is spent in the plankton or falling outside the size that defines the meiofauna at certain life history stages. Conversely, holomeiobenthoic organisms remain components of the meiofauna

throughout their entire lifecycle, *i.e.* they are sediment bound and always lie within the size range that defines meiofauna. Although many taxa constitute this latter group, the Nematoda is typically by far the most abundant and diverse, usually comprising between 60 and 90 % of the total number of individuals in a system and certain studies recording up to 130 species (Sharma & Webster, 1983; Pastor de Ward, 1998; Coull, 1999; Nicholas & Hodda, 1999; Vanhove *et al.*, 1999; Nicholas & Trueman, 2005).

1.1.2 Nematodes as bioindicators

Kennedy & Jacoby (1997), Bongers & Ferris (1999) and Coull (1999), among others, have promoted the use of the characteristics of nematode assemblages as bioindicators of the environmental conditions in aquatic ecosystems, pointing out that nematodes have the advantage of usually being in greater density and diversity than any of the other components of the meiofauna and typically being relatively immobile and thus not able to move far from a particular area. Furthermore, nematodes are ubiquitous, being present in both polluted and unpolluted sediments. In comparison to benthic macroinvertebrates, which are more commonly used as bioindicators, Kennedy & Jacoby (1997) considered that nematodes are more useful for this purpose because their small size makes sampling easier and more rapid, and the necessary samples comprise only a very small part of the total population.

The species composition of nematode assemblages has been found to change in response to various natural environmental gradients over scales ranging from very broad (latitudinal) to microscale habitat levels. Among the various environmental factors that influence the characteristics of meiofaunal assemblages, Coull (1999) considered that sediment particle size, salinity and temperature are the three most important.

1.1.2.1 Macroscale spatial gradients

Boucher (1990), Lambshead *et al.* (2000) and Mokievsky & Azovsky (2002) all found that the diversity of nematodes was related to latitude, with assemblages from temperate areas being more diverse than those in equatorial or polar regions. However, Rex *et al.* (2001) considered that the factor(s) responsible for the above trends remain ambiguous, reflecting the limited number of studies on the subject and that Gobin & Warwick (2006) found no evidence of latitudinal trends.

Cook *et al.* (2000) and Grémare *et al.* (2002) reported differences in both nematode abundance and diversity across the depth gradient of a continental shelf. The latter authors demonstrated that nematode densities in sediments were lower but more consistent in sediments in water depths greater than 200 m than in shallower waters. Lambshead (2003) attributed the negative correlation of nematode densities with depth over this scale to a decline in the abundance of primary producers available as a food source. Udalov *et al* (2005) offers corroboration to the assertion of Lambshead (2003), speculating that the decreasing trend in mean nematode size and density with increasing depth may be as a result of correlations with other environmental variables which also vary with depth rather than the direct effect of depth *per se*, *e.g.* sediment characteristics or trophic conditions.

1.1.2.2 Influence of salinity gradients

The generalised "model" developed by Remane (1934) for the effects of a salinity gradient on the species richness of benthic invertebrates in the Baltic Sea, postulated that species richness was greatest in marine waters, moderate in freshwater and least in waters with salinities between 5 and 8. Heip *et al.* (1985) and Attrill (2002) considered that the approach of Remane (1934) was not entirely appropriate for estuarine conditions, as it did not account for the variation that occurs in salinity over time. To address this issue, Attrill (2002) examined the relationship between species richness and

variation in salinity, rather than with average salinity. This latter author found that the diversity of assemblages was highest in the more stable marine waters than in areas where salinity was most variable and then increased in consistently fresh water. Ferrero *et al.* (2008) later tested Attrill's (2002) model and confirmed its validity. Other workers have found that the densities of nematodes followed similar trends, *i.e.* were greatest in stable marine waters and least in areas where salinities varied markedly (McArthur *et al.*, 2000; Yamammuro, 2000). Furthermore, the species compositions of nematode assemblages have been found to change progressively along spatial salinity gradients and undergo pronounced changes with sudden very and marked environmental alterations (Nicholas *et al.*, 1992; Armenteros *et al.*, 2006).

1.1.2.3 Tidal zonation

The area at a land / water interface comprises both submerged and emmersed zones, furthermore, when the body of water experiences tidal fluctuations, a third zone is present *i.e.* the intertidal. The vastly different physicochemical regimes of these three zones have very distinct effects on the biota which inhabit this area. Indeed, the concept that biotic assemblages change along a gradient perpendicular to the waterline has been recognised by authors studying many different organisms (*e.g.* Warwick, 1971; Blome, 1982; Hodda & Nicholas, 1985; Alongi, 1987c; Nicholas, 2001; Carlson *et al.*, 2006). Zonation within the intertidal area, *i.e.* between the high and low tide marks, is the most commonly studied gradient in this area in relation to nematode assemblages (*e.g.* Hodda & Nicholas, 1985; Alongi, 1987c; Olafsson, 1995; Ndaro & Olafsson, 1999; Gwyther, 2003). The trend toward intertidal studies is largely a product of the large expanses of such areas present in macrotidal regions. In microtidal regions, such as south-western Australia, the intertidal zone is very narrow and difficult to subdivide. Thus subtidal, intertidal and supratidal become much more relevant and useful units of study (*e.g.* Hourston *et al.*, 2005).

1.1.2.4 Vertical zonation

The majority of free-living aquatic nematodes can only inhabit those layers of sediment where oxygen is biologically available (Coull, 1999). Furthermore, within this oxic layer, most nematodes aggregate towards the water / sediment interface where oxygen concentrations are greatest (McLachlan, 1978; Heip *et al.*, 1985; Eleftheriou & McIntyre, 2005). As for densities, the species composition of nematode assemblages exhibit fine scale vertical zonation often on the scale of millimetres (Joint *et al.*, 1982; Steyaert *et al.*, 2003). Such zonation may also be attributed to the effects of variations in factors such as food availability, sediment granulometry and interspecific interactions such as competition and predation (Heip *et al.*, 1985; Steyaert *et al.*, 2003).

1.1.2.5 Sediment characteristics

The species compositions of nematode assemblages have been shown to be related to sediment grain-size composition and percentage organic matter (Heip *et al.*, 1985; Austen & Warwick, 1989; Muthumbi *et al.*, 2004). In their review of ecological studies on marine nematodes, Heip *et al.* (1985) concluded that the densities of marine nematodes were greatest in fine sediments and thus negatively correlated with grain-size. However, coarse sediments were identified as containing more diverse assemblages, while finer sediments were typically dominated by a few common species. Warwick (1971) found that the nematode taxa that were associated with coarse sediments often had thicker and more ornamented cuticles than those in silts and also were usually more setose. Heip *et al.* (1985) suggested that the possession of a robust cuticle is an adaptation for living in unstable coarse grained sediments.

1.1.2.6 Microhabitats

The small size of nematodes, together with their high densities in aquatic sediments has provided the opportunity of exploring microscale patchiness (1-10 cm) in characteristics of assemblage (Findlay, 1981). The main factor responsible for this variation is sometimes immediately evident. For example, the elevated densities of nematodes that are often present around the burrows of macrofaunal organisms reflect localised increases in oxygen concentrations (Heip *et al.*, 1985; Alongi, 1986) or particulate organic material (Dauwe *et al.*, 1998) . However, in many cases, the factors influencing the patchiness could not be determined. For instance, in contrast to their hypothesis, Pinckney & Sandulli (1990) failed to find a relationship between the densities of nematodes on an intertidal sandflat and the densities of microphytobenthic algae.

1.1.2.7 Seasonal variation

Seasonal variation in nematode assemblages has been studied in a range of environments, including mangroves (Alongi, 1987c), saltmarshes (Eskin & Coull, 1987), seagrass beds (Fisher, 2003), sandy nearshore marine waters (Nicholas, 2001), intertidal sandflats (Yodnarasri *et al.*, 2008) and the deep sea (Shimanaga *et al.*, 2004). In their review, Heip *et al.* (1985) attributed seasonal variations in the characteristics of meiofaunal assemblages to the influence of temperature, *i.e.* densities are typically lower in cooler periods than warmer periods as a result of the effects of temperature on both metabolic rate and reproductive success. Generation times for free-living aquatic nematodes typically range from days to months, while for certain species it may be up to a year (Platt & Warwick, 1988), however, this is highly dependent on characteristics of their environment (Warwick, 1981). In their laboratory studies, Moens & Vincx (2000b; 2000a) found that the generation times of common estuarine nematode species were greatly extended at lower temperatures

(2-10 times longer at 5 ºC than at 25 ºC) and that the rates of nutrient assimilation and respiration began to decrease at temperatures below *ca* 25 ºC. Furthermore, since many nematode species are selective feeders (Wieser, 1953; Moens & Vincx, 1997), the species composition of their assemblages are likely to change seasonally when the composition of their food sources underwent seasonal variations. In this context, it is relevant that the densities of certain nematode feeding guilds have been shown to be related to variations in the abundances of the food sources of those groups (Tietjen, 1969; Skoolmun & Gerlach, 1971; Austen & Warwick, 1995).

1.1.2.8 Small scale temporal variation

Due to the fact that meiofaunal nematodes are typically sediment bound and undergo relatively limited movement, variations in their community compositions over a short time scale (*i.e.* hours) tend to be small. However, some studies have identified changes in the vertical distributions of nematodes in relation to tidal cycles (Palmer, 1988; Steyaert *et al.*, 2001). Steyaert *et al.* (2001) found that certain species of nematodes migrated towards the sediment surface during inundation of a tidal flat and then returned when the tide was low, while the reverse pattern occurred with other species. The latter authors speculated that the tidal cycle of pore-water drainage and seep, changes in temperatures and migration of prey all contributed to those trends.

1.1.2.9 Anthropogenic influences

Research into anthropogenic influences on meiofaunal assemblages have focused particularly on the effects of organic enrichment, hydrocarbon spills, as well as various other specific contaminants and toxins, *e.g.* metals and metallic compounds (review by Coull & Chandler, 1992). The latter authors concluded that, in the majority of cases, the diversity of meiofauna declines in the presence of organic pollutants and, depending on the severity of the pollution and type of environment, may lead to either an increase or decrease in densities. The deleterious effects of copper, lead, zinc, iron and cadmium

contaminations on meiofaunal organisms have been documented by several authors in both ecological and experimental studies (Austen *et al.*, 1994; Somerfield *et al.*, 1995; Gyedu-Ababio & Baird, 2006).

The toxic compound Tribuyltin (TBT) is of particular relevance to aquatic organisms since it has been used extensively as an anti-foulant on boats. TBT, often in the form of paint chippings, is a common contaminant of sediment in areas of high boat usage such as harbours and marinas. Normally, this organo-tin compound breaks down relatively quickly in water, but when adsorbed onto sediment grains takes much longer to degrade (Neumann, 1970). Several workers have shown that TBT, even when present at relatively low concentrations, has significant detrimental effects on meiofaunal density, diversity and composition (Austen & McEvoy, 1997; Dahllöf *et al.*, 2001; Schratzberger *et al.*, 2002).

1.2 Review of Australian nematological studies

Greenslade (1989) provided a comprehensive checklist of the nematode species recorded in Australian marine and estuarine environments prior to 1989 and also gave details of the relevant taxonomic publications of nematodes. Since that publication, a considerable number of Australian taxonomic papers have been produced, (*e.g.* Greenslade & Nicholas, 1991; Nicholas, 1993; Blome & Riemann, 1994; Gourbault & Vincx, 1994; Stewart & Nicholas, 1994; 1995; Nicholas, 1996; Nicholas, 2002; Nicholas & Trueman, 2002). These taxonomic studies have obviously benefited the quality of many ecological studies on nematodes, as they have facilitated the identification of many of the key species.

As a result of the Belgian Expedition to the Great Barrier Reef in 1967, a series of publications were produced on the nematofauna of the region, the majority of which focussed only on taxonomy. The twelfth article however, *i.e.* Decraemer & Coomans (1978), provided a brief ecological description of the nematofauna in the mangroves and adjacent beach on Lizard Island (Great Barrier Reef).

McLachlan (1978; 1985) and McLachlan & Hesp (1984) studied meiofaunal assemblages at a high taxonomic level, with nematodes being only considered at the phylum level. Each study explored how the composition of the meiofaunal assemblage varied in response to a single environmental variable, *i.e.* redox discontinuity (McLachlan, 1978), stranded macrophyte accumulations (McLachlan, 1985) and mesoscale beach formations (McLachlan & Hesp, 1984).

Alongi (1986) also conducted a study on the Great Barrier Reef, which examined the distribution and trophic structure of nematode assemblages as they related to the functional zones of a carbonate sand reef. The same author also produced a series of publications on tropical estuarine meiofauna; two of which considered nematodes at a phylum level only (Alongi, 1987a; 1987c) and a third which investigated the distribution of nematode species among estuaries and intertidal zones in the same region (Alongi, 1987b; 1990).

The meiofauna of the Hunter River Estuary in eastern Australia were the subject of a series of studies by Hodda & Nicholas (1985; 1986a; 1986b). The first study examined the spatial distribution of major meiofaunal taxa as well as certain abundant nematode species within the intertidal area, and determined that the densities were most closely correlated with elevation above low tide mark and depth within the sediment, but neither with salinity nor the median grain-size of the sediment. Using the same study

area, Hodda & Nicholas (1986b) assessed seasonal variation of the major meiofaunal taxa as well as the nematode assemblages specifically, but found that while measurable temporal trends existed in the density, diversity and trophic characteristics of the nematode assemblages, they were not consistent across locations and were attributable to "non-seasonal environmental changes". Hodda & Nicholas (1986a) used k-dominance plots based on nematode species diversity to make comparisons between the nematofauna at sites contaminated with metallic pollutants with relatively pristine sites. However, the outcomes of this latter study were inconclusive and that there were many factors which confounded assessment of metallic pollution in this estuary. Hodda (1990) reanalysed the above data, along with new data from two other estuaries to assess the variation in nematode assemblages at three spatial scales. He concluded that the spatial variations were most related to oxygen penetration, organic content and grain-size composition of the sediment.

Warwick *et al.* (1990) examined the effects of localised physical disturbance by soldier crabs on the nematode assemblages of a Tasmanian beach. This study found that the composition of the nematode assemblage changed in response to this mechanical disturbance.

Nicholas *et al.* (1991) determined that the density and trophic composition of the nematode fauna in sediments in a temperate mangrove mudflat in the Clyde Estuary (New South Wales) changed both vertically throughout the sediment and among intertidal zones. Nicholas *et al.* (1992) examined the effects on the nematofauna of barrages in the Murray River Estuary, which restrict river flow and create periodic freshwater pulses when the river flow is seasonally high. Assemblages were found to be significantly different above and below the barrages, and furthermore, sudden seasonal decreases in salinity were found to cause very high mortality, presumably as a result of osmotic stress on individuals.

In the past decade, research into the ecology of nematodes in estuaries has been developed in two different geographical regions of Australia. The first centred on the estuarine mangrove habitats of the Barwon River in Victoria in temperate Australia (Gwyther & Fairweather, 2002, 2005, Gwyther 2003). These workers studied various aspects of colonisation of mangrove pneumatophores by meiofauna at a phylum level and also at a species level for nematodes, focusing on the ways in which the composition of those communities changed over time. In her study of nematodes in mangrove leaf-litter, Gwyther (2003) found that the diversity but not density of nematodes was related to the state of leaf degradation, and that the species and feeding guild compositions of the assemblages were influenced by location within the intertidal zone.

The second geographical region in which nematode research has been in conducted in estuaries in recent years is located further north near Townsville in tropical northern Australia. These studies demonstrated that certain characteristics of the nematode assemblages in mangrove and seagrass areas differed, and also changed during the year, which were presumed to reflect differences in food sources and variations in temperature and salinity (Fisher, 2003; Fisher & Sheaves, 2003).

Nicholas & Hodda (1999), Nicholas (2001) and Nicholas & Trueman (2005) are the only workers to have recently studied nematode assemblage at a species level in nearshore marine sediments in Australia. These studies, which were undertaken on a temperate, moderately exposed beach on the New South Wales coastline, demonstrated that the density and species composition of the nematode assemblages underwent seasonal changes and changed in response to extreme wave action which remodelled the beach. The abundances and compositions of the nematode fauna in the low, mid and high tidal regions of this beach were also found to differ markedly. Analysis of the data derived from the above beach and other beaches in widely-distributed regions of

Australia indicated that species richness provides an adequate index of biodiversity for the free living nematodes of these beaches.

Recently, Dye (2005) and Dye & Barros (2005) studied the meiofaunal assemblages of the intermittently closed and open lakes and lagoons (ICOLLs) on the mid New South Wales coast. Trends in the compositions of the meiofauna at high taxonomic levels were considered in relation to locations within each lake and whether the lakes were open or closed, and artificially or naturally breached. The contributions of nematodes to the meiofaunal assemblages declined from the mouths to the more isolated areas of these lakes. Importantly from a management point of view, the characteristics of the meiofaunal assemblages of artificially and naturally breached lakes did not differ.

1.3 Habitat classification

1.3.1 The need for habitat classification schemes

The highly productive estuarine environment and nearshore marine areas rank among the most sensitive of aquatic environments and are also particularly susceptible to anthropogenic perturbation (Schleske & Odum, 1961; McLusky & Elliott, 2004). These systems and their catchments are often intensively used by human populations, thus leading to potential conflict between a range of commercial and recreational activities and the need to conserve the biotic integrity of the those environments (Hodgkin, 1994; Edgar *et al.*, 2000). In order to balance these requirements, environmental managers require a sound scientific basis for developing their policies and making decisions.

The management of aquatic systems has often been based on an *ad hoc* "scenery over science" approach (Hackman, 1993; Roff & Taylor, 2000), in which areas that contain environments or biota with high aesthetic value are designated as having a high conservation priority. Although this approach to management has the advantage of protecting the visible and charismatic elements of an ecosystem, it is not based on a

robust scientific identification of those components that are critical for optimal ecosystem function. In contrast, habitat classification schemes provide a systematic and rigorous method for determining the range and characteristics of habitats within a given area or ecosystem. In addition to providing reliable habitat inventories, such schemes also provide a foundation for understanding the relationships between biota and their habitats. Consequently, such schemes can ultimately be developed as tools for predicting what type of biota are likely to be found at any site within a system, while minimising the need for further extensive fieldwork. This has many applications for both environmental managers and ecologists (*c.f.* Valesini *et al.*, 2003 and Valesini *et al.*, 2004).

1.3.2 Types of classification schemes

Existing habitat classification systems for nearshore marine and estuarine waters encompass a range of different spatial resolutions and levels of complexity. In their simplest form, they can be based on a subjective separation of sites into one of a series of broad and obvious classes, *e.g.* sand *vs* seagrass (York *et al.*, 2006) or sheltered *vs* exposed (Fowler-Walker *et al.*, 2006). This type of simple classification is of use when the habitat groups are very distinct and the aims for the dependent study are very clear, as in the examples above. However, many management plans and ecological studies require a more quantitative and detailed designation of habitats than just a subjective separation based on a single variable. Many such schemes have been developed for coastal and estuarine waters (*e.g.* Banks & Skilleter, 2002; Madden *et al.*, 2005; Hume *et al.*, 2007).

One of the most common architectures for habitat classification schemes is that of the hierarchical system, whereby broad levels of the classification contain a series of nested classification units (*e.g.* Allee *et al.*, 2000; Madden *et al.*, 2005). One of the greatest advantages of these systems is that the step-wise nature of their classification process

allows the inclusion of a large number of characteristics while allowing the user to consider only a few at each step of the process. However, these schemes have the potential to become highly complex with many levels, and as a result, become difficult to apply. Furthermore, some hierarchical systems can produce far more habitat types than are either practical or relevant (*e.g.* Merkel & Associates, 2003)

Many published habitat classification schemes employ "enduring environmental variables" as classification criteria, since such variables are often simple to measure from remotely sensed sources (*e.g.* aerial photographs and maps), the results remain valid over time, and the variables only need to be quantified once. In contrast, some classifications also use non-enduring environmental variables, or characteristics of the flora and / or fauna as classification criteria since they quantify or are directly linked to the important biotic ecosystem components (*e.g.* Connor *et al.*, 1996). The main disadvantage of using non-enduring and / or biotic variables is that extensive data collection is required over appropriate temporal scales before suitable information can be gleaned for use in habitat classification. Such an approach usually requires a great expenditure of resources for collection and analysis before the classification can be constructed, particularly in the case of small and mobile biota.

The variables used in various habitat classification schemes may be defined as either quantitative or qualitative. The major advantage of using quantitative classification variables is that it allows the statistical demonstration of significantly different habitat types, *i.e.* each habitat type is significantly different from every other habitat type. Furthermore, it allows statistical comparisons with quantitatively defined biotic assemblages. When qualitative variables are used, such statistical validation of the habitat types is not possible (*e.g.* Howes *et al.*, 1994), which precludes statistical matching of the biotic assemblages (*e.g.* Zacharias *et al.*, 1999; see Subsection 4.4.1).

1.3.3 Ideal characteristics of habitat classification schemes

From the consideration of the aforementioned main points, it is proposed that the most rigorous and useful schemes for classifying nearshore marine and estuarine habitats, and thus providing a basis for predicting the biota that are likely to be found in each habitat should have the following four characteristics:

(i) *Based on enduring environmental characteristics.* The use of enduring environmental characteristics for classifying habitats minimises the amount of field time and effort required for and is thus more efficient than quantifying the biota. In most cases, the values for such variables can be extracted from a database, measured from a map or quantified from an aerial photograph.

(ii) *Quantitative.* Habitat classification systems constructed from quantitative data are unambiguous, repeatable and provide a basis for testing statistically whether the resultant separation of habitat types is valid. Furthermore, such schemes also allow statistical examination of the extent to which the characteristics of the biotic assemblages and those of the habitat types are related.

(iii) *User-friendly.* The structure of the scheme should be easy to understand and use, and the data employed should be relatively easy to acquire.

(iv) *Predictive.* The classification system should allow the habitat type of any site, not employed to produce the original habitat classification, to be easily and accurately predicted. This is of particular importance for ongoing management and the assessment of aquatic systems.

1.4 General aims

The general aims of this study are five-fold, each of which corresponds to a different chapter of this thesis. The specific hypotheses which are used to explore these broad aims are detailed in each of the relevant chapters.

Chapter 2

To determine the extent of the match between the spatial differences in the characteristics of the nematode assemblages in nearshore marine waters along the lower west coast of Australia with the habitats identified in those waters by Valesini *et al.* (2003). Furthermore, to determine the relative extent of differences in the composition of the nematode assemblages among habitat types, seasons and shore-perpendicular zones.

Chapter 3

To determine if the characteristics of the nematode assemblages in nearshore subtidal sediments of the Swan River Estuary changed spatially along the length of the estuary and / or temporally among seasons over the course of a year, and to ascertain whether any such changes are related to particular environmental characteristics.

Chapter 4

To devise and implement a quantitative, readily usable and flexible habitat classification scheme based on enduring environmental variables, for classifying the habitat types within estuaries of south-western Australia.

Chapter 5

To identify and formally describe a significant number of the new nematode species which may be found during the course of the preceding ecological studies of the nematode assemblages in nearshore marine and subtidal estuarine waters in south-western Australia.

Chapter 6

To determine if the characteristics of the nematode assemblages in subtidal nearshore sediments differ significantly among upper and lower regions of the Swan River Estuary and nearby marine areas. Furthermore, to assess the spatial differences in the composition of the nematode assemblages throughout the Swan River Estuary as determined in Chapter 3, in the context of the habitat classification scheme developed in Chapter 4.

Chapter 2 Ecology of free-living nematodes in nearshore coastal waters of south-western Australia

2.1 Introduction

Meiofauna are often the most abundant metazoans in the soft sediments of estuaries and nearshore marine waters (Giere, 1993). Since these invertebrates make a substantial contribution to the diets of larger organisms, they constitute a crucial component of food chains and play an important role in nutrient recycling in these waters (Gee, 1989; Coull, 1990; Kennedy & Jacoby, 1997). Despite their importance, there are far less data on the diversity and ecology of the meiofauna than the macrofauna, mainly reflecting a bias towards studying organisms that are sufficiently large for ready identification (Giere, 1993; Coull, 1999). The most numerically important phylum of the meiofauna in nearshore marine and estuarine waters is the Nematoda, whose individuals often outnumber those of all other meiofaunal taxa collectively (Heip *et al.*, 1985; Coull, 1999). Most of the studies on the ecology of the Nematoda in temperate nearshore marine and estuarine waters have been conducted in the Northern Hemisphere (*e.g.* Wieser, 1959a; Warwick, 1971; Platt, 1977; Warwick & Gee, 1984; Soetaert *et al.*, 1995; Tita *et al.*, 2002; Yodnarasri *et al.*, 2008). The only studies of the ecology of nematodes in temperate coastal marine waters of the Southern Hemisphere, that are based on separation at the species level, are those of Wieser (1959b) in Chile, and of Warwick *et al.* (1990), Gwyther & Fairweather (2002), Nicholas (2001) and Nicholas & Hodda (1999) in eastern Australia.

The densities, diversities and / or compositions of nematode assemblages have been related to individual factors, such as sediment grain size (Wieser, 1959a; Warwick, 1971; Alongi, 1986; Coull, 1988; Ndaro & Olafsson, 1999), organic content (Warwick, 1971; Moens *et al.*, 1999), extent of oxygenation of the sediment (Coull, 1988) and degree of exposure to wave action (Wieser, 1959a; 1959b). However,

no attempt has been made to explore statistically whether the compositions of the nematode assemblages in a region are related to habitat type, where the habitat types have been distinguished quantitatively on the basis of differences in the values for a suite of environmental characteristics. Since the compositions of the nematode assemblages are good indicators of variations in environmental conditions (Warwick, 1971; Alongi, 1986; Tita *et al.*, 2002), they provide a good "model" for investigating the extent to which biotic criteria differ among habitat types.

During the present study, focus was placed on determining the extent to which the species richness, density and composition of nematode assemblages in bare sand sediments on the heterogeneous and microtidal lower west coast of Australia are influenced by habitat type, time of year (season) and tidal zone. Nematode assemblages were thus sampled seasonally at three nearshore habitat types, which had been shown to differ, in particular, in the extent to which they were exposed to wave activity and to which they contained seagrass (Valesini *et al.*, 2003). Our results were used to test the following hypotheses.

(i) The diversity and density of nematodes will be greatest in the habitat type that provides the most stable substrate for benthic organisms, *i.e.* the one most highly sheltered from wave activity and which contains areas of dense seagrass.

(ii) The composition of the nematode assemblages will vary among habitat types due to the affinity of certain species for particular environmental conditions. In particular, it is hypothesised that the prevalence of epistrate grazers (sensu Wieser, 1953) will be greatest in sheltered habitats, in which areas the microphytobenthos is likely to be most abundant (Fielding *et al.*, 1988).

(iii) Seasonal changes in environmental characteristics such as wave activity and water temperature will be accompanied by changes in diversity, density and species composition of the nematode assemblages.

(iv) As tidal action in south-western Australia is limited, the characteristics of the nematode fauna in nearshore waters will not vary markedly in zones perpendicular to the shore.

2.2 Materials and methods

2.2.1 Sampling regime and laboratory procedures

The nematode assemblages in three zones (A, B and C) in the sandy substrates at each of three habitat types, designated as 1, 2 and 6 by Valesini *et al.* (2003), were sampled during the day in each season between the summers of 2000 and 2001 (Fig. 2.1). Habitat type 1 is the most highly sheltered from wave activity and contains areas of dense seagrass within 50 m of the shoreline and further offshore, whereas habitat type 6 is the most exposed to wave activity and supports no attached vegetation. Habitat type 2 is intermediate between habitat types 1 and 6 in its characteristics, being moderately sheltered from wave activity and containing sparse patches of seagrass within 50 m of the shore and also further offshore. The sediment grain size profile for each of the three habitat types was very similar, with the vast majority of grains in each habitat type separating out on mesh sizes of 125, 250 and 500 μm, with their contributions on those mesh sizes at the different habitat types ranging between 21-32 %, 38-45 % and 24-29 %, respectively (Wildsmith *et al.*, 2005). When present, seagrass beds comprised mixed stands of *Posidonia australis*, *P. sinuosa, Amphibolis antarctica* and *A. griffithii.* Zone A (upper swash) is located between the high-water mark on the beach-face and the effluent line (*i.e.* the highest point of groundwater discharge on the beach-face), and is thus submerged for only part of each tidal cycle. Zone B (lower swash) is situated between the effluent line and the lower limit of the wave swash, and thus its interstitial

environment, unlike that in Zone A, remains saturated. Zone C (subtidal) is located in approximately 1 m of water at low tide and is thus permanently submerged throughout the tidal cycle.

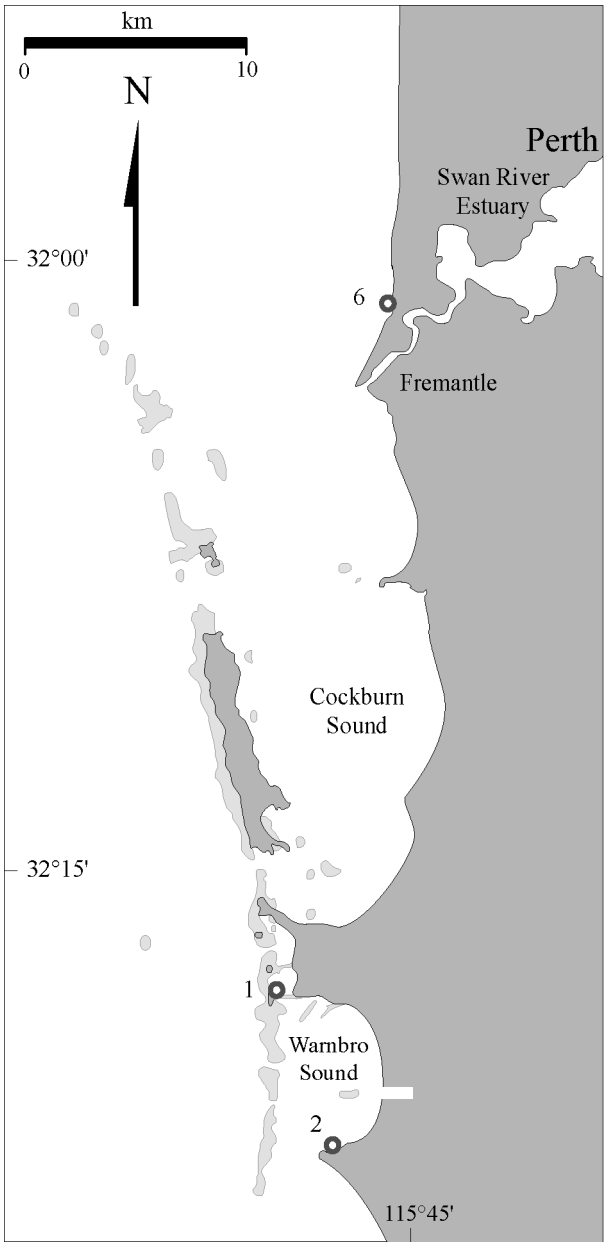

Figure 2.1 Map of the Perth metropolitan coast in the south-western Australia, showing the location of the three sites at which nematode assemblages were sampled, and which represented habitat types 1, 2 and 6 as classified by Valesini *et al.* (2003). Light grey areas denote significant areas of subtidal reef.

Five randomly-located sediment cores were collected during the day from bare sand substrate in each zone in each habitat type and in each of the five seasons of sampling. On each sampling occasion, the samples from each zone in each habitat type were taken from within an area of *ca* 700 m^{-2} and were always separated by a distance of at least

2 m. Sampling, which was always undertaken at least 3 m from any seagrass that was present, was spaced over two weeks in the middle of each season to reduce the chance of bias occurring through the collection of sample under atypical environmental conditions. The perspex corer was 3.2 cm in diameter, 11 cm high and sampled an area of 8.03 cm^2. Since nematodes can penetrate to substantial depths in sandy substrates (Nicholas & Hodda, 1999), the densities of nematodes that we have calculated from each core are probably an underestimate of those that would have been present. However, since the sediment compositions of the sandy substrates in each of the three habitat types is very similar (Wildsmith *et al.*, 2005) the comparisons made among the relative densities of nematodes in the three habitat types are likely to be broadly applicable. Each sediment core was immediately fixed in 5 % formalin / seawater solution. The sediment from each core was passed through two nested sieves, the first made of 500 μm mesh and the second of 63 μm mesh, a procedure which excluded benthic macroinvertebrates but retained the meiofauna. The meiofauna were removed from the remaining fine sediment by flotation in the colloidal silica solution Ludox ™, which separated out the organic fraction. Each sample was rinsed in water and preserved in 70 % ethanol, and then diluted to 20 ml, from which five 1 ml subsamples were extracted without replacement. The nematodes in each subsample were isolated under a binocular dissecting microscope and those in each group of five subsamples were pooled. These pooled samples were placed in a 10 % glycerol / water solution, which was evaporated for 12 hours at 60 ºC, leaving the nematodes in anhydrous glycerol. The nematode samples were mounted in anhydrous glycerol on microscope slides, which were then sealed with paraffin wax and slide sealant (Platt & Warwick, 1988).

Nematodes were identified to nominal species level using a compound microscope at magnifications of between 100 and 1000 x and employing mainly the keys in Platt & Warwick (1988) and Warwick *et al.* (1998). The number of each nematode

species in each sample was then converted to a density, *i.e.* number of individuals 10 cm^{-2}.

2.2.2 Statistical analyses

The species richness and density of nematodes in the replicate samples were subjected to Analysis of Variance (ANOVA) to determine whether those variables differed significantly among habitat types, seasons and zones. To determine whether a variable required transformation in order to conform to the assumption of normality and constant variance and, if so, which type of transformation was required, the relationship between $\log_{10}$ of the standard deviations and $\log_{10}$ of the means of the replicates for that variable was examined (Clarke & Gorley, 2001). The results demonstrated that, prior to subjecting the above variables to ANOVA, the number of species and density both required log-transformation. The H_0 for the ANOVA, namely that the means for each of the above dependent variables did not differ significantly among the independent variables, was rejected when the significance level (P) was < 0.05. Each replicate sample was considered to be independent of all other samples and all main effects were regarded as fixed.

The compositions of the nematode assemblages were analysed using routines in the PRIMER 5.2 statistical package (Clarke & Gorley, 2001). The $\log_{10}$ transformed densities of the various nematode species, derived from replicate samples from each zone in each habitat type in each season, were used to construct Bray-Curtis similarity matrices. These matrices were subjected to non-metric multidimensional scaling (nMDS) ordination.

One-way and two-way crossed Analysis of Similarity (ANOSIM) tests were used to determine whether the faunal compositions of samples from the various habitat types, seasons and zones differed significantly among those *a priori* groups. In each ANOSIM test, the null hypothesis that there were no significant differences among groups was

rejected if the significance level (P) was < 0.05. When significant differences were detected among *a priori* groups, the R-statistic was used to determine the extent of those differences, and Similarity Percentages (SIMPER) was employed to elucidate which species typified each of those groups and distinguished between each pair of groups.

All ordination plots, ANOSIM tests and SIMPER employed replicates except for the initial ordination plot, which compared the overall compositions in each of the habitat types (Fig. 2.3) and for clarity was constructed using the means of each set of replicate samples.

2.3 Results

2.3.1 Nematode assemblages

More than 15 000 nematodes, representing 75 species, were recorded in the 225 cores collected during this study (Table 2.1). The number of species was greatest at the highly sheltered habitat type 1 (62) and least at habitat type 6 (44) which was the most exposed to wave activity. Moreover, the total number of nematodes collected from habitat type 1 was *ca* 5-6 times greater than at each of the other two habitat types (Table 2.1). Eight species contributed more than 5% each to the total number of individuals in habitat types 1 and 2, and four species exceeded this level of contribution in habitat type 6. The dominant species, *i.e.* those contributing >10 % to the overall number of nematodes, differed among the three habitat types. Thus, *Paracomesoma sipho* (14.9%), *Mesacanthion* sp. nov. (13.3 %) and *Daptonema* sp. (10.8 %) dominated the nematode assemblage at habitat type 1, whereas *Chromadorita* sp. (21.2 %) and *Trileptium* sp. (12.6 %) were the most abundant species at habitat type 2, and *Gonionchus australis* (31.9 %), *Theristus* sp. 1 (17.7 %) and *Onyx potteri* sp. nov. (13.1 %) constituted the majority of nematodes at habitat type 6 (Table 2.1).

	Habitat type 1			Habitat type 2			Habitat type 6		
	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk
Paracomesoma sipho	28.2	14.9	1	2.3	7.2	5	1.5	3.7	5
Mesacanthion sp. nov.	25.1	13.3	2	0.5	1.5	12	0.5	1.2	13
Daptonema sp.	20.5	10.8	3	2.1	6.7	6	0.5	1.3	12
Chromadorita sp.	12.6	6.7	4	6.7	21.2	1	0.3	0.7	20
Neochromadora sp.	12.3	6.5	5	0.1	0.4	26	0.1	0.2	35
Trissonchulus sp.	10.9	5.8	6	0.2	0.5	24	0.3	0.8	17
Dichromadora sp.	10.8	5.7	7	0.3	1.0	19	0.4	1.0	15
Theristus sp. 1	9.5	5.0	8	2.5	7.7	3	7.1	17.7	2
Trileptium sp. nov.	-	-	-	4.0	12.6	2	0.3	0.7	19
Enoplus sp.	0.1	0.1	54	2.3	7.3	4	0.1	0.2	32
Metadesmolaimus sp.	1.1	0.6	23	1.6	5.1	7	0.7	1.7	10
Viscosia sp.	0.7	0.4	33	1.6	5.1	8	0.4	1.0	14
Gonionchus australis	1.1	0.6	26	0.4	1.1	18	12.8	31.9	1
Onyx potteri sp. nov.	0.3	0.1	48	0.4	1.4	14	5.2	13.1	3
Rhabditis sp. 1	1.7	0.9	19	0.4	1.4	15	2.3	5.8	4
Bathylaimus australis	2.1	1.1	16	0.9	2.9	9	1.2	3.0	6
Ascolaimus australis sp. nov.	0.3	0.1	45	-	-	-	0.9	2.2	7
Nudora sp.	-	-	-	0.1	0.4	30	0.8	2.0	8
Enoplolaimus aff. *litoralis*	0.1	<0.1	57	0.1	0.2	42	0.8	2.0	9
Viscosia sp. 2	0.8	0.4	31	0.5	1.7	11	0.6	1.5	11
Metoncholaimus sp.	0.6	0.3	36	0.1	0.2	38	0.4	1.0	16
Epacanthion georgei	-	-	-	-	-	-	0.3	0.8	18
Graphonema sp. 2	1.5	0.8	21	0.4	1.4	16	0.3	0.7	21
Marylynnia annae	7.3	3.9	9	0.1	0.2	33	0.3	0.7	22
Trefusia sp.	2.0	1.1	18				0.3	0.7	23
Microlaimus sp. 1	2.9	1.5	13	0.7	2.3	10	0.2	0.5	24
Spirinia laevis	1.7	0.9	20	0.1	0.3	31	0.1	0.3	26
Camacolaimus sp.	0.7	0.4	32	0.2	0.6	22	0.1	0.2	30
Pomponema sp.	3.4	1.8	12	0.1	0.2	34	0.1	0.2	31
Graphonema sp. 1	0.9	0.5	28	0.5	1.5	13	0.1	0.2	33
Theristus sp. 2	1.1	0.6	27	0.2	0.6	21	0.1	0.2	34
Metalinhomoeus sp.	5.1	2.7	10	-	-	-	0.1	0.2	38
Parapinnanema sp.	3.9	2.1	11	-	-	-	0.1	0.2	39
Catanema australis sp. nov.	2.9	1.5	14	-	-	-	0.1	0.2	40
Trochaimus sp.	2.1	1.1	17	-	-	-	0.1	0.2	41
Halanonchus sp.	-	-	-	0.4	1.3	17	-	-	-
Paralinhomoeus sp. 1	1.5	0.8	22	0.2	0.6	20	-	-	-
Microlaimus sp. 3	0.3	0.2	44	0.2	0.6	23	-	-	-
Chromadorina sp.	2.4	1.3	15	0.1	0.4	27	-	-	-
Microlaimus sp. 2	1.1	0.6	24	0.1	0.2	35	-	-	-
Gomphionema typicum	1.1	0.6	25	-	-	-	-	-	-
Mean density of nematodes		189			32			40	
Number of species		37			33			35	

Table 2.1 The mean density ($\overline{X}$; numbers 10 cm^{-2}), percentage contribution to each core (%) and rank by abundance (Rk) of each of the nematode species in samples collected at habitat types 1, 2 and 6. Only those species which contributed > 5 % to the total density in at least one of the habitat types are presented.

2.3.2 Number of species, density and species relatedness

Three-way ANOVA demonstrated that both the number of species and density of nematodes differed significantly among habitat types, seasons and zones. However, there were significant two-way interactions between habitat type and season for both of these dependent variables, and also between habitat type and zone for the latter of those variables (Table 2.2).

	df	Number of species		Density		Δ+		Λ+	
Main Effects									
Habitat type(H)	2	4.046	***	14.945	***	6724.207	***	459955.587	***
Season (S)	4	1.690	***	6.264	***	8620.598	***	371577.687	***
Zone (Z)	2	0.236	**	1.269	***	435.596	ns	19032.328	ns
Two-way interactions									
HxS	8	0.137	**	0.678	***	1759.237	**	68434.869	*
HxZ	4	0.083	ns	0.638	**	1394.130	ns	11171.414	ns
SxZ	8	0.013	ns	0.111	ns	442.552	ns	13373.236	ns
Three-way interactions									
HxSxZ	16	0.059	ns	0.273	ns	671.578	ns	31578.360	ns

Table 2.2 Mean squares and significance levels for three-way ANOVA of the number of species and density of nematodes, and Δ+ and Λ+ for samples from zones A, B and C at habitat types 1, 2 and 6 in each season between summer 2000 and summer 2001. df = degrees of freedom. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns = not significant.

For both number of species and density, the mean squares for habitat type were far greater than those for the other two main effects, which, in turn, were greater than those for the interactions. The mean number of species and densities were greatest at habitat type 1 in each season and typically markedly so (Figs 2.2a, c). In each habitat type, the values for both of these biotic variables declined to a minimum in winter and then increased during spring and summer 2001. The significant habitat type x season interactions were attributable largely to the fact that the values for these two variables increased between summer 2000 and autumn in habitat types 1 and 6, whereas the reverse was true in habitat type 2 (Figs 2.2a, c). The mean number of species in zone C was significantly greater than in either zones A or B (Fig. 2.2b). The mean densities of nematodes in each zone at habitat type 1 were far greater than those in the corresponding zones at habitat types 2 and 6 (Fig. 2.2d). However, while the mean density was greatest in zone C at habitat types 1

and 6, and particularly in the first of these habitat types, it was greatest in zone A in habitat type 2 which accounts for the habitat type x zone interaction (Fig. 2.2d).

