UNIVERSITY OF CALIFORNIA
RIVERSIDE

Nematode Ecological Study Of A Wetland Restoration-In-Progress At Bolsa Chica, California: Implications For Biomonitoring

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology and Organismal Biology

by

Ian William King

December 2011

Dissertation Committee:
Dr. Paul De Ley, Chairperson
Dr. Michael Allen
Dr. William Walton

The Dissertation of Ian William King is approved by:

Committee Chairperson

University of California, Riverside

ACKNOWLEDGEMENTS

My thanks go first and foremost to my supervisor, Dr. Paul De Ley. His guidance, insight, encouragement and unfailing good humour were essential to the completion of this dissertation.

After serving as Oral Qualifying Exam Committee members, Drs. Michael Allen and William Walton kindly agreed to continue as Dissertation Committee members. I'd like to thank them for their help in both capacities.

Various members of the De Ley lab were always generous with their time and help. I'd like especially to thank Dr. Irma Tandingan De Ley, and three undergraduate assistants – Allison Bistline, Jennifer Chung and Cymphonee Robinson.

Thanks to the members of the Baldwin lab. Dr. James Baldwin was always willing to provide assistance in any way he could, as both a colleague and a department chair, as well as making the unforgettable "Handel Joke". I appreciate the guidance and friendship that came from hours spent together at the microscope with Dr. Manual Mundo-Ocampo. Thanks to Dr. Erik Ragsdale and Stephanie Samtoy for their friendship and stimulating conversations, and most importantly, for introducing me to "Los G's". Tiago Pereira has helped me understand aspects of nematode ecology through our conversations, fuelled by his generous supply of coffee.

My sincere thanks go to Kelly O'Reilly (California Department of Fish and Game) for furnishing access to Bolsa Chica, and for giving me a better understanding

of the wetlands through our conversations. Grace Adams and her excellent staff at the Bolsa Chica Conservancy were very helpful, and gave me an annual opportunity to remind myself why I got into Nematology by allowing me to present a display at their Earth Day festivities.

I'd like to thank Dr. Andrew Brooks, director of the Carpinteria Salt Marsh Reserve, for granting me