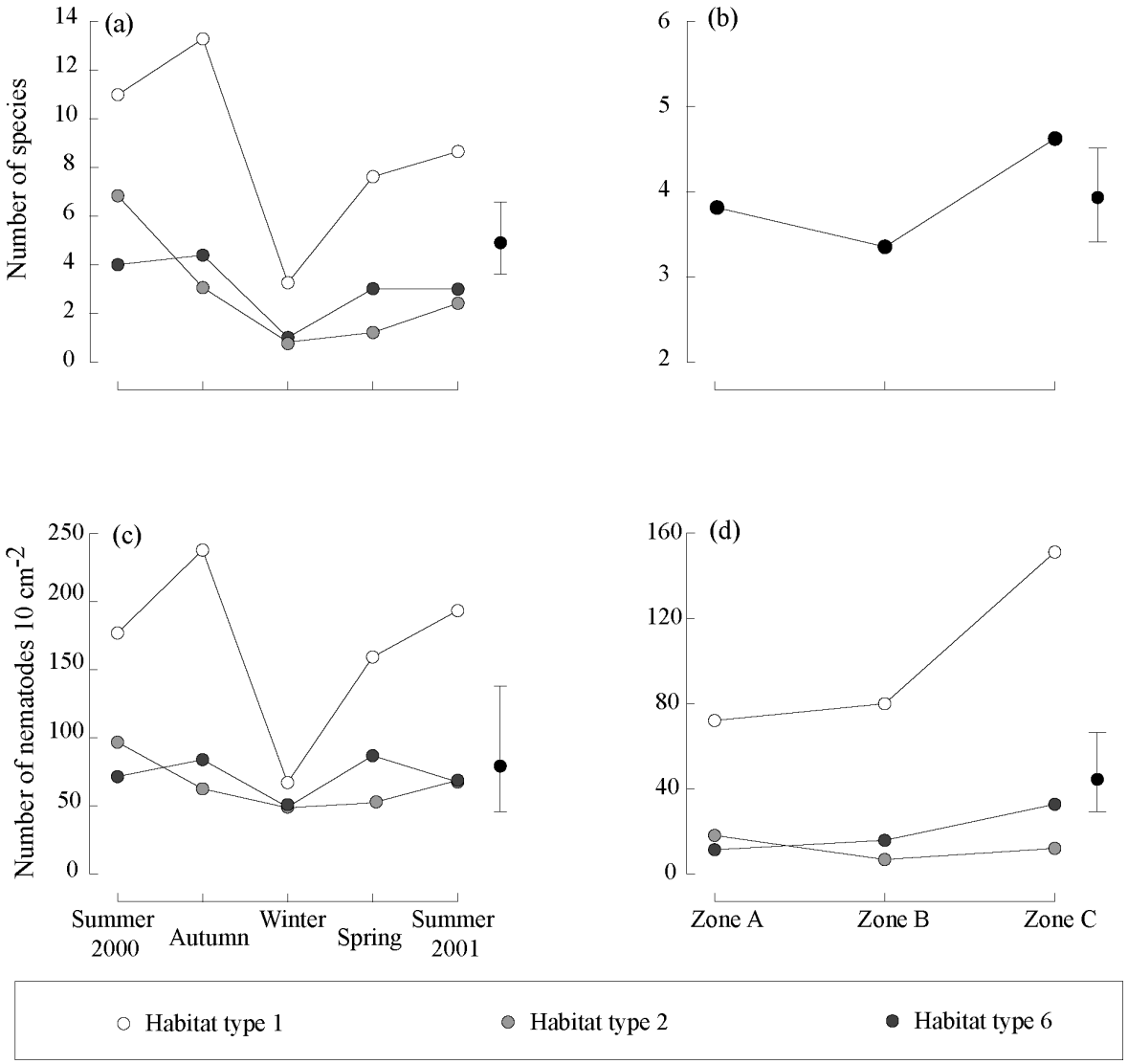

Figure 2.2 Mean number of nematode species at (a) each habitat type in each season and (b) in each zone. Mean density of nematodes at (c) each habitat type in each season and (d) in each zone at each habitat type. Points represent the back transformed marginal means used by ANOVA, and the overall mean ± 95 % CI for each variable is shown on the right of its respective plot.

2.3.3 Comparison of nematode assemblages among habitat types

When the mean densities of the various nematode species recorded in each zone at each habitat type during each season were subjected to nMDS ordination and coded for habitat type, the samples from each habitat type, and particularly from habitat type 1, formed relatively discrete groupings (Fig. 2.3). One-way ANOSIM showed that the overall compositions of the nematode assemblages differed significantly among habitat types

(P = 0.001) and that the extent of those differences were greater than those among seasons and zones, *i.e.* R-statistics = 0.233, 0.111 and 0.011, respectively. Pairwise comparisons demonstrated that the differences in species compositions were greatest for habitat types 1 *vs* 6 (R-statistic = 0.361) and least for habitat types 2 *vs* 6 (R-statistic = 0.161).

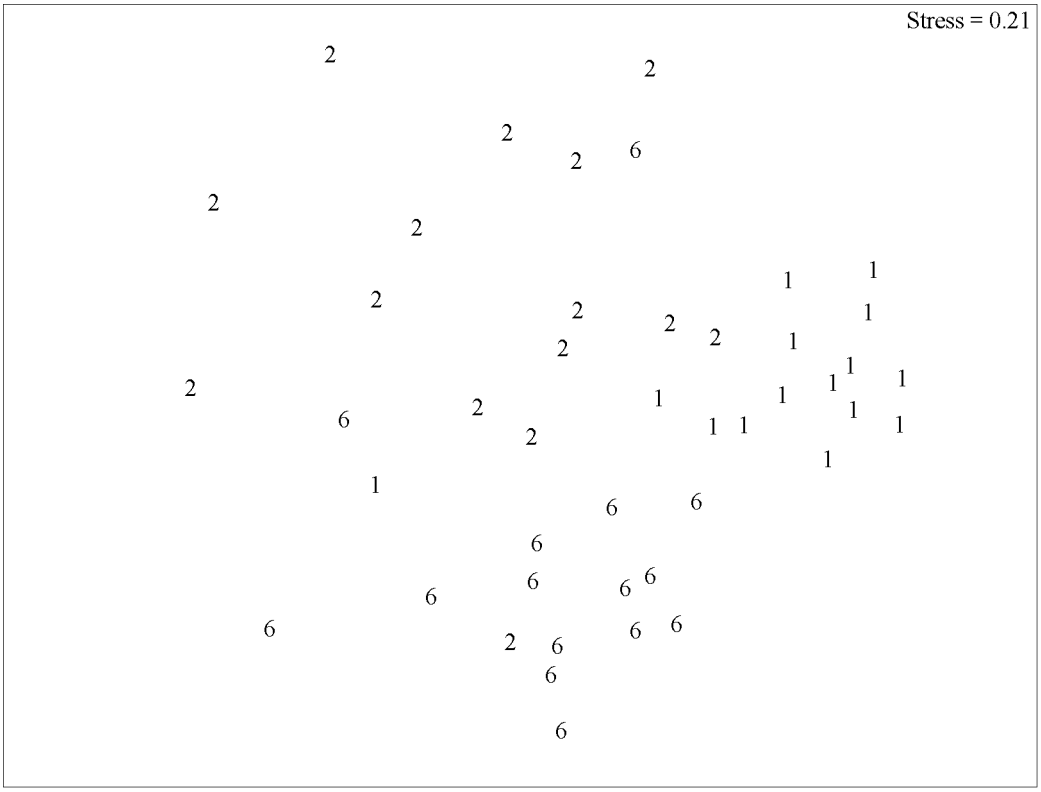

Figure 2.3 Three-dimensional nMDS ordination of the mean densities of nematode species in samples collected at habitat types 1, 2 and 6, in zones A, B and C during all seasons between summer 2000 and summer 2001, coded for habitat type.

SIMPER demonstrated that the nematode fauna at habitat type 1 was distinguished from that at habitat type 6 by relatively greater numbers of *Mesacanthion* sp. 1 *Paracomesoma sipho*, *Daptonema* sp., *Dichromadora* sp., *Marylynnia annae* and *Nanolaimoides decoratus* and by relatively lower numbers of *Gonionchus australis* (Table 2.3). *Thersitus* sp. 1 and *P. sipho* were the only species that contributed to the suites that typified both habitat types 1 and 6, and the latter species was far more important at the first of these habitat types (Table 2.3). These latter two ubiquitous species were also among the species that typified habitat type 2, the others being *Trileptium* sp. nov. and *Enoplus* sp., neither of which were typifying species for either habitat types 1 or 6.

	Habitat type 1	Habitat type 2	Habitat type 6
Habitat type 1	*Paracomesoma sipho* *Mesacanthion* sp. nov. *Theristus* sp. 1 *Dichromadora* sp. *Marylynnia annae* *Pomponema* sp.		
Habitat type 2	*Mesacanthion* sp. nov. (1) *Paracomesoma sipho* (1) *Daptonema* sp. (1) *Dichromadora* sp. (1) *Marylynnia annae* (1) *Pomponema* sp. (1)	*Paracomesoma sipho* *Theristus* sp. 1 *Trileptium* sp. nov. *Enoplus* sp.	
Habitat type 6	*Mesacanthion* sp. nov. (1) *Paracomesoma sipho* (1) *Daptonema* sp. 1 (1) *Dichromadora* sp. (1) *Marylynnia annae* (1) *Pomponema* sp. (1) *Gonionchus australis* (6)	*Paracomesoma sipho* (2) *Bathylaimus australis* (6) *Onyx potteri* (6) *Gonionchus australis* (6) *Theristus* sp. 1 (6)	*Theristus* sp. 1 *Onyx potteri* *Gonionchus australis* *Bathylaimus australis* *Paracomesoma sipho*

Table 2.3 Species determined by SIMPER as those most responsible for typifying the nematode assemblages at habitat types 1, 2 and 6 (non-shaded boxes) and for distinguishing between the nematode assemblages at each pair of those habitat types (shaded boxes). The habitat types at which distinguishing taxa were most abundant are provided in bracketed superscripts.

Two-way crossed ANOSIM tests for season x zone in each habitat type showed that the species composition at two habitat types were influenced significantly by both of those variables whereas the assemblage at habitat type 2 wwas only significantly influenced by season (Table 2.4). Thus, subsequent analyses of nematode compositions among habitat types were carried out separately for each season x zone combination.

	Habitat type 1	Habitat type 2	Habitat type 6
Season	0.313 ***	0.229 **	0.265 ***
Zone	0.209 ***	0.087 ns	0.181 ***

Table 2.4 Global R-statistics for two-way crossed ANOSIM tests for the compositions of the nematode assemblages for season x zone in each habitat type. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

nMDS ordination of the densities of the species recorded in each zone in each season showed that the samples from the different habitat types usually formed tight and relatively discrete groups (Fig. 2.4). This was particularly the case in zone C during the summers of 2000 and 2001 (Figs 2.4c and o) and in zone B during spring and summer 2001 (Figs 2.4k and n). One-way ANOSIM tests demonstrated that the species compositions of nematodes differed significantly among habitat types in each season x zone combination (P = 0.001-0.009), except for in both zones A and B during winter (Table 2.5). The associated Global R-statistic values were usually greatest for zone C, especially during summer in both 2000 and 2001, and were > 0.350 in the majority of cases in the other two zones.

2.3.4 Seasonal and zonal comparisons of nematode assemblages in each habitat type

When the data for each habitat type was analysed separately, the Global R-statistic values for the nematode compositions among seasons were greater than those for zones, ranging from 0.224-0.331 and 0.081-0.195, respectively (Table 2.5).

Pairwise ANOSIM comparisons demonstrated that, in habitat type 1, the composition in each of the five seasons was significantly different from that in each other season (P = 0.001-0.018). The R-statistic values for the pairwise comparisons among seasons in that habitat type ranged from 0.185 for summer 2000 *vs* autumn to 0.544 for summer 2000 *vs* summer 2001. In habitat type 2, each pairwise comparison between the compositions in the five seasons was significantly different (P = 0.001-0.027), except for spring *vs* autumn and winter and for autumn *vs* winter. The R-statistic value was greatest for summer 2000 *vs* winter, *i.e.* 0.457. All pairwise comparisons between seasons were significant (P = 0.001-0.031) in habitat type 6, except for summer 2000 *vs* autumn, with the R-statistic being greatest for winter *vs* spring, *i.e.* 0.450.

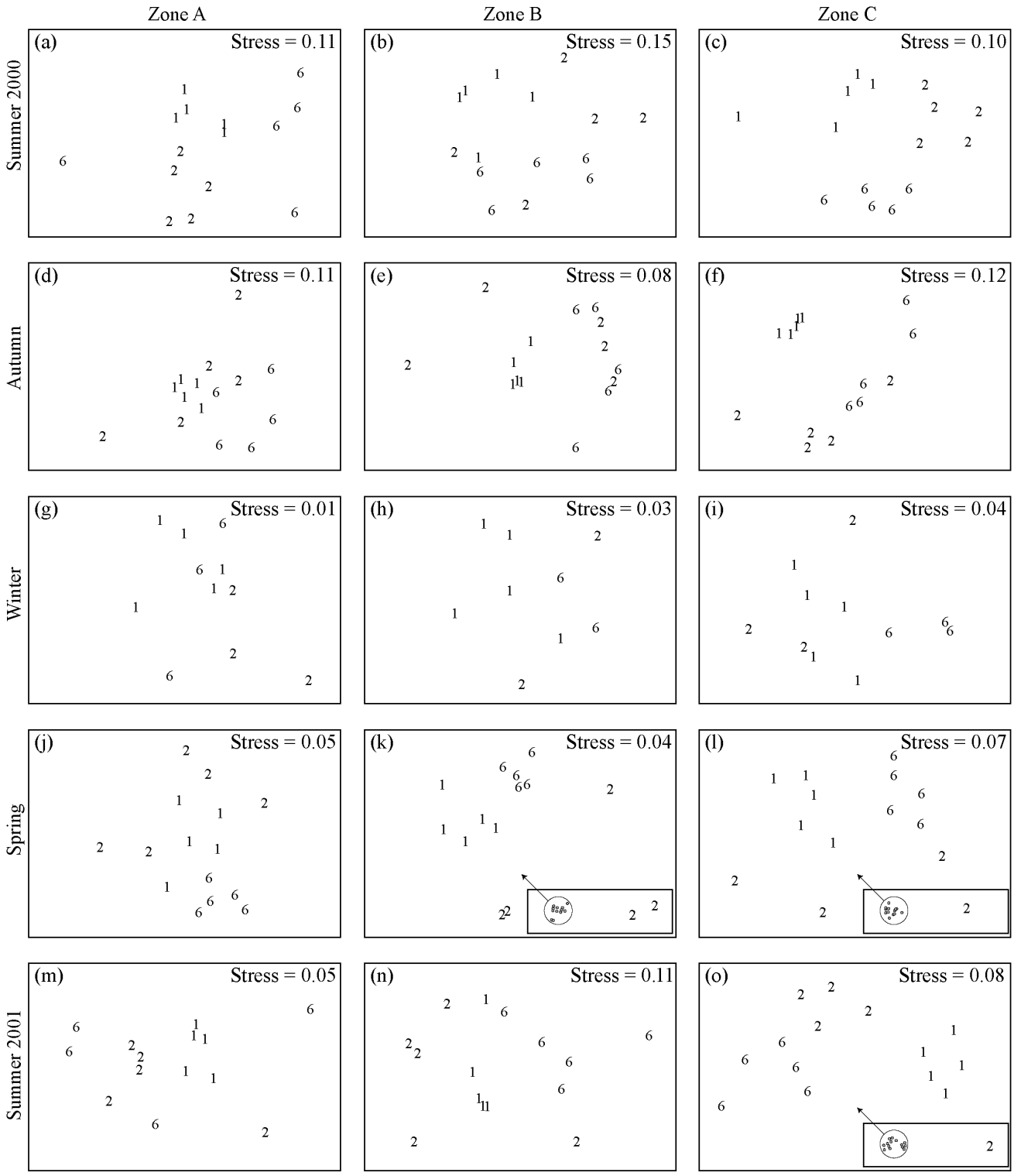

Figure 2.4 Two-dimensional nMDS ordinations of the densities of nematode species in samples collected in zones A, B and C during summer 2000 (a, b and c), autumn (d, e and f), winter (g, h, and i), spring (j, k and l) and summer 2001 (m, n, and o) coded by habitat type.

Pairwise ANOSIM comparisons also demonstrated that, in habitat type 1, the nematode assemblages in zone C were significantly different from those in both zones A and B (P = 0.001 and 0.004, respectively), while those in the latter two zones were not significantly different from each other. In habitat type 2, significant differences were found only between the nematode assemblages in zones A and C (P = 0.037), whereas, in habitat type 6 the assemblages in each zone were significantly different from that in each of the other two zones (P = 0.001-0.037). In habitat types 1, 2 and 6, the R-statistic for the comparison between zones was greatest in the case of zones A *vs* C, with R-statistic values of 0.334, 0.107 and 0.263, respectively.

		Zone A		Zone B		Zone C	
Summer 2000	Global	0.542	***	0.358	**	0.789	***
	1 *vs* 2	0.648	**	0.472	*	0.704	**
	1 *vs* 6	0.448	**	0.584	**	0.804	**
	2 *vs* 6	0.544	**	0.074	ns	0.884	**
Autumn	Global	0.301	**	0.245	***	0.592	***
	1 *vs* 2	0.286	**	0.348	*	0.648	**
	1 *vs* 6	0.496	**	0.608	**	0.842	**
	2 *vs* 6	0.224	*	0.152	ns	0.204	ns
Winter	Global	0.053	ns	0.188	ns	0.510	**
	1 *vs* 2					0.244	ns
	1 *vs* 6					0.723	*
	2 *vs* 6					0.672	*
Spring	Global	0.452	***	0.565	***	0.512	***
	1 *vs* 2	0.096	ns	0.478	**	0.268	**
	1 *vs* 6	0.622	**	0.880	**	0.948	**
	2 *vs* 6	0.600	**	0.514	**	0.432	**
Summer 2001	Global	0.382	**	0.468	**	0.732	***
	1 *vs* 2	0.554	**	0.384	*	0.672	**
	1 *vs* 6	0.531	**	0.584	**	1.000	**
	2 *vs* 6	0.081	ns	0.456	*	0.464	**

Table 2.5 R-statistics and significance levels for one-way ANOSIM tests on the compositions of nematode assemblages in each season x zone combination, both globally and for each pair of habitat types. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

2.4 Discussion

2.4.1 Number of species and densities

Seventy five species of nematodes were recorded during the present study, only three of which, at that time, were able to be assigned to previously described species. In contrast, all but two of the 75 species recorded during this study were able to be allocated to described genera. However, only 20 of the 62 genera recorded were also found during studies carried out on sandy beaches at a similar latitude in temperate eastern Australia (Nicholas & Hodda, 1999; Nicholas, 2001). Yet, most of the genera recorded on the lower west coast have been previously found in one or more of a wide range of marine and estuarine environments in Australia (Greenslade, 1989).

2.4.2 Comparisons of nematode assemblages among habitat types

Our data demonstrate that, in each season and zone, the mean number of species and density of nematodes were both consistently greater at habitat type 1 than at habitat types 2 and 6. This is almost certainly due to the fact that, as a result of relatively low wave energy, habitat type 1 provides a physically stable environment which is particularly conducive to colonisation by meiofauna (Bell & Sherman, 1980; Palmer, 1986). The shallow, sheltered conditions characteristic of habitat type 1 would also favour the growth of microphytobenthos (Fielding *et al.*, 1988), which are a primary food source for nematodes (Moens & Vincx, 1997).

The significant difference that was found between the compositions of the nematode assemblages in the three habitat types, parallels the differences found among those of the ichthyofaunas in four habitat types in the same region, including habitat types 1 and 2 (Valesini *et al.*, 2004). The results of SIMPER, demonstrated that four of the six most important typifying species at habitat type 1, *i.e. Paracomesoma sipho*, *Dichromadora* sp., *Marylynnia annae* and *Nanolaimoides decoratus* sp., belonged to genera whose buccal armature is considered to be adapted for feeding on benthic diatoms

(Moens & Vincx, 1997). Furthermore, the densities and percentage contribution of both *Neochromadora* sp. and *Trissonchulus* sp., which are also presumed to be diatom feeders, were far greater at habitat type 1 than at the other two habitat types. The far greater densities of these six nematode species at habitat type 1 would be consistent with the conclusion that the abundance of microphytobenthos would be likely to be greatest in this type of sheltered habitat (Fielding *et al.*, 1988).

In contrast to the situation at habitat type 1, three of the five most important typifying species of the nematode assemblage at the relatively exposed habitat type 6 belong to genera which are regarded as deposit feeders, *i.e. Theristus* sp.1, *Gonionchus australis* and *Bathylaimus australis* (Moens & Vincx, 1997). Thus, at this habitat type, where the greater turbulence due to wave action would presumably discourage the growth of microphytobenthos, there appears to have been selection for nematodes that are adapted to ingesting bacteria and detritus rather than diatoms.

The two most important typifying species in habitat type 2, *i.e. Paracomesoma sipho* and *Theristus* sp. 1, were the most important species at habitat types 1 and 6, respectively. This implies that both algal and bacterial food sources are readily available to nematodes at habitat type 2. However, *Enoplus* sp. and *Trileptium* sp. nov., which both belong to the predator functional feeding group and characterised neither of the other two habitat types, were important typifying species in habitat type 2.

The differences in the compositions of the nematode assemblages among the three habitat types concur with Alongi (1986), Warwick *et al.* (1990), Dittmann (2000) and Fisher (2003) who also found that such assemblages are valuable indicators of environmental conditions in Australian waters. The species composition of nematode assemblages have often been shown to be related to sediment grain-size (Wieser, 1959a; Warwick, 1971; Alongi, 1986; Coull, 1988; Ndaro & Olafsson, 1999). However, the

compositions of the nematode fauna in the three habitat types did differ, particularly in the case of habitat type 1 *vs* 6, even though the sediment compositions in those habitat types were very similar (Wildsmith *et al.*, 2005).

2.4.3 Comparisons of nematode assemblages among seasons and zones

The mean number of species and densities of nematodes in each habitat type underwent pronounced declines during winter. Indeed, a number of cores collected in this season yielded no nematodes, particularly in the case of those taken in the two intertidal zones (A and B) at the two most exposed habitat types. The declines in these two biotic parameters in winter, which parallels those found in a similar environment on the east coast of Australia (Nicholas & Hodda, 1999), suggest that a large number of nematodes die and/or their reproductive success declines dramatically during this season, when water temperatures fall to their mean seasonal minima of *ca* 16.5 °C. This view is consistent with the findings of Heip *et al.* (1985) and Moens & Vincx (2000a), who concluded from laboratory studies that the generation time of certain nematode species increased at low water temperatures. It is probably also relevant that in winter, the interstitial environment becomes less stable due to a marked increased in wave turbulence and thus presumably becomes much less hospitable. Furthermore, the microphytobenthos, a major food source of nematodes (Moens *et al.*, 1999), will presumably decline in abundance during that season.

The marked increases that occurred during spring in the densities and number of species of nematodes in habitat types 1 and 6 demonstrate that the assemblages of nematodes in these environments are capable of rapid recovery when environmental conditions become favourable. The compositions of the nematode assemblages at these two habitat types were also particularly discrete in spring, which can be attributed to particularly rapid increases in the numbers of certain typifying species, such as *Mesacanthion* sp. nov., *B. australis* and *P. sipho* at habitat type 1 and *G. australis* and *Theristus* sp. 1 at habitat type 6.

The mean number of species and mean densities were significantly greater in zone C than in zones A and B, probably due to zone C being permanently submersed and thus providing a more stable environment for nematodes than those in the two intertidal zones. The composition of the nematode assemblages along the lower west coast of Australia varied only slightly among zones, and thus to a lesser degree than in the eastern Australian study of Nicholas & Hodda (1999). This difference may reflect the fact that although the latter region is still considered microtidal, the mean tidal height is about 60 % greater than that on the lower west coast of Australia.

2.5 Conclusions

The results of our study indicate that nematode assemblages in nearshore waters on the lower west coast of Australia are strongly influenced by habitat type and thus by differences in the values for the suite of enduring physical characteristics that distinguish the various habitat types. The influence of season on the characteristics of nematode assemblages in these waters was less pronounced than habitat type but greater than that of zone. Thus, hypotheses i, iii were both confirmed as the nematode assemblages differed among both site and seasons. Likewise hypothesis ii was also confirmed with epistrate grazing nematode species being most prevalent in the sheltered and low energy habitat. Hypothesis iv was found to be partly correct, with nematode assemblages showing significant differences among zones at two of the three habitat types. However, the extent of those inter-zone differences were much less than ether habitat type or seasonal differences.

Chapter 3 Spatial and seasonal variations in the ecological characteristics of the free-living nematode assemblages in a microtidal estuary

3.1 Introduction

Nematodes, which occur in every habitat that can support life, are the most numerous of all metazoans and one of the most diverse metazoan taxa in marine waters (Platt & Warwick, 1980). Free-living nematodes play an important functional role in aquatic ecosystems (Platt & Warwick, 1980; Coull, 1999). For example, they are of major energetic importance in benthic environments as they represent a significant part of the diet of many aquatic organisms (Gee, 1989; Coull, 1990) and facilitate the mineralisation of organic matter (Coull, 1999; Riera & Hubas, 2003). Furthermore, as nematodes are highly habitat specific (Findlay, 1981; Joint *et al.*, 1982), changes in the composition of their assemblages are good indicators of when changes are occurring in the environment, either naturally (McLachlan, 1978; Guo *et al.*, 2001; Nozais *et al.*, 2005) or as a result of anthropogenic activities (Coull & Chandler, 1992; Kennedy & Jacoby, 1997; Bongers & Ferris, 1999).

The environmental resources of free-living nematodes are partitioned among species in different ways, and this is particularly true of its food resources, a feature reflected in the high degree of feeding selectivity exhibited by the various species (Moens & Vincx, 1997). As the latter selectivity is reflected, in turn, by marked differences in the physiognomic characteristics of the buccal cavities of nematode species, the particular characteristics of each species can be used to deduce the functional feeding group to which that species belongs (Wieser, 1953; Moens & Vincx, 1997). The densities of one of these groups, *i.e.* epistrate grazers, often peak at the time of year when the microphytobenthos is also at its maximum (Tietjen, 1969; Skoolmun & Gerlach, 1971; Hodda & Nicholas, 1986b).

The species composition of nematodes in the macrotidal estuaries of temperate regions in the northern hemisphere have been shown to be influenced by salinity and the characteristics of the sediment (Warwick, 1971; Austen & Warwick, 1989; Soetaert *et al.*, 1995). Although the density and diversity of nematodes were also found to be related to salinity in certain areas of these estuaries (Soetaert *et al.*, 1995), this was not universally the case in this region (Warwick, 1971; Warwick & Price, 1979; Austen & Warwick, 1989). Furthermore, the conclusions drawn as to whether the species compositions of nematodes in these macrotidal temperate estuaries undergo conspicuous seasonal changes have not been unanimous (c.f. Tietjen, 1969; Warwick, 1971). The only study to have specifically explored the ways in which the nematode faunas of a microtidal southern hemisphere estuary vary spatially and seasonally are those of Hodda & Nicholas (1985; 1986b). In their study of the fauna in the intertidal zone of the mangrove-dominated Hunter River Estuary in temperate eastern Australia, those workers found that the densities were correlated most closely with elevation above low tide mark and pollution levels but neither with salinity nor the median grain-size of the sediment. Although the densities and species compositions at the different sampling sites changed during the year, they did not follow a consistent pattern across all sites, which was attributed to the influence of "non-seasonal environmental changes". In another study, Hodda (1990) concluded that variations among nematode faunas at sites in the Hunter River Estuary and two other estuaries on the same coast were related to oxygen penetration, organic content and grain-size composition of the sediment.

The large estuaries of south-western Australia, such as that of the Swan River, and many of those in south-eastern Australia and southern Africa, are microtidal and often comprise three morphologically distinct regions (Chalmer *et al.*, 1976; Bird, 1984; Potter *et al.*, 1990). The lower estuary consists of a short and narrow entrance channel,

which opens into a large and wide central basin region that represents the middle estuary. The latter area is fed by tributary river(s), the downstream reaches of which are saline and constitute the upper estuary. The categorisation of the Swan River Estuary into these three regions was based not only on morphological distinctions, but also on the pattern of distribution of the various species of benthic macroinvertebrates (Chalmer *et al.*, 1976). Subsequent work showed that the compositions of the ichthyofauna, particularly in the upper region of that estuary, undergo pronounced changes between the wet (low salinity) and dry (high salinity) periods of the year (*e.g.* Loneragan & Potter, 1990). Other studies on the Swan River Estuary have shown that the densities of microphytobenthos were greatest in the lower estuary (Masini & McComb, 2001) and that the amount of particulate organic material was greatest in the upper estuary (Wildsmith, 2008), both of which will inevitably influence the structure of the lower trophic levels in those regions.

As the majority of rainfall, and thus also of freshwater discharge, occurs in winter and early spring in south-western Australia, the salinity in the estuaries of this region declines markedly during that period and then increases slowly during the subsequent months as salt water gradually penetrates upstream (Stephens & Imberger, 1996). However, because tidal action within these estuaries is weak, the salinity at any given site does not undergo marked changes during any tidal cycle. These characteristics contrast markedly with those normally found in macrotidal temperate regions of the northern hemisphere, which tend to be funnel-shaped and not divided into distinct regions, and undergo marked changes in salinity and water level during each tidal cycle.

The present study on the Swan River Estuary represents the first concurrent exploration of the ways in which the density, diversity, species composition and trophic categories of the nematode fauna of the nearshore subtidal sediments of a microtidal estuary vary spatially and temporally. The results, which are likely to be applicable to other

permanently-open microtidal estuaries in southern Australia and southern Africa, were used to test the following two hypotheses.

(i) Biotic variables, such as density, number of species, species composition and trophic structure, change progressively throughout the estuary as environmental variables such as salinity, the amount of organic material and the amount and type of potential food change along the same spatial axis. However, there will also be a tendency for some regional differentiation in the above biotic variables as the broad biological and environmental characteristics of the lower, middle and upper estuary differ.

(ii) The changes that occur throughout the year in variables such as temperature and salinity and the potential food sources for nematodes is reflected in cyclical seasonal changes in species compositions and the trophic characteristics of the nematode assemblages.

3.2 Materials and methods

3.2.1 Sampling regime

The Swan River Estuary is located on the microtidal lower west coast of Australia (Fig. 3.1). The very small tidal regime means that there is essentially no intertidal area throughout most of the length of this estuary. The estuary contains a small amount of seagrass and macroalgae in the channel and basins and riparian vegetation in the upper reaches and no mangroves.

Nematodes were sampled at 12 shallow (< 2 m deep) sites that were located at intervals throughout the length of the Swan River Estuary (Fig. 3.1) each of which comprised the area within a 100 m radius of a designated point on the shoreline. Five randomly-located replicate samples were extracted from the sub-tidal sediment at each site, in the middle each of the four seasons between summer 2004/5 and spring 2005.

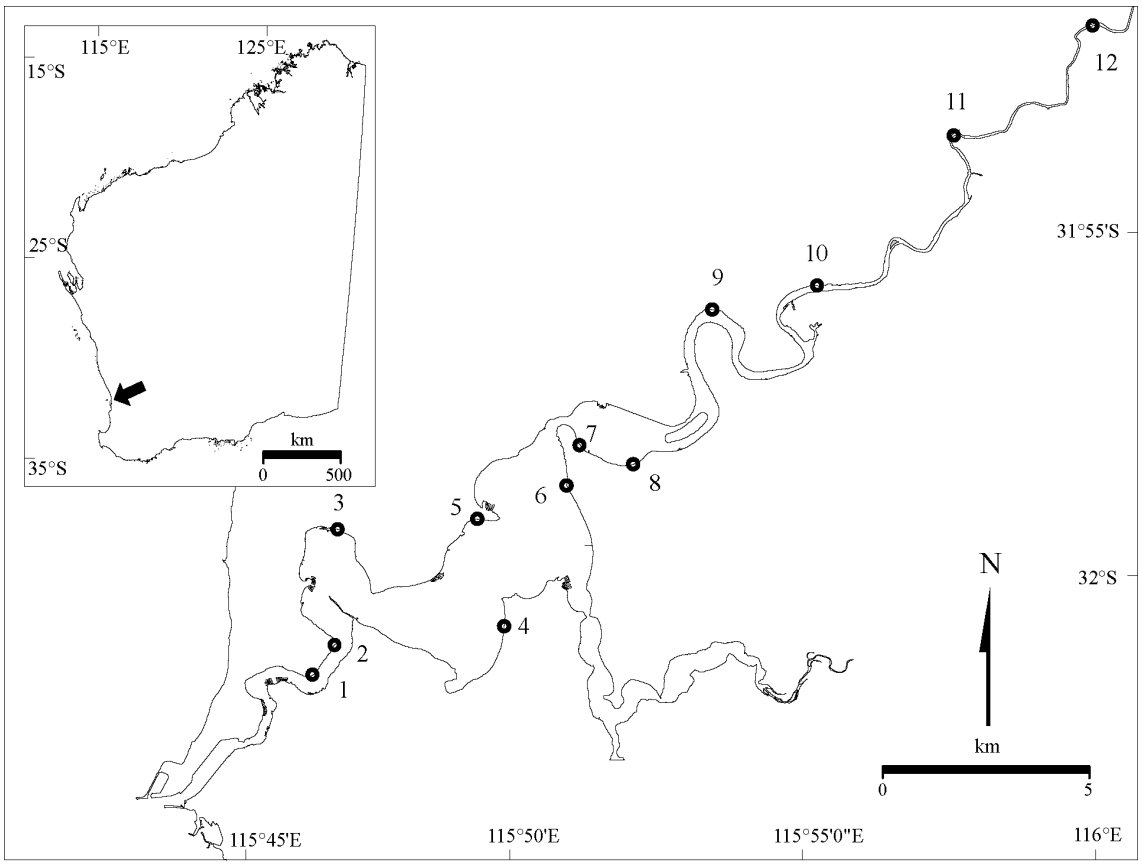

Figure 3.1 Map of the Swan River Estuary, showing the location of the 12 sites at which nematode assemblages were sampled. The arrow in the inset shows the location of this estuary within Western Australia.

Samples were collected using a steel corer, which was 3.57 cm in diameter (*i.e.* covering a surface area of 10 cm^2) and sampled to a depth of 10 cm. In each season, three of the replicate samples at a site were collected on one occasion and the other two at least two weeks later. Each sediment core was immediately fixed in a 5 % formalin / estuary water solution.

The same corer was used to obtain three additional sediment samples from each site in each season for determining (i) the depth at which the colour of the sediment changed from light to dark (henceforth referred to as the transition zone), (ii) the percentage particulate contribution of organic material (%POM) and (iii) the composition of the grain sizes in the sediment. Note that the depth at which the sediment changes colour from light to dark, which is the point where ferric iron is reduced to ferrous iron (Sikora & Sikora, 1982), is deeper than the point at which oxygen is biologically

available (Heip *et al.*, 1985). Thus, the entire oxic layer of the sediment, and therefore the vast majority of free-living nematodes, were sampled (Heip *et al.*, 1985).

A further three sediment cores were extracted from each site in each season using disposable plastic corers, 2.8 cm in diameter and 5 cm long, to provide material for calculating the chlorophyll concentration at those sites. The cores were immediately wrapped in aluminium foil to exclude light, stored on ice and then frozen.

Temperature, salinity and dissolved oxygen concentration were recorded in the middle of the water column in three regions of each site on each sampling occasion using a YSI 556 Multi-Parameter Handheld Meter.

3.2.2 Laboratory procedures

The nematodes were separated from the sediment in the cores using the laboratory procedures described in Hourston *et al.* (2005), except that a decantation step was added prior to using Ludox ™ to increase the efficiency with which nematodes were removed from the sediment. Thus, each sample was suspended in 800 ml of tap water and the larger sediment grains briefly allowed to settle, after which the remaining suspension was decanted through nested sieves of 500 and 63 μm mesh. The sand from which the suspension was decanted was subjected to the above procedure four more times to ensure that all nematodes had been removed. The organisms were separated from the fine sand and debris particles remaining on the 63μm sieve using Ludox ™ and preserved in 70 % ethanol. The procedures for isolating, subsampling, mounting and identifying the nematodes are given in Hourston *et al.* (2005). Counts were conducted on sub-samples, generally comprising one quarter of each core. The total number of each nematode taxon in each sample is expressed as a density, *i.e.* number 10 cm^{-2}.

Each nematode species was assigned to one of the four following functional feeding groups designated by Wieser (1953) on the basis of buccal cavity morphology.

1A; Species without a buccal cavity or with only a narrow tubular buccal cavity and which ingest particles of bacterial size, *i.e.* the selective deposit feeders. 1B; Species with a large buccal cavity that is not armed with teeth, *i.e.* non-selective deposit feeders. 2A; Species having a buccal cavity armed with small or moderate sized teeth, *i.e.* epigrowth or diatom feeders. 2B; Species with large teeth or jaws, *i.e.* predators / omnivores.

Each of the cores taken for determining %POM and sediment grain size was dried at 100 ºC, weighed to the nearest 0.01 g and ashed at 550 ºC to remove any organic material. Each core was then reweighed to enable the weight and thus %POM to be calculated (Heiri *et al.*, 2001). The ashed sample was wet-sieved through a 63 μm mesh, dried at 70 ºC and reweighed to determine the weight of fines (< 63 μm). The remaining sediment was then wet-sieved through six nested sieves (2000, 1000, 500, 250, 125 and 63 μm) and each fraction dried at 70 ºC and weighed to enable its contribution to the total core weight to be determined.

The top *ca* 1 cm of the cores collected for chlorophyll analysis was ground in 30 ml of acetone using a mortar and pestle. The resultant slurry was centrifuged and the light absorbances of the supernatant at wavelengths of 664, 647 and 630 ηm measured using a Hitachi U-1100 spectrophotometer. The concentrations of chlorophyll fractions a, b and c were determined from those absorbances and summed (Parsons *et al.*, 1984).

The sediment from which the chlorophyll had been extracted was dried and weighed, thereby enabling the concentration of chlorophyll to be expressed as mg g^{-1} of sediment.

3.2.3 Univariate statistical analyses

Two-way Analyses of Variance (ANOVA) were performed to determine whether the following environmental and biotic variables differed significantly among the 12 sites and four seasons, with site and season both considered fixed factors. The environmental

variables were water temperature (°C), salinity and dissolved oxygen concentration in the water column (mg L^{-1}), and transition zone depth (cm), POM (%) and chlorophyll concentration in the sediment (mg g^{-1}), while the biotic variables were density and number of species of nematodes. Salinity was measured using the practical salinity scale and is thus dimensionless. All univariate analyses were conducted using the SPSS v 15 statistical software package. Prior to subjecting the data for each dependent variable to ANOVA, the relationship between the mean and standard deviation of each set of replicate samples was examined to determine whether these variables required transformation to meet the test assumptions of normality and constant variance required by ANOVA. In those instances where this was not the case, the appropriate transformation was then applied (Clarke & Gorley, 2006). The H_0 for all ANOVAs, *i.e.* that significant differences did not exist among *a priori* groups, was rejected if the significance level (P) was < 0.05.

3.2.4 Multivariate statistical analyses

All of the following multivariate statistical analysis and routines were carried out using the PRIMER v 6 statistical package (Clarke & Gorley, 2006).

3.2.4.1 Sediment grain-size composition

The means of the sediment grain size fractions in the three replicate samples obtained from each site in each season were analysed using Principal Component Analysis (PCA) to visually explore the extent of any differences among sites and seasons, and to identify which granulometry fractions were primarily responsible for any such differences. Two-way crossed ANOSIM was conducted using a Euclidian dissimilarity matrix constructed from the same data to ascertain whether the grain-size compositions differed significantly among sites and / or seasons.

3.2.4.2 Comparisons of the species compositions among sites and seasons

The densities of each nematode species in each replicate sample from each of the 12 sites in each season were fourth-root transformed. The means of the transformed densities for each site in each season were used to construct a Bray-Curtis similarity matrix, which was then subjected to group-averaged hierarchical cluster analysis and non-metric multidimensional scaling (nMDS) ordination. The samples were coded for site to assess visually the extent to which the compositions of the samples from the various sites were similar or different.

A second Bray-Curtis matrix, constructed from the densities of each species in the individual replicate samples, was subjected to a two-way crossed Analysis of Similarity (ANOSIM) test to ascertain whether the species compositions of the nematode assemblages differed significantly among sites and / or seasons. For this and all subsequent ANOSIM tests, the null hypothesis that there were no significant differences among groups was rejected if the significance level (P) was < 0.05. The R-statistic values determined by ANOSIM for comparisons between those a priori groups that were significantly different were used to ascertain the degree to which those groups were dissimilar. R-statistic values approaching unity demonstrate that the compositions of the groups are very different, while those close to zero show that they are very similar (Clarke, 1993). When ANOSIM detected significant differences among a priori groups, two-way crossed site by season Similarity Percentages (SIMPER) was used to determine the species that typified those groups and those which distinguished each group from each of the others (Clarke, 1993).

As species composition was influenced significantly by both site and season (see Subsection 3.3.4), the matrix constructed from the replicate data for the fauna at each of the 12 individual sites was subjected separately to nMDS ordination to show the relationships between the faunal compositions in the four seasons without the

counfounding influence of site. Using the same Bray-Curtis similarity matrices, one-way ANOSIMs were then used to explore whether the species compositions at each site differed among seasons, and if so, the extent of those seasonal differences. To explore the effects of site in each season, nMDS ordinations were constructed for each of the four seasons using the mean densities of each species in each set of five replicate samples for each site. This enabled the question of whether nematode composition changed sequentially along the length of the estuary to be explored visually. The matrices constructed from the replicate data in each season were then subjected to one-way ANOSIMs to determine whether the species composition differed among sites in each season and, if so, the extent of those site differences.

3.2.4.3 Relationships between nematode fauna composition and environmental variables

Analyses were conducted to determine the extent to which the pattern of spatial differences in the suite of aquatic variables (salinity, temperature and dissolved oxygen concentration) and "non-granulometric" sediment variables (chlorophyll, POM and transition zone depth) matched that exhibited by the nematode fauna. BioEnv, employing mean values for each variable in each season and all seasons collectively, was used to elucidate which of the three aquatic variables, or combination of those variables, was best correlated with nematode compositions, both overall and in each season (Clarke & Ainsworth, 1993). This process was then repeated for the three "non-granulometric" sediment variables. Note that, because there were only 12 sites, only 12 points were available for the matching procedure and that it was thus inappropriate to consider more than three variables in any single test (Clarke *et al.*, 2008). Since the composition of the suite of sediment grain sizes has been reported to influence the composition of nematode assemblages (Coull, 1988), the RELATE procedure was used to determine the extent to which nematode composition is correlated with granulometry as a whole. RELATE was also used to determine the correlation between the patterns of

similarity of nematode assemblages and the matrix of the geographical distance (not ranked distance) among sites As both the BioEnv and RELATE routines require complete complementarity of samples, the data for replicates for each of the above variables for each site were averaged. Spearman rank correlation (ρ) was employed as the matching co-efficient in the procedures and the null hypothesis, that there is no match in the rank order arrangement of samples between pairs of matrices, was rejected if P was < 0.05.

The mean compositions of the functional feeding groups at the 12 sites in each of the four seasons were subjected to PCA to investigate the extent to which the functional feeding groups of nematodes are distributed according to site in the estuary and which groups were mainly responsible for any spatial and / or temporal differences.

The Euclidian Distance matrix constructed from the compositions of the four functional feeding groups in replicate samples at each site in each season was subjected to two-way crossed ANOSIM to determine whether trophic composition was significantly influenced by site and / or season and, if so, the relative extent of those influences.

3.3 Results

3.3.1 Environmental data

Two-way ANOVA demonstrated that water temperature, salinity, dissolved oxygen concentration, transition zone depth, %POM and sediment chlorophyll concentration were each significantly influenced by site and also by season in all cases except %POM (Table 3.1). There was a significant interaction between site and season for all variables apart from %POM. For each of the variables for which season was significant, the mean squares were greater for that factor than those for site, except in the case of dissolved oxygen, and also than those for the interactions.

The mean seasonal temperature at all sites except 5 was always greatest in summer, followed by spring and then autumn and winter (Fig. 3.2a). The same trends were exhibited at site 5, except that the temperature in summer was unusually low and less than during spring, which helps account for the significant interaction between site and season. Although the temperature in summer, and to a lesser extent spring, tended to increase with distance from estuary mouth, the reverse was the case during autumn and to a greater extent winter. The mean seasonal water temperatures ranged from a low of 13 °C at site 12 in winter to 30 °C at site 6 in summer. The mean seasonal salinity at

	Site (Si)		Season (Se)		Si x Se Interaction		Residual
Factor type	Fixed		Fixed		Fixed		
Degrees of freedom	11		3		33		192
Abiotic							
Temperature	4.559	***	554.658	***	5.375	***	1.455
Salinity	766.811	***	2450.548	***	20.488	***	11.755
Dissolved oxygen	0.483	***	0.269	*	0.205	***	0.031
Transition zone	23.009	***	42.341	***	9.596	***	4.087
%POM	21.099	***	0.855	ns	1.502	ns	1.230
Chlorophyll concentration	283.584	***	459.235	***	102.635	*	62.928
Biotic							
Density	11.447	***	7.343	***	1.749	***	0.473
Number of species	1.837	***	0.266	ns	0.440	***	0.123

Table 3.1 Mean squares and significance levels for two-way ANOVAs of the five abiotic and two biotic variables recorded at twelve sites in each of the four seasons between summer 2004/5 and spring 2005. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

each site was almost invariably greater in summer than autumn, which in turn was greater than in spring and winter (Fig. 3.2b). The magnitude of differences in this variable also decreased with distance from estuary mouth, with values in summer and autumn declining from close to that of full strength seawater (35) at the downstream sites to about 13 at the most upstream site and those for winter and spring declining from 23-28 near the estuary mouth to *ca* 3 at the head of the estuary. Dissolved oxygen (DO) concentrations were always greatest during spring at sites 1-6, and generally during winter at sites 7-12, and were lowest during summer at 8 of the 12 sites

(Fig. 3.2c). Furthermore, the DO concentrations in each season were greatest at sites 4, 5 or 6, which are relatively shallow and exposed to wave energy.

The transition zone depth in each season showed no clear tendency to change progressively throughout the estuary or to differ in a consistent manner among seasons (Fig. 3.2d). However, the transition zone depths at sites 3-7 were always least in summer and were relatively small in all seasons at site 12. Although the concentration of sedimentary chlorophyll at any given site varied considerably among seasons, it was always greatest in spring at the four most downstream sites, with values at two of those sites in spring being among the three greatest recorded for any site in any season (Fig. 3.2e). The mean %POM to the sediment at sites 1-7 lay between 0.8 and 1.5 %, which was less than those at all sites further upstream, where it peaked at 4.0 % at site 9 and 5.2% at site 12 (Fig. 3.2f).

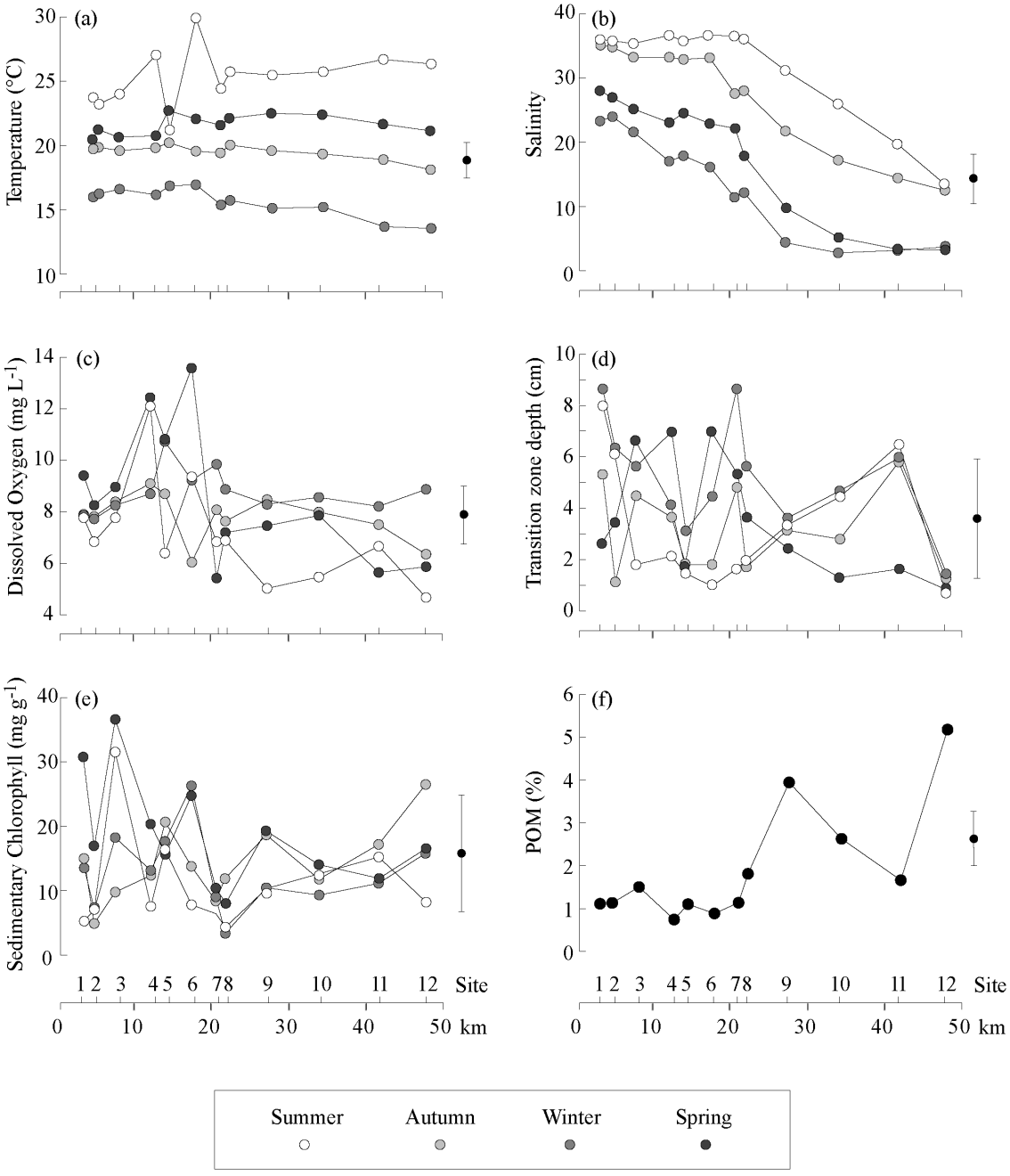

Figure 3.2 Mean values for (a) temperature, (b) salinity, (c) dissolved oxygen concentration, (d) depth of transition zone, *i.e.* the point where the sediment changes from light to dark in colour, and (e) total sedimentary chlorophyll at each of the twelve sites in each season, and (f) %POM at each site *vs* the distance of each site from the estuary mouth along the midline of the estuary (km). In this Figure and Figure 3.4, points represent the back transformed marginal means used by ANOVA, and the overall mean ± 95 % CI for each variable is shown on the right of its respective plot.

Two-way crossed ANOSIM, using the matrix derived from replicate samples, showed that sediment granulometry differed significantly among sites ($P < 0.001$, R-statistic = 0.723), but not seasons. Principal Components Analysis (PCA) of the data for the various sediment grain size fractions, demonstrated that 84 % of the total variation was encompassed by principal component axes 1 and 2. Although samples from certain site(s), *e.g.* 4, 6-8, 10 and 11, showed a very marked tendency to group together on the PCA plot, the samples from the various sites were not arranged sequentially on that plot according to distance from the estuary mouth (Fig. 3.3). The orientation of the eigenvectors demonstrated, for example, that the group of sediment samples from sites 10 and 11 contained large contributions of the coarsest grain-size fractions (500 µm and 1 and 2 mm), whereas those from sites 6, 7 and 8 comprised greater amounts of the 250 µm fraction.

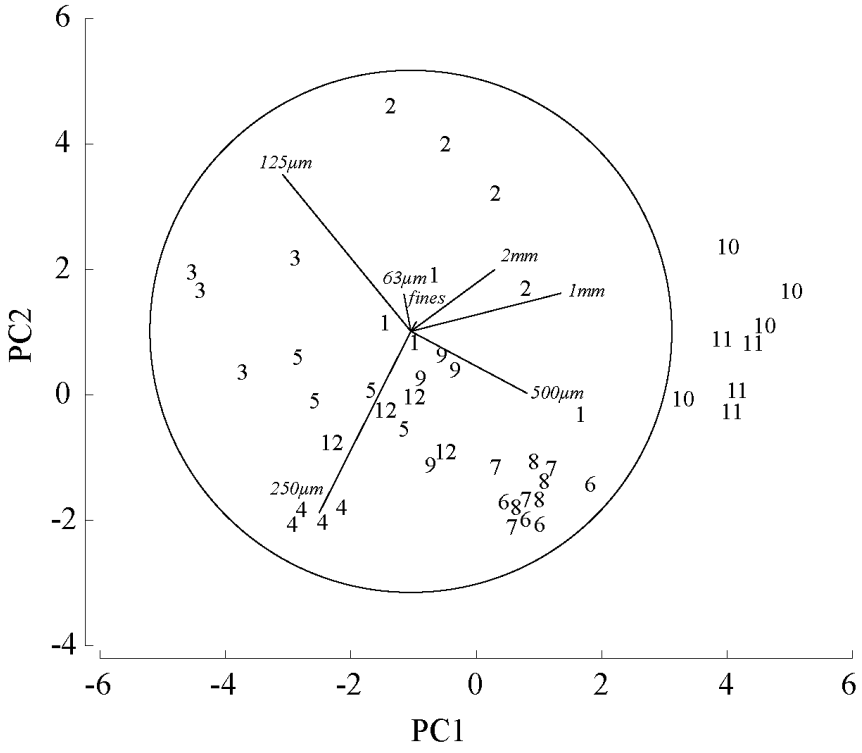

Figure 3.3 Principal component analysis plot derived from the mean percentage contributions of the various grain-size fractions to the sediment samples obtained from each site in each season. Principal component axes 1 and 2 collectively accounted for 84.4 % of the total variation present.

3.3.2 Densities and percentage contributions of the nematode species at the twelve sites

Over 150 000 nematodes belonging to 76 species were collected during seasonal sampling between summer 2004 and spring 2005 of the sub-tidal sediments of the twelve sites that were distributed throughout the Swan River Estuary. The mean density (No. 10 cm $^{-2}$) of nematodes, derived from pooled data for all seasons, decreased in a largely sequential manner from 1139 at site 1, close to the estuary mouth, to a minimum of 160 at site 9, the most downstream site in the upper estuary and then increased to between 297 and 668 at the three uppermost sites (Table 3.2). The number of species recorded at the 12 sites also declined in an essentially sequential manner from sites 1 and 2 (49) to site 10 (21), and then increased slightly further upstream (Table 3.2).

The dominant species at sites 1-6 in the lower and middle reaches of the estuary were similar (Table 3.2). Thus, in terms of abundance, *Spirinia parasitifera* ranked first at sites 1, 2, 3, 5 and 6, where it contributed between 26 and 44 % to the total number of individuals, and *Theristus* sp. 2 always ranked in the top five species at sites 1-6 (5-16 %). *Comesoma arenae* also made substantial contributions at sites 1, 2 and 4 (7-9 %) and the same was true of *Bathylaimus australis* at sites 2 and 3 (6 and 11 %) and *Chromadorina* sp. at sites 1 and 2 (5 and 18 %).

Although *Dichromadora* sp. made a considerable contribution to the number of individuals at site 1 (8 %), it tended to be more common further upstream where it ranked in the top five contributors at each of sites 4-9 (5-29 %). Similarly, while *B. australis* and *Viscosia glabra* were present in moderate numbers at some of the downstream sites, they made their greatest contributions further upstream at sites 7 (35 %) and 10 (21 %), respectively (Table 3.2).

		Site																																	
		1			2			3			4			5			6			7			8			9			10			11			12		
	FFG	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk
Spirinia parasitifera	1A	499	44	1	227	26	1	327	35	1	29	3	11	271	34	1	256	41	1	11	3	7	18	6	6	20	8	4	1	<1	15	<1	<1	17	2	<1	14
Theristus sp. 2	1B	141	12	2	48	5	5	151	16	2	120	11	2	67	8	3	65	10	2	16	5	6	5	2	14	4	2	11	-	-	-	-	-	-	-	-	-
Dichromadora sp.	2A	96	8	3	24	3	9	46	5	6	331	29	1	84	11	2	53	9	3	31	9	3	24	8	4	14	5	5	8	5	7	11	3	7	15	2	7
Comesoma arenae	1B	78	7	4	82	9	3	<1	<1	20	76	7	5	26	3	10	7	1	15	1	<1	16	<1	<1	21	2	<1	14	-	-	-	1	<1	16	1	<1	15
Chromadorina sp.	2A	61	5	5	162	18	2	21	2	10	48	4	9	15	2	13	9	1	14	7	2	8	5	2	13	2	<1	15	4	2	10	2	<1	15	<1	<1	16
Bathylaimus australis	1B	18	2	10	53	6	4	106	11	4	3	<1	20	1	<1	20	12	2	11	117	35	1	13	4	8	7	3	8	-	-	-	<1	<1	19	-	-	-
Nannolaimoides decoratus	2A	18	2	10	14	2	13	116	12	3	66	6	6	31	4	9	30	5	5	2	<1	12	1	<1	18	2	<1	17	3	2	11	17	5	5	47	7	3
Halichoanolaimus duodecimpapillatus	2B	25	2	8	20	2	10	50	5	5	47	4	10	34	4	8	26	4	6	2	<1	14	<1	<1	20	2	<1	16	3	2	13	5	1	10	10	2	9
Viscosia glabra	2B	31	3	7	20	2	12	24	3	9	108	10	3	36	5	7	40	7	4	<1	<1	18	10	3	10	4	1	12	33	21	1	5	1	12	4	<1	13
Metadesmolaimus sp. 1	1B	35	3	6	47	5	6	36	4	7	95	8	4	46	6	6	18	3	9	26	8	4	20	7	5	-	-	-	<1	<1	17	-	-	-	-	-	-
Terschellingia sp. 1	1A	13	1	15	20	2	11	33	4	8	24	2	12	52	7	4	10	2	12	<1	<1	17	2	<1	17	1	<1	18	9	6	6	5	2	9	4	<1	12
Pontonema sp. 1	2B	1	<1	26	2	<1	23	<1	<1	17	50	4	8	48	6	5	21	3	8	17	5	5	17	6	7	4	2	10	11	7	5	4	1	13	6	<1	11
Theristus sp. 1	1B	1	<1	27	<1	<1	26	4	<1	12	-	-	-	3	<1	17	26	4	7	81	24	2	79	27	1	93	36	1	29	18	2	176	54	1	404	60	1
Pierrickia sp. nov.	1A	-	-	-	<1	<1	27	-	-	-	<1	<1	25	11	1	14	5	<1	16	<1	<1	21	37	13	2	11	4	6	26	17	3	6	2	8	43	6	4
Gomphionema typicum	2A	<1	<1	29	-	-	-	1	<1	13	14	1	13	20	3	11	<1	<1	20	2	<1	15	32	11	3	-	-	-	-	-	<1	-	-	<1	-	-	-
Metadesmolaimus sp. 2	1B	-	-	-	-	-	-	-	-	-	3	<1	18	-	-	-	10	2	13	4	1	11	5	2	12	39	15	2	13	8	4	27	8	2	21	3	6
Metalinhomoeus sp.	1B	14	1	13	8	<1	16	<1	<1	20	-	-	-	11	1	15	<1	<1	23	<1	<1	19	8	3	11	32	12	3	7	4	8	20	6	4	60	9	2
Parodontophora aurata sp. nov.	2B	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	24	-	-	-	2	<1	13	11	4	9	9	4	7	3	2	12	26	8	3	25	4	5
Monhysterida sp. 4	1B	3	<1	21	-	-	-	6	<1	11	-	-	-	-	-	-	<1	<1	22	6	2	10	3	<1	16	6	2	9	1	<1	16	5	1	11	14	2	8
Parascolaimus brevisetus sp. nov.	1B	-	-	-	-	-	-	<1	<1	20	-	-	-	-	-	-	<1	<1	23	<1	<1	22	<1	<1	23	3	1	13	7	4	9	13	4	6	9	1	10
Oncholaimus domesticus	2B	2	<1	25	7	<1	17	1	<1	15	<1	<1	23	-	-	-	-	-	-	7	2	8	5	2	15	-	-	-	3	2	14	3	<1	14	<1	<1	16
Neochromadora sp. 1	2A	2	<1	23	45	5	7	<1	<1	20	3	<1	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	18
Paracomesoma sipho	2A	6	<1	19	1	<1	24	1	<1	14	13	1	14	<1	<1	22	3	<1	17	<1	<1	23	-	-	-	-	-	-	-	-	-	<1	<1	18	-	-	-
Camacolaimus sp.	1A	9	<1	16	8	<1	14	-	-	-	<1	<1	24	<1	<1	23	1	<1	19	<1	<1	19	<1	<1	23	<1	<1	19	-	-	-	<1	<1	20	-	-	-
Subsphaerolaimus sp. nov.	1B	-	-	-	-	-	-	<1	<1	17	-	-	<1	16	2	12	-	-	-	-	-	-	<1	<1	25	-	-	-	<1	<1	18	-	-	-	-	-	-
Epacanthion georgeii	2B	2	<1	24	5	<1	19	-	-	-	12	1	15	<1	<1	24	<1	<1	25	-	-	-	-	-	-	<1	<1	19	-	-	-	-	-	-	-	-	-
Chromadorida 2	2A	13	1	14	37	4	8	<1	<1	17	2	<1	22	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	21	-	-	-	-	-	-	-	-	-
Pomponema sp. 1	2A	5	<1	20	7	<1	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	19	-	-	-	-	-	-	-	-	-	-	-	-
Onyx cephalispiculus sp. nov.	2A	16	1	12	3	<1	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	22	-	-	-	-	-	-	-	-	-	-	-	-
Pseudochromadora cazca	2A	2	<1	22	<1	<1	25	-	-	-	57	5	7	3	<1	18	17	3	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Choniolaimus papillatus	2B	<1	<1	29	<1	<1	27	-	-	-	5	<1	17	7	<1	16	2	<1	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neochromadora sp 2	2A	<1	<1	29	-	-	-	1	<1	15	9	<1	16	-	-	-	<1	<1	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Daptonema sp.	1B	<1	<1	28	5	<1	19	<1	<1	20	2	<1	21	2	<1	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Monhysterida sp. 3	1B	20	2	9	-	-	-	-	-	-	-	-	-	<1	<1	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pomponema sp. 2	2A	7	<1	17	4	<1	21	-	-	-	<1	<1	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mesacanthion sp. nov.	2B	6	<1	18	8	<1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean total density (No. 10cm^{-2})		1138.9			877.5			931.4			796.7			618.8			335.4			297.5			256.8			160.0			328.3			668.4			297.5		
Total number of species		49			49			30			41			36			31			25			27			24			21			25			22		

Table 3.2 Functional feeding group (FFG) of each nematode species, and the mean density ($\overline{X}$; No. 10 cm^{-2}), percentage contribution (%) and rank by abundance (Rk) of those species at each of the twelve sampling sites in the Swan River Estuary, derived from pooled data for all seasons. The five most abundant species at each site have been shaded. Only those species which contributed > 0.5 % to the total density in at least one of the sites are presented..

The nematode assemblages at the upper estuary sites (7-12) were all dominated by *Theristus* sp. 1, where this species always ranked either first or second (Table 3.2). This was particularly the case at the uppermost site (12), at which this species comprised 60 % of all individuals. Other species that contributed substantially to the nematode assemblages in upstream areas were *Metadesmolaimus* sp. 2 at sites 9-11 (8-15 %), *Pierrickia* sp. nov. at sites 8, 10 and 12 (6-17 %), *Metalinhomoeus* sp. at sites 9, 11 and 12 (6-12 %), *Nannolaimoides decoratus* and *Parodontophora aurata* sp. nov. at sites 11 and 12 (5-7 % and 4-8 %, respectively) and *Gomphionema typicum* at site 8 (11 %).

3.3.3 Density and number of species

Two-way ANOVA demonstrated that the density and number of species of nematodes differed significantly among sites and that the former also differed significantly among seasons (Table 3.1). Although there was a significant interaction between site and season for both of these biotic variables, the mean squares for each of these interactions were far less than those for the main effects that were significant. The densities tended to be greater at sites 1-6 than at 7-10 and then increased at the uppermost sites, and were also greater in spring than in the other seasons at eight of the 12 sites (Fig. 3.4a). However these trends were not entirely consistent, as is reflected in the significant interaction between site and season for this variable. The number of species showed a similar overall trend to density and thus declined progressively from lower estuary sites (except site 3) to sites 8 and 9 and then showed a slight increase. The significant interaction between site and season reflects the fact that this biotic variable did not obviously tend to follow the same order in terms of season at each site and, atypically for the lower reaches, was low in all seasons at site 3 (Fig. 3.4b).

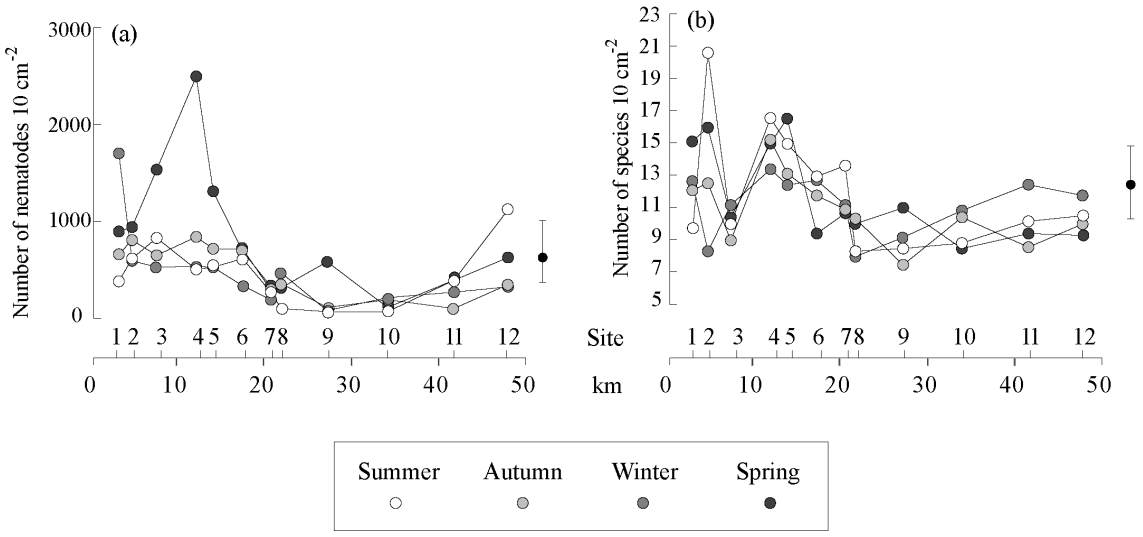

Figure 3.4 Mean values for (a) density and (b) number of nematode species at each sampling site in each season *vs* the distance of each site from the estuary mouth along the midline of the estuary (km).

3.3.4 Comparisons of nematode assemblages among sites and seasons

Two-way crossed ANOSIM, using the matrix constructed from the replicate densities of nematode species obtained from each site in each season, demonstrated that species composition differed significantly among both sites and seasons ($P = 0.001$) and that the differences were greater for site (R-statistic = 0.834) than for season (R-statistic = 0.592). Pair-wise tests showed that the composition of the assemblages at each site was significantly different from that at each of the other sites ($P = 0.001$). Furthermore, the R-statistic values demonstrate that the compositions of the nematode faunas at sites located relatively close to each other tended to be far more similar to each other than were those that were further apart, *e.g.* the R-statistic for sites 1 *vs* 2 was 0.304, whereas that for sites 1 *vs* 12 was 0.996. Likewise, R-statistic values showed that the compositions in widely-separated seasons, *i.e.* summer *vs* winter and spring *vs* autumn (0.728 and 0.634, respectively) were greater than those between consecutive seasons (< 0.610).

In the dendogram produced by Cluster Analysis of the similarity matrix constructed using the means for the densities of the nematode species at each site in each season, the samples separated, at a similarity level of 33 %, into two discrete groups, *i.e.* those from sites 1-6 and 7-12 (Fig. 3.5a). Furthermore, the samples from a particular site or neighbouring sites tend to cluster together. The clear separation between the samples from upstream and downstream sites is present in dendograms produced using data for each season separately (Figs not shown). On the nMDS ordination plot, derived from the same matrix, the samples show a very marked tendency to progress from left to right according to their distance from the estuary mouth (Fig. 3.5b).

The site component of the two-way crossed SIMPER showed that the assemblages at many sites, or pairs of sites, contained a species that both characterised and consistently distinguished the assemblage at that site from those of all other sites. Thus, this role was performed by *Comesoma arenae* at sites 1 and 2, *N. decoratus* at site 3, *Pseudochromadora cazca* at site 4, *Halichoanolaimus duodecimpapillatus* at sites 5 and 6, *Metadesmolaimus* sp 1 at site 7, *Bathylaimus australis* at sites 7 and 8, *Gomphionema typicum* at site 8, *Pierrickia* sp. nov. at site 10, *Parodontophora aurata* sp. nov. at site 12 and by *Metalinhomoeus* sp. at sites 11 and 12. Three species made an important contribution to the distinction between the samples for sites in different regions of the estuary on the ordination plot shown in Fig. 3.5, namely, *Spirinia parasitifera* at sites 1-6 and 8, *Theristus* sp. 2 at sites 3-6, and *Theristus* sp. 1 at sites 7-12. The seasonal component of the two-way crossed SIMPER showed more subtle trends than that for the site component.

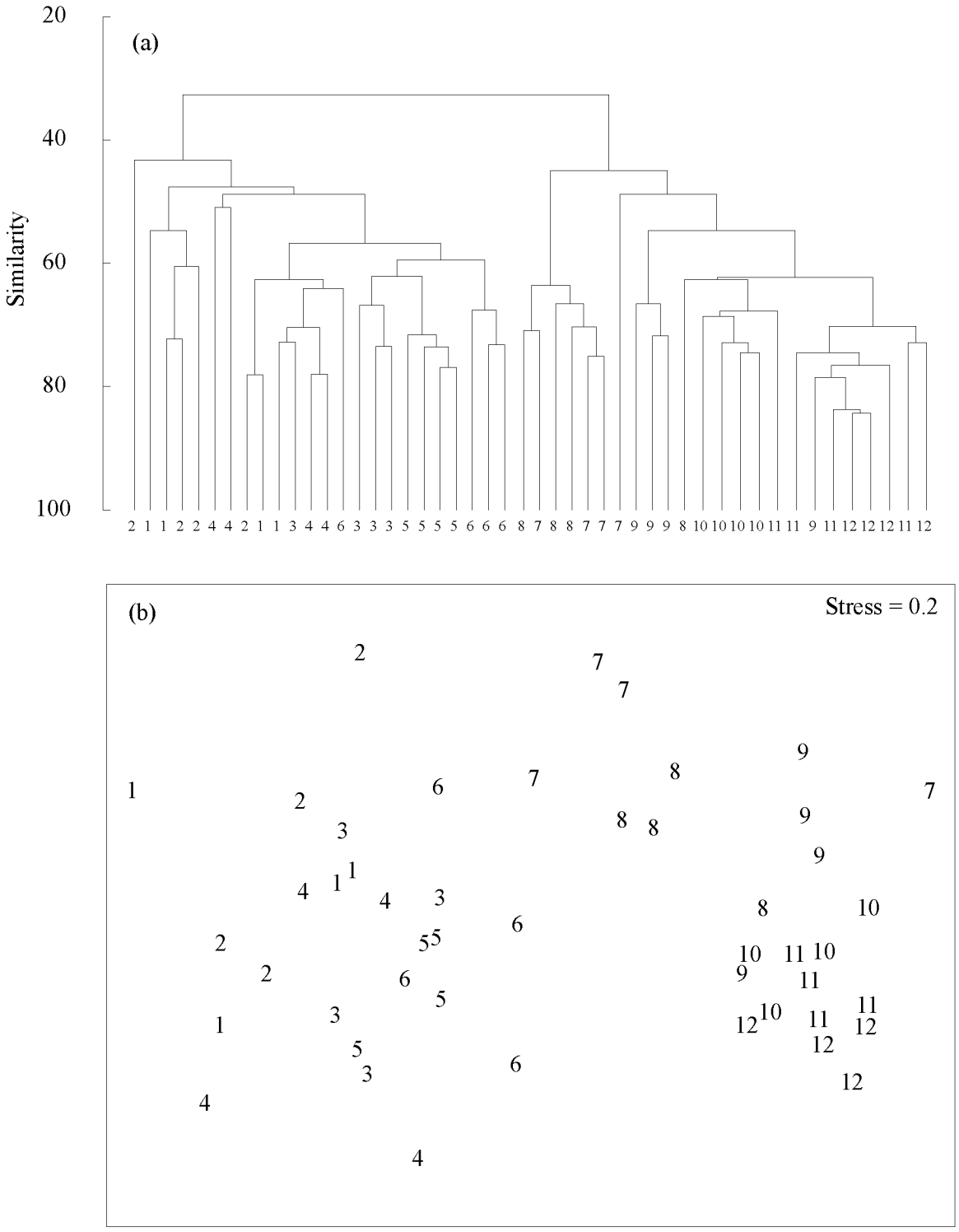

Figure 3.5 (a) Dendogram produced by Cluster Analysis and (b) two-dimensional nMDS ordination of the similarity matrix constructed from the mean densities of each nematode species at sites 1-12 in the Swan River Estuary in each season between summer 2004/5 and spring 2005.

The nematode fauna present in each season was characterised by a similar suite of species and those in any given season tended to be distinguished from those of the faunas in each of the other seasons by differences in the densities of those same species. For example, *Dichromadora* sp., which distinguished the fauna in each season from that in every other season, occurred in greater densities in spring than winter, which in turn were greater in summer and then autumn. Likewise, *N. decoratus* was most common during spring, followed by summer, winter and finally autumn, while *Chromadorina* sp. was most prevalent in spring, then summer, autumn and winter. Note that the above three species each attained their highest densities during spring and that each belong to the epistrate grazing functional feeding group.

One-way ANOSIM tests demonstrate that the compositions of the nematode assemblages differed significantly among seasons at every site ($P = 0.001$), being particularly well defined at site 6, for which the R-statistic was greatest, *i.e.* 0.852 (Fig. 3.6f). The magnitude of the seasonal differences tended to be greater in the middle estuary, with the R-statistics for sites 4, 6, 7 and 8 being the largest and always > 0.700. On the separate nMDS ordination plots for each of the twelve sites, the samples for each season often show a marked tendency to form distinct groups (Fig. 3.6). The samples for successive seasons often underwent clear progressive cyclical changes on the ordination plots, a feature well displayed on the plots for sites 1, 6, 10, 11 and 12.

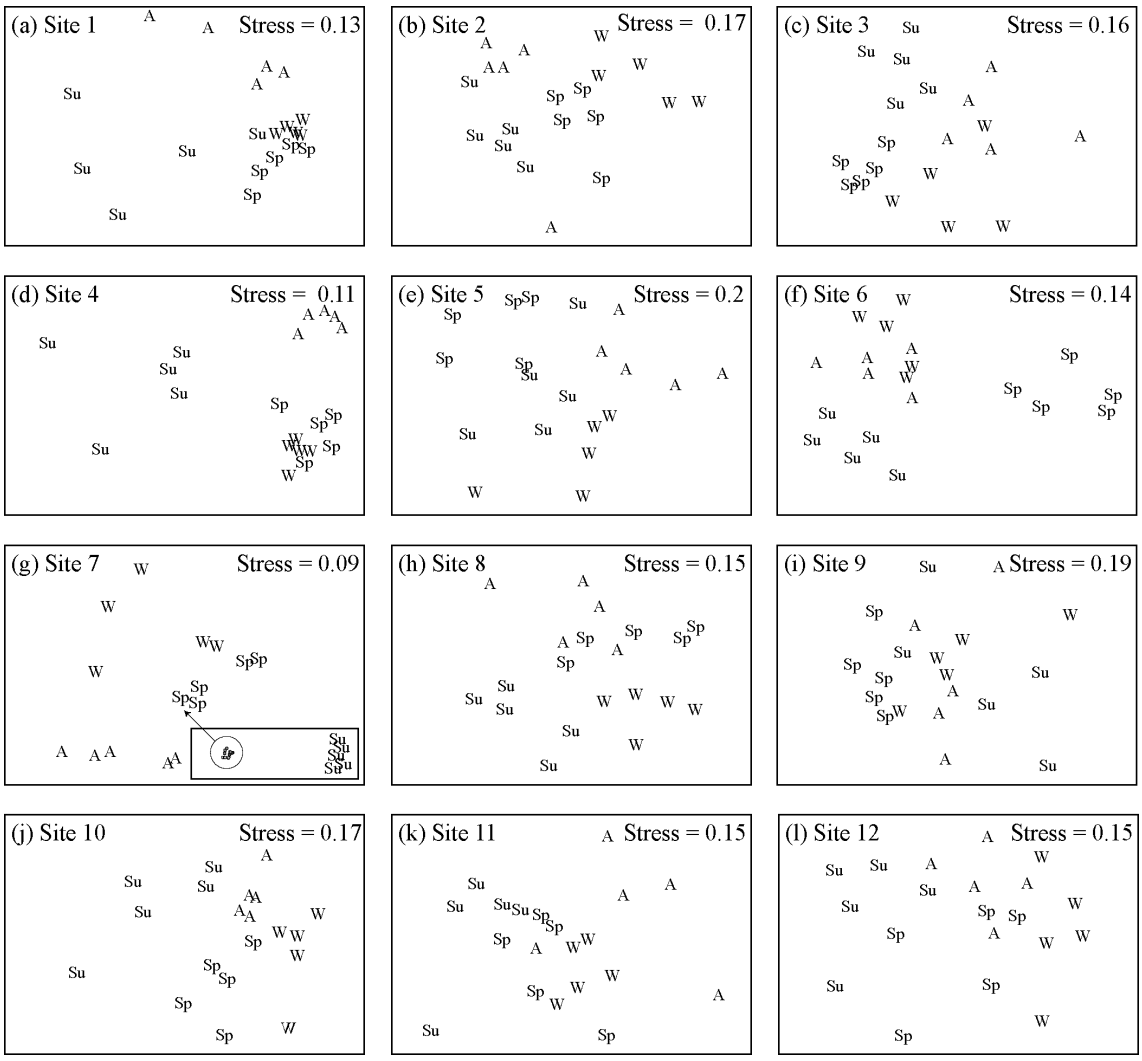

Figure 3.6 Two-dimensional nMDS ordinations of the matrices constructed from the densities of each nematode species in the five replicate samples collected from each of the sites (1-12) in each season between summer 2004/5 and spring 2005. Samples are coded for season.

3.3.5 Relating nematode assemblage patterns to environmental characteristics

When the matrices constructed from the mean densities of the nematode species at the 12 sampling sites were subjected to ordination, the points for the samples at the various sampling sites in each season progressed in a largely linear manner on the resultant plots according to location of site in the estuary (Fig. 3.7). Thus, on each ordination, those from the most downstream sites lie on the left, while those from the most upstream sites lie on the right, with the distance between points for sites 6 and 7 always being relatively large, and the greatest for any pairs of sites in three of the four seasons. RELATE tests demonstrated that the patterns of differences in the compositions of the nematode assemblages among sites were significantly correlated with distance from

estuary mouth (P = 0.001), both overall (ρ = 0.764) and in each season, with that in spring exhibiting the closest match, (ρ = 0.805), followed by autumn (ρ = 0.801), winter (ρ = 0.739) and then summer (ρ = 0.582). The relatively lower correlation in summer largely reflects the fact that the point for site 7 lies well to the right of those for all other sites (Fig. 3.7a).

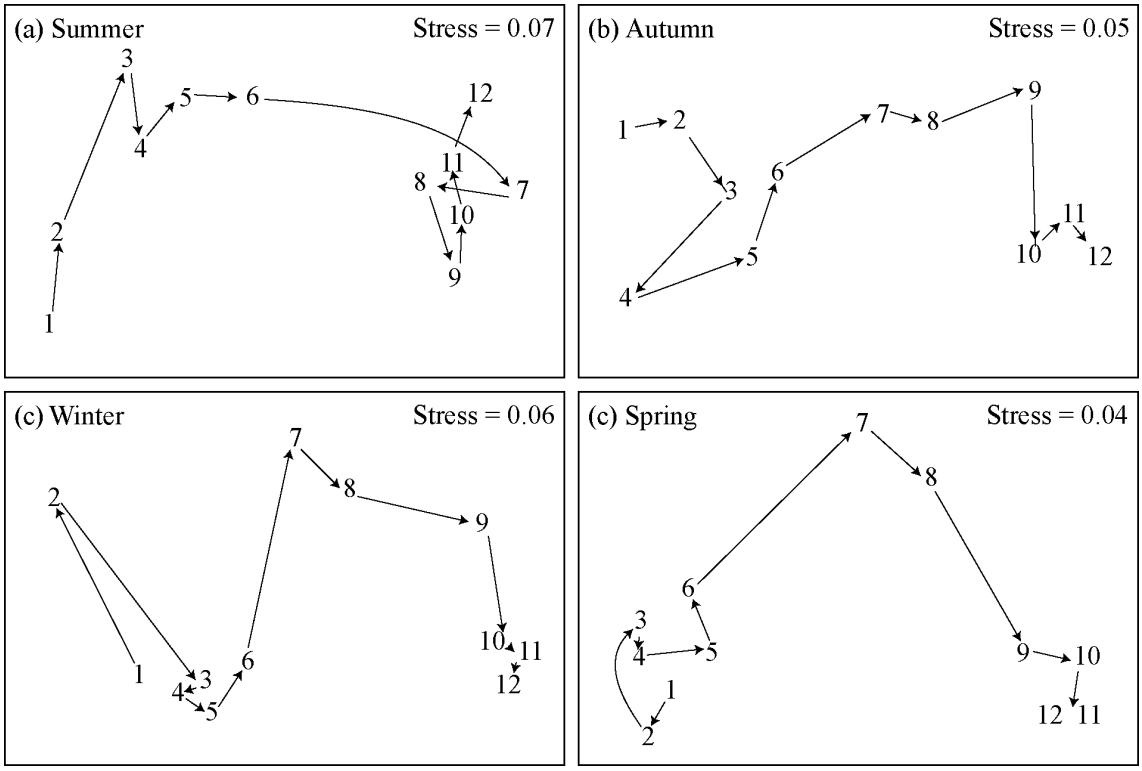

Figure 3.7 Two-dimensional nMDS ordinations of the matrices constructed from the mean densities of each nematode species at each of the twelve sites in each season between summer 2004/5 and spring 2005. Samples are coded for site.

The use of BioEnv demonstrated that, among the aquatic variables, the pattern of rank-order of similarity in the compositions of the nematode assemblages at the various sites was significantly and most highly correlated overall with that of salinity, (ρ = 0.775, P = 0.010), and that the correlation was not improved by the inclusion of temperature and/or dissolved oxygen concentration. Furthermore, salinity was also identified as the best matching variable in separate tests for autumn (ρ = 0.828, P = 0.010), winter (ρ = 0.866, P = 0.010) and spring (ρ = 0.859, P = 0.010), but not summer where no significant match was found (P = 0.440), with the improvement in

match in the former three seasons being negligible by including one or both of the other aquatic variables. In the case of the non-granulometric sedimentary variables, BioEnv demonstrated that the pattern of rank-order of similarity of the compositions of the nematode assemblages was significantly correlated overall with %POM ($\rho = 0.335$, $P = 0.040$), but not with transition zone depth or chlorophyll concentration. Seasonal tests for the non-granulometric sedimentary variables showed that a combination of %POM and chlorophyll concentration provided the best match with the compositions of the nematode assemblages in each season, but that the correlation was significant in only autumn and spring ($P < 0.05$), and that those correlations were always lower than 0.390.

The RELATE test demonstrated that the pattern of rank-order of similarity between the compositions of the nematode assemblages at the 12 sites was significantly correlated with that for the grain size compositions of the sediments ($P = 0.001$) but that the degree of correlation was relatively low ($\rho = 0.353$).

3.3.6 Analysis of functional feeding groups

Two-way crossed ANOSIM demonstrated that the contributions of the four Functional Feeding Groups (FFGs) differed significantly among sites and seasons (both $P = 0.001$), with the R-statistic being greater for site (0.541) than season (0.361). Sixty three of the 66 pairwise comparisons among sites were significant, and in general, were greatest between sites that were widely separated, with those for each of sites 1 and 2 *vs* each of sites 9-12 being greater than *ca* R = 0.500. The R-statistics for seasonal pairwise comparisons demonstrated that the FFG composition of the samples from spring were the most distinct, with values for spring *vs* each other season always exceeding 0.450 and being greater than between any other two seasons.

When the mean contributions of the various functional feeding groups at the 12 sites in the four seasons were subjected to PCA, the 2-dimensional plot encompassed 84 % of the total variation in the data (Fig. 3.8). The samples from sites 7-12 formed a group in, or close to, the upper right quadrant of the PCA plot and were positively correlated with the eigenvector for the 1B FFG (Fig. 3.8a). In contrast, the majority of the samples for sites 1-6 were widely distributed through the other three quadrants of the plot and were thus more positively correlated with the eigenvectors for the 1A and 2A FFGs. The four seasonal samples from each of sites 7-12 showed a much greater tendency to group together than did those from each of sites 1-6. When the data were coded for season, 11 of both the autumn and winter samples and 9 of the 12 summer samples lay in the upper half of the plot, whereas 7 of the 12 spring samples lay in the bottom half of the plot (Fig. 3.8b)

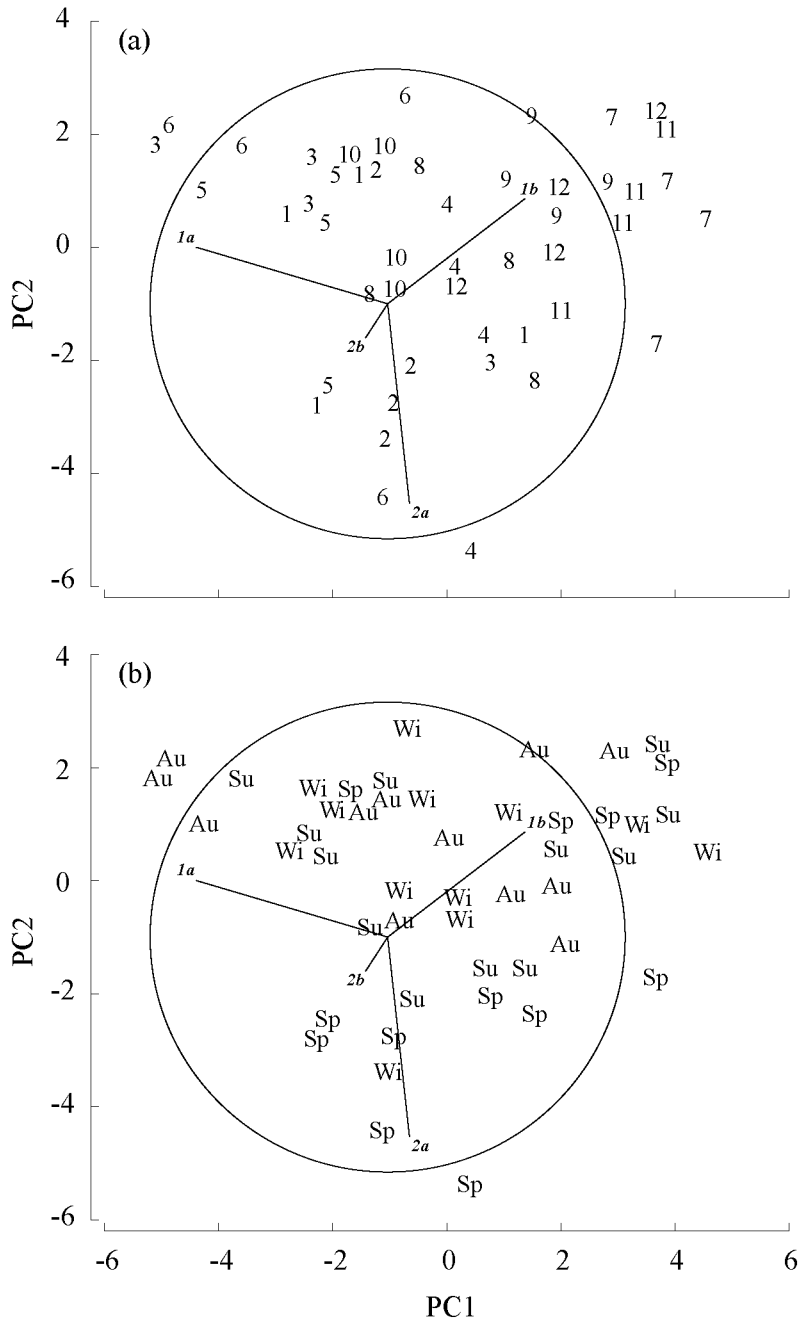

Figure 3.8 Principal component analysis plot derived from the mean percentage contributions of each of the four functional feeding groups to the total number of nematodes in the samples at each site in each season. Samples have been coded for (a) site and (b) season. Principal component axes 1 and 2 collectively accounted for 84.5 % of the total variation present.

3.4 Discussion

This study is the first to explore statistically the ways in which the densities, numbers of species and species compositions of nematode assemblages in subtidal waters differ throughout the length of a microtidal estuary and the extent to which those three biotic variables change in this type of estuary during the year. This approach enabled emphasis then to be placed on exploring which environmental variables were mainly responsible for influencing the spatial and temporal distributions of those nematode assemblages. This study also represents the first time that the trophic compositions of nematode assemblages numerous sites throughout an estuary have been analysed, with a view to elucidating how the contributions of the various functional feeding groups change with location and season and which factors are responsible for any such changes.

3.4.1 Spatial variation

Our results demonstrated that, while the species compositions of the nematode assemblages in the Swan River Estuary are influenced markedly by both site and season, the first of these factors is by far the most important. In the context of site, it is relevant that the ordination plots shown in Fig. 3.7 demonstrate that, in each season, species composition changes progressively along the estuary. This point is emphasised by the R-statistic values for comparisons between the compositions of assemblages at the proximal and distal ends of the estuary being close to 1, whereas those for the compositions at sites close together were far lower. The very pronounced differences in the compositions at the extreme ends of the estuary are reflected in the horseshoe-like distribution of the samples on the ordination plot that utilised data for all four seasons (Fig. 3.5). This pattern of distribution reflects the fact that the mechanics of the ordination procedure are influenced by the very high dissimilarity between samples at the extremes in a gradient (Seber, 1984; Clarke & Gorley, 2006). As with nematodes, the species composition of the benthic macroinvertebrate and fish faunas in the lower

and upper reaches of the Swan River Estuary were shown to differ markedly (Loneragan & Potter, 1990; Wildsmith, 2008).

The change in the species compositions of nematodes through the estuary was shown by BioEnv to be closely correlated with changes in salinity, thereby paralleling the situation with nematode faunas in macrotidal estuaries in Europe (Warwick, 1971; Austen & Warwick, 1989). However, Cluster Analysis emphasised that the compositions at the various sites constituted two very distinct groups, namely those in the entrance channel and lower basin (sites 1-6) *vs* those in the upper basin and riverine parts of the estuary (sites 7-12). This regional distinction, with the point of separation occurring where the estuary becomes very constricted and thus separates the large downstream and upstream basins, parallels that found with the fish faunas that are likewise located in nearshore waters (Loneragan *et al.*, 1989). As salinity influences the composition of nematode faunas, it is relevant that, in each season apart from summer, salinity declines progressively and markedly upstream of site 6 and that it exhibits the greatest intra-annual variation at sites 7-9. It is therefore suggested that the species that characterise the fauna of the assemblages upstream of site 6 are particularly well adapted to the osmotic stress produced by the lower and more variable salinities that are found in that part of the estuary. The conclusion that variation in salinity is important in influencing the composition of the nematode fauna of the Swan River Estuary parallels that drawn by Armenteros *et al.* (2006) for meiofaunal assemblages in the mangroves of a Cuban gulf that exhibited spatial differences in salinity.

The distinction between the compositions at sites 1-6 and 7-12 was shown by SIMPER, and by the data for the percentage contributions and ranking by abundance for each species in Table 3.2 to be due to marked differences in the abundances of certain species. This shift was due to in particular to a pronounced decline in the importance of *Spirinia parasitifera* and *Theristus* sp. 2 and to a marked increase in the importance of

Theristus sp. 1. Thus, *S. parasitifera* and *Theristus* sp. 2 contributed 30 and 11 % respectively, to the total number of nematodes recorded at sites 1-6, but only 6 and 3 %, respectively, to those collected from 7-12, whereas *Theristus* sp. 1 contributed only 4 % to the numbers at sites 1-6 compared with as much as 36 % at sites 7-12. Although *S. parasitifera* is a very cosmopolitan species, it was not recorded during extensive studies of the sediments in nearshore waters along the coast outside the Swan River Estuary, as were neither of the other two species (Hourston *et al.*, 2005).

Although salinity was the most important of the factors which influenced the species compositions of nematode assemblages in the Swan River Estuary, those compositions were also influenced by sediment granulometry and %POM. In the case of the macrotidal Exe estuary (UK), a combination of transition zone depth, salinity and granulometry (as reflected by median particle size) of the sediment provided the strongest relationship with the species composition of nematode assemblages (Warwick, 1971; Clarke & Ainsworth, 1993). In macrotidal estuaries, the strong tidal activity typically leads to the development of a strong spatial gradient in sediment composition, with sediments at the mouth being coarse and well-oxygenated, whereas those at the head are finer and poorly oxygenated (McLusky & Elliott, 2004). However, our data show that grain-size composition does not change in a comparable sequential manner along the Swan River Estuary, presumably reflecting variations among locations in such factors as flow, soil type and extent and type of vegetation. This accounts for changes in the compositions of the nematode assemblages along the estuary being far less strongly correlated with that of granulometry than with that of salinity, which does change progressively with increasing distance from estuary mouth.

The positive correlation between the compositions of the nematode assemblages and %POM reflects the fact that the fauna in the lower and upper halves of the estuary constituted relatively distinct groups and that the levels of POM are far greater in the

upper part of the estuary. This point further emphasises that there is a regional component to the pattern of distribution of nematode species within the overall trend for compositions to change sequentially along the longitudinal axis of the estuary.

It was conspicuous that the number of nematode species at site 3 was relatively low in all seasons as this ran counter to the strong trend for the values at the other downstream sites (1, 2, 4 and 5) to be high. This apparent anomaly can almost certainly be explained by the fact that Tributyl-tin, which is known to be toxic to nematodes (Schratzberger *et al.*, 2002), was present in high levels in nearby sediments, having been used as an antifoulant on boats in a nearby marina (Shute, 2007).

The tendency for the number of nematode species in the Swan River Estuary to decrease with declining salinity and then to show a slight increase at the lowest salinities broadly parallels the Remane paradigm that was based on an analysis of the changes in the number of species along a salinity gradient in the Baltic Sea (Remane, 1934). It also parallels the trends exhibited by nematodes in a Japanese coastal lake system that is connected to the sea (Yamammuro, 2000) and by the meiofauna in the Thames Estuary in the United Kingdom (Attrill, 2002). However, Attrill (2002) concluded that the diversity of subtidal meiofauna (and macrofauna) of the Thames estuary was influenced more by variation in salinity than by absolute salinity, which parallels our conclusions regarding species composition (see earlier). The importance of the magnitude of change in salinity was also considered by Armenteros *et al.* (2006) to influence meiofaunal community structure in a tropical mangrove system to a greater extent than does absolute salinity *per se*.

3.4.2 Seasonal variation

The high Global R-statistic of 0.592 in the global two-way crossed ANOSIM with site shows that species composition undergoes considerable seasonal variation. Furthermore, the distributions of the samples for the four seasons on the ordination plots shown in Fig. 3.6 demonstrate that the composition of the nematode fauna at the 12 sites in the Swan River Estuary undergo progressive cyclical changes during the year, thereby paralleling the situation with the fish faunas in nearshore waters of the same estuary (Hoeksema & Potter, 2006). Variations in the composition of the fish fauna of the Swan River Estuary are due, in part, to interspecific differences in the timing of recruitment of juveniles, often as a result of differences in the temperatures that stimulate spawning in the various species.

As reproduction, maturation and development of two co-occurring nematode species were shown to be differentially influenced by temperature (Moens & Vincx, 2000a), the changes in species composition of the nematode fauna of the Swan Rive Estuary may likewise reflect temperature-related differences in the reproductive success and / or recruitment of the various species. However, since salinity had a differential impact on the viability of juveniles of the two species studied by Moens & Vincx (2000a), changes in this environmental variable may also influence the relative abundances of the various species in the Swan River Estuary and thus also contribute to the cyclical changes in the composition of its nematode assemblages. Seasonal variation in salinity is also likely to be a more important factor in influencing seasonal changes in nematode assemblages in microtidal estuaries such as that of the Swan River, more so than in macrotidal estuaries where the shorter-term variation in salinity over diurnal and lunar time-scales may be greater in magnitude than the seasonal changes. The seasonal changes in the composition of the nematode assemblages partly reflected the marked increase that occurred in the population of the epistrate grazing species *Dichromadora* sp.,

Nanolaimoides decoratus and *Chromadorina* sp. in spring, when the density of microphytobenthos peaks in the Swan River Estuary (Masini & McCoomb, 2001; Montani *et al*. 2003).

The very conspicuous seasonal changes undergone by the species compositions of nematode assemblages throughout the Swan River Estuary parallel the situation recorded in two macrotidal estuaries in the north-eastern region of the United States (Tietjen, 1969). Furthermore, the seasonality in the two North American estuaries, and also in an estuary in Germany (Skoolmun & Gerlach, 1971), likewise reflected increases in the densities of epistrate-grazing species in spring and / or summer, when benthic microflora production was at its maximum. However, conspicuous seasonal variations were not found in the nematode assemblages of two macrotidal estuaries in Europe (Warwick, 1971; Warwick & Price, 1979). Although the composition of the nematode assemblages in the Hunter River estuary, which is located in eastern Australia at a similar latitude to the Swan, varied through the year, the trends were not consistent across all study sites, which was attributed to differences in non-seasonal environmental changes in the estuary (Hodda & Nicholas, 1986b). Our ability to demonstrate that nematode compositions underwent cyclical seasonal changes in the Swan River Estuary and identification of the species responsible for those changes benefited greatly from the development of nMDS ordination plots, ANOSIM tests and SIMPER, which were not available to some earlier workers. It would be worthwhile to use contemporary multivariate statistical techniques to explore whether the compositions of nematode assemblages in all types of estuaries typically vary seasonally.

3.4.3 Trophic structure

Because free-living species of nematode tend to be selective in the food they ingest, the presence of large amounts of a particular type of food at a locality would favour colonisation by species that belonging to the particular trophic group or groups most able to exploit it. The distributions of the seasonal samples for the 12 sites on the PCA plot, derived from the percentage contributions of each of the four Functional Feeding Groups (FFGs), suggest that the types of food ingested by the nematode species in the downstream and upstream reaches of the estuary differ markedly. The great importance of non-selective deposit-feeding species, such as *Theristus* sp. 1 to the nematode fauna upstream of site 7 presumably reflects the presence of greater amounts, and sometimes markedly so, of their main food source (POM) in the sediments of those sites than in those further downstream. In comparison to the situation upstream, the trophic compositions of the nematode assemblages in the lower reaches was far more seasonally variable, with those in spring being the most distinct and characterised by epistrate grazers such as *Dichromadora* sp., *Nannolaimoides decoratus* and *Chromadorina* sp.. This peak in abundance of epistrate feeders in the lower estuary corresponds, both temporally and spatially, to where the concentration of sedimentary chlorophyll and therefore density of microphytobenthos was greatest. The co-occurring density peaks of microphytobenthos and epistrate grazing nematodes is consistent with the proposal that the members of this feeding group are more susceptible to variation in their food source than the other three groups (Tietjen, 1969; Austen & Warwick, 1995).

3.5 Conclusions

In summary, this study has demonstrated that the species composition of nematode assemblages of the Swan River Estuary changes largely in a linear manner along the length of this system and that these changes are closely related to the overall decline in salinity and, to a lesser extent, the increase in the percentage of organic material in the sediment, with increasing distance from the estuary mouth. Species composition is also moderately related to sediment grain-size composition, even though that variable does not change in a similarly progressive manner. This suggests that certain species prefer certain sediment types which, through differences in factors that affect granulometry, tend to differ among areas in this microtidal estuary. The density and number of species tended to decline with increasing distance from estuary mouth, to reach a minimum in the area where salinity started decreasing markedly and exhibited the greatest intra-annual variation, and then rose slightly in the uppermost reaches. The spatial and temporal variations recorded in the compositions of the different functional feeding groups were related to differences in the relative abundances of the different types of food present.

Chapter 4 Classification of habitats in the Swan River Estuary

4.1 Introduction

Estuaries in urbanised settings, such as the Swan River Estuary, are very susceptible to a range of detrimental anthropogenic influences caused by local industrial, agricultural and recreational activities that are undertaken on their waters and in their catchments. For this reason, environmental managers and ecologists need reliable baseline data on the biotic and abiotic characteristics of these systems, and effective tools for evaluating (i) the ecological impacts of proposed anthropogenic or natural environmental changes, and (ii) the effectiveness of conservation efforts. Such information is crucial for establishing effective environmental policies and guiding management actions.

A reliable classification of the habitat types present in an estuary is a crucial tool for environmental managers. It provides an inventory of the environmental diversity of a system, information on the ways in which the various habitat types differ and a sound basis for establishing the links between estuarine habitats and biota. Furthermore, it provides a sound framework for extrapolating such knowledge for a limited area over a larger one.

The ideal habitat classification scheme should have certain characteristics, *i.e.*, it needs to be quantitative, based on enduring environmental criteria, user friendly and predictive. The scheme devised by Valesini *et al.* (2003) for nearshore marine waters along the lower west coast of Australia satisfies all of these criteria and for this reason has been used as a basis for the current scheme.

The main aim of the scheme developed by Valesini *et al.* (2003) was to "develop a user-friendly but quantitative approach [to] enable the different types of habitats in nearshore coastal waters of a region to be readily identified". To achieve this, quantitative measurements of a suite of enduring environmental characteristics, *i.e.* those that are not subject to considerable temporal variation, were used to classify an environmentally-diverse range of nearshore sites into one of six habitat types that differed mainly in their exposure to wave activity and contributions of different substrate / submerged vegetation types. This classification can be tailored to local conditions and is thus transferable or readily adaptable to any nearshore marine region. Furthermore, a method for predicting the habitat type of any other nearshore site of interest along that coastline (*i.e.* one outside those used to create the classification) was developed for use by managers and scientists working in those waters.

The use of enduring environmental characteristics as the basis for classifying habitat types is a very important characteristic of the present system that has carried through from that of Valesini *et al.* (2003). It ensures that the classification of sites remains valid over time, *i.e.* if a classification is based on non-enduring characteristics then when those characteristics change, so too will the classification. Furthermore, enduring characteristics can usually be measured remotely from existing data sources such as aerial photographs and bathymetric charts. These remote data sources are often readily available and relatively less expensive than the filedwork required to collect environmental or biotic data.

As with the approach developed by Valesini *et al.* (2003), the current scheme aims to develop a quantitative and user-friendly method for classifying nearshore sites into appropriate habitat types, using measurements for a range of enduring environmental characteristics that can be measured from mapped or remotely-sensed data sources. However, this approach differs fundamentally from that earlier scheme in terms of

particular aspects of its underlying philosophy and the statistical techniques employed to achieve the classification. Thus, whereas Valesini *et al.* (2003) employed a partially subjective *a priori* framework to initially assign sites to habitat types, the present scheme is completely quantitative and employs the relatively new multivariate statistical technique "Similarity Profiles" (SIMPROF; Clarke & Gorley, 2006) to identify groups of sites that do not differ significantly in their environmental characteristics, and thus represent distinct habitat types.

The aim of this study was to develop a scheme to quantitatively classify the spectrum of environmentally-diverse nearshore sites in an estuarine environment into their appropriate habitat types. The Swan River Estuary, located in south-western Australia, has been used as a case study for this purpose. The criteria for the classification system is that it be based on the quantitative measurements for a suite of enduring environmental variables and that those variables can be easily obtained from mapped data sources. It is envisaged that this approach could be adopted as-is or tailored specifically for classifying the habitat types in estuaries elsewhere in the world.

The Biological validity of the habitat classification scheme has not been assessd in this chapter. Validation has been undertaken using nematode assemblages as the test faual group, the documentation of which is contained in Chapter 6 of this thesis.

The study contaned in this chapter was carried out by a team of workers, each of which had specific roles. The other workers involved in this study are listed as authors on the resultant publication, *i.e.* Valesini *et al* (in press). Although I am capapable of reproducing all of the methodologies presented in this chapter, I did not personally conduct them in this instance. The sections of methodologies that I did not personally perform are the raster analyses in subsection 4.2.4.2 and parts of the statistical analyses in subsection 4.2.6.

4.2 Materials and methods

4.2.1 Site description

Climate

South-western Australia has a Mediterranean climate, experiencing hot dry summers and cool wet winters. The distinct seasonality is driven by cyclical changes in the position of the mid-latitudinal cyclonic / anticyclonic pressure cell systems. During the summer, the climate of Perth, the major city in the region, is dominated by high pressure anticyclonic cells located at 37-38 ºS, which bring hot, dry air from over the desert interior of the continent westwards to the coast, resulting in low precipitation (October-March average total rainfall = 109.8 mm, *ca* 15 % of total) and high temperatures (average daily maximum 28.2 ºC). A regular local diurnal wind pattern is also prevalent in which the hot dry easterly air flow in the morning is replaced by a cool south-westerly breeze in the afternoon. Winter weather patterns are dictated by the low pressure cyclonic cells which migrate northwards to 29-32 ºS, effectively displacing the summer anticyclones. These pressure cells can result in strong onshore winds ranging in direction from the north to the southwest and bring cool wet air from over the ocean. These onshore winds are responsible for the lower temperatures (average daily maximum 20.7 ºC) and the onshore airflow propagates the majority of the regions annual rainfall (April-September average total rainfall = 676.1 mm, *ca* 85 % of total) (Valesini *et al*., 2003, BoM 2008).

Morphology

The Swan River Estuary covers an area of *ca* 50 km^2 and has a catchment covering an area of *ca* 122 000 km^2. It carries an estimated mean annual river flow of 624 GL, making it one of the largest estuaries in south-western Australia (Hodgkin & Hesp, 1998). It is permanently open to the ocean, which contrasts with

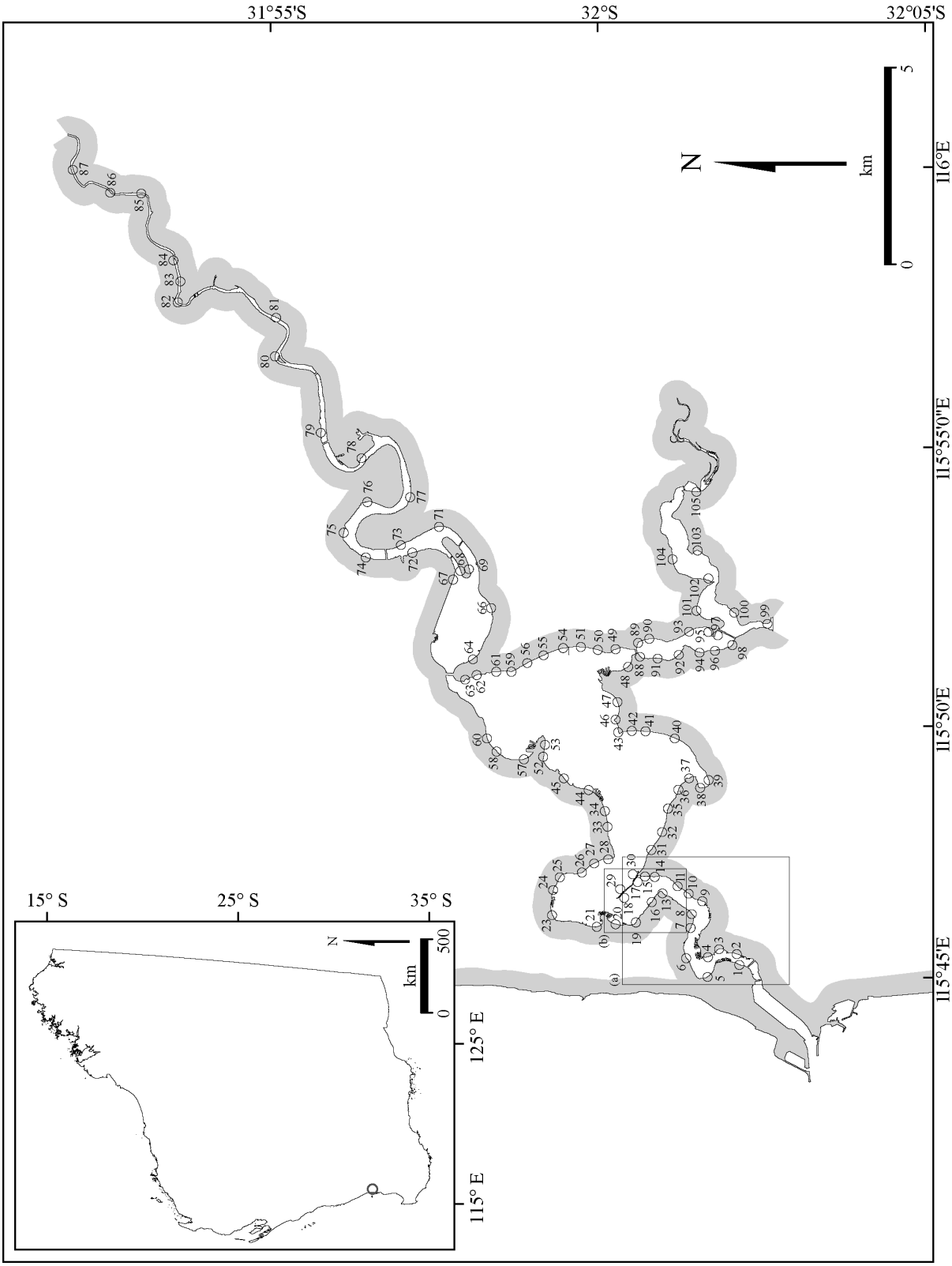

Figure 4.1 Map of the Swan River Estuary showing the location of the 101 sites used for the habitat classification. Note that the areas bounded by boxes denote locations of subsequent maps used to illustrate various environmental variables. Thus, the area in (a) appears in more detail in Figures 4.4a-c and (b) in Figure 4.4d.

many other south-western Australian estuaries, which are either seasonally, normally or permanently closed by a sand bar across the mouth. While the permanent exchange between the Swan River Estuary and the ocean was historically due to its relatively high natural flow rate and the low energy marine environment near its mouth, the entrance to this system has since been highly modified to accommodate the major international shipping port of Fremantle (Hodgkin & Hesp, 1998).

The Swan River Estuary is a drowned river valley estuary and thus lies roughly perpendicular to the coast, (Hodgkin & Hesp, 1998). It contains three morphologically and hydrodynamically distinct regions; (i) the tidal portion of the two primary tributaries (the Swan and Canning Rivers, the former of which contributes most of the freshwater flow), (ii) a large central lagoonal basin, and (iii) a narrow entrance channel (Chalmer *et al.*, 1976). The basin area comprises Perth Water, into which the Swan River flows, and the far larger Melville Water, which receives discharge from both tributaries. The two basins are demarcated by a distinct constriction between them (Fig. 4.1). Depths vary markedly throughout the system generally being 1-5 m deep, with the deepest point (21 m) occurring in the entrance channel.

Geology

The catchment geology of the Swan River Estuary comprises two main parts, namely the aeolian / alluvial Swan Coastal Plain (SCP), on which the estuarine section of the system is located, and the archaic Yilgarn Block, which supports the majority of the catchment area. Dividing the above two features is the Darling Scarp.

The Swan Coastal Plain is an area is between 15 and 25 km wide and extends 1000 km along the lower west coast of Australia from the Murchison River to Cape Leeuwin. It is primarily composed of two archaic dune systems, the Bassendean Sands, which were formed during the Pleistocene, and the younger Spearwood Dunes, which formed

during the Holocene and are now partially lithified into an eolianite termed Tamala limestone. The riverine and basin areas of the estuary lie primarily on Bassendean Sands, while the channel bisects the Spearwood Dunes. Localised deposits of alluvial clay ("Guilford Clay") can also be found close to the base of the Darling Scarp.

Hydrology

The nearshore waters along the lower west coast of Australia are subject to only small semidiurnal tidal fluctuations, with a mean spring tidal range of *ca* 0.6 m (Hamilton *et al.*, 2001). Within the Swan River Estuary, these microtidal fluctuations are attenuated to *ca* 80 % of that in nearby coastal waters, and decrease in height by a further 1 % km^{-1} upstream. Thus, astronomical tides do not greatly influence the hydrology of the estuary. Water level fluctuations are influenced more by changes in barometric pressure and storm surges (up to 1 m), which can persist for between 5 and 15 days (Hamilton *et al.*, 2001; Hamilton *et al.*, 2006). The extended duration of these conditions means that the constrictive effect of the entrance channel is diminished and that the system receives the full amount of fluctuation.

The Swan River Estuary, like most other estuaries in the region, experiences extreme seasonal changes in hydrology, which is due largely to the highly seasonal rainfall and subsequent freshwater inflow into its tributaries (Spencer, 1956; Stephens & Imberger, 1996; Hamilton *et al.*, 2006). During summer, the majority of the estuary is saline, experiencing salinities that range from of *ca* 35 in the lower reaches to *ca* 5 at a distance of 40 km upstream, with some areas becoming hypersaline. These conditions are due to the encroachment upstream of a marine-water salt wedge during periods of low river flow. When the river flow increases during winter, the resultant fresh water forms a layer over the more dense saline water in the upper reaches of the estuary, causing a distinct halocline. This stratification leads to deoxygenation of the lower saline water mass. As the water flow increases further, the lower water mass is

pushed downstream and partially mixed with the fresh water, with the result that the estuary is effectively fresh to within 5 km of the ocean, with saline pockets remaining only in the deeper holes. When the rainfall subsides during spring, the estuary progressively returns to its summer state of predominantly saline water (Kurup *et al.*, 1998; Thompson, 2001; Thompson & Tracey, 2002).

Substrate and submerged vegetation

The macroscopic aquatic flora of the Swan River Estuary primarily comprises; (i) three species of seagrass, *Halophila ovalis*, *Zostera mucronata* and *Ruppia megacarpa*, the first of which is by far the most dominant, and (ii) a diverse assemblage of macroalgae (Astill & Lavery, 2004a). Beds of *H. ovalis* occur to a depth of *ca* 2.5 m throughout the entrance channel, Melville water and the lower reaches of the Canning River. The total area covered by this seagrass species in the Swan River Estuary is estimated to be between 4.6 and 5 km^2 (Connell & Walker, 2004). While *Z. mucronata* and *R. macrocarpa* are not as abundant as *H. ovalis*, they are found in localised beds in the lower and middle estuary, respectively.

While 36 species were recorded during a recent survey of the macroalge taxa of the Swan River Estuary, the two species *Gracilaria comosa* and *Cystoseira trinodis* were the most abundant (Astill & Lavery, 2004a; 2004b). The former species is often found free-floating over shallow soft substrate in the basin / lower river reaches, while the latter is usually attached to rocky substrates in the entrance channel and basin areas.

The unvegetated substrates of the Swan River Estuary are mainly unconsolidated and range from calcareous biogenic sands and degraded limestone outcrops in the lower and middle reaches, to fine sands and muds in the riverine reaches. Additionally, certain areas of the Swan River Estuary have other localised substrate types including bare rock, heavy clay, coarse granite gravel, or archaic bivalve beds.

Uses and Anthropogenic Modifications

The catchment of the Swan River Estuary, and indeed the estuary itself, has undergone considerable anthropogenic modifications since initial European settlement in the early 1800s. The estuary is now surrounded by the capital city of Perth, which has a population of *ca* 1.44 million (Australian Bureau of Statistics, 2006). In addition to the urban and industrial activities associated with this city, the catchment of the Swan River Estuary contains extensive agricultural activity. The estuary itself has also been modified in numerous ways, such as by the construction of seawalls, many marina developments and an extensive stormwater drainage system. The most obvious anthropogenic modification, the Fremantle shipping harbour, is the largest commercial operation within the system, with 25.5 million tonnes of trade goods with a value of *ca* $1.8 billion passing through it in 2004/05 financial year (Fremantle Ports, 2007). Other commercial uses of the estuary include a small commercial fishery for species such as Black Bream (*Acanthopagrus butcheri*), Blue Manna crabs (*Portunus pelagicus*) and Sea Mullet (*Mugil cephalus*) (Fletcher & Santoro, 2008).

Environmental management

Environmental management of the Swan River Estuary falls under several different jurisdictions ranging from international treaties to local governments. International jurisdiction over this system resides in The Convention on Wetlands of International Importance especially as Waterfowl Habitat (Ramsar convention), the China Australia Migratory Bird Agreement (CAMBA) and the Japan Australia Migratory Bird Agreement (JAMBA), all of which aim to conserve habitats for migratory birds. Governance at the federal level stems from the Environmental Protection and Biodiversity Act (1999), which contains provision for the State-governed Swan River Trust through the Environmental Protection (Swan and Canning Rivers) Policy (1998) and the Swan River Trust Act (1976). Another state-level management authority with

governance over the estuary and its surrounds is the Department of Environment and Conservation, which administers the Swan Estuary Marine Park. Local governments manage issues within their jurisdiction, such as storm water discharge, drainage, urban planning and foreshore restoration and management.

4.2.2 Study sites

In order to capture the range of environmental diversity found throughout the Swan River Estuary, 101 nearshore sites were selected from the area between the estuary entrance, just upstream of the commercial port, to the limit of the tidal influence in both the Swan and Canning Rivers (Fig. 4.1). Each site was defined by a point on the estuary shoreline and all of the waters, substrate and submerged vegetation within a 100 m radius of that point, up to a depth of 2 m. The maximum depth of 2 m was chosen because this was approximately the depth at which (i) the substrate could be reliably viewed from an aerial photograph and (ii) waves approaching the shoreline began interacting with the substrate.

4.2.3 Enduring environmental variables selected for classification

Thirteen enduring environmental variables were measured at each site to provide a quantitative dataset for classifying the 101 study sites into habitat types (Table 4.1). Each of these variables, which were measured from mapped sources (see subsection 4.2.4) belonged to one of three major groups. The first group, "location", reflected the position of each site throughout the estuary with respect to its vicinity to marine and freshwater inputs. It was considered to be a surrogate for a large suite of water quality variables, such as salinity, dissolved oxygen, temperature, turbidity and force of tidal / riverine flow. The second group comprised seven variables and quantified the extent to which each site was exposed to wave energy. The first five of these exposure variables were fetches, one for each of the four cardinal directions and a fifth (direct fetch) that was oriented perpendicular to the shoreline. These fetches

reflected the exposure of each sites to wave energy that was generated by local winds. The sixth variable in this group was the direct distance from the shore to the 2 m depth contour, *i.e.* the wave shoaling margin, while the seventh variable was the average slope of the substrate. These latter two variables reflected the extent of shoaling and attenuation as waves approach the shoreline. The third group of environmental variables quantified the areas occupied by the various types of substrate / submerged aquatic vegetation (SAV) at each site. Six categories were distinguished, namely bare substrate, submerged vegetation, rock, emergent vegetation that extended into the nearshore water (*e.g.* reeds), snags (submerged tree branches) and ancient beds of large bivalve shells (*Katelyesia* sp.). Seagrass and macroalgae were combined into a single category (submerged vegetation) because their spectral signatures could not be reliably separated using the aerial photograph.

Variable	Variable type	Transformation	Weighting
Distance from estuary mouth	Location	√	100.00
MEF (Direct)	Exposure	√	14.28
MEF (North)	Exposure	√√	14.28
MEF (East)	Exposure	√√	14.28
MEF (South)	Exposure	√√	14.28
MEF (West)	Exposure	√√	14.28
MED (2 m depth contour)	Exposure	√√	14.28
Slope	Exposure	√√	14.28
% submerged vegetation	Substrate / SAV	√	20.00
% rock	Substrate / SAV	√	20.00
% snags	Substrate / SAV	√	20.00
% riparian vegetation	Substrate / SAV	√	20.00
% bivalve beds	Substrate / SAV	√	20.00

Table 4.1 The category (location, exposure or substrate / SAV type), transformation and weighting factor for each of the 13 enduring environmental variables used to classify nearshore habitat types in the Swan River Estuary. SAV = Submerged Aquatic Vegetation, MEF = Modified Effective Fetch, MED = Modified Effective Distance

4.2.4 Measurement of enduring environmental variables using GIS

Each of the 13 enduring environmental variables recorded at the 101 nearshore study sites were measured from either a high resolution aerial photograph of the estuary (0.4 m x 0.4 m pixels) obtained from the Department of Land Information and taken in December 2003, or from a Digital Elevation Model (DEM) produced from depth

soundings obtained from the Department of Planning and Infrastructure. These measurements were made using either the ArcGIS 9.1 or Idrisi v.14 Geographical Information Software (GIS) packages. ArcGIS was used primarily to determine values for vector-based distance variables, (*i.e.* fetches, distance to the 2 m contour and distance from estuary mouth, while Idrisi was employed to extract values for the raster-based variables, *i.e.* contributions of the various substrate / SAVs and slope. Figures 4.2 and 4.3 are flow diagrams of the procedures implemented within the vector and raster analyses, respectively. To ensure compatibility between GIS programs and among files, all data files were created in, or reprojected into, Universal Transverse Mercator (UTM 50S) projection using the Geodetic Datum of Australia, 1994 (GDA94).

4.2.4.1 Vector Analyses

The following three data files were used to calculate the distance variables for each study site, *i.e.* fetches, distance to 2 m contour and distance from the estuary mouth.

(i) A point file containing the easting and northing co-ordinates and unique identifiers for all study sites throughout the estuary. These coordinates were obtained in the field using a hand-held Global positioning System (GPS) with an accuracy of *ca* 3 m (Magellan Meridian Gold with dGPS correction).

(ii) A polyline file of the estuary coastline, including islands and structures such as bridges and jetties. This file was created by digitising lines along the land-water interface visible on the aerial photograph (see Fig. 4.2).

(iii) A polyline file of the location of the 2 m depth contour throughout the estuary, created in the raster analysis subsection (4.2.4.2) from the Digital Elevation Model (DEM ; see Fig. 4.3).

Fetches

All five fetches measured at each site (*i.e.* N, S, E, W and direct), were represented by their respective Modified Effective Fetch (MEF) value, rather than just a distance along a single bearing. Modified Effective Fetch is essentially an average of several fetch lines that deviate either side of the desired fetch direction within a constrained arc and was used in order to obtain a more robust measure of each fetch. This measure reduces the potential for relatively microscale features (*e.g.* rocky outcrops) to have a disproportionately large effect on the fetch measurement, which is particularly pertinent in estuaries such as the Swan River that contains many small structures such as jetties and marinas.

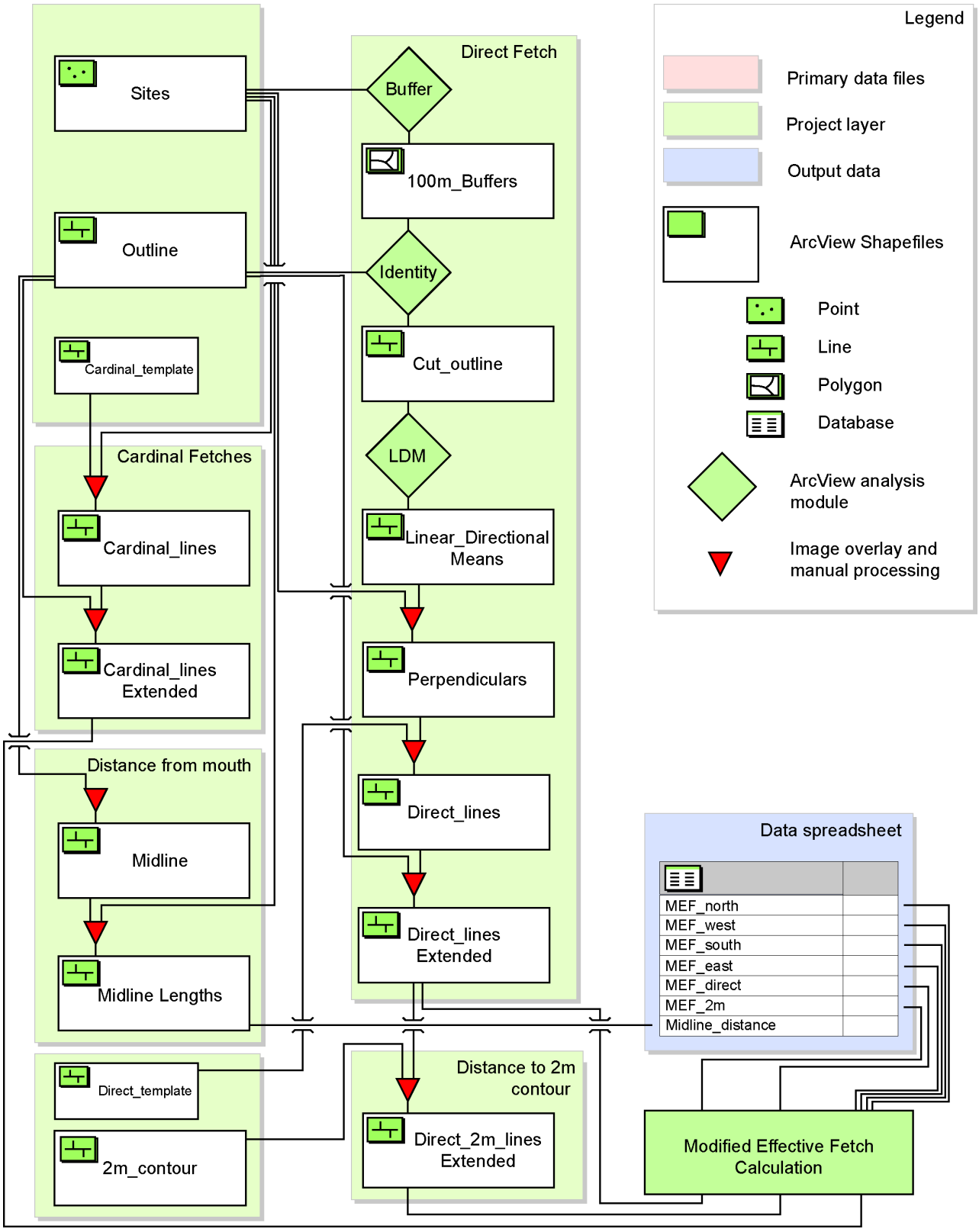

Figure 4.2 Flow diagram of the vector analyses undertaken to measure fetches, distance to the 2 m contour and distance from estuary mouth at each of the nearshore study sites in the Swan River Estuary, including the procedures followed, modules used and files produced.

The formula used to calculate MEF was based on that described in CERC (1977)

$$MEF = \frac{\Sigma (X_i \times \text{Cos } \gamma_i)}{\Sigma \text{ Cos } \gamma}$$

where X_i is the length of fetch line i and γ_i is the angle of deviation of that line from the direction for which the MEF is being calculated.

The above methodology employed by CERC (1977) was adjusted for this study to provide a more robust measure of MEF. Thus, whereas those workers used only three component fetch lines oriented 45º apart, nine component fetch lines at 9º increments from the bearing of interest were employed in this study. This modification was made because the methodology used by CERC (1977) was designed for marine systems, and the likelihood of individual fetch lines encountering microscale features were considered to be greater in the estuarine environment.

For each calculation of MEF, the lengths of the nine component fetch lines were effectively weighted by their deviation from the desired direction and averaged to produce a single measurement. For example, to calculate the MEF in a westerly direction (*i.e.* 270º), the lengths (X_i) for fetch lines 306º ($\gamma = 36$), 297 º ($\gamma = 27$), 288º ($\gamma = 18$), 279º ($\gamma = 9$), 270º ($\gamma = 0$), 261º ($\gamma = 9$), 252º ($\gamma = 18$), 243º ($\gamma = 27$) and 234º ($\gamma = 36$) would be used in the above calculation, along with their respective angles of deviation (γ_i).

To determine the MEF for the four cardinal directions at each site, each of the component fetch lines were extended from a site, over water, to the point at which they first intersected the opposite shore or any other emergent structure. Any lines that lay

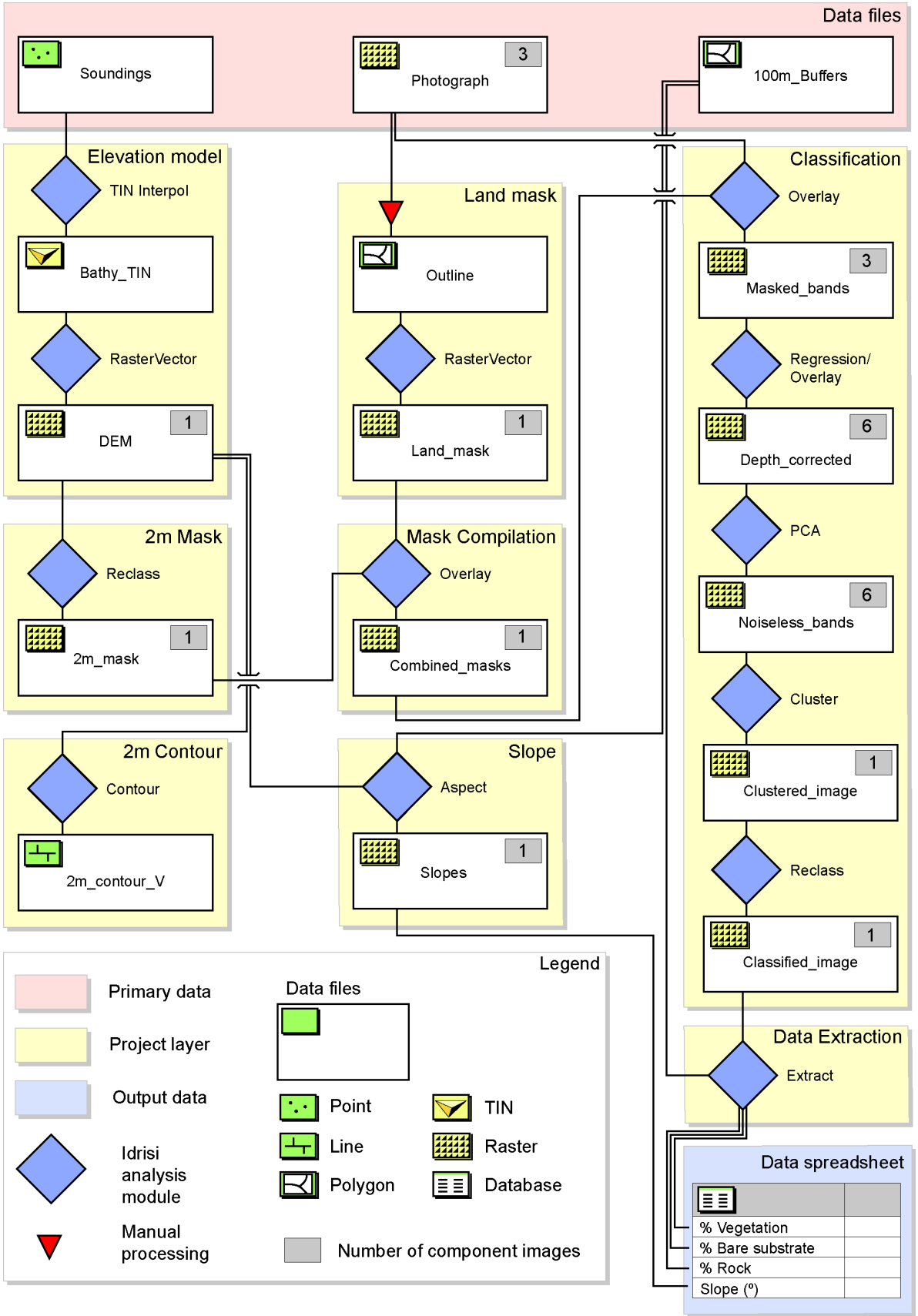

Figure 4.3 Raster image processing flow diagram flow diagram for the raster analyses to quantify the percentage contribution of each of the substrate / SAV classes and the average slope at each of the nearshore study sites in the Swan River Estuary including the procedures followed, modules used and files produced.

entirely on the land were deleted and their length recorded as zero. The lengths of the remaining lines were calculated using the XTools plug-in module "Calculate length" and then exported to MS Excel, where they were substituted into the above formula (Fig. 4.4a).

Calculation of the direct fetch firstly required the aspect (*i.e.* facing direction) of each site to be determined. This was achieved by establishing the average orientation of the coastline within the 200 m diameter of each site, then constructing a perpendicular line to it. The MEF of that line was then calculated as described above (Fig. 4.4b).

Wave shoaling margin

The distance from the midpoint on the shore of each site to the wave shoaling margin (*i.e.* the 2 m depth contour) was determined by overlaying the nine component fetch lines employed in the MEF calculation for Direct fetch with the polyline of the 2 m contour and then trimming those lines at the point which they first intersect the contour line. In those cases where a fetch line did not intersect the 2 m contour it was left extended to the opposite shoreline. As for the above MEF calculations, the lengths of the nine component lines were measured and the modified effective distance to the 2 m depth contour was determined (Fig. 4.4c).

Distance from estuary mouth

In order to determine the distance of each site from the estuary mouth, a line that ran along the middle of the longitudinal axis of the estuary was firstly constructed. This midline was created by finding the midpoint between opposing banks at a series of locations along the length of the estuary, then linking these points to form a continuous line. For each site, the smallest distance from its midpoint to the midline was determined, and the distance along the midline from that intersection to the estuary mouth was then calculated (Fig. 4.4d).

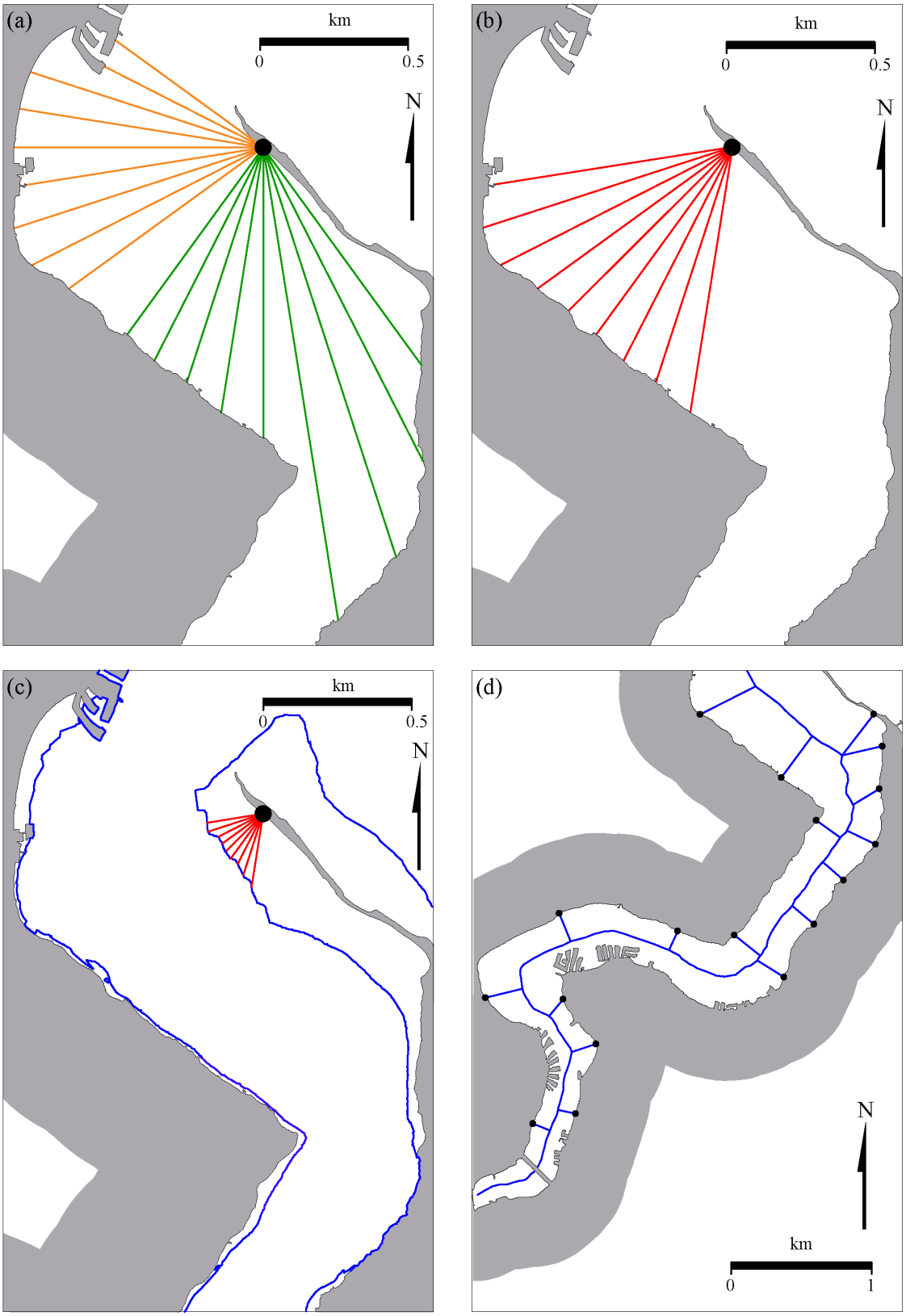

Figure 4.4 Map of the Point Walter region of the Swan River Estuary showing, for site number 18 (a) the fetch lines used to calculate the MEFs for the cardinal directions (b) the fetch lines used to calculate the direct MEF, (c) the 2m depth contour and the fetch lines used to calculate the modified effective distance to the 2m contour, and (d) the channel section showing sites 1-17, the midline used to calculate the distance from the estuary mouth.

4.2.4.2 Raster Analyses

The three primary datafiles that were used for the measurement of the raster-based environmental variables for each site, *i.e.* substrate / SAV and slope were as follows:

(i) A vector point file containing the latitude, longitude and depth values for points throughout the estuary *i.e.* X, Y and Z coordinates, respectively;

(ii) The georeferenced aerial photograph mosaic of the Swan River Estuary; and

(iii) A vector polygon file containing arcs of 100 m radius, which delineated the area occupied by each site. This file was created during the vector analyses in the previous sub-section.

Slope

A Triangulated Irregular Network (TIN) file was created from the datafile containing the X, Y and Z coordinates using the "TIN" analysis module. This file was then converted to a continuous raster surface with the module "TINSurf" to produce a DEM of the Swan River Estuary. The polyline file that specified the location of the 2 m depth contour was then created from the DEM using the "Contour" module. The average slope of the substrate within (º) within each site was then calculated using the "Aspect" module. These data were then exported into MS Excel.

Photograph processing

Identifying and quantifying the contributions of the various substrate and submerged vegetation categories at each site comprised two main activities, namely (i) classifying the various categories from the aerial photograph, and (ii) groundtruthing the identity of those categories in the field to verify the accuracy of the photograph classification.

Classification of the photograph

The first step in classifying the photograph involved excluding those parts of the image that lay outside the area of interest for the classification, *i.e.* all terrestrial areas and waters > 2 m deep. Using both the estuary outline and the DEM, a Boolean mask was created to isolate the relevant parts of the image. This was achieved by assigning pixels within the areas of interest a value of 1, and those in the areas to be excluded a value of 0. The mask and image were then multiplied together creating a new image where the areas of interest retained their original value, and everything else had a value of 0.

The second stage of image processing was to remove any 'noise' from the image, *i.e.* spectral information on the image that is not a genuine reflection of the environment, but is instead due to sensor error. This process involved firstly creating separate images from the original image that represented the red, green and blue component bands of the colour spectrum (RGB bands) and then subjecting those component images to an unstandardised Principal Components Analysis (PCA). The first two principal component (PC) images collectively explained 87 % of the total spectral variation present in the image, and were thus considered to reflect genuine information. The third PC image explained only 13 % of the variation, and was considered to represent predominantly noise.

In order to obtain new bands that did not contain noise, the eigenvectors for PCs 1 and 2 were used to back-transform each of the three PC images. As a final step in the noise removal process, the values on each of the new noiseless bands were stretched to occupy the range 0-256 used for byte data.

The final stage of the image processing involved removing the influence of water depth on the amount of light which reached the substrate surface. This was achieved using the method detailed by Mumby & Edwards (2000). Thus, pixels of a uniform substrate type at varying depths were chosen from the image and their values recorded for all three bands. These values were $\log_{10}$ transformed and the data for all possible pairs of bands were plotted on separate bi-plots. Note that six separate bi-plots were produced since the data for each band was treated as both the dependent and independent variable (*i.e.* I *vs* J and J *vs* I). These data were subjected to least squares regression and the slope of the resultant line was treated as the coefficient of attenuation for each pair of bands (k_I/k_J). The following formula was then employed to produce six new depth-invariant bands.

$$\text{DIB I}vs\text{J} = \ln(\text{band}_I) - ((k_I/k_J) \times \ln(\text{band}_J))$$

where DIB I*vs*J is the new depth invariant band created from bands I and J, and (k_I/k_J) is the regression coefficient specific to those two bands.

The module "CLUSTER" was then used to perform an unsupervised classification on the data for the six processed images to define 10 classes of pixels that differed in their peak spectral value. Each pixel was then assigned to one of these classes on the basis of which peak its value most closely corresponded to. This process condensed the data for the six depth-invariant bands into a single image where each pixel had only one of 10 values. The identity of these classes was unknown at this stage.

A field regime was then carried out to determine (i) the identity of the 10 benthic classes, and (ii) whether any of these classes comprised the same substrate / SAV class. This exercise demonstrated that the 10 classes could be condensed into just three, which represented either bare substrate, rock or vegetation (*i.e.* seagrass or macroalgae).

The module "Reclass" was then used to manually reclassify each of the 10 pixel classes into one of the three broader categories, *i.e.* bare substrate, vegetation and rock. This produced the final map of the substrate / SAV classes for the Swan River Estuary.

Groundtruthing of substrate and submerged vegetation type

A groundtruthing exercise was carried out to assess the accuracy of the three-class substrate / SAV map described above. Prior to undertaking this field work, a subplot of 5 m radius was selected within each of the benthic classes present at each site from the classified image. The XY coordinates of the centre of each subplot were entered into a GPS, the subplots were then visited and the actual substrate / SAV type recorded. To obtain an estimate of the accuracy of the GIS classification, both overall and for each substrate / SAV class, the number of correctly classified subplots was expressed as a percentage of the total number of subplots. Overall, the classification derived from the classified image identified the correct benthic class in 74.4 % of cases. Submerged vegetation was the most accurately classified class (94.2 %), followed by bare substrate (70.5 %). Rock was the least accurately classified category (50.5 %), which, when incorrectly classified, was usually identified as bare substrate.

The percentage contribution of each substrate / SAV class to the total substrate area at each site was determined, and the resultant data exported to the multivariate datasheet in Excel.

Supplementation of GIS data with field data

The tannin-stained and turbid waters of the upper estuary precluded the nearshore substrates in those areas from being classified from the aerial photograph. Thus, the percentage contributions of the various substrate / SAV classes were evaluated entirely by fieldwork.

At each site in the upper section of the estuary, nine transects that ran perpendicular to the shoreline and extended to the 2 m depth contour were created. They were spaced 20 m apart, in order to cover the entire 200 m length of shoreline of each site. The percentage cover of any substrate / SAV class within a 2 m swath along each transect was visually estimated by snorkellers and values for each class were then averaged across all transects. Three new substrate classes were detected in the upper estuary, namely riparian vegetation, bivalve beds and snags (*i.e.* submerged tree branches).

The data from the field-based analyses were added into the final database which expressed, for every site, a measurement for each of the 13 enduring environmental variables.

4.2.5 Data pre-treatment

The data for the 13 enduring environmental variables at the 101 nearshore sites were subjected to the following routines in the multivariate statistics package PRIMER v 6 (Clarke & Gorley, 2006) to identify those groups of sites that were significantly distinct in terms of their suite of environmental characteristics, and thus comprised habitat types.

Prior to multivariate analyses, Draftsman plots were used to assess whether the data for each of the environmental variables conformed to a normal distribution, and whether any pairs of variables were highly correlated. The resultant scatterplots of each pair of variables facilitated visual detection of whether the distribution of the data was notably

skewed, which in turn provided a basis for selecting an appropriate transformation to ameliorate any such effect. The data transformations applied to each variable are presented in Table 4.1. Percentage cover of bare substrate was the only environmental variable that was highly correlated with several others, *i.e.* most other substrate / SAV variables, and was thus excluded from subsequent analyses.

As the environmental variables were measured in several different units, it was essential to normalise the data prior to further analysis, *i.e.* independently for each variable, the mean was subtracted from each sample and then divided by the standard deviation. This procedure placed the data for every variable on the same scale, irrespective of its unit of measurement (Clarke & Gorley, 2006).

Lastly, to ensure that each of the three main categories of variables, *i.e.* location, exposure to wave activity and substrate / SAV type contributed equally to the habitat classification, a weighing procedure was carried out. Thus, each of these three categories was considered to contribute an arbitrary proportion of 100 % to the data matrix, *i.e.* a total of 300. Each variable was then weighted according to the total number of variables that belonged to its category. For example, as seven variables comprised the exposure category, each variable in that category was assigned a weight of 100/7 = 14.28 %. The weight assigned to each variable is presented in Table 4.1.

4.2.6 Classification of habitat types

The pre-treated environmental data set was used to create a dissimilarity matrix using the Manhattan distance measure, which contained pair-wise resemblances for each of the 101 sites. The resemblance matrix was then subjected to group-average, hierarchical agglomerative clustering (CLUSTER). The Similarity Profiles test (SIMPROF) was undertaken simultaneously with CLUSTER to identify those groups of sites that did not differ significantly in their values for the various environmental variables, and thus comprised habitat types. The SIMPROF routine tests for the presence of any significant structure within a group of samples that have no *a priori* grouping framework. It achieves this by comparing the actual profile of resemblances between all pairs of samples ranked from lowest to highest with those for a large number of permuted profiles that have been generated from random permutations of the same data (Clarke & Gorley, 2006). The test statistic (π) is calculated by summing the absolute differences between the actual and permuted profiles. When coupled with CLUSTER, a SIMPROF test is calculated at each node of the dendogram to assess if there is any significant structure among the samples below that node. The H_0 that there are no significant differences in the environmental characteristics among sites (*i.e.* no significant "structure"), was rejected if the associated significance level (P) was < 1 %. When the null hypothesis was rejected for a test at one of the nodes, all of the samples below that juncture were considered to represent a habitat type.

4.3 Results

The CLUSTER procedure, coupled with the SIMPROF test, yielded a classification that divided the 101 nearshore sites into 18 statistically-distinct habitat types, *i.e.* groups that did not contain significant environmental differences among sites (Fig. 4.5). The notation of these habitat types reflects their distance from the estuary mouth, *i.e.* A is the closest to the mouth while R is the furthest upstream.

At the highest level of dissimilarity, CLUSTER and SIMPROF distinguished a habitat comprised of the eight sites located in the upper Swan River (habitat type R) from those in the rest of the estuary, followed by a group of two sites located further down stream (habitat type P; Figs 4.5 and 4.7). The next node in the classification separated two broad groups of habitat types located in the lower Swan and Canning rivers and the basins (Q and G-O) from those in the lower most reaches of the main basin and the channel (A-F).

The first broad group of habitat types was subsequently split into two groups. The first of these contained only habitat type Q, which comprised 17 sites located in the middle to lower reaches of both rivers. The second group represented habitat types in the lower reaches of the Canning Rivers and the basins, and contained three splits, all at a similar level of dissimilarity, thus producing four groups of habitat types. The first contained habitat types on the southern side of the main basin (G, I, J and K), the second comprised habitat types in the lower Canning River and the small basin at the base of the Swan River (M, N, and O), and the third and fourth comprised habitat types on the eastern (L) and northern banks (H) of the main basin, respectively.

Within the second broad group of habitat types, habitat type B was firstly split away from the remaining channel sites, followed by the group of sites representing habitat type A in the lowest reaches of this region of the estuary. The remainder of this group

was split into four habitat types, two of which were located in the middle channel (C and D), and the other two in the upper channel (E and F).

The nMDS plot of the 101 sites, created from the same resemblance matrix used to create the dendrogram in Fig. 4.5, shows clearly the relationship among the habitat types (Fig. 4.6). In particular, habitat types R, P, and Q, which are located in the riverine reaches, form very distinct groups on the right of the plot, while B, and to a lesser extent A, C and D, which are all located in the channel form relatively distinct groups on the left. The large group of sites located in the centre of the plot represent habitat types E-O, each of which is found in the basins and lower reaches of the rivers

To identify the main differences in the enduring environmental characteristics that distinguished the various habitat types, the mean value for each environmental variable at each habitat type was standardised against the largest value for that variable across all habitat types, *i.e.* essentially expressing it as a percentage of the maximum value encountered for that variable. These values were plotted for each habitat type, thus producing an environmental "profile" for each (Fig. 4.8). It is evident from these profiles that habitat type R, which had the least resemblance to all other habitat types (Fig. 4.5), was distinct because it was located the greatest distance from the estuary mouth, had very small fetches in all directions, a very narrow shoaling margin and contained the largest percentage of snags and emergent vegetation. Like habitat type R, sites for habitat type P were also located relatively large distances from the estuary mouth, typically had small fetches in all directions and lacked submerged vegetation. However, habitat type P was distinct mainly because substrate comprised substantial proportions of large bivalve shells, unlike the situation at any other habitat type.

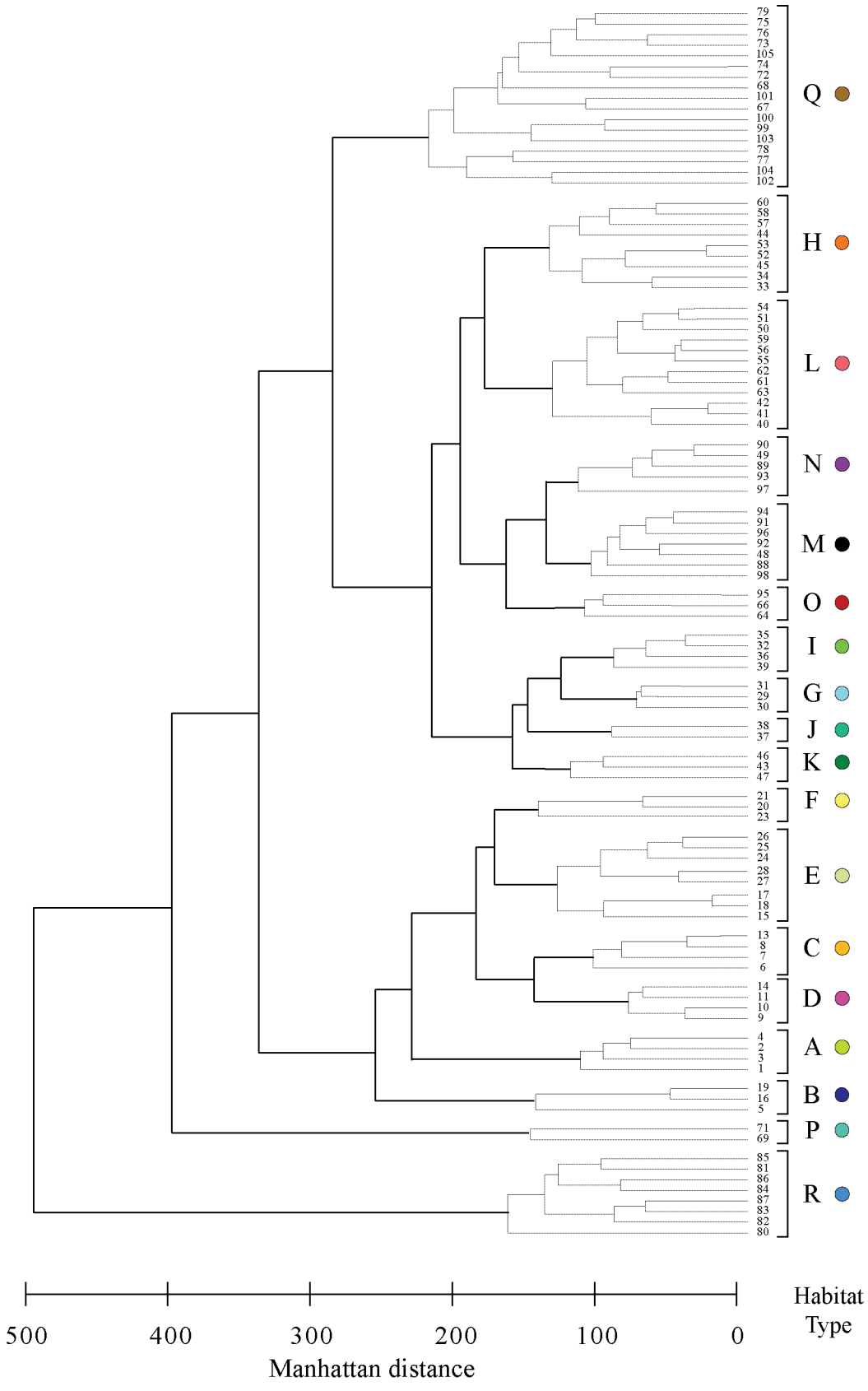

Figure 4.5 Dendogram produced by CLUSTER showing the similarity of the 101 classification sites in the Swan River estuary, based on the full suite of 13 environmental variables. For those groups for which SIMPROF could detect no significant structure, the dendogram is marked in fine lines. For those divisions denoting significantly different groups the dendogram is marked in bold.

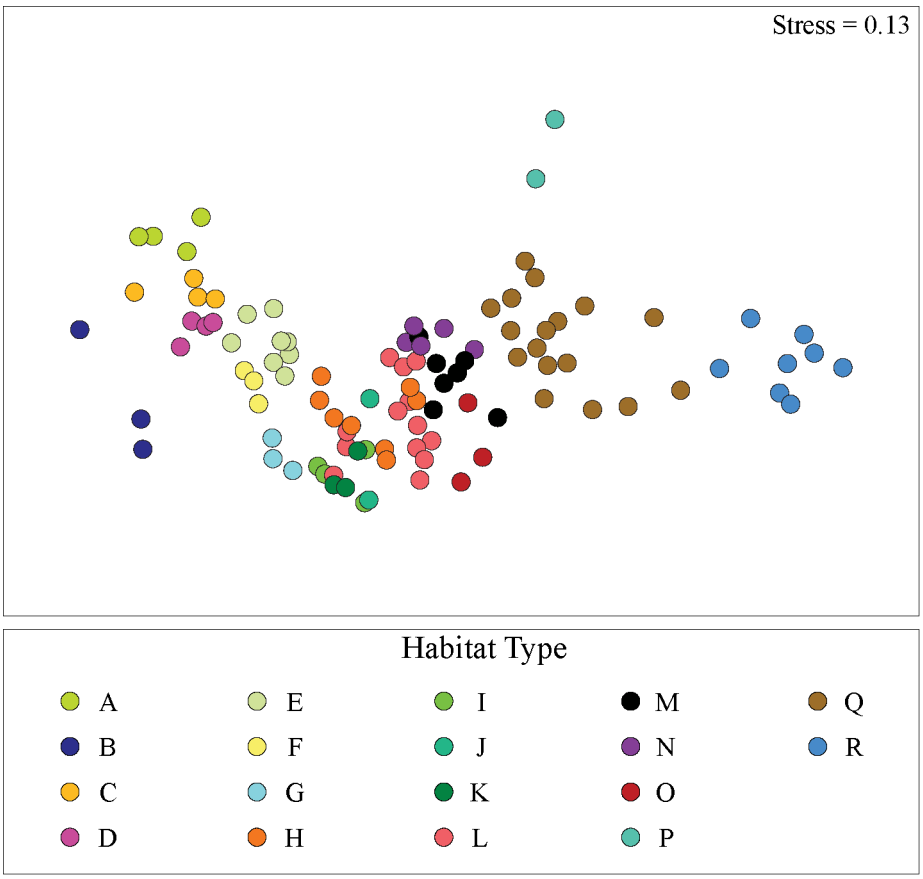

Figure 4.6 Two-dimensional nMDS solution showing the similarity of the 101 classification sites in the Swan River Estuary, based on the full suite of 13 environmental variables. Sites are coded for the significantly different habitat type groups defined by the SIMPROF classification.

Like habitat type R, the fetches in all directions and the width of the shoaling margin of habitat type P were relatively small. Habitat type Q, in contrast, was characterised by the presence of substantial amounts of snags and emergent vegetation and like habitat types R and P, lacked submerged vegetation and had relatively short fetches in all directions.

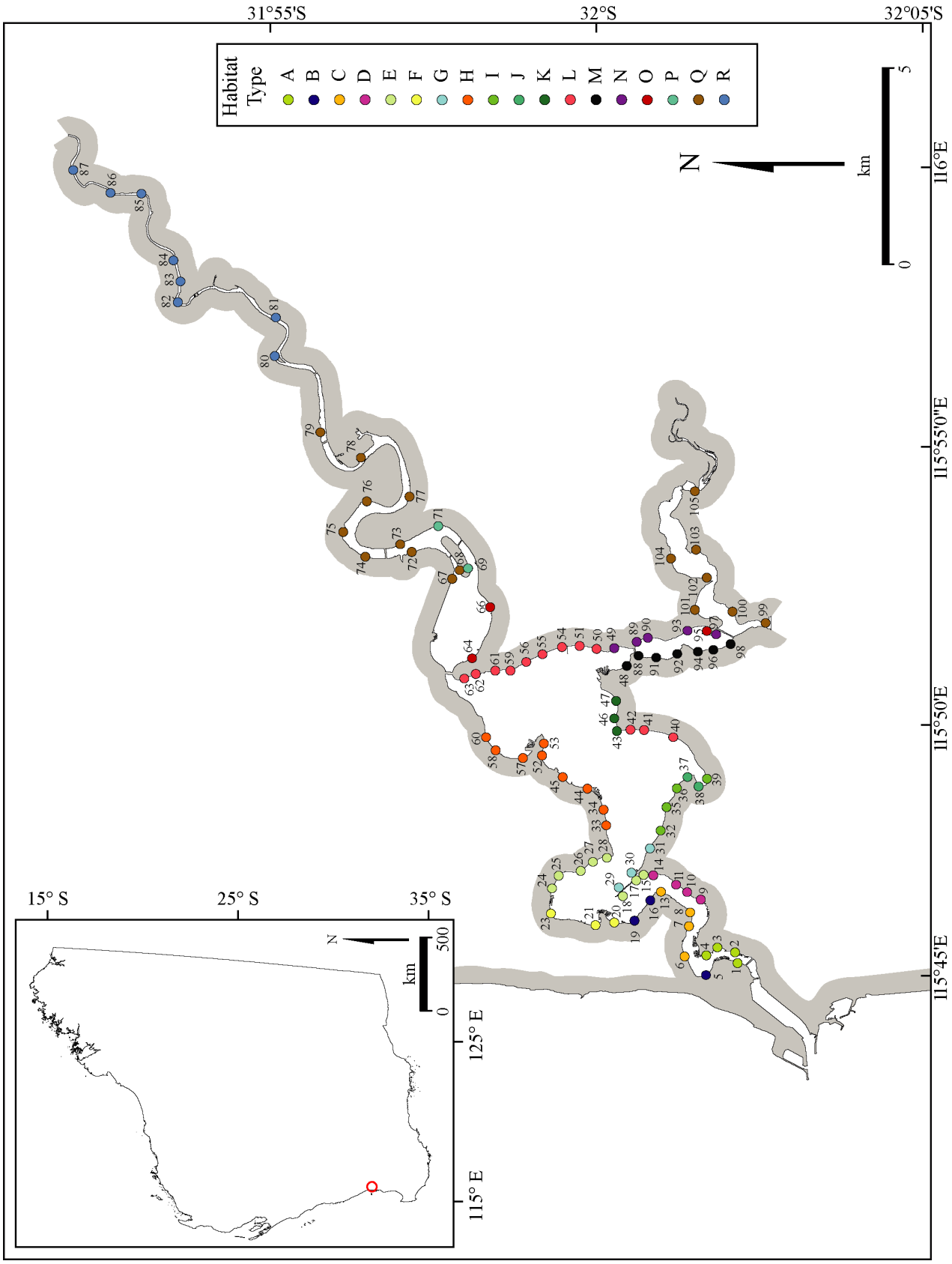

Figure 4.7 Map of the Swan River Estuary showing the location of the 101 sites used for the habitat classification and their classification into one of the 20 habitat types as designated by CLUSTER and SIMPROF.

Habitat types M, N and O all occurred in the lower Canning River or at the base of the Swan River and were thus located similar distances from the estuary mouth. M and N were found exclusively in the Canning River and typically had small to moderate fetches in most directions, a relatively short distance to the 2 m contour, and moderate amounts of submerged vegetation. M was distinguished from N by the relatively north to east fetches *vs* south to west fetches. Habitat type O was distinguished from the above two habitat types mainly by its very wide shoaling margin.

Basin habitat types G, I, J and K all had moderate to large direct and northerly fetches and contained small to moderate quantities of submerged vegetation. They were discriminated from each other by differences in their distance to the 2 m contour, relative contribution of rock to the substrate and other dominant fetch directions. The other basin habitat types, H and L, were characterised mainly by their large southerly and westerly fetches, respectively.

The channel habitat types (A-F) were each characterised by relatively short distances from the estuary mouth, typically small to moderate fetches in most directions, short distances to the wave-shoaling margin and, with the exception of habitat type B, large amounts of submerged vegetation. Distinction among these habitat types reflected mainly differences in substrate type, slope and dominant fetch direction. For example, habitat type B contained the largest quantities of rock and, while patches of this substrate type were present at habitat types C-E, it was completely lacking at habitat types A and F. Furthermore, whereas habitat types B and C had steeply sloping substrates, those at habitat types A, E and F were relatively shallow.

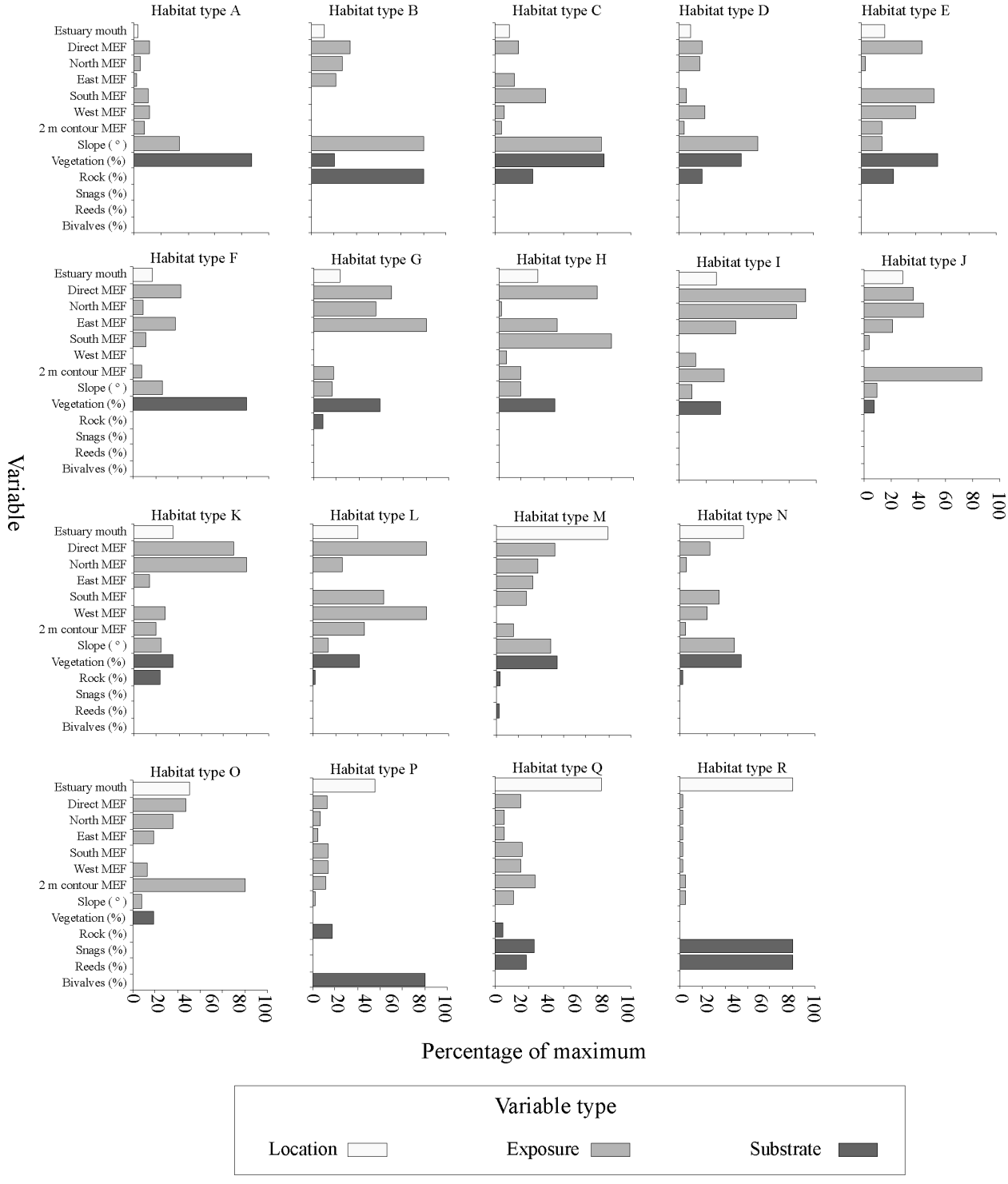

Figure 4.8 Profile graphs of the 13 variable types for the 20 habitat types found in the Swan River Estuary (A-R). For each variable, the bar represents the mean value for the sites in that habitat type, expressed as a percentage of the largest value of that variable found across all habitat types.

4.4 Discussion

The overarching aim of this component of the study, namely to devise a quantitative approach for classifying nearshore habitat types within south-western Australian estuaries based on measurements for a suite of enduring environmental variables at a diverse range of sites, has been successful.

Inspection of the environmental profiles for the range of nearshore habitat types identified throughout the Swan River Estuary demonstrated that the groups of sites produced by the classification scheme were logical and intuitive. For example, all of the habitat types located in the circular main basin were discriminated mainly on the basis of their fetch directions (*e.g.* habitat types H, I and L) and / or substrate types (*e.g.* habitat type K). Similarly, habitat types in the narrow upper riverine reaches, which had similar small fetches in all directions, were differentiated mainly on the basis of their distance from the estuary mouth (*e.g.* habitat types Q and R). Furthermore, although the channel (A-F) and riverine habitat types (O, P, Q and R) all typically had small fetches in most directions and narrow wave-shoaling margins, they were well discriminated by their very different distances from the estuary mouth and characteristic substrate / submerged aquatic vegetation (SAV) types.

4.4.1 Comparison with existing habitat classification schemes

The most common method for classifying habitat types in aquatic environments is through the use of a hierarchical system. Such systems function by dividing the area of interest into successively smaller areas on the basis of differences in their physical or, in some cases, biological characteristics. Three of the best known hierarchical systems have been developed in America in conjunction with the National Oceanic and Atmospheric Administration (NOAA) to enable the classification of aquatic habitats across that continent in a standardised manner, *i.e.* Cowardin *et al.* (1979), Allee *et al.* (2000) and Madden *et al.* (2005). The latter two schemes represent a

progression in methodology on the preceding scheme(s). Other important hierarchical systems include those developed by the Joint Nature Conservation Committee for the United Kingdom (JNCC; Connor *et al.*, 1996), the European Nature Information System (EUNIS; EEA, 2008), within which that of Connor *et al.* (1996) is nested, and the Integrated Marine and Coastal Regionalisation of Australia (IMCRA) system (Commonwealth of Australia, 2006).

One of the greatest strengths of hierarchical classification systems is that they can be applied to a wide range of spatial scales. Thus, by using broad criteria at higher levels, such schemes can effectively divide large areas into manageable portions and employ, within each of these, a finer suite of criteria to distinguish habitats at successively finer scales. A second strength of hierarchical systems is their step-wise approach to defining habitat types. Thus, the sequential nature of the decision-making process allows many different variables to be incorporated into the scheme, while allowing the end user to consider only one or two at a time.

Despite the above advantages of hierarchical classification systems, three major problems are commonly associated with this approach, particularly in earlier schemes. These include the following:

(i) The unwieldiness of a very large number of hierarchical levels and thus habitat types;

(ii) The use of qualitative and / or non-enduring classification criteria; and

(iii) The absence of statistical testing to verify that the resultant habitat types are significantly different.

When applied at a broad scale, hierarchical systems function very effectively to divide up large areas, but must have many nested levels to be able to discriminate habitat types at a biologically-relevant scale. This often makes these schemes complex and cumbersome, a point illustrated very well in the classification by Merkel & Associates (2003) for the marine region around San Diego. This system contains 15 hierarchical levels, each based on different characteristics, which ultimately define local-scale habitat types. In addition, the classification system used for that study employs "modifiers" at each level, which makes the number of possible habitat types very large. In contrast, the CMECS scheme (Madden *et al.*, 2005) has only six hierarchical levels, but also includes modifiers and descriptors to enhance the resolution of each hierarchical level. Unfortunately this has resulted in the same problem as previous systems with the very large number of choices making the system very complex.

The use of qualitative descriptors in habitat classification systems introduces the problem of their subjective interpretation and thus inconsistencies among users in the resultant classification. An example of the potential ambiguity introduced by subjective descriptors is provided in one of the lower levels of the hierarchical scheme developed by Merkel and Associates (2003), in which the user is required to identify an area as "stable" or "unstable" based on the extent of any "ecological seasonal changes". Varying interpretation of such criteria by different users can lead to considerable inconsistency in the resultant classification, particularly when the classification area is large and / or many workers are involved. The classification system developed in the current study addresses this problem by using quantitative measurements for all criteria, thus eliminating any ambiguity from the classification process.

The use of non-enduring variables for habitat classification presents two issues. Firstly, the classification does not remain valid over time and thus needs to be redetermined for each temporal unit of interest. For example, Madden *et al.* (2005) employs salinity as a descriptor for certain aquatic habitat types and, although this criterion may be appropriate for waters which do not experience marked salinity variation, it is not appropriate for environments such as estuaries, where it may change seasonally or even daily. Secondly, non-enduring variables usually need to be measured directly in the field and over a sufficient period to capture the range of their temporal variability. In most cases, this requires a significant amount of resources and may even be logistically impossible in some cases. The use of enduring environmental variables as surrogates for non-enduring ones (*e.g.* distance from estuary mouth as a surrogate for salinity such as in the present classification), allows the researcher to circumvent the above problems. Thus, the resultant classification remains valid over a long period and the variables employed in the scheme are relatively easy to measure accurately from remotely-sensed sources (*e.g.* Roff *et al.*, 2003).

Certain classifications systems, *e.g.* that of Connor *et al.* (1996), have encountered similar problems to those described above by employing biota as classification criteria rather than environmental variables. The rationale for using this approach is that the resultant "biotopes", *i.e* "…the combination of habitat together with its recurring associated biological community", are directly relevant to the biota they support and are thus readily interpretable, *e.g.* a "kelp" biotope. However, the use of biota as the basis of a habitat classification scheme also results in classification units that are likely to be subject to temporal change and requires a large amount of resources to undertake the necessary field-work before any classification can be initiated.

Many habitat classification systems lack any sort of statistical demonstration that the habitat types derived from the scheme are significantly different from each other. That is, areas that have similar characteristics are assumed to belong to the same habitat and, more importantly, each habitat is assumed to differ from "adjacent" habitats. There is thus no reliable way to ascertain whether any of the habitat types derived from these schemes are redundant and/or whether some habitat types contain sufficient variability to justify further division. In some cases, the inability to test for significant differences among habitat types stems from the fact that the classification critera are not fully quantitative. For example, Howes *et al.* (1994) was unable to test statistically for differences among habitat types as they were not based on quantitative criteria. Zacharias *et al.* (1999) then investigated variation in the biotic assemblages with respect to those habitat types and found that the biota were indistinguishable across three of the "different" habitat types. Unfortunately, the lack of significant biotic differences cannot be definitively explained because the validity of the three habitat types cannot be statistically determined, *i.e.* the environmental characteristics of the three habitat types may not have been statistically different, and thus their initial division redundant.

Some quantitative classification systems have partially addressed the above problem of statistically defining habitat types by subjecting the data for the classification criteria to a multivariate clustering procedure (*e.g.* Edgar *et al.*, 2000). However, habitat types defined in this manner are done so at an arbitrary level of resemblance among samples, and thus there is no guarantee that they actually differ statistically. The current classification has used not only quantitative measurements of environmental variables to characterise the various habitat types, but also a statistical test at each node of the clustering process to statistically define those groups of sites that form significantly different habitat types. There is thus no arbitrary decision rule imposed on the

classification process, nor a requirement to test an *a priori* classification of sites, which may not be accurate.

4.4.2 Comparisons with the habitat classification scheme developed by Valesini *et al.* (2003)

The classification scheme developed during this study is based partly on that developed by Valesini *et al.* (2003) for nearshore waters along the lower west coast of Australia. They thus share the common characteristic of employing measurements for enduring environmental variables as classification criteria for habitat types, including those that reflect exposure to wave energy and substrate / SAV type. However, the present system represents a significant change in methodology from Valesini *et al.* (2003), due partly to its adaptation to the estuarine environment but also to the recent availability of the multivariate statistical routine "SIMPROF" of Clarke and Gorley (2006).

The greatest difference between the approach of the present habitat classification scheme and that developed by Valesini *et al.* (2003) is that it has eliminated the subjective component in that latter scheme. Thus, Valesini *et al.* (2003) initially assigned each study site to one of six *a priori* habitat types, based on a subjective assessment of its level of exposure to wave activity and the main types of substrate / SAV it contained. Although these habitat types were then validated statistically by a demonstration that the values for their suite of enduring environmental criteria differed significantly, the scheme did not address whether any particular site may have been better assigned to another habitat type. The need for any such subjective *a priori* classification was effectively eliminated in the present study through the use of SIMPROF (Clarke & Gorley, 2006). When coupled with a clustering procedure, this routine tests whether groups of samples at each node of the resultant dendogram differ significantly from each other, and thus whether they form homogeneous groups (habitat

types in this case). This allowed habitat types in the Swan River Estuary to be identified without imposing any *a priori* classification framework.

In order to make the scheme appropriate for use in estuarine environments, which typically experience distinct gradational changes in physico-chemical parameters along their length, the addition of an enduring environmental variable that reflected those gradients was required. Thus, the distance of each site from the estuary mouth was measured to serve this purpose, and act as a surrogate for salinity, temperature and dissolved / particulate organic load which typically vary along the longitudinal axis of estuaries.

4.4.3 Transferability of the habitat classification scheme

One of the most desirable characteristics of an aquatic habitat classification scheme is the ability to be applied to other areas beyond that of the initial classification, *i.e.* to be transferrable. While the results of the present classification system are relevant only to the Swan River Estuary, the general approach to habitat classification developed in this study can easily be applied to any estuary in south-western Australia, or indeed, elsewhere in the world. Thus, the approach is entirely flexible in that the particular environmental variables that may be important in any estuary of interest can easily be incorporated in the scheme, just as irrelevant ones can be omitted.

When the current classification scheme is applied to a series of estuaries, their habitat types may not be directly comparable across systems because the classification variables relevant to each estuary may differ. However, it is important to recognise that this approach to habitat classification can still facilitate valid comparisons among habitat types in different estuaries, providing that a uniform suite of enduring variables is used to describe the environmental variation among sites in all of the estuaries being considered. It is noteworthy that many of the 13 enduring environmental variables used

to define the habitat types in the Swan River Estuary are also relevant for the definition of habitat types in many other estuarine systems.

In order to facilitate meaningful comparisons among habitat types across several estuaries, additional variables to quantify biologically relevant differences which distinguish the estuaries as a whole can be included, *e.g.* latitude and longitude of sites, or a score reflecting the degree of connectivity between the estuary and ocean. This will allow the classification to account for the physical separation of sites in different estuaries but also assess the similarity of sites across those boundaries.

Lastly, the present habitat classification system is also sufficiently flexible to allow it to be applied to environments outside estuaries, *e.g.* in marine, freshwater and even terrestrial environments, as long as the suite of variables employed to distinguish habitat types are biologically-relevant, quantitative, temporally enduring and able to be accurately measured from mapped sources.

4.4.4 Future research and developments

The major future development for the present habitat classification system is the development of a predictive component, so that any unclassified nearshore site of interest can be quantitatively assigned to one of the existing habitats. Considering the quantitative and statistically-robust nature of the classification system, there are currently two viable alternatives for producing this tool.

(i) A predictive "grid" *sensu* Valesini *et al.* (2003); or

(ii) A dichotomous key containing threshold values for particular enduring environmental variables.

The habitat classification scheme presented in Valesini *et al.* (2003) contained a "nearest-replicate grid" that could be used to predict quantitatively the habitat type of previously unclassified nearshore marine sites along the lower west coast of Australia. This grid was created by subjecting the data for the enduring environmental variables at the range of study sites to a Principal Components Analysis (PCA) and then dividing the resultant two-dimensional PCA space into areas occupied by the sites representing each habitat type. It also contained an area "outside of previous experience" to which sites belonging to a habitat that had not been identified in that study would be assigned. The resultant plot allowed any new site to be assigned to a habitat by determining its values for the suite of enduring environmental variables from mapped data, inserting those values into the linear equations that defined the principal components 1 and 2, and then plotting the resultant co-ordinates on the PCA grid. However, Valesini *et al.* (2003) reported that this method, while reasonably reliable, still misclassified some habitat types when interrogated using a jack-knifing procedure.

The second of the above options is currently being pursued and is detailed in an in-prep publication by Valesini *et al.*. This method involves a novel use of "LINKTREE", another relatively new multivariate routine (Clarke *et al.*, 2008), to create a binary "linkage-tree" whose terminal nodes each represent one of the habitat types in the Swan River Estuary, or indeed, any estuary of interest. At each intermediate node of the linkage-tree, one or more enduring environmental variable(s) and threshold value(s) are specified, thus providing a "decision rule" for choosing the path through the tree branches. Any new site can be assigned to its appropriate habitat type by measuring the requisite suite of environmental variables from mapped data and then following the chain of decision rules through the series of nodes until a terminal group (habitat type) is reached. This approach has the advantage over the previous method of having no complicated calculations or plots, and no potential for misclassification. This style of

predictive tool incorporates one of the greatest strengths of hierarchical classification systems, *i.e.* the user is able to determine the appropriate habitat type of a new site by making a series of choices and considering very few variables at each stage. The difference is that both the habitat types and the threshold values have been determined statically in the present system, not arbitrarily as for most hierarchical systems.

Chapter 5 Descriptions of ten new species of free-living aquatic Nematoda from south-western Australia

5.1 Introduction

A total of 119 species were found during the current intensive ecological studies of the free-living aquatic nematodes in marine and estuarine sediments of south-western Australia. The majority of these species are undescribed. Formal taxonomic descriptions of ten of these species are presented in this chapter. These ten species were chosen because they encompass all of the new species that were encountered in the Axonolaimidae Filipjev 1918 (four species) and Desmodoridae Filipjev 1922 (six species). Furthermore, several of the newly-described species were shown to be important discriminating taxa for the assemblages, or to contribute substantially to the total number of individuals at certain sampling locations.

Although the four species of Axonolaimidae all belong to the subfamily Axonolaiminae, each belongs to a different genus, *i.e. Ascolaimus*, *Parascolaimus*, *Odontophora* and *Parodontophora*. Of the six new species within Desmodoridae, one belongs to the Desmodorinae (*Bolbonema*), two to the Spirininae (two *Onyx* species) and three to the Stilbonematinae (one each of *Eubostrichus*, *Catanema* and *Leptonemella*). The description and diagnosis of each species contains the type locality, habitat and etymology of that species. Holotype and paratype material for each of the species has been deposited at the West Australian Museum, the registration numbers for which are included in the description of each species.

5.2 Materials and methods

The methodology for the collection, preservation, mounting and identification for individuals is detailed in Chapters 2 and 3, as well as in Hourston *et al.* (2005). Reference slides for the type material of each species were prepared by transferring specimens from mixed assemblage slides to individual wet mount (100 % glycerol) species slides, and sealed with slide sealant. All drawings were made using an Olympus CH40 compound light microscope with incorporated drawing tube at either 400x or 1000x magnification. Hand-drawn images were scanned and subsequently edited using the Adobe Illustrator CS2 software package.

5.3 Descriptions

Class ADENOPHOREA
Order Monhysterida
Family Axonolaimidae Filipjev 1918
Subfamily Axonolaiminae Filipjev 1918
Genus *Ascolaimus* Ditlevsen 1919
***Ascolaimus australis* sp. nov.**
Figures 5.1a-c; Table 5.1

Type material

Holotype male, 1 paratype male, 1 paratype female, 1 paratype juvenile.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7450.

Description of holotype male

The body is slender, of medium length and predominantly transparent. The fine striations on the cuticle are light but distinct and are present along almost the entire length of the body, beginning at the posterior margin of the amphid. Six minute labial papillae are located at the extreme anterior of the head, followed by a ring of four relatively long cephalic (17 μm) setae. Located in line with the cephalic setae are four files of somatic setae (10 μm) which continue along the length of the oesophagus, alternating in their position of insertion between the dorso-lateral and ventro-lateral files (see Figure 5.1a).

The buccal cavity is typical of the genus *Ascolaimus*, being double-conical in shape, weakly sclerotised and unarmed. The narrow oesophagus has no swellings or bulbs, but does have a small indistinct cardia. The amphid is a relatively large circular loop located over the posterior cone of the buccal cavity.

The spicules are short and arcuate, describing an even arc of about 90° and taper to a simple point distally. Proximally, they are cephalated and appear to have a twist so that

the cephalation is directed dorso-laterally. The gubernaculum encases the distal tips of the spicules and has a substantial dorso-caudal apophysis. Testes are paired and outstretched. No supplementary organs are present. The tail is short, conical and bears no terminal setae.

Description of paratype female

Female specimens are very similar to males, exhibiting the same diagnostic structures including buccal cavity, and amphid structure, as well as setation patterns. The ovaries are paired, opposed and outstretched.

Habitat

Near-shore, sandy marine sediments of south-western Australia, most commonly located in sub- and supra-tidal sediments on moderately exposed beaches. Type specimens were collected from supra-tidal sediments at Leighton Beach, Western Australia (habitat type 6 *sensu* Chapter 4) during February 2001.

Differential diagnosis

The genus *Ascolaimus* contains only one other valid species, *i.e. A. elongatus* (Bütschli 1874), which has been has been reported from many locations throughout Europe and North America in many separate articles, many of which provide detailed illustrations. While some small details of *A. elongatus* differ among depictions, this species' spicules invariably have distinctive barbed distal ends and spade-shaped proximal cephalation (De Coninck & Schuurmans-Stekhoven, 1933; Gerlach, 1953; Warwick *et al.*, 1998). Both distinctive characteristics of the above species are lacking in the presently described species. The spicules of *A. australis* sp. nov. have simple straight distal tips and rounded cephalations.

Etymology

Since the only other *Ascolaimus* species (*A. elongatus*) has, to date, only been found in northern hemisphere localities, *A. australis* sp. nov. has been named in contrast, and to reflect its southern hemisphere type locality.

		Holotype	Par. ♀	Par. ♂
Length (mm)		1.186	1.376	1.355
a		49.4	49.1	56.4
b		8.8	9.3	8.7
c		15.2	16.9	16.1
Head diameter		10	10	13
Setae:	Cephalic	12	15	20
	Somatic	6	6	5
Buccal cavity L/W		13/4	14/5	13/5
Amphid:	from ant	5	8	9
	L/W	7/7	7/7	7/8
	cbd	12	14	18
Nerve ring:	from ant	72	80	88
	cbd	22	23	22
Oesophagus length		134	147	155
Max. diameter		24	28	24
Vulva:	from ant	-	780	-
	V%	-	57	-
	cbd	-	28	-
Spicule (arc)		34	-	31
ABD		25	20	22
Tail length		78	81	84

Table 5.1 Morphological measurements for the holotype of *Ascolaimus australis*, as well as those for the male and female paratype specimens. All measurements are in µm unless otherwise stated in Tables 5.1-5.4 and 5.6-5.11. a = body length / maximum body width, b = body length / oesophagus length, c = body length / tail length, L = length, W = width, cbd = corresponding body diameter, from ant = distance from anterior, V% = percentage of vulva distance from anterior to total body length, ABD = anal body diameter. All abbreviations used are consistent across Tables 5.1-5.4 and 5.6-5.11.

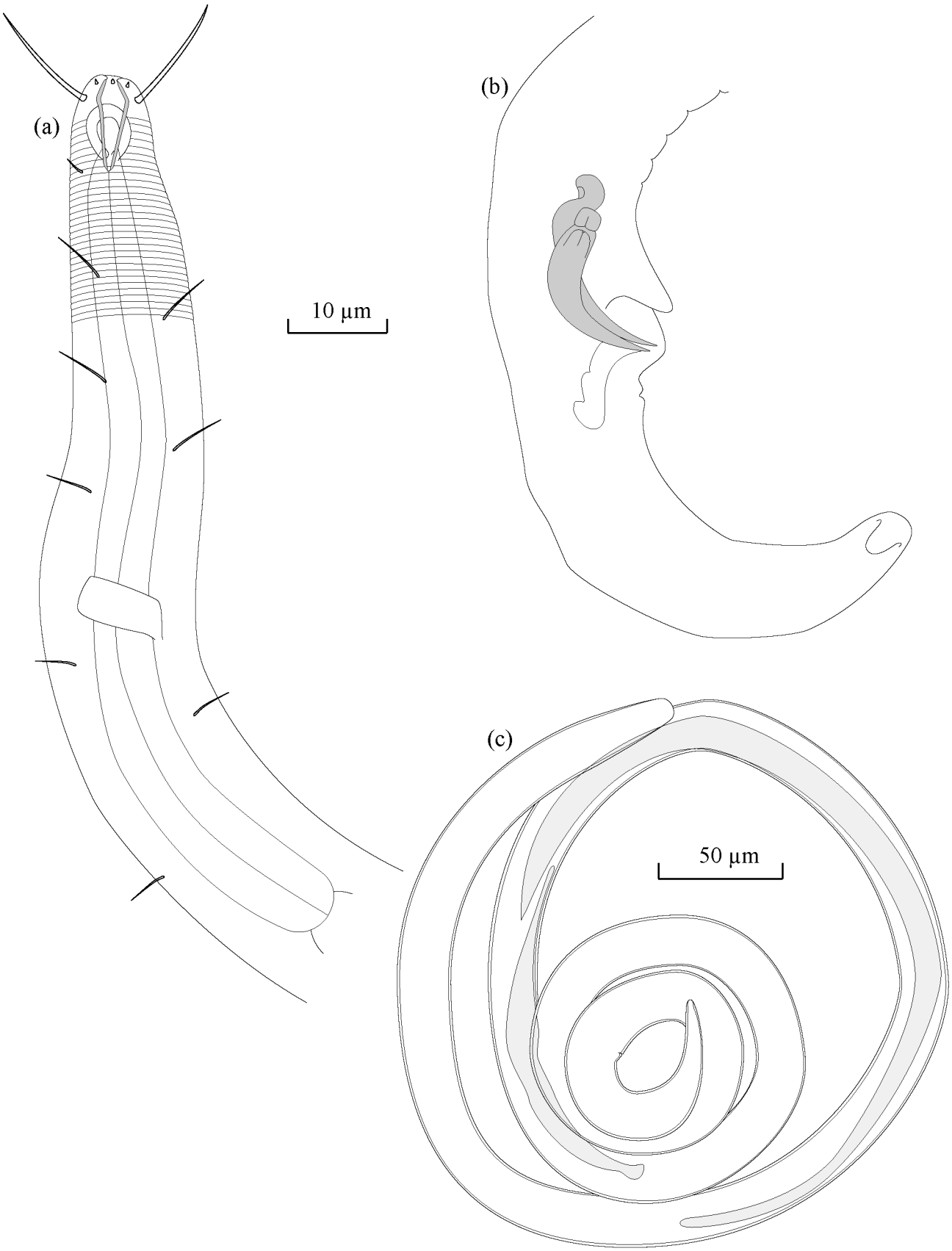

Figure 5.1 *Ascolaimus australis* sp. nov.: (a) head and oesophageal region of holotype; (b) caudal region of holotype; (c) total body of paratype male.

Genus *Parascolaimus* Wieser 1959
***Parascolaimus brevisetus* sp. nov.**
Figures 5.2a-d; Table 5.2

Type material

Holotype male, 2 paratype males, 4 paratype females.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7451.

Description of holotype male

The body is slender, medium to small in size and lacks colouration. The cuticle bears fine, indistinct striations which are only visible on the posterior half of the body and the tail. Somatic setation is sparse, and the setae are short (4 μm). Typical of the genus, this specimen has a lightly sclerotised, double-conical buccal cavity containing labial "claws" in the anterior part, *sensu* Wieser (1959a). The amphid is a circular loop located 7 μm from the anterior, level with the posterior cone of the buccal cavity. The cephalic setae are relatively short and are located in front of the amphids, 5 μm from the anterior. Additionally, four pairs of cervical setae are present. The oesophagus has a very slight posterior bulb and a distinct cardia.

The spicules are slim and moderately arcuate with distinctive rounded cephalations. The form of the gubernaculum is characteristic of the genus comprising two parts; a dorsal part with an apophysis, and a paired tubular part lying parallel to the distal end of the spicules. No preanal supplementary organs can be distinguished, although the presence of minute structures may be surmised from the "wrinkled" appearance of the cuticle in the preanal region. Notably, two files of sub-ventral setae (*ca* 7 μm long) extend along the posterior part of the body, and onto the tail. The tail is short, conical and has a very

slight terminal swelling. No terminal setae were present. The testis is large, single and outstretched, and the distinct spherical spermatozoa are readily visible.

Description of paratype female

This species is monomorphic with the females resembling the males in all aspects including the sub-ventral and cervical setation. The paired ovaries are opposed and outstretched.

Habitat

This species is present in the mid to upper reaches of the Swan River Estuary. It can be found in sediment types ranging from clay / mud to coarse sand, but occurs more commonly in the latter. Type specimens were collected from Fish Market Reserve (Site 11 *sensu* Chapter 3).

Differential diagnosis

Parascolaimus brevisetus sp. nov. can be assigned to *Parascolaimus* on the basis of the generic defining features as described by Wieser (1959a) *i.e.* 'claw-like' structures in the buccal cavity, and a complex, two-part gubernaculum. Within the genus, the present species can be easily discriminated from the other four described species in that the length of the cephalic setae (5 µm) are less than one quarter the length of that described for *P. tau* Wieser 1959 (36-47 µm), *P. ungulatus* Belogurov & Kartavtseva 1975 (26-40 µm), *P. amphidoporus* Wieser 1959 (*ca* 60 µm) and *P. proprius* Belugurov & Kartavtseva 1975 (*ca* 40 µm). Furthermore, males of the present species possess distinctly rounded cephalate spicules whereas those of the other four species are either simple, spade-shaped or slightly cephalate. Additionally, the amphids of the present species (50 % CBD) are smaller than those of *P. tau* (75 % CBD), larger than those of *P. ungulatus* (25 % CBD) and *P. proprius* (30 % CBD), and of a different shape to *P. amphidoporus*.

Etymology

P. brevisetus has been named for one of the primary features which discriminates it from other *Parascolaimus* species, *i.e.* its relatively much shorter cephalic setae.

		Holotype	$\overline{X}$ ♀	$\overline{X}$ ♂
Length (mm)		1.035	0.975	0.883
a		41.4	32.7	39.5
b		6.0	7.8	6.3
c		11.0	9.5	9.9
Cephalic setae		5	4	5
Somatic setae		3	3	4
Buccal cavity L/W		15/4	15/4	13/4
Head diameter		9	8	9
Amphid:	from ant	7	7	6
	L/W	6/6	5/5	5/5
	cbd	11	11	11
Oesophagus length		182	126	142
Nerve ring:	from ant	88	78	79
	cbd	20	19	18
Max. diameter		25	21	20
Vulva:	from ant	-	532	-
	V%	-	53.6	-
	cbd	-	29	-
Spicule (arc)		26	-	26
ABD		22	18	19
Tail length		94	102	89

Table 5.2 Morphological measurements for the holotype of *Parascolaimus brevisetus* sp. nov., as well as the averages for male and female paratype specimens.

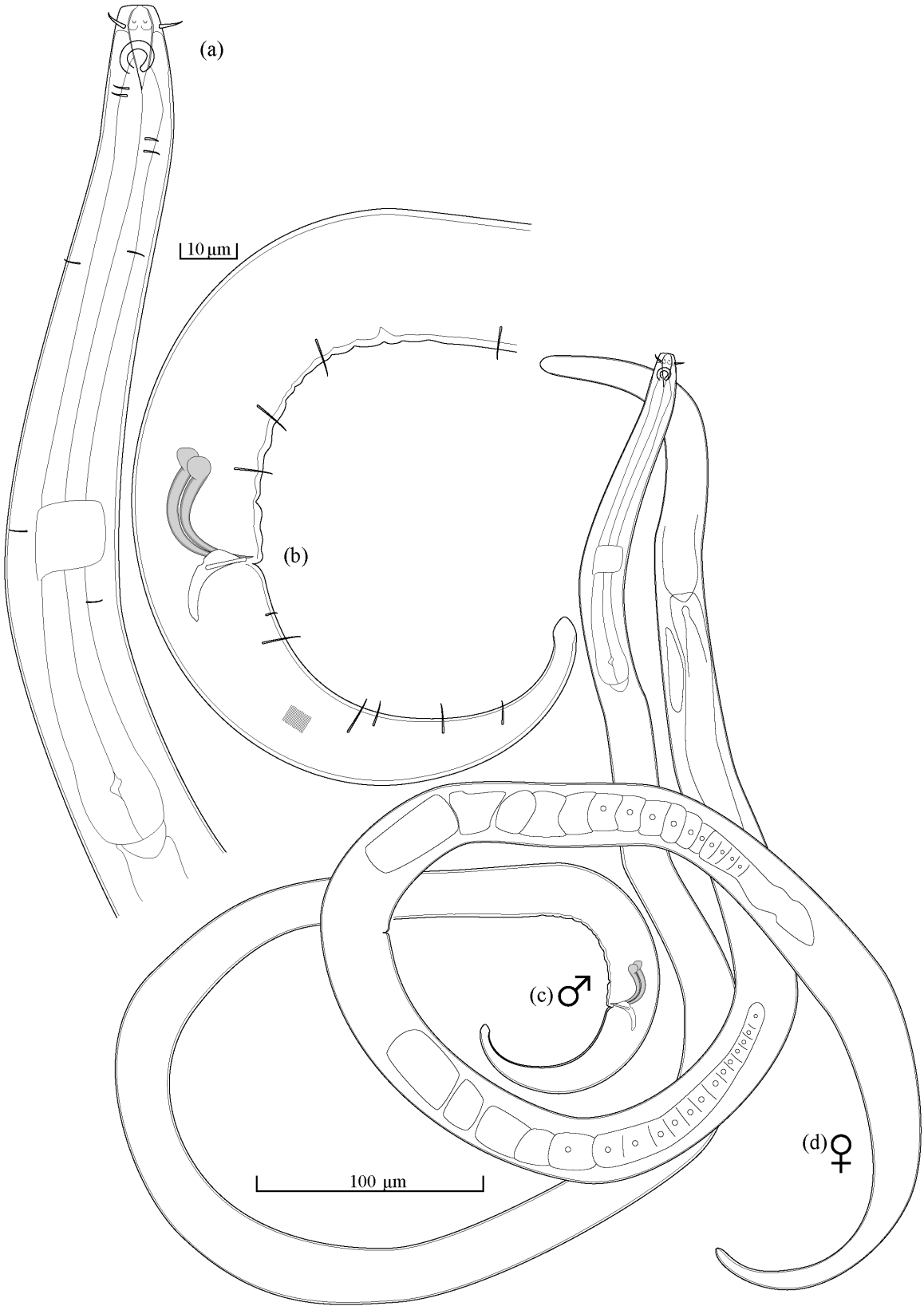

Figure 5.2 *Parascolaimus brevisetus* sp. nov.: (a) head and oesophogeal region of holotype; (b) caudal region of holotype; (c) total body of holotype; (d) total body of paratype female.

Genus *Odontophora* Bütschli 1894
***Odontophora serrata* sp. nov.**
Figures 5.3a-d; Table 3

Type material

Holotype male, 1 paratype male, 1 paratype female.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7452.

Description of holotype male

Odontophora serrata sp. nov. is a long, slender nematode, the cuticle is smooth over most of the body, with fine annulations visible only on the tail. The anterior setation comprises four crowns, one labial, two cephalic and one sub-cephalic. The anterior-most crown comprises six minute labial papillae circling the oral opening. The anterior cephalic crown of six long setae are located level with the posterior margin of the amphid, while the posterior cephalic crown comprises four short setae located *ca* 4 µm behind the anterior crown. The subcephalic crown is located a further 4 µm behind the posterior cephalic crown, and comprises four long setae. Four rows of cervical setae are present, which extend along the length of the oesophagus. The remainder of the body bears no somatic setation until the caudal region.

The amphid is a small rounded loop and in the case of the holotype, has a long, extruded corpus gelatum.

The structure of the buccal cavity is typical of the genus *Odontophora*, *i.e.* conical with the posterior walls strongly cuticularised and containing six well developed odontia. Characteristicly of *O. serrata* sp. nov., each odontium bears three pairs of projections giving it a serrated appearance. Located between the odontia are cutitularised, triangular shaped plates with fine striations / denticles on their anterior edge. The oesophagus is

cylindrical and does not have an anterior or posterior bulb but does have a cardia. The nerve ring is located *ca* 70 % along the length of the oesophagus, and the excretory pore is level with the subcephalic crown of setae.

The caudal region bears four submedian rows of stout setae. Two pairs of conspicuous setae are located sub-terminally on the dorso-lateral surfaces which are very long (anterior and posterior pair are *ca* 32 and 64 µm, respectively). The well developed spicules are strongly arcuate, with a distinct, *ca* 90° angle. The proximal end has only slight cephalation and is reflexed dorsally. The gubernaculum is cuticularised and conspicuous, with a long dorsally-directed apophysis. Seventeen small precloacal supplements are present on the ventral surface, anterior to the cloaca. They comprise small papillae, each pierced by a fine pore. The testis is single, outstretched and lies to the left of the intestine.

Description of paratype female

The general body form, anterior setation, amphid and buccal cavity of the female are identical to that of the male. The caudal setation of the female is much sparser and lacks the long conspicuous subterminal setae described from the holotype male. The ovaries are paired, opposed and outstretched, and the mature eggs are large (44 x 160 µm), elongate and very conspicuous on the paratype female.

Habitat

O. serrata sp. nov. is regularly found in calcareous sediments within both marine and estuarine environments of south-western Australia, but is usually only present in low numbers. The type specimens were collected during February 2005, from Chidley Point in the lower Swan River Estuary (site 2 *sensu* Chapter 3).

Differential diagnosis

Jensen & Gerlach (1976) have discussed the difficulties involved in separation of the large number of species in this genus which differ only in minor features. They describe the dententiculate projections on the odontia of *O. bermudensis* Jensen & Gerlach 1976 as peculiar and suggest that their presence is best seen on specimens on which the odontia are protruded, and that the form of the odontia in descriptions where this is not the case is unknown. However, the three pairs of lateral projections on each odontium of *O. serrata* sp. nov. are very obvious even on specimens where they are not protruded and we do not feel that they could have been overlooked if this species had been found and described earlier. There are three other species on which similar projections on the odontia have been identified, *i.e. O. villoti* Luc & De Coninck, 1959 and *O. paravilloti* Blome 1982 which have one pair of projections on each odontium (three-lobed) and *O. bermudensis* which has two pairs (five-lobed). In contrast, *O. serrata* sp. nov. has three pairs of projections on each odontium (seven-lobed). Species in this group also share the presence of a pair of long dorsolateral setae near the tail tip, but none so long as *O. serrata* sp. nov..

Etymology

This species had been named for its characteristic odontia, which have a distinctly serrated appearance due to the projections along their edges.

		Holotype	Par. ♀	Par. ♂
Length (mm)		3.08	3.086	3.061
a		64.1		66.5
b		18.9	19.2	19.8
c		29.3	30.2	24.6
Head diameter		26	30	31
Setae:	Cephalic 1	36	32	37
	Cephalic 2	8	6	8
	Subcephalic	33	31	30
	Subterminal	64	-	65
Amphid:	from ant	6	2	3
	L/W	6/6	7/7	8/7
	cbd	25	30	27
Nerve ring:	from ant	115	114	113
	cbd	36	44	38
Oesophagus length		163	161	154
Max. diameter		48	63	46
Vulva:	from ant	-	1.7	-
	V%	-	55	-
	cbd	-	63	-
Spicule (arc)		50	-	52
ABD		41	36	40
Tail length		105	102	124

Table 5.3 Morphological measurements for the holotype of *Odontophora serrata* sp. nov., as well as those for the male and female paratype specimens.

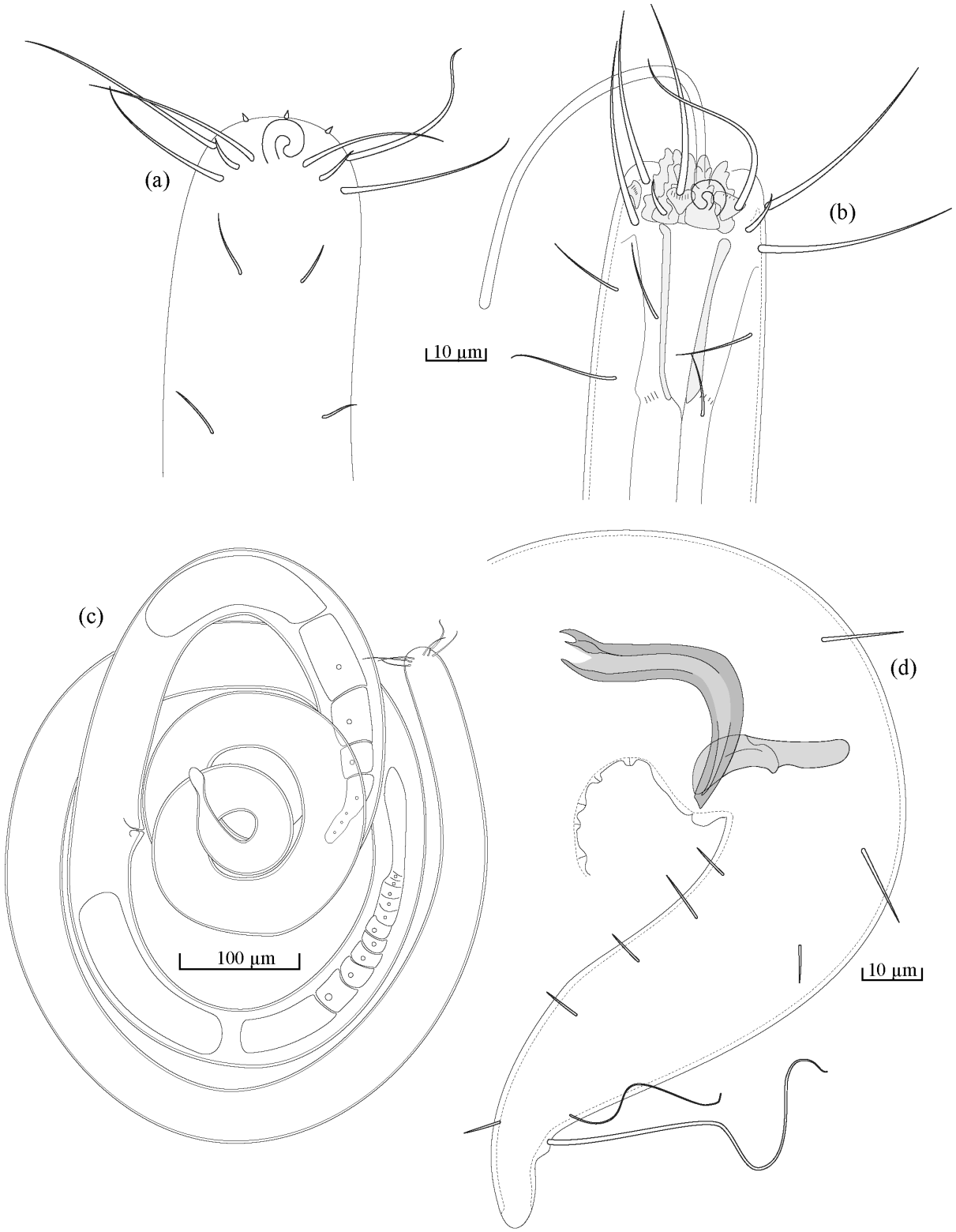

Figure 5.3 *Odontophora serrata* sp. nov.: (a) head of paratype female, external only; (b) head of holotype, internal and external; (c) total body of paratype female; (d) caudal region of holotype.

Genus *Parodontophora* Timm 1963
***Parodontophora aurata* sp. nov.**
Figures 5.4a-d; Tables 5.4 and 5.5

Type material

Holotype male, 1 paratype male, 2 paratype females.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7453.

Description of holotype male

A medium sized nematode which is reasonably stout for its length. It has light striations over the entire body, and is generally dark amber in colour. Six minute labial papillae and four short cephalic setae are present, as well as four files of short opisthocephalic setae (sensu Wu *et al.*, 2000). The dorso-lateral opisthocephalic files each comprise 3-4 setae, while the ventro-lateral files comprise only 2 each. Some additional sparse setation is evident along the oesophageal region. The buccal cavity is relatively long and narrow, with parallel and strongly sclerotised walls. Six simple but well developed odontia are located at the anterior of the buccal cavity, level with the cephalic setation. The crook-shaped amphid is positioned entirely over the buccal cavity and does not extend onto the oesophageal region, with the shorter leg measuring *ca* 70 % the length of the longer leg. The oesophagus is narrow, does not surround the buccal cavity and is slightly thicker posterior to the nerve ring, but does not display any distinct oesophageal bulb.

The spicules are arcuate and slender with a distinct split in the cephalation. The gubernaculum is approximately triangular with a narrow dorso-caudal apophysis. No precloacal supplements are present. The tail is conical and bears two sub-ventral files of post anal setae. The two testes are outstretched.

Description of paratype female

The females of this species are stouter than the males (De Man a ♂ = 40, ♀ = 30) but otherwise share all diagnostic characters. The vulva is located at *ca* 50 % of the body length. The ovaries are paired, opposed and outstretched.

Habitat

Parodontophora aurata sp. nov. is relatively common in sediments at upper estuarine / riverine locations in the Swan River Estuary. It does not exhibit any marked preference for sediment type and is found in types ranging from gravels to fine clay / mud. The holotype specimen was collected at Fish Market Reserve (site 11 *sensu* Chapter 3).

Differential diagnosis

Parodontophora aurata sp. nov. can be distinguished from the majority of other congeneric species by the fact that the two arms of the amphid are not extremely different in length, and the longer arm does not extend past the base of the buccal cavity, such as the case for *P. wuleidaowanensis* Zhang, 2005. Of the remaining species, a group of morphologically similar species was identified by Wu *et al.* (2000) in which *P. aurata* sp. nov. should also now be included. The latter authors present a table summarising the distinguishing characteristics for those five allied *Parodontophora* species. It is evident from Table 5.5 that although comparable values for most characters can be found among the other five species, the particular combination of characters found for *P. aurata* sp. nov. is unique.

Etymology

This species has been named for the distinct and consistent colouration of individuals, which ranges from light yellow through to dark amber.

		Holotype	$\overline{X}$ ♀	Para ♂
Length (mm)		0.17	1.240	1.220
a		39	32.6	41.8
b		8.2	7.9	8.1
c		9.1	10.4	9.5
Head diameter		18	14	15
Setae: Cephalic		8	8	7
Opisthocephalic		6	6	6
Cervical		6	6	6
Buccal cavity L/W		33/6	31/8	37/6
Amphid:	from ant	5	4	4
	dorsal arm	20	17	19
	ventral arm	14	12	15
	cbd	18	14	15
Nerve ring:	from ant	94	96	88
	cbd	29	30	28
Oesophagus length		142	157	152
Max. diameter		30	39	29
Vulva:	from ant		643	-
	V%		52	-
	cbd		39	-
Spicule (arc)		40	-	37
ABD		26	24	27
Tail length		129	121	130

Table 5.4 Morphological measurements for the holotype of *Parodontophora aurata* sp.nov., as well as for the male paratype and average female paratype specimens.

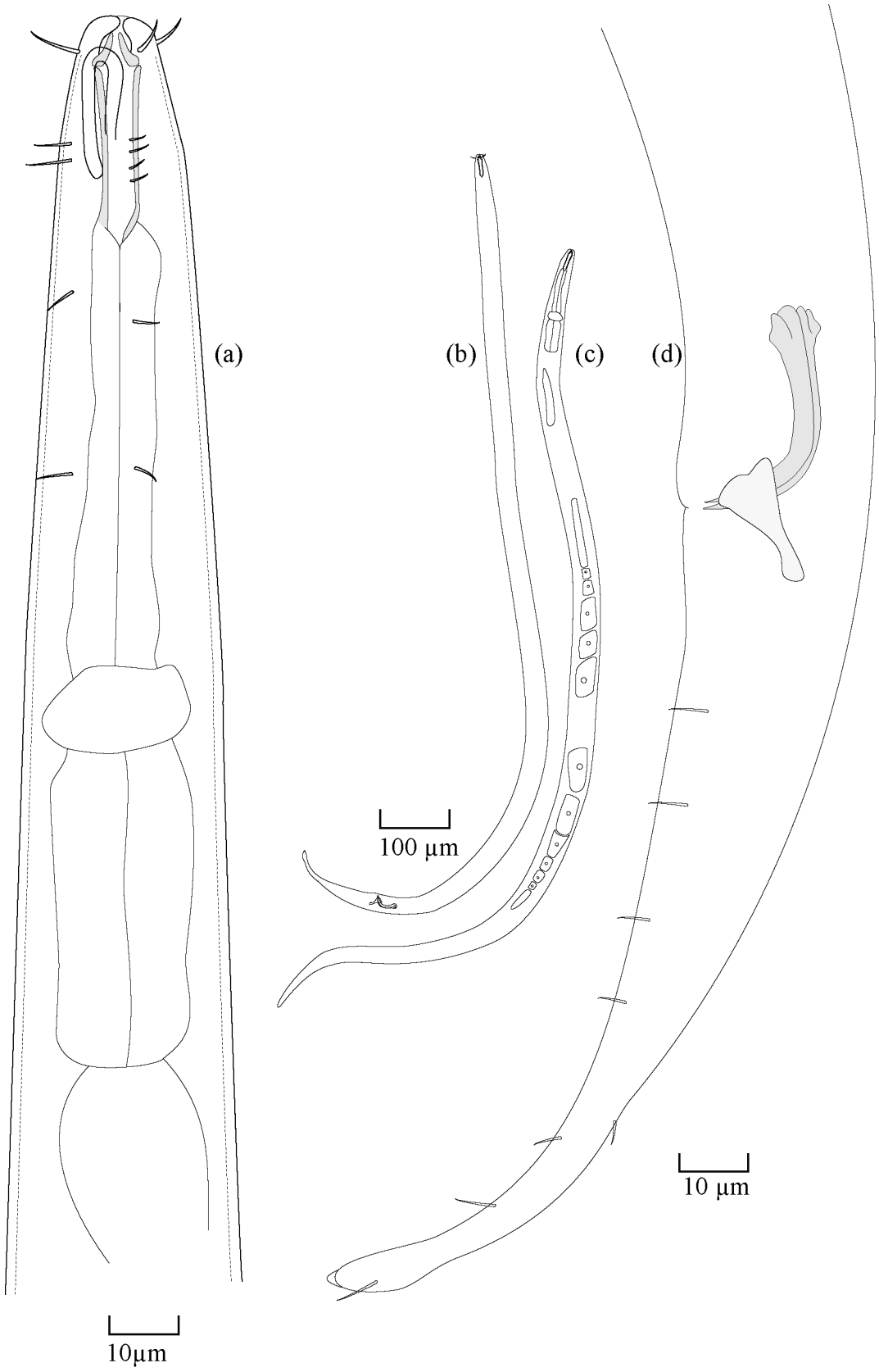

Figure 5.4 *Parodontophora aurata* sp. nov.: (a) head of holotype; (b) total body of holotype; (c) total body of paratype female; (d) caudal region of holotype.

	Parodontophora aurata sp. nov.	*Parodontophora marina*	*Parodontophora quadristicha*	*Parodontophora breviamphida*	*Parodontophora marisjaponici*	*Parodontophora limnophilla*
Cephalic setae length (μm)	7	6-7.5	10.5	7-8	7	4
Opisthocephalic setae length (μm)	7	?	?	?	3.6-4.0	2
Opisthocephalic setae arrangement	(4D-2V)2	(3D-1V)2	(4D-3V)2	(1D-2V)2	(3D-1V)2	(3D-1V)2
Amphidial dorsal/ventral arm	72 %	>65 %	?	<50 %	80 %	50 %
Position of excretory pore	mid-stoma	mid-stoma	mid-stoma	anterior stoma	anterior stoma	anterior stoma
Renette cell/ oesophagus length	36 %	45-57 %	50-55 %	40 %	35-40 %	34-47 %

Table 5.5 An adaptation of Table 2 as presented in Wu *et al.* (2000) with *Parodontophora aurata* sp. nov. included for comparison. In those situations where a character is similar for both *P. aurata* sp. nov. and another species, that character has been highlighted for both those species.

Order Chromadorida Filipjev 1922
Family: Desmodoridae Filipjev 1922
Subfamily: Desmodorinae Filipjev 1922
Genus: *Bolbonema* Cobb 1920
***Bolbonema spiralis* sp. nov.**
Figures 5.5 a-c, Table 5.6

Type material

Holotype male, 6 paratype males, 6 paratype females.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7454.

Description of holotype male

A medium sized nematode with a distinctly annulated cuticle. Annulations are coarse and irregular in the cervical region, gradually becoming fine and regular posteriorly. The body bears eight files of somatic setae, two sub-dorsal, two sub-ventral and four sub-lateral, spaced regularly along the entire body length. Somatic setae are arranged alternately long (*ca* 22 μm) and short (*ca* 4 μm), and finish just posterior to the cloaca. The cephalic capsule is smooth, distinct from the body and divided into two parts that are separated by a deep but only semi-complete abscission, located about 30 % from the anterior and interrupted by the amphid. The relatively large spiral amphid (11 μm, two turns) is located 5 μm from the anterior and the posterior edge is aligned with the base of the cephalic capsule. Six papilliform labial setae occur around the oral opening and four cephalic setae (9 μm) are situated level with the centre of the amphid. Four short (4 μm) sub-cephalic setae are present, just posterior to the cephalic capsule, and just before the anterior-most ring of eight long (16 μm) somatic setae. The buccal cavity is minute and the oesophagus is relatively short and narrow with a distinct oesophageal bulb without cardia. The spicules are 48 μm long, forming a simple arc,

with some cephalation present. The gubernaculum is poorly defined and is small and simple, with a small, slender dorsal apophysis. The single testis is outstretched. There are no supplements and the tail is short, conical and without terminal setation.

Description of paratype female

Female *Bolbonema spiralis* sp. nov. are approximately the same length as males but have a significantly greater maximum girth. The amphids have slightly less than two turns but have the same form as the males. In all other respects the sexes are monomorphic. The ovaries are paired, opposed and reflexed.

Habitat

All type specimens are from near-shore, sandy marine sediments within Mangles Bay, Western Australia (February 2004). Additional individuals have also been recorded from the marine reaches of the Swan River Estuary in Western Australia.

Differential diagnosis

Verschelde *et al.* (1998) ascribe two other species to the genus *Bolbonema*, namely *B. brevicolle* Cobb 1920, and *B. longisetosum* Jensen 1985. The latter species has longitudinally oval amphids describing a single loop in the male, with distinct pre- and post-cloacal supplements. *B. brevicolle* is rather poorly described by Cobb (1920) on the basis of a single female specimen, the amphids of which are in the form of a single circular loop like those depicted by Jensen (1985) for a juvenile *B. longisetosum*. The synonomy of *B. brevicolle* and *B. longisetosum* cannot be ruled out on the basis of the published taxonomic literature, however, both differ from *B. spiralis* sp. nov., which has distinctly spiral amphids with two turns. *B. spiralis* sp. nov. also differs from *B. longisetosum* in the absence of any pre- or post-cloacal supplements as well as the form of its spicules and gubernaculum.

Etymology

The specific name refers to the spiral nature of the amphids, which are a primary diagnostic feature within the genus.

		Holotype	$\overline{X}$ ♀	$\overline{X}$ ♂
Length (mm)		1.093	1.056	1.035
a		32.1	28.8	37.1
b		11.5	11.6	10.5
c		12.8	12.4	11.3
Head diameter		19	17	17
Setae:	Cephalic	9	10	10
	Subcephalic	4	4	4
Amphid:	from ant	5	4	4
	L/W	11/11	9	11/11
	cbd	19	17	17
Nerve ring:	from ant	56	60	60
	cbd	26	25	24
Oesophagus length		94	91	95
Max. diameter		34	36	28
Vulva:	from ant	-	547	-
	V%	-	51	-
	cbd	-	34	-
Spicule (arc)		48	-	48
ABD		18	17	19
Tail length		84	86	91.2

Table 5.6 Morphological measurements for the holotype of *Bolbonema spiralis* sp. nov., as well as the average measurements for the male and female paratype specimens.

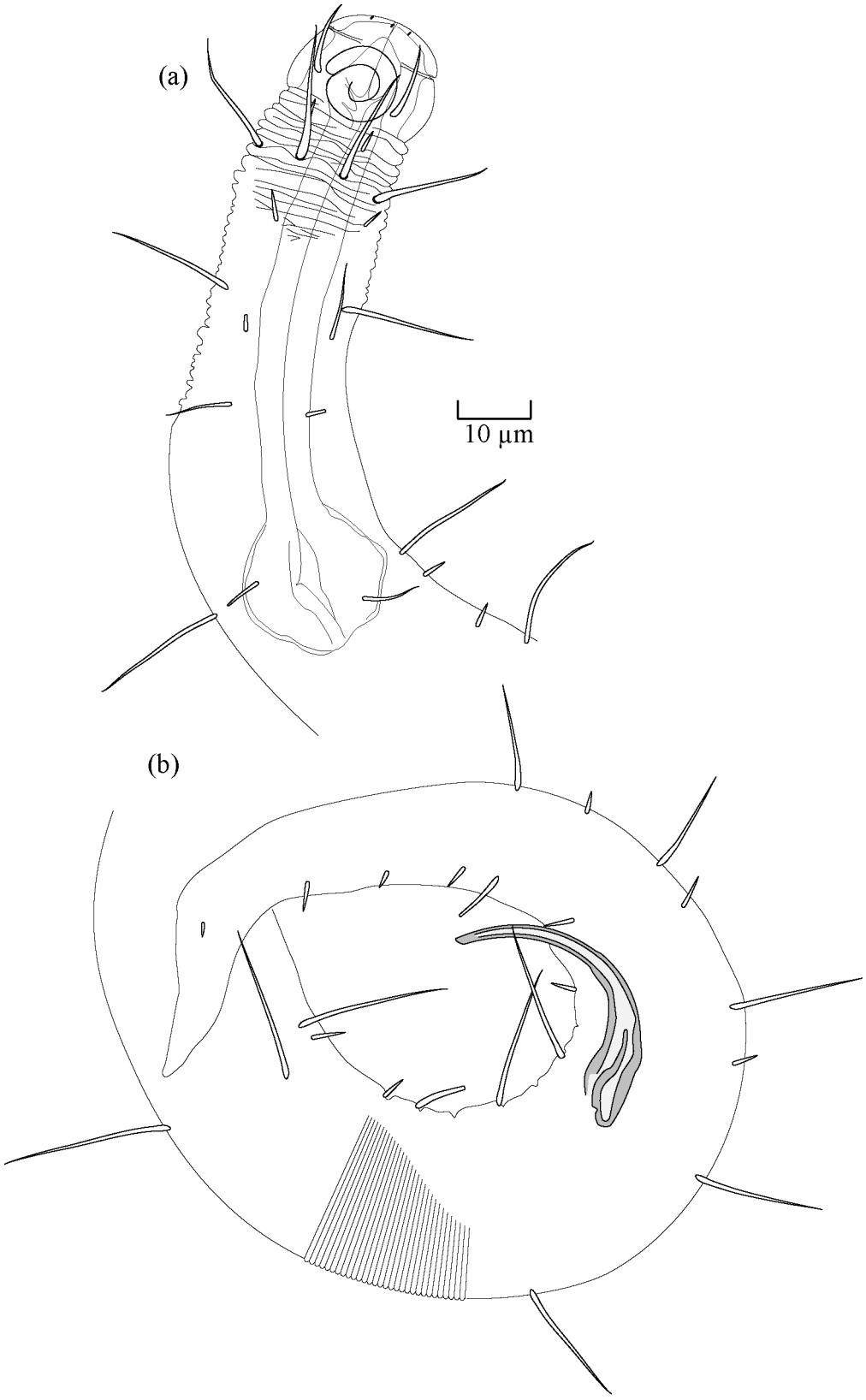

Figure 5.5 *Bolbonema spiralis* sp. nov.: (a) head and oesophageal region of holotype; (b) caudal region of holotype.

Subfamily: Spiriniinae Gerlach and Murphy 1965
Genus: *Onyx* Cobb 1891
***Onyx cephalispiculus* sp. nov.**
Figures 5.6a-c, Table 5.7

Type material

Holotype male, 7 paratype males, 5 paratype females.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7455.

Description of holotype male

The holotype is a medium length nematode with a very finely and regularly striated cuticle. General colouration is red / brown and very short, fine setae occur irregularly over the body. Four, more concentrated, fields of irregularly spaced short setae are evident in the cervical region on the dorsal, ventral and lateral surfaces. The simple loop amphids are located at the extreme anterior of the head, and occupy *ca* 25 % of the head diameter.

Setation comprises six labial setae around the oral opening, four cephalic setae just anterior to the amphid, eight subcephalic setae just posterior to the amphid, and some irregular setation behind this.

The dorsal tooth is typical of *Onyx* spp. being long, hollow and sclerotised. Immediately anterior to the point of the dorsal tooth, the buccal cavity is strongly sclerotised, forming a thick ring or band. The entire buccal cavity and tooth apparatus is surrounded by a well developed anterior oesophageal bulb. The posterior oesophageal bulb is relatively elongated, with a lightly sclerotised inner lining, and exhibits the lacunae described for *O. macramphis* Blome and Riemann 1994. The distinct nerve ring is located *ca* 50 % along the length of the oesophagus.

The 24 precloacal supplements are distinctly S-shaped and are more or less uniform in size. In paratype males the number of supplements varies between 17 and 24. The spicules are well defined and characteristically arcuate and cephalated, with the cephalation being bilobed. The tail is relatively short (112 μm) and bears two subventral files of somatic setae, as well as a conspicuous cluster of short setae just posterior to the cloaca on the ventral surface. No terminal setae are present and the testis is single and outstretched.

Description of female specimens

Male and female specimens do not display any remarkable dimorphism aside from the females being slightly less hirsute than the males. The ovaries are paired, opposed and reflexed.

Habitat

All type specimens for *Onyx cephalispiculus* sp. nov. were collected from the subtidal sediments at the predominantly marine environment of the lower reaches of the Swan River Estuary (sites 1 and 2 *sensu* Chapter 3).

Differential diagnosis

An annotated list of *Onyx* species is presented in Blome and Reimann (1994) which presents a summary of the diagnostic characters of the eight species previously described for that genus, to which *O. perfectus* (Cobb 1891), and now *O. cephalispiculus* sp. nov. (and *O. potteri* sp. nov., see later) should now be added.

Onyx cephalispiculus sp. nov. most resembles *Onyx sagittarius* (Gerlach 1950) in that the males of both species possess up to 24 strongly cuticularised, S-shaped, precloacal supplements; a feature which distinguishes these two species from all of the other known species of this genus. *O. cephalispiculus* sp. nov. can be discriminated from *O. sagittarius* by the shapes of both the spicules and gubernaculum. The spicules of

O. cephalispiculus sp. nov. are strongly arcuate and cephalated, with the cephalation being abscised laterally giving it a bi-lobed appearance while those of *O. sagittarius* are non-cephalated and not as strongly arcuate. Also, the gubernaculum and its dorsal apophysis are larger and more robust for the present species than depicted for *O. sagittarius*. Furthermore, the post cloacal, subventral cuticular elevations noted for *O. sagittarius* are absent on the present species. The fields of short setae in the cervical region of individuals of both sexes are a unique feature of this species.

Etymology

The species name "cephalispiculus" reflects the highly distinctive cephalations of this species' spicules which are unlike those of its congeners.

		Holotype	$\overline{X}$ ♀	$\overline{X}$ ♂
Length (mm)		1.20	1.24	1.24
a		21	22	25
b		5	6	6
c		11	12	12
Head diameter		34	33	32
Setae:	Labial	5	5	5
	Cephalic	13	15	13
	Subcephalic	7	9	9
Amphid:	from ant	1	0	1
	L/W	6/7	5/7	5/7
	cbd	24	19	18
Nerve ring:	from ant	115	107	108
	cbd	47	50	47
Oesophagus length		235	213	206
Max. diameter		58	57	51
Vulva:	from ant	-	705	-
	V%	-	0.57	-
	cbd	-	57	-
Spicule (arc)		69	-	71
No. of supplements		24	-	21
ABD		47	35	47
Tail length		112	100	107

Table 5.7 Morphological measurements for the holotype of *Onyx cephalispiculus* sp. nov., as well as the average measurements for the male and female paratype specimens.

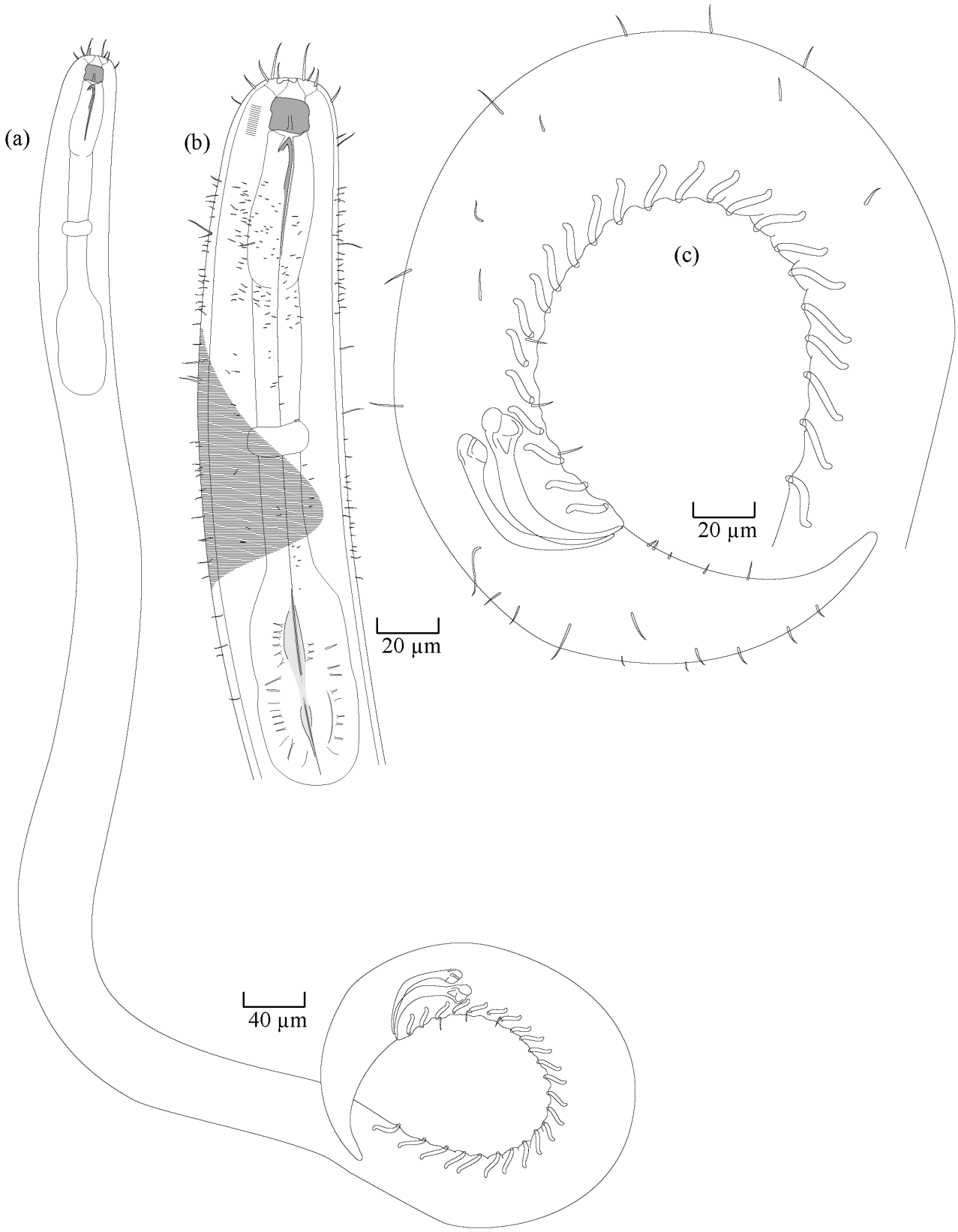

Figure 5.6 *Onyx cephalispiculus* sp. nov.: (a) total body of holotype; (b) head and oesophageal region of holotype; (c) caudal region of holotype.

***Onyx potteri* sp. nov.**
Figures 5.7a-d, Table 5.8

Type material

Holotype male, 1 paratype female, 4 juveniles.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7456.

Description of holotype male

The holotype specimen is moderately long and slim with a dark brown colouration and irregular short sparse somatic setation. The cuticle striations are fine and distinct, and begin just anterior to the posterior border of the amphid and terminate 9 μm from the tail tip. The amphids are conspicuous, anteriorly placed, and form a spiral with 2.75 turns. The cephalic setation is in two crowns, the anterior comprising six short setae, and the posterior, four longer setae. There is a circle of eight medium length setae located level with the posterior border of the amphid, and a few longer setae scattered over the oesophageal region. The buccal cavity is cup-shaped and lightly cuticularised, with distinct rugae just inside the oral opening. The dorsal tooth is typical of the genus *Onyx*, being long, hollow and sclerotised. The oesophagus exhibits two distinct muscular bulbs, one at the anterior surrounding the dorsal tooth, and the other at the posterior end. The posterior oesophageal bulb is long and lacks any internal cuticularisation or lacunae. The nerve ring is indistinct, but is visible around the isthmus between the two oesophageal bulbs.

A file of setae is present on the dorsal surface of the caudal region, continuing down the relatively short tail. The setae comprising the above file are all very short (3 μm) with the exception of two on the tail which were much longer (20 μm) Furthermore, a single very small post-cloacal thorn is visible on the ventral surface of the tail.

Ten distinct tubular precloacal supplements are present on the holotype. The posterior eight supplements are all well defined, closely spaced and located on a particularly thick section of cuticle. The anterior two supplements are less distinct, more widely spaced and the surrounding cuticle is noticeably thinner than for the previous group. The moderately sclerotised spicules are slender and strongly curved with a simple distal tip and a small cephalation at the proximal ends. Just below the proximal cephalations there is a distinct constriction in the spicules, which appears more strongly sclerotised than the rest of the structure. The gubernaculum is oval, surrounds the distal tip of the spicules, and has a strongly sclerotised posterior margin.

Description of female specimens

The female has the same general body shape as the male. The amphids are of similar dimensions as the male and describe a spiral of 2.5 turns. Likewise, the cephalic / cervical setation has the same arrangement and lengths as for the male. The ovaries are paired, opposed and reflexed, with each uterus containing a single large and elongate egg (*ca* 80 x 25 μm).

Habitat

The type specimens, including the holotype were collected from nearshore marine waters along the lower west coast of Western Australia during the study by Hourston *et al.* (2005). This species was most common in the calcareous sediments of the relatively high energy nearshore site, Leighton Beach (habitat type 6 *sensu* Chapter 2).

Differential diagnosis

According to the annotated list of *Onyx* species in Blome and Reimann (1994) only one other congener also has ten tubular precloacal suppliments, *i.e. O. dimorphus* (Gerlach 1963). *O. dimorphus* is further described as having sexually dimorphic amphid

structure, and depicted as relatively hirsute. This is in contrast to *O. potteri* sp. nov. in which the sexes have similar amphids, and all specimens are comparatively glabrous. Furthermore, there are no described species within this genus that have similarly shaped spicules with the distinct constriction below the cephalation.

Etymology

This species is dedicated to Prof. Ian Potter for his guidance in this work and his major contribution to marine and estuarine research in Western Australia.

		Holotype	Par. ♀	$\overline{X}$ Juv
Length (mm)		1112	776	729.5
a		39.7	26.8	28.6
b		8.1	5.6	6.4
c		13.7	12.9	11.4
Head diameter		20	21	21
Setae:	Labial	4	3	3
	Cephalic	14	11	10
	Subcephalic	9	6	7
Amphid:	from ant	-	2	3
	L/W	8/8	8/8	7/8
	cbd	21	18	19
Nerve ring:	from ant	63	67	61
	cbd	23	28	23
Oesophagus length		137	138	114
Max diameter		28	29	25
Vulva:	from ant	-	426	-
	V%	-	55	-
	cbd	-	29	-
Spicule (arc)		50	-	-
ABD		27	23	20
Tail length		81	60	63

Table 5.8 Morphological measurements for the holotype of *Onyx potteri* sp. nov., as well as those for the female paratype, and the averages of the juvenile specimens.

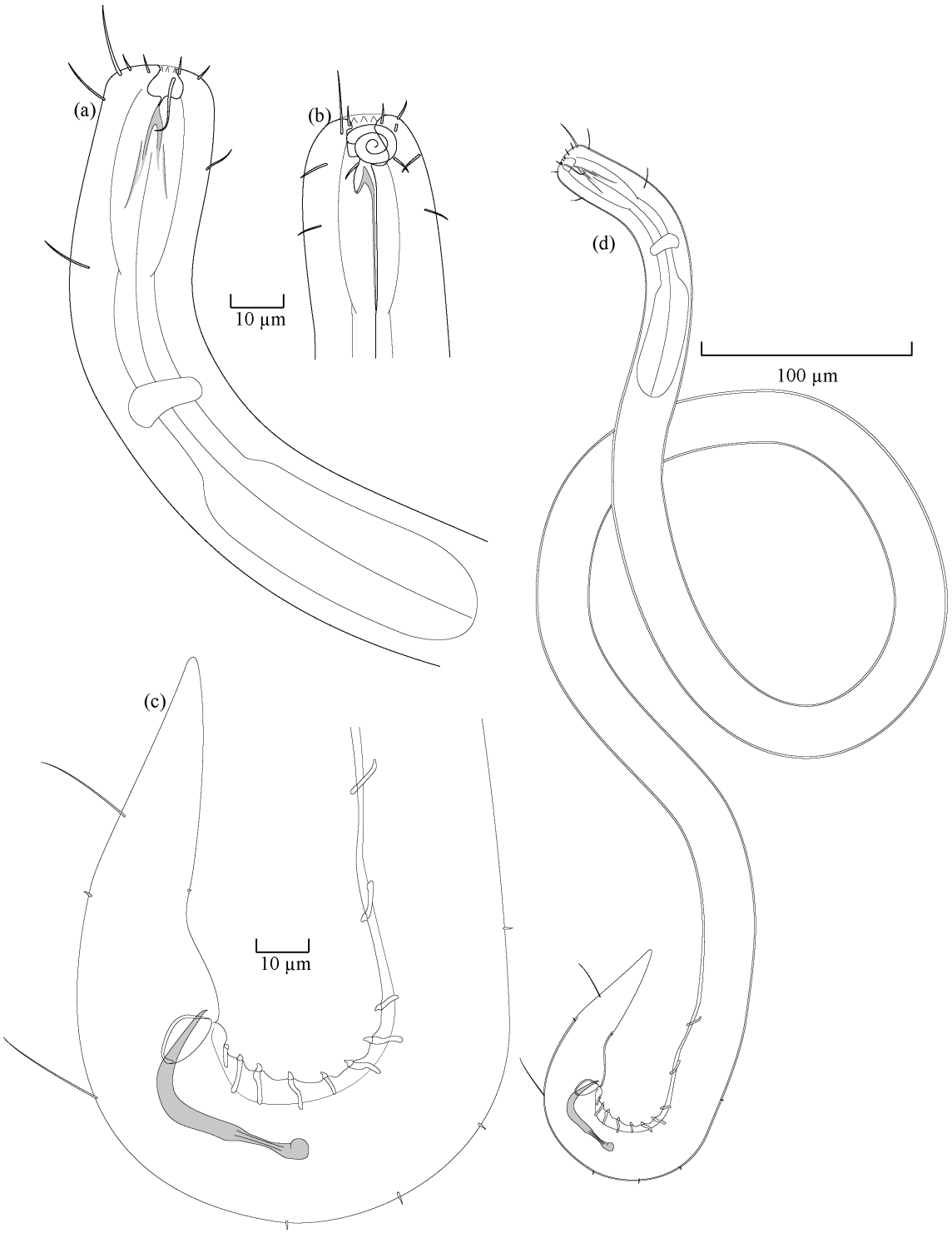

Figure 5.7 *Onyx potteri* sp. nov.: (a) head and oesophageal region of holotype; (b) head of paratype female; (c) caudal region of holotype; (d) total body of paratype male.

Subfamily: Stilbonematinae Chitwood 1936
Genus *Eubostrichus* Greeff 1969
***Eubostrichus otti* sp. nov.**
Figures 5.8a-d, Table 5.9

Type material

Holotype male, 3 paratype females 3 juveniles.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7457.

Description of holotype male

The body is extremely long, narrow and colourless. The cuticle ornamentation comprises indistinct and very fine striations which begin level with the anterior of the amphid and finish just short of the tail tip. As is common among members of the Stilbonematinae, parts of the cuticle of the holotype for *E. otti* sp. nov. is covered with ectosymbiotic bacteria which, in the case of this species, are crescent shaped. The bacterial coat of the holotype specimen covers only small areas of the body, however inspection of the paratype material suggests that in life, the coat would have covered the majority of the body.

The large amphids are round loops. The buccal cavity is minute and the majority of the oesophagus is very thin and poorly muscled. The oesophageal bulb is round and reasonably well developed but has no observable cardia. The tail is relatively short, cylindrical, and tapers quickly at its extremity.

Cephalic setation comprises eight relatively long setae (15 μm) which are evenly spaced around the circumference of the head, 4 μm from the anterior and inserted level with the anterior margin of the amphid. The subcephalic setae comprise two distinct crowns, the first of only four setae, which are a similar length to the cephalic setae but inserted

beside the amphid in a sublateral position (8 μm from the anterior). The posterior crown of eight shorter (14 μm) sub-cephalic setae is located 22 μm from the anterior. No somatic setation is evident on those areas where the bacterial coat is missing. Distinctive ventral setation is present around the cloaca and on the tail. Six pairs of 5-8 μm setae are present in this area, five pairs sub-ventrally, and one laterally. The sub-ventral setae comprise one pair in front of the cloaca (10 μm pre-anally) and another four on the tail (26, 36, 48 and 67 μm post-anally). The single pair of lateral setae is located 55 μm posterior to the cloaca. Additionally, a pair of double subterminal setae (10 μm) is also present 5 μm from the tip of the tail on the lateral surfaces. The pair of double setae is similar to the condition described for *E. topiarus* Berger *et al.* 1996.

The spicules are arcuate, 43 μm long (arc) and slim but well defined. The proximal end of each of the spicules is cephalate, with a conspicuous notch. The gubernaculum is small and narrow, but is well defined, with a very conspicuous and distinctive sickle-shaped dorsal apophysis. The single testis is outstretched.

Description of female specimens

The general body form and primary descriptive features such as setation, cuticular ornamentation and epibionts are all shared by both males and females. However, several dimorphic characters distinguish the sexes of this species. Firstly, females of this species are substantially longer than are the males and have a longer tail relative to their body length than males but a narrower ABD. Females also lack the distinctive caudal setation displayed by the males. The amphids of the female and juvenile specimens are substantially smaller than those of the holotype male; consequently, the anterior crown of sub-cephalic setae is located beside the amphids for the male, but at the posterior edge of the amphids for the rest of the individuals (Figs 5.8a-b). The paired ovaries of the females are equal in size, opposed and reflexed.

Habitat

All of the type material, including the holotype, for *Eubostrichus otti* sp. nov. was collected from sediment cores taken from the saline, lower / middle reaches of the Swan River Estuary in Melville Water. Individuals of this species were present in only a few samples and never in large quantities. Specimens were found in sediment types ranging from coarse biogenic sands to fine muds.

Differential diagnosis

Of the members of the subfamily Stilbonematinae, *Eubostrichus otti* sp. nov. most resembles *E. topiarius*, sharing features such as amphid, spicule and gubernaculum structure, cuticle ornamentation, and crescent-shaped epibionts. However these two species are distinct. By far the most conspicuous discriminating feature is the size of adults, which is up to 6 mm in *E. topiarius* while the largest individual found for the presently described species was only 3.2 mm. Additionally, the different arrangement of anterior setation is an important divergence. The cephalic setation of *E. topiarius* is arranged with an anterior crown of 4, followed by another of 8 (*i.e.* 4+8). On *E. otti* sp. nov., the arrangement of the two cephalic crowns is reversed with the anterior comprising 8 and the posterior crown 4 setae (8+4). Two other similar species include *E. longisetosus* Berger *et al.* 1996 and *E. africanus* Muthumbi *et al.* 1995, however, these two species also differ in terms of the arrangement of their cephalic setation (4+4).

It should be noted that one of the juvenile paratype specimens of *E. otti* hosted two separate forms of epibionts. While most individuals of this species were covered with crescent-shaped epibionts, a single juvenile individual was covered by this form only on the posterior half of the body, while the anterior half was instead covered with a coccoid species of epibiont, similar to those displayed by *Catanema australis* sp. nov. (see later).

Etymology

This species is named after Prof. Jörg Ott in recognition of his pioneering work with Stilbonematids.

		Holotype	$\overline{X}$ ♀	$\overline{X}$
Length (mm)		3.23	2.86	1.84
a		134.5	108.8	95.1
b		27.3	28.9	20.2
c		36.2	32.	21.3
Head diameter		18	17	14
Setae:	Cephalic	15	17	10
	Subcephalic 1	15	12	8
	Subcephalic 2	14	10	11
Amphid:	from ant	2	1	2
	L/W	16/12	7	7/ 8
	cbd	18	17	14
Nerve ring:	from ant	64	67	61
	cbd	22	23	18
Oesophagus length		118	99	90
Max. diameter		24	26	19
Vulva:	from ant	-	1.60	-
	V%	-	56	-
	cbd	-	26	-
Spicule (arc)		43	-	-
ABD		24	17	13
Tail length		89	90	85

Table 5.9 Morphological measurements for the holotype of *Eubostrichus otti* sp. nov., as well as the average measurements for the female and juvenile paratype specimens.

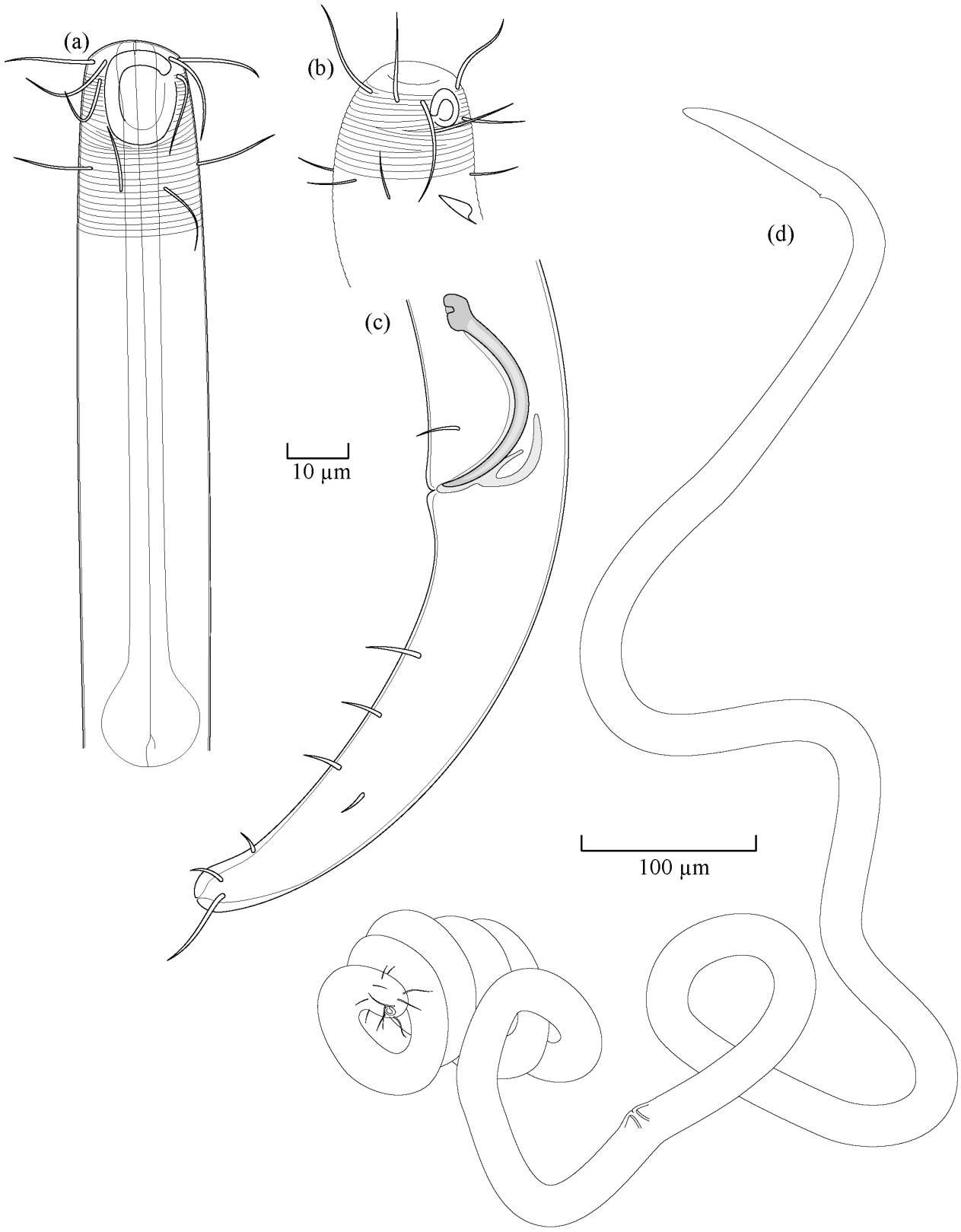

Figure 5.8 *Eubostrichus otti* sp. nov.: (a) head and oesophageal region of holotype; (b) head of paratype female; (c) caudal region of holotype; (d) total body of paratype female.

Genus: *Catanema* Cobb, 1920
***Catanema australis* sp. nov.**
Figures 5.9a-f, Table 5.10

Type material

Holotype male, 3 paratype males, 3 paratype females.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7458.

Description of holotype male

The holotype is very tightly coiled and extremely elongate. Its general colouration is dark brown and the cuticle is covered with a thick layer of coccoid epibionts which obscures any surface ornamentation that may be present. Two crowns of cephalic setae are present, both located at the extreme anterior of the head. The four setae comprising the anterior crown are relatively long (15 μm), while the eight of the posterior crown are only half that length (7 μm). A single crown of four short (4 μm) cervical setae is located approximately 20 μm from the anterior. No other somatic setation is evident along the length of the body. The form and size of the amphids could not be determined due to their location at the extreme anterior of the head. In most specimens an extruded corpus gelatum is observable.

The buccal cavity is minute and unarmed but is enclosed within a muscular anterior oesophageal bulb (diagnostic of the genus *Catanema*). The remainder of the oesophagus is relatively short and narrow, with a poorly defined posterior bulb. The nerve ring is located *ca* halfway along the oesophagus and is small and poorly discernable.

Glandular sensory organs (GSOs), similar to those described for *Eubostrichus topiarus* by Berger *et al.* (1996) are present but are not associated with hollow spines as were those that latter species. GSOs tend to be more numerous, larger and more closely

spaced on the ventral surface in the cervical region of the holotype and paratype males, than on the rest of the body.

The evenly arcuate spicules of the holotype for *Catanema australis* sp. nov. are short, very slim and distinctly cephalated proximally. The gubernaculum is small and bears a squarish caudal apophysis. Just anterior to the cloaca on the ventral surface, a file of four short (3 μm) single spines is present. Posterior to the cloaca, four pairs of sub-ventral spines are evenly spaced along the tail. The testis is single and outstretched.

The coccoid epibionts covering the cuticle of the holotype form a largely continuous layer, one cell thick, over almost the entire organism, with only the extremities exposed.

Description of female specimens

In general, male and female specimens are similar, but with mature females tending to be longer than the males (> 3 mm). Furthermore, the females lack the caudal spines where are present on the males. The ovaries are paired, opposed and reflexed. The GSOs are more numerous in females than in males, and are especially large and more closely spaced on the ventral surface near the vulva as well as in the cervical region.

Habitat

Catanema australis sp. nov. occurs in marine sediments, along the coast of south-western Australia, being more common in sheltered, low energy environments such as those at Penguin Island (habitat type 1 *sensu* Chapter 2), where the holotype specimen was collected. See locality details in Hourston *et al.* (2005) where this species is incorrectly labelled as "*Leptonemella* sp."

Differential diagnosis

Catanema australis sp. nov is most similar to the described species *C. exilis* (Gerlach, 1963) in the extreme anterior position of the amphids (anterior to the 4 long

cephalic setae) and the fact that the subcephaic and subamphidial seate (terminology of Platt & Zhang, 1982) comprise a single circle of 8 at the same level. The form of the amphids cannot be ascertained, but their location is indicated by the origin of the corpus gelatum, which is extruded in most specimens. Furthermore, *C. exilis* has seven pairs of prominent subventral tubular supplements on the male tail, whereas *C. australis* sp. nov. has only four pairs of "normal" setae or spines.

Etymology

This species is named to reflect its southern hemispheric distribution in comparison to the northern hemispheric distribution of its described congeners.

		Holo.	$\overline{X}$ ♀	$\overline{X}$ ♂
Length (mm)		2.166	3.002	2.268
a		77	97.9	84.2
b		26	28.7	24.8
c		25	33.6	26.9
Head diameter		19	22	20
Setae:	Cephalic 1	15	13	11
	Cephalic 2	6	4	5
	Cervical	4	3	3
Nerve ring:	from ant	52	57	51
	cbd	24	26	23
Oesophagus length		82	105	91
Max. diameter		28	30	27
Vulva:	from ant	-	30	-
	V%	-	606	-
	cbd	-	30	-
Spicule (arc)		38	-	36
ABD		26	20	26
Tail length		87	89	84

Table 5.10 Morphological measurements for the holotype of *Catanema australis* sp. nov., as well as the average measurements for the male and female paratype specimens.

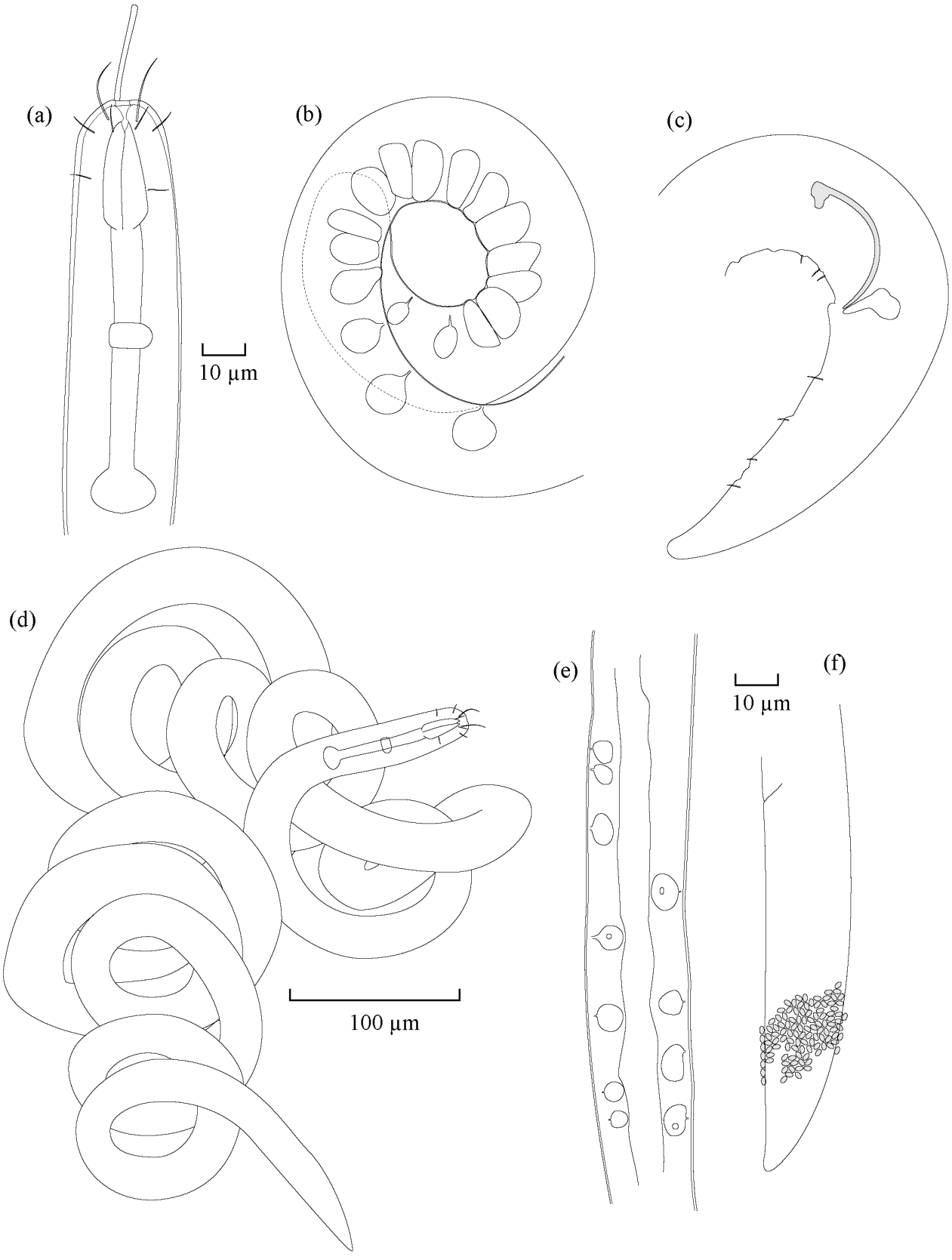

Figure 5.9 *Catanema australis* sp. nov.: (a) head and oesophageal region of paratype female; (b) head and cervical region of holotype showing GSOs; (c) caudal region of holotype; (d) total body of paratype female; (e) paratype female mid-body showing GSOs; (f) caudal region of paratype female showing epibionts.

Genus: *Leptonemella* Cobb 1920
***Leptonemella peronensis* sp. nov.**
Figures 5.10a-d, Table 5.11

Type material

Holotype male, 1 damaged paratype male, 1 paratype female.

Type material deposited at the Western Australian Museum.

Registration Number WAM V7459.

Description of holotype male

The body is very long and narrow with a strong brown colouration and a coarsely and regularly striated cuticle. Anteriorly, it possesses a distinct, smooth cephalic capsule which bears two cephalic, one cervical and possibly one labial crowns of sensory setae (The invagination of the cephalic capsule does not allow for examination of any labial setation). The cephalic crowns comprise four relatively long setae anteriorly (23 μm) and six slightly shorter setae more posteriorly (13 μm). The cervical crown is located at the base of the cephalic capsule and comprises eight short setae (4 μm). The amphids were also unable to be observed due to the invagination of the head.

The spicules are strongly arcuate and 1.4 ABD long (arc) with a distinct cephalation. The gubernaculum is narrow, without an apophysis and lies parallel to the posterior half of the spicules. The tail is relatively short (86 μm) with the cuticular striations covering all except the last 25 μm of the tail. Two pairs of short (6 μm) subventral spines are located anterior to the cloaca and another three pairs post-cloacally. Additionally, two pairs of sub-terminal setae are situated 12 μm from the tip of the tail on the sub-ventral surfaces.

In contrast to many other species within the Stilbonematinae, the type specimens of the present species lack any sheath of epibionts. It is not implicit that this sheath does not

occur on individuals of this species, only that it is absent for the described individuals in their fixed and mounted state.

Description of female specimens

The single female specimen has a similar general body morphology, cuticular ornamentation and setation pattern as the male but with some notable differences. The most apparent difference is the discrepancy in total length, with the female measuring 3.1 mm in comparison the males length of 2.04 mm. Although the female was much longer, the maximum diameters of both individuals were comparable (*ca* 30 μm) resulting in a much greater DeMan a value for the female (103) than the male (68). Likewise, the tail of the female is relatively longer than that of the male (♀abd = 4.3 *vs* ♂abd = 3), and has no sub-ventral pairs of setae.

Habitat

Type specimens were collected from a low energy, shallow, near-shore marine environment at Point Peron, south-western Australia (February 2004). The sediment was relatively coarse and calcareous, and contained appreciable amounts of organic material (seagrass detritus).

Differential diagnosis

This species is similar to *Leptonemella granulosa* Boucher 1975 in many respects. The only appreciable differences are the surface texture of the cephalic capsule, the tail length and the circum cloacal setation. *L. peronensis* sp. nov. does not have the noticeably granular cephalic capsule which is characteristic of *L. granulosa*. Furthermore, *L. peronensis* sp. nov. has a somewhat shorter tail than that described for *L. granulosa*, particularly in the case of the males where it is only 3 ADB in comparison to the 5.6 ABD. The holotype of *L. granulosa* is depicted as having 7 pairs of short post-cloacal spines on the subventral surfaces and none anterior to the cloaca, whereas

L. peronensis sp. nov. has two pre-cloacal pairs of setae, only four post-cloacal pairs and a double pair near the tail terminus.

Etymology

This species is named after its type locality, *i.e.* Point Peron, Western Australia.

		Holo	Par. ♀
Length (mm)		2.040	3.113
a		68.1	103.7
b		20.6	27.1
c		25.2	30.2
Head diameter		24	26
Setae:	Cephalic 1	23	23
	Cephalic 2	13	18
	Cervical	4	12
Nerve ring:	from ant	47	
	cbd	28	
Oesophagus length		99	115
Max. diameter		30	30
Vulva:	from ant	-	2
	V%	-	53
	cbd	-	28
Spicule (arc)		40	-
ABD		27	24
Tail length		81	103

Table 5.11 Morphological measurements for the holotype of *Leptonemella peronensis* sp. nov., as well as those for the female paratype specimen.

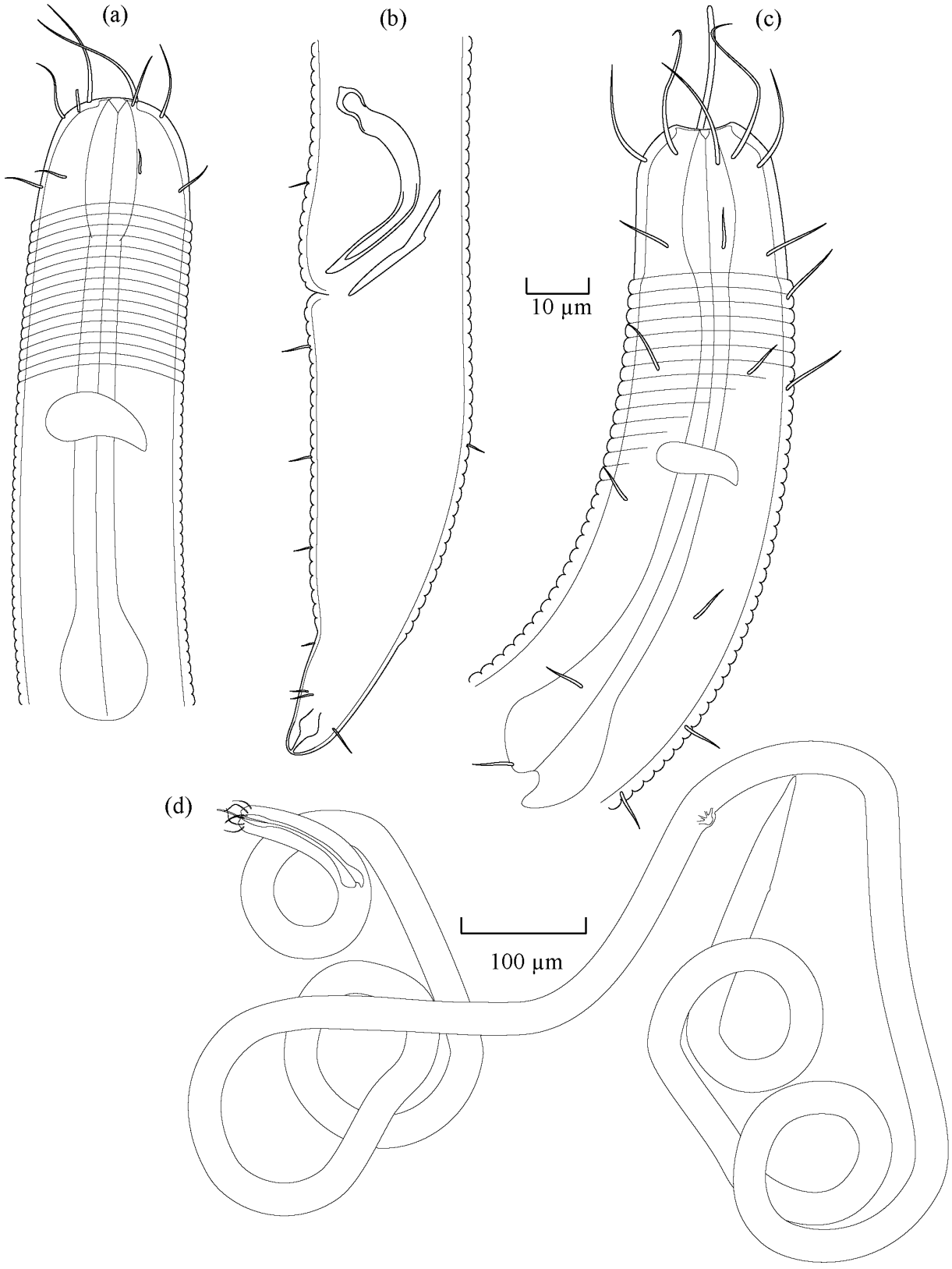

Figure 5.10 *Leptonemella peronensis* sp. nov.: (a) head and oesophageal region of holotype; (b) caudal region of holotype; (c) head and oesophageal region of paratype female; (d) total body of paratype female.

5.4 Discussion

The description of the ten nematode species provided in this chapter make an important contribution to our knowledge of the taxonomy of free-living nematodes in the sediments of coastal and estuarine waters in Australia and will be invaluable for ecological studies that incorporate this phylum. This contribution is particularly relevant considering the paucity of taxonomic publications on this group in south–western Australia (*i.e.* Inglis, 1969; 1970; 1971).

The present ecological studies have highlighted that many nematode species of this region remain undescribed. In particular there are several undescribed species within the Chromadoridae, Comesomatidae, Oncholaimidae, Thoracostomopsidae and Xyalidae which were numerically important as well as being of value characterising the nematofauna of certain regions in south–western Australia.

A special acknowledgement must be made at this point to the undergraduate students of the Marine and Estuarine Biology field trip 2005, who collected several individuals of the type material for *Bolbonema spiralis* sp. nov. and *Leptonemella peronensis* sp.nov..

Chapter 6 Comparisons and conclusions

6.1 Comparison of marine and estuarine nematode assemblages

6.1.1 Introduction

Microtidal nearshore marine and estuarine areas represent very different environments for free-living nematodes in terms of both their physico-chemical characteristics and the food sources they contain. For example, the spatial and temporal variations in water chemistry are far more variable in estuaries than in nearshore areas, and are a constant and considerable stressor for the nematodes inhabiting that environment (Heip *et al.*, 1985; Forster, 1998). Conversely, physical stability is often much greater in estuaries as they are typically fetch limited and their morphology tends to preclude ocean swell. In contrast, nearshore marine waters are generally more exposed to both swell and local wind waves, which cause scouring and sediment resuspension. In terms of their food resources, estuaries are among the most productive aquatic ecosystems (Schleske & Odum, 1961; McLusky & Elliott, 2004), while the productivity of nearshore marine areas is often much lower (McLusky & Elliott, 2004). Furthermore, the type and source of productivity may vary considerably between these two environments, with higher energy marine environments tendending to promote phytoplankton while estuaries may produce more macrophytes and microphytobenthos in addition to phytoplankton (McLusky & Elliott, 2004). Additionally, the source and type primary production in estuaries may considerably affect the detrital food resources.

After reviewing the literature, both (Heip *et al.*, 1985) and Mclusky & Elliot (2004) conclude that in general, the numbers of species within estuaries is less than that within either the sea or freshwater, and that this lower diversity is largely due to the fact that only certain species have the potential to withstand the variable physico-chemical regimes in those environments. This conclusion is consistent with those of

Remane (1934), Atrill (2002) and most recently Ferrero *et al.* (2008). In contrast, the densities of organisms are often markedly greater in estuarine than marine environments, reflecting the greater productivity of the former environment (Heip *et al.*, 1985; McLusky & Elliott, 2004). Thus, the relatively few species that can tolerate the dynamic conditions of estuaries are able to exploit the abundant resources of those systems and therefore may attain very high densities.

The diverse range of environmental conditions found throughout the Swan River Estuary was shown to be reflected in both spatial and temporal differences in species richness (number of species), density and composition of the resident nematode fauna (see Chapter 3). Those analyses demonstrated that species richness and density was moderate in the freshwater environment of the uppermost sites of the estuary, they fell to their minima in the region where salinity varied most, and then increased dramatically to their highest levels in the marine-dominated lower estuary. They also showed that the compositions of the nematode assemblages changed progressively along the length of the estuary.

Through following the same progression, it is hypothesised that that species richness will be higher in marine areas than in even the most downstream locations within the estuary, while the opposite it true of densities as a reflection of the level of productivity which is typically greater in estuaries than nearshore marine waters. Furthermore, the composition of the nematode assemblages will change progressively, with those in the marine environment being most similar to those of the lower estuary and least like those in the riverine, upper estuary. Since environmental conditions are so diverse along the length of the estuary, the variation in the composition of the nematode assemblages is likely to be much greater among the estuarine samples than across the marine samples. Previous analyses carried out during this study clearly demonstrated that the changes in species compositions of the nematode assemblages are progressive along the length of

the Swan River Estuary (Chapter 3). However, there was also particularly a distinct natural division between the compositions of the assemblages at the lower (1-6) and upper (7-12) estuary sites.

There is particular value in ascertaining statistically, whether the spatial distribution of the nematofauna can be explained by differences in a suite of environmental criteria that can be readily and accurately mapped. If a significant and reliable match is obtained, there is thus a sound basis for predicting, quantitatively, the characteristics of the nematofauna that are likely to occur at any site of interest along the length of an estuary, simply by ascertaining the environmental characteristics of that site. This has many applications for both managers and ecologists working in estuaries. The habitat classification scheme developed in chapter 4 of this thesis provides an ideal basis for determining whether the spatial distribution of the nematode fauna in the Swan River Estuary can be related to differences in values for temporally enduring environmental variables, and thus whether this relationship can be used in a predictive manner.
A statistical match between the nematode assemblages and enduring environmental variables provides a foundation for developing similar approaches in other estuaries.

The particular hypotheses addressed during this component of the study are as follows:

(i) The numbers of nematode species are greater in nearshore marine waters along the lower west coast of Australia than in either upper or lower regions of the Swan River Estuary, while the reverse is true for the nematode densities.

(ii) The species composition of the nematode assemblages changes in a progressive manner from marine waters to lower estuary then upper estuary, reflecting progressive changes in the physico-chemical environment. These progressive changes in assemblage composition also exist at genus and family levels, albeit to a lesser extent.

(iii) The species composition of the nematode assemblages varies much more among estuarine than nearshore marine sites, as a result of the far greater variations in physico-chemical conditions in the former environment.

(iv) The proportions of the four Functional Feeding Groups (FFGs) within the nematode fauna vary among the marine and two estuarine regions, reflecting differences in the dominant food sources at each location.

(v) The compositions of the nematode assemblages at sites representing the same habitat type within the Swan River Estuary (*sensu* chapter 4) are more similar to each other than are those between sites belonging to different habitat types.

6.1.2 Materials and methods

6.1.2.1 Data and data pretreatment

The datasets used in this chapter for the comparisons of the nematofauna in marine and estuarine waters were the same as those employed in Chapters 2 and 3. However, as only subtidal areas were sampled in the estuary, the samples from only subtidal marine substrates were used for subsequent comparisons. Likewise, to ensure comparability, only the first four seasons of the marine samples, which corresponded to the four seasons of estuarine data, were used in the following analyses. All analyses were conducted using the PRIMER v 6 statistical package (Clarke & Gorley, 2006) with the PERMANOVA module (Anderson *et al.*, 2008). As the nematode assemblages from the lower (1-6) and upper (7-12) estuarine sites formed distinct groups (see Chapter 3), the division of those groups was considered to represent an *a priori* factor (*i.e.* region) in the present analyses. All three marine sites were considered to collectively represent a third region.

6.1.2.2 Univariate statistical analyses

Euclidian distance matrices, constructed separately for both numbers of species and densities of nematodes in each replicate sample, were subjected to Permutational Multivariate Analysis of Variance (PERMANOVA) to determine whether each of these variables differed among the three regions. This 3-way test included the following main effects and their associated interactions; region (fixed), site nested within region (random) and season (fixed). Since differences in the nematofauna among sites and seasons have already been considered for both the marine and estuarine environments in previous chapters, only the results for the region factor were of specific interest in the present analyses. However, both of the former factors were included to:

(i) determine the relative importance of the region factor by assessing the magnitude of its component of variation relative to those of the other factors, and

(ii) achieve the most appropriate unit of replication to assess differences among regions.

Prior to subjecting the data for species richness and density of nematodes to PERMANOVA, the relationship between the $\log_{10}$ of the mean and the $\log_{10}$ of the standard deviation for each set of replicate samples was examined to determine whether the data required transformation to meet the test assumption of constant variance. The results of this procedure showed that the numbers of species did not require transformation, but that densities required a $\log_{10}$ transformation as in previous chapters. The null hypothesis (H_0) that significant differences did not exist among *a priori* groups was rejected if the significance level (P) was < 0.05.

6.1.2.3 Multivariate statistical analyses

The Bray-Curtis similarity matrix constructed from the $\log_{10}$ transformed densities of the various nematode species in replicate samples at each site in each season was

subjected to PERMANOVA to determine whether the compositions of the assemblages varied significantly among the three regions. The design of this test was identical to that employed for the univariate tests described in the previous subsection. PERMANOVA was employed rather than ANOSIM, as in previous chapters, as the latter test cannot examine three factors simultaneously and / or test for significant interactions between those factors. In order to quantify the pairwise differences among regions, a two-way crossed region x season ANOSIM was employed. A two-way crossed region x season SIMPER analysis was then used to ascertain which taxa were most responsible for distinguishing the assemblages between each pair of regions. This latter analysis was carried out on the mean abundance of each species in the different assemblages at each site in each season in order to emulate the appropriate level of replication for examining regional differences. A non-metric Multidimensional Scaling (nMDS) ordination, produced from Bray-Curtis similarity matrix created from the mean densities of the various species at each site in each season, were used illustrate differences in the compositions of the nematofauna among regions

Furthermore, to assess whether the nematofaunal compositions of the samples differed among regions, once the influence of density had been removed, the densities of the individual species were standardised across samples by their maxima, and a new Bray-Curtis similarity matrix constructed. PERMANOVA, SIMPER and nMDS were each repeated as described above.

Each of the above multivariate analyses were repeated after the data for the species compositions had been aggregated to the genus and then family levels, and also according to functional feeding group (FFG), in order to assess the extent to which nematode assemblages separated among regions across a range of taxonomic levels and a single functional level. Instead of nMDS, the standardised data were subjected to Principal Components Analysis (PCA) to illustrate any differences in the composition

of the FFGs of nematodes among the three regions and to determine which groups were mainly responsible for any such differences using the eigenvectors in association with the PCA plot.

6.1.2.4 Relationships between nematode assemblages and habitat types

The 12 nearshore sites sampled in the Swan River Estuary were assigned to their appropriate habitat type using the classification procedure described in Chapter 4. They represented seven habitat types, five of which comprised two sites, while the remaining two comprised only one site, *i.e.* sites 1 and 2 belonged to habitat type C, 4 and 6 to L, 7 and 8 to O, 9 and 10 to Q and 11 and 12 to R, while sites 3 and 5 belonged to habitat types E and H, respectively.

In order to assess the extent to which the classification of habitat types provided a sound basis for reflecting spatial differences in the composition of the nematode assemblages, the Bray-Curtis similarity matrix constructed from the mean densities of the various nematode species recorded at each site in each season was subjected to a two-way crossed site x season ANOSIM. The resultant pairwise comparisons were used to ascertain whether the nematofaunal compositions at sites assigned to the same habitat type differed significantly (*i.e.* $P < 0.05$), with the R-statistic values for all pairwise comparisons being used to gauge the relative extent of intra- *vs* inter-habitat type differences. Two-way crossed site x season SIMPER was then used to determine which species were most responsible for discriminating between the assemblages at each habitat type.

RELATE was used to assess the degree of correlation in rank order arrangement between the Manhattan resemblance matrix constructed from the mean values of the enduring environmental variables used to characterise each habitat type, and the Bray-Curtis similarity matrix constructed from the mean densities of the nematode

species recorded at each habitat type. This test was carried out for nematofaunal data that had been averaged over all seasons, and also for that data recorded in each season separately. Spearman rank correlation (ρ) was employed as the matching co-efficient in the procedures and the H_0, that there is no match in the rank order arrangement of samples between pairs of matrices, was rejected if P was < 0.05.

6.1.3 Results

A total of 119 species of nematodes comprised the nematode faunas in the sediments of the two regions of the Swan River Estuary and the nearby marine region, with only 8 of those species which made significant contributions to the fauna being recorded in all three regions (Table 6.1). A further three species were present in all three regions, but only occurred in very low densities. Seventy five nematode species were found in marine sediments, of which 57 were not found in estuarine sediments. The lower and upper estuary regions were each inhabited by 58 and 38 species, respectively, 18 and 3 of which were restricted to each of these regions. The suite of the five most abundant species in each region showed no overlap with the equivalent suite for the other two regions. The average density of nematodes varied greatly among regions, with that in the marine sediments being less than 1/10 of that in the lower estuary and only 1/4 of that in the upper estuary (Table 6.1 and Figure 6.1).

6.1.3.1 Differences in species richness and density among regions

PERMANOVA demonstrated that both species richness and density of nematodes varied significantly among regions, sites and seasons and that all interactions between those main effects were also significant (P = 0.001). However, for both variables, the component of the overall variation attributable to region was at least twice as great as that for any other main effect or interaction. Both number of species and density of nematodes were lowest in the marine region and greatest in the lower estuary in every season, with only minor differences in the magnitude of these trends being responsible for the small but significant region x season interaction in each case.

	Marine			Lower Estuary			Upper Estuary		
	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk	$\overline{X}$	%	Rk
Paracomesoma sipho	11.1	12.7	1	-	-	-	-	-	-
Daptonema sp 1	9.9	11.3	2	-	-	-	-	-	-
Mesacanthion sp nov	8.4	9.6	3	2.4	0.3	25	-	-	-
Theristus sp 3 (marine)	6.3	7.2	4	-	-	-	-	-	-
Gonionchus australis	5.9	6.8	5	-	-	-	-	-	-
Spirinia parasitifera	-	-	-	268.0	29.7	1	8.8	2.6	11
Dichromadora sp 2	-	-	-	105.8	11.7	2	17.1	5.0	6
Theristus sp 2	-	-	-	98.7	10.9	3	4.2	1.2	16
Chromadorina sp 1	0.9	1.1	21	52.8	5.8	4	3.4	1.0	19
Metadesmolaimus sp 1	0.6	0.7	28	46.2	5.1	5	7.7	2.3	12
Theristus sp 1	-	-	-	5.9	0.6	16	143.6	42.1	1
Bathylaimus australis	1.6	1.8	15	32.4	3.6	10	23.0	6.7	2
Metalinhomeous	2.0	2.3	11	5.5	0.6	17	21.2	6.2	3
Pierrickia sp nov	-	-	-	2.8	0.3	22	20.8	6.1	4
Metadesmolaimus sp 2	-	-	-	2.1	0.2	26	18.1	5.3	5
Parodontophora sp nov	0.0	0.0	62	0.0	0.0	58	12.6	3.7	7
Nanolaimoides decoratus	-	-	-	45.9	5.1	6	12.1	3.5	8
Pontonema	-	-	-	20.5	2.3	12	9.8	2.9	9
Viscosia glabra	-	-	-	43.1	4.8	8	9.3	2.7	10
Gomphionema typicum	0.4	0.4	34	6.3	0.7	15	5.6	1.6	14
Parascolaimus sp nov	0.4	0.5	30	0.1	0.0	52	5.4	1.6	15
Terschillingia	-	-	-	25.5	2.8	11	3.8	1.1	17
Halichoanolaimus quatrodecimpapillatus	-	-	-	33.7	3.7	9	3.7	1.1	18
Comesoma arenae	-	-	-	44.8	5.0	7	1.0	0.3	21
Neochromadora sp 1	4.4	5.1	7	8.3	0.9	14	0.1	0.0	30
Pseudochromadora cazca	-	-	-	13.1	1.5	13	-	-	-
Dichromadora sp 1	4.5	5.1	6	-	-	-	-	-	-
Chromadorita sp 1	3.1	3.5	8	-	-	-	-	-	-
Marylynnia annae	2.7	3.1	9	-	-	-	-	-	-
Thalassironus sp 1	2.6	3.0	10	-	-	-	-	-	-
Rhabditis sp 1	1.9	2.2	12	-	-	-	-	-	-
Microlaimus sp 1	1.6	1.9	13	-	-	-	-	-	-
Onyx sp nov	1.6	1.8	14	-	-	-	-	-	-
Parapinnanema sp 1	1.4	1.6	16	-	-	-	-	-	-
Pomponema sp 1	1.2	1.4	17	-	-	-	-	-	-
Catanema sp nov	1.2	1.3	18	-	-	-	-	-	-
Viscosia sp 1	1.1	1.2	19	-	-	-	-	-	-
Graphonema sp 2	1.0	1.1	20	-	-	-	-	-	-
Total mean density (no. 10 cm^{-2})		87.4			903.1			340.7	
Total number of species		75			58			38	

Table 6.1 The mean density ($\overline{X}$; numbers 10 cm^{-2}), percentage contribution (%) and rank by abundance (Rk) of the nematode species recorded in the marine region and the upper and lower estuarine regions. The five most abundant species in each region have been shaded. Only those species which contributed greater than 1 % to the total number of individuals in any region have been presented.

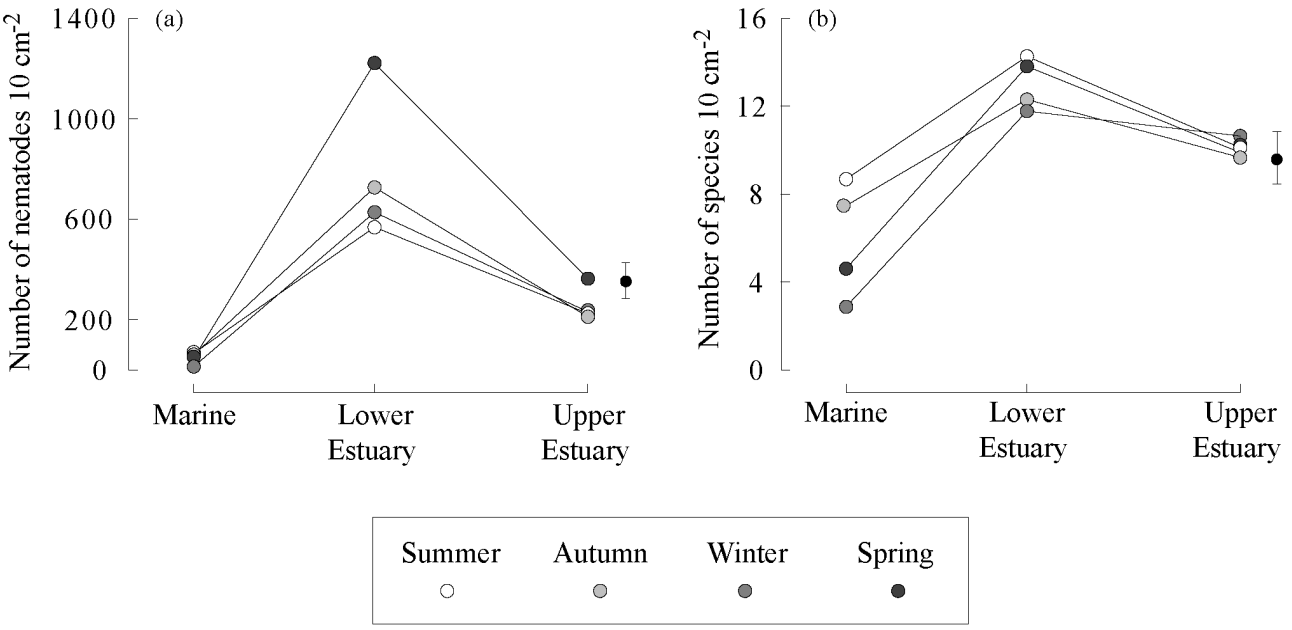

Figure 6.1 Mean values for (a) number of nematode species and (b) density in each region in each season. For the sake of clarity the overall mean ± 95 % confidence interval for each variable is shown to the right of its respective plot.

6.1.3.2 Differences in nematofaunal compositions among regions

Species level

PERMANOVA demonstrated that the species composition of the nematode assemblages differed significantly among all main effects and interaction terms. However, as described above for density and species richness, the regional factor explained the majority of the variability among samples, accounting for more than twice that of the next most important factor (site). Pair-wise comparisons between each pair of regions demonstrated that the composition in each region was significantly different from that in each of the other two regions ($P = 0.001$), and that the assemblages from the two estuarine regions were more similar to each other than either was to that of the marine region.

The nMDS ordination plot, constructed using the mean densities of species recorded at each site in each season, clearly illustrated that the compositions of the samples from the two estuarine regions were far more similar to each other than those of the marine region (Fig. 6.2a). Furthermore, the points for the samples from each of the estuarine

regions formed much tighter groups than those from the marine region. The same trends were evident on the ordination conducted on the species level data after the densities in each sample had been standardised (Fig. 6.2b).

SIMPER determined that the nematode assemblages in mean samples from the two estuarine regions were discriminated from each other primarily by the species *Spirinia parasitifera* and *Theristus* sp. 2, both of which occurred in greater densities in the lower estuary, and also *Theristus* sp. 1, which was more common in the upper estuary. These three species were also identified in previous analyses as responsible for discriminating between assemblages at sites from the lower and upper estuary (e.g Subsection 3.3.4). Comparisons between the assemblages in the marine region *vs* the upper and lower estuarine regions demonstrated that there was almost an almost complete divergence in species composition. Thus, the ten species which most consistently distinguished the assemblages found in the two estuarine regions from that in the marine regions were all either totally absent from, or much less abundant in, marine sediments. The most important of those species were *Spirinia parasitifera*, *Theristus* sp. 2 and *Halichoanolaimus duodecimpapillatus* for the lower estuarine region, and *Theristus* sp. 1 and *Dichromadora* sp. 1 for the upper estuarine region.

Higher taxonomic levels

Analysis of the nematode assemblages in the marine and two estuarine regions at the generic and family levels showed very similar trends to those exhibited at the species level. Thus, PERMANOVA again demonstrated that the region, site and season main effects and their interactions were all significant (P = 0.001- 0.002), and that region was still by far the most important factor, followed by site within regions. Pair-wise ANOSIM comparisons showed that the assemblages in the marine region differed more from those in both of the estuarine regions at both generic (R-statistic = 0.802-0.835) and family (R-statistic = 0.664-0.709) levels than did they did between the two

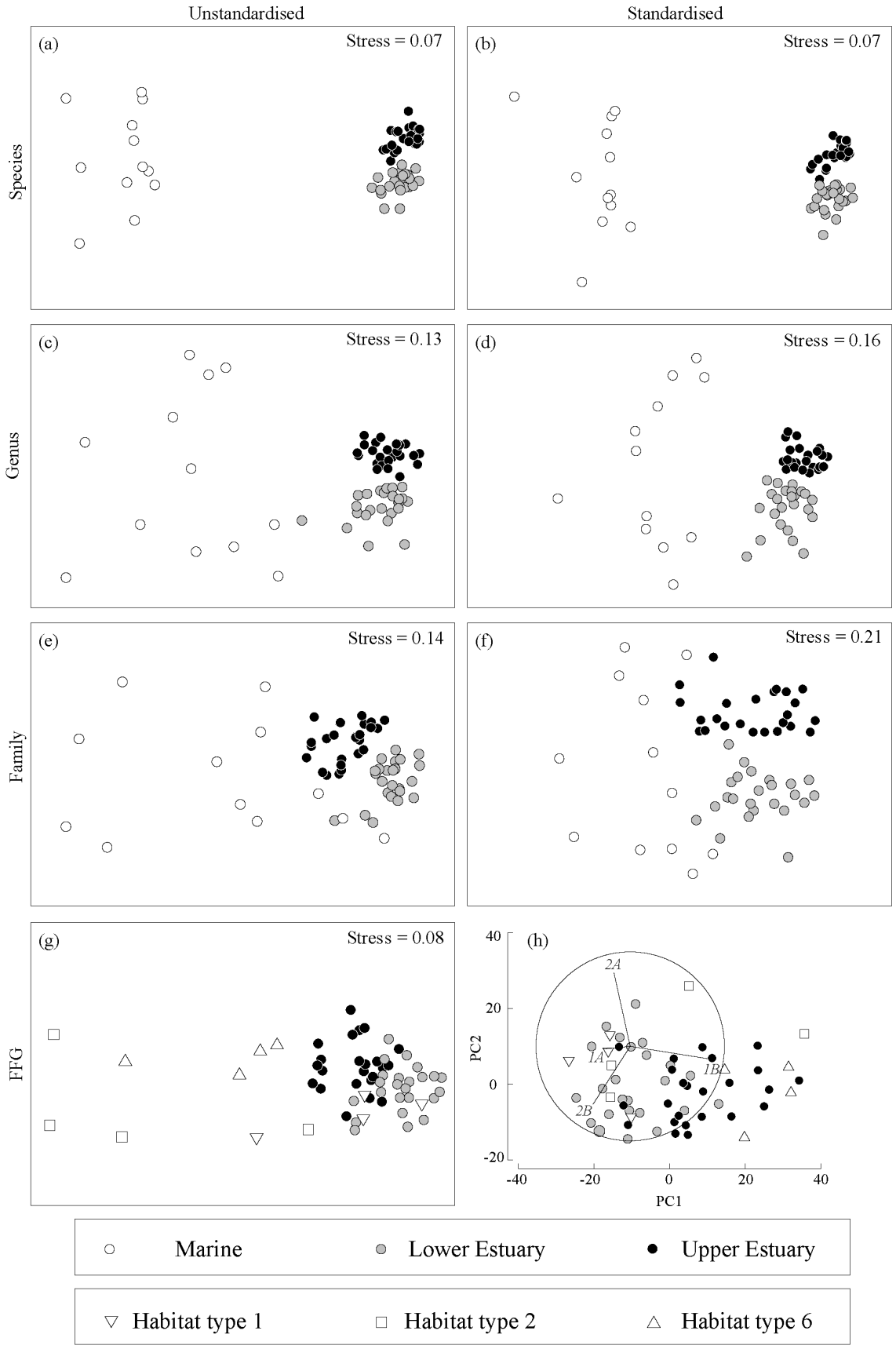

Figure 6.2 Two-dimensional nMDS ordinations of the mean densities of the various nematode taxa at each site in each season in marine and estuarine sediments, coded for region. The nematode taxa were represented by the species, genus, family and FFG levels on plots (a), (c), (e), and (g), respectively. Ordinations based on standardised data, representing species, genus and family taxonomic levels are shown in (b), (d), and (f), respectively. (h) PCA plot constructed from the standardised densities of the four FFGs, coded for season, and in the case of marine samples, site.

estuarine regions (genus R-statistic = 0.477; family R-statistic = 0.475). ANOSIM at the family level determined that the compositions of the assemblages in the marine regions were slightly more similar to those of the upper estuary than the lower estuary. However, differences among regions were still very large even at that highest taxonomic level.

The nMDS ordinations derived from the generic (Fig. 6.2c) and family (Fig. 6.2e) level assemblage data both show that the points representing each region formed relatively discrete groups, but the extent of that distinction decreases with increasing taxonomic level. Thus, the nMDS ordination of generic level data exhibited a similar arrangement of points to that of the species level data, with those points for the upper and lower estuary regions forming tight groups towards the upper right and lower right of the plot, respectively, and well to the right of those from the marine region (Fig. 6.2c) Samples from the marine region were still widely dispersed, but located closer to those of the estuarine regions than was the case on the ordination derived from species level data (*c.f.* Figs 6.2a, c). Likewise, the ordinations on the family level ordination plot (Fig. 6.2e) showed similar patterns but with samples being less tightly clustered. The plots constructed from standardised data at the generic (Fig. 6.2d) and family (Fig. 6.2f) levels still showed that the groups of points from each of the three regions were distinct and that the degree of distinction decreased with increasing taxonomic level. Furthermore, the samples from the lower and upper estuary were noticeably more dispersed on the family level plot than on previous plots (*c.f.* Figs 6.2f *vs* a-e).

At the genus level, SIMPER identified that *Spirinia*, *Comesoma*, *Halichoanolaimus* and *Chromadorina* were all much more abundant in the lower than upper estuary, while *Theristus* was slightly more abundant in the upper than lower estuary. Much greater densities of *Spirinia, Theristus, Metadesmolaimus* and *Halichoanolaimus* consistently discriminated samples from the lower estuary from those in the marine region, while

Theristus, Metadesmolaimus, Pierrickia and *Dichromadora* were all more abundant in the upper estuary than marine region. At the family level, SIMPER identified that the Desmodoridae and Chromadoridae were more abundant in the lower estuary, while the Xyalidae were slightly more abundant in the upper estuary. The comparison between lower estuarine and marine regions demonstrated that the Desmodoridae, Xyalidae, Chromadoridae and Oncholaimidae were all far more abundant in the former region. Representatives of the Xyalidae and Oncholaimidade were also more prevalent in the upper estuary than in the marine region. In contrast, the Chromadoridae were more abundant in marine than upper estuarine sediments.

Functional Feeding Groups

When the mean contributions of the four FFGs at each site in each season were subjected to PERMANOVA, the results were remarkably similar to those described above for each of the three taxonomic levels. *i.e.* all main effects and interactions were significant ($P < 0.005$) and, of those factors, region accounted for by far the greatest portion of the total variation. Pair-wise ANOSIM comparisons showed that significant differences in the FFG composition existed between all pairs of regions, with the lower *vs* upper estuary being most similar and the lower estuary *vs* marine being the most divergent.

The nMDS ordination constructed from the average densities of the four functional feeding groups at each site in each season shows essentially the same trends as those produced from the density data that had been aggregated to family level (*c.f.* Figs 6.2e, g). Thus, the upper and lower estuary samples formed groups on the right of the ordination plot, with only minimal overlap, while the samples from the marine waters were highly dispersed and intermingled to some extent with those from the lower estuarine region. To further elucidate the relationships between those intermingling points, the points for the marine samples were coded for their constituent sites (see

Chapter 2). Thus, the marine points that intermingled with the lower estuary points all belonged to habitat type 1. SIMPER identified that the densities of all four FFGs were greater in estuarine than marine samples.

In order to further elucidate the relative importance of the various FFGs to the assemblages in each region, the density data were standardised, then subjected to PCA (Fig. 6.2h). Principal components 1 and 2 encompassed 85 % of the total variation among samples, and the resultant plot demonstrated that the groups of samples from the upper and lower estuarine regions remained relatively distinct from each other , while those from the marine region were scattered across the majority of the plot (Fig. 6.2h). When the marine samples were coded for the three constituent habitat types, those from habitat type 1 were found to be interspersed with the samples from the lower estuary sites while those from the habitat type 6 intermingled with those from the upper estuary The 1B FFG had the strongest (positive) relationship with PC1 thus demonstrating that samples from the upper estuary, and habitat type 6 in the marine region contained the highest proportion of this FFG. The majority of the variation along PC2 was attributable to the 2A and 2B, which showed positive and negative relationships, respectively. Variation along this PC largely reflected differences in FFG composition among seasons (data not shown).

6.1.3.3 Relationships between nematode assemblages and habitat types

Two-way crossed site x season ANOSIM demonstrated that the composition of the nematofaunal assemblages within the Swan River Estuary differed significantly between all pairs of sites ($P = 0.001$). However, the R-statistics for comparisons between sites assigned to the same habitat type ranked among the 10 smallest out of 66 comparisons in four out of the five cases, *i.e.* 0.304 (C), 0.371 (R), 0.509 (Q) and 0.683 (O). In contrast, that for habitat type L was considerably higher (0.929). Therefore, intra-habitat type differences in nematofaunal composition were almost invariably smaller than inter-habitat type differences.

The nMDS ordination plot constructed from the mean of densities of nematode species recorded at each site in each season is the same as that presented in Chapter 3 (Fig. 3.5b), but with each sample coded for habitat type rather than site (Fig. 6.3). Distinct groupings on the basis of habitat type can be clearly discerned, particularly at the upstream habitat types O, Q and R and, to a lesser extent, the most downstream habitat type (C).

The two-way crossed site x season SIMPER that was presented in Chapter 3, has also been employed in this chapter to elucidate which species were most responsible for distinguishing the various habitat types. Thus, *Comesoma arenae*, *Bathylaimus australis*, *Metalinhomeous* sp. and *Pontonema* sp. 1were particularly characteristic of the faunas at habitat types C, O, R and Q, respectively.

RELATE demonstrated that the pattern of spatial differences among the enduring environmental characteristics of the seven habitat types was significantly correlated with that of the nematode assemblages ($P = 0.001$), and that those correlations were very strong, both overall ($\rho = 0.806$), and in each season ($\rho = 0.700$-0.834).

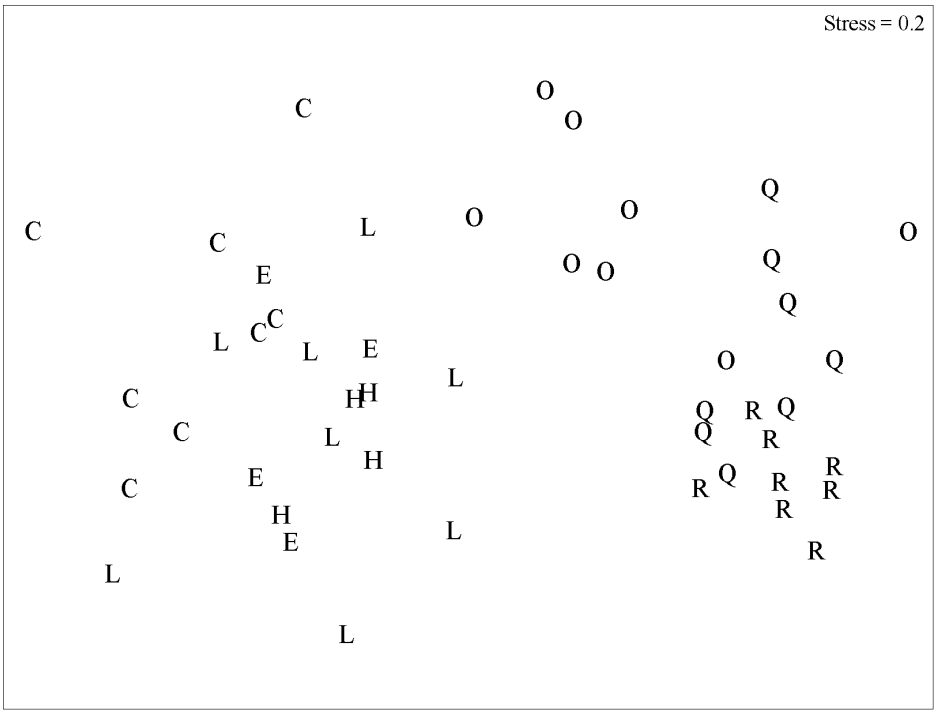

Figure 6.3 Two-dimensional nMDS ordination of the similarity matrix constructed from the mean densities of the various nematode species at sites 1-12 in the Swan River Estuary in each season between summer 2004/5 and spring 2005, coded for habitat type.

6.1.4 Discussion

6.1.4.1 Differences in nematode assemblages among marine and estuarine regions

The total number of nematode species found throughout the year in nearshore marine sediments along the lower west coast of Australia was 75, compared with only 68 and 38 species in the lower and upper regions of the Swan River Estuary, respectively. Thus, the hypothesis tha species richness is higher in marine than estuarine regions has been confirmed. However, fewer species were present, on average, in each replicate core collected from marine sediments (5) than in those from either the lower (13) or upper (10) estuarine regions. The apparent dichotomy between the total species richness *vs* the average species richness reflects the fact that the compositions of the marine assemblages are much more variable than those in the estuarine areas and the lower average abundances. The densities of nematodes exhibited the hypothesised trend,

i.e. they were considerably lower in the marine environment than in both the lower and upper estuary.

The compositions of the nematode assemblages in the three regions were all significantly different from each other at all levels of taxonomic resolution, *i.e.* species, genus and family. Indeed the patterns of the relationships among samples collected from the different regions were remarkably similar at all three taxonomic levels assemblages thus paralleling the conclusions of Herman & Heip (1988) and Warwick (1988) for meiofaunal and macrofaunal assemblages, respectively who found that a large proportion of assemblage structure was apparent even at higher taxonomic levels. However, the expected sequential change in assemblage composition from upper estuary, to lower estuary, then to the marine region was not observed. Indeed, the composition of the nematofaunal assemblages in marine samples were very divergent, and approximately equally so, from those of the two estuarine regions at all levels of taxonomic aggregation and for both unstandardised and standardised data. Furthermore, the differences between the assemblages in marine and estuarine sediments were much greater than between those of the two estuarine regions, even though those latter two regions span salinities from freshwater all the way to almost marine. Thus, the finding that the nematode faunas in the estuarine regions are more similar to each other than to that of the marine region is similar to that found for density, but is contrary to the hypothesis that variation in nematode assemblages will be greater across the breadth of samples from the estuarine than marine environment

At the finest taxonomic resolution, the marked distinction among regions is due to very few of the species occurring in all three, or even in two of the three locations. However, the presence of similar divisions at the generic and family levels indicates that there are fundamental differences among the assemblages. The fact that such trends are still evident even when standardised data were employed, demonstrates that these

assemblage differences are not just caused by the dramatic differences in species densities. In terms of family-level composition, samples from the upper estuary tended to be distinguished from the lower estuary and marine sites by greater abundances of Xyalidae, while the marine region had large amounts of Chromadoridae compared with the upper estuary. The lower estuary was still distinct from the upper estuary and marine regions through its greater densities of most families. However, samples from this region were distinguished by relatively high densities of Chromadoridae, Xyalidae, Desmodoriae and Oncholaimidae even though the former two taxa also characterised the upper estuarine sites

It is apparent from the nMDS ordinations of data at all taxonomic levels that, in addition to being very divergent from the estuarine samples, the nematode assemblages in samples from the marine region are far more widely dispersed. Even when the data were aggregated to the family level and standardised to remove the effect of density, the dispersion of points for marine samples was considerably greater than for both estuarine regions combined.

A possible explanation for this finding is that the combination of greater overall species richness and lower density has resulted in assemblages in the marine environment which are very patchy. Thus, the comparatively few individuals found in the marine environment belong to such a diverse range of taxa that, even even among replicate cores collected at the same location and time, there are considerable differences in species composition. In comparison, the relatively lower total species richness coupled with the much greater densities found in samples from the estuarine regions, means that each species is represented by far more individuals and thus the assemblages are much less variable.

The dichotomy between marine and estuarine assemblages was still evident when densities of the functional feeding groups at each site in each season were analysed. However, when standardised, the division between marine and estuarine samples was no longer evident while the upper *vs* lower estuary distinction was still very apparent.

The arrangement of the points on the PCA plot for the four FFGs shows that the nematode assemblage at the most sheltered marine habitat type (1) has a similar trophic composition to that of the lower estuary, *i.e.* the assemblages in both regions contain significant proportions of epistrate grazing (2A) species. This is likely to be due to the fact that both locations are relatively shallow, have low wave energy and mostly marine environments which promotes the growth of microphytobenthos, the preferred food source of this trophic group (Fielding *et al.*, 1988). The fact that the trophic composition of the assemblages at the highest energy marine habitat type (6) is most similar to that in the upper estuarine region is unusual since these two locations are the most divergent in their physico-chemical characteristics. Both locations contained relatively large proportions of the non-selective (1B) FFG, which indicates that detrital material and microorganisms are likely to be the dominant food source available at both of these locations. Such a convergence is likely to be a consequence of the inhibition of benthic primary productivity in both of these regions. Thus, in the high energy marine environment, growth of microphytobenthos is reduced by the constant scouring and resuspension of the sediment, whereas in the turbid and tannin-stained conditions, found in the upper Swan River Estuary, light penetration is likely to be the limiting factor (Fejes *et al.*, 2005; Loiselle *et al.*, 2007).

6.1.4.2 Relationships between nematode assemblages and habitat types

The compositions of the nematode assemblages were generally found to exhibit greater significant differences between sites assigned to different habitat types, than between sites assigned to the same habitat type, *i.e.* inter-habitat type differences were greater

than intra-habitat type differences. However, the fact that the nematode assemblages exhibited significant, albeit smaller, inter-site differences within the habitat types considered environmentally homogeneous indicates that there are factors which affect the composition of these assemblages that are not represented in the classification.

RELATE demonstrated that the pattern of spatial differences among the enduring environmental characteristics used to define the habitat types was significantly and extremely well matched with that exhibited by the nematofauna, both overall and in each season. Such findings provide sound evidence that such a classification scheme could be used to reliably predict the composition of the nematode assemblage that are likely to be found at any site in the Swan River Estuary.

In summary, the habitat classification scheme developed for the Swan River Estuary captures a considerable portion of the environmental variability, which affects the composition of the nematode assemblages. This is evidenced by the significant differences among assemblages at different habitat types as well as the significant and close match between the patterns of environmental and assemblage characteristics. However, it must also be recognised that the classification does not fully encompass all of relevant environmental variability, which is responsible for the finer level of assemblage variability at the intra-habitat type scale.

6.2 General conclusions

The body of work contained in this thesis has contributed to three major areas of the field of meiofaunal nematology.

Firstly, this thesis has contributed to the knowledge of the ecology of free-living aquatic nematodes in microtidal marine and estuarine environments (Chapters 2, 3 and 6), employing a dataset, that is spatially and temporally comprehensive. This has allowed detailed and robust univariate and multivariate statistical analyses to ascertain the nature of the spatial and temporal differences in the characteristics of the nematofauna in these environments. Analyses of spatial and temporal factors were conducted in a concurrent manner and were integrated with investigations of relationships between characteristics of the biota and environment.

Secondly, this thesis has provided taxonomic descriptions of ten new nematode species (Chapter 5). The free-living nematode assemblages have been very poorly studied in Western Australia and thus these new descriptions represent a substantial contribution to the study of these neglected benthic communities. Furthermore, whereas the only previous taxonomic studies in this region provide descriptions from opportunistically collected samples (*i.e.* Inglis, 1969; 1970; 1971), the present study incorporates samples from a diverse range of aquatic habitats and a full suite of seasons, thus providing a much broader inventory of the local taxa.

Lastly, the use of aquatic habitat classification systems to predict spatial differences in biotic assemblages have typically been in relation to assemblages such as fish and occasionally macroinvertebrates, but to date, not to meiofaunal or nematode assemblages. The studies contained in this thesis have not only constructed a robust and useful habitat classification system for microtidal estuaries, but have demonstrated its use for predicting spatial differences in highly habitat-specific nematode assemblages.

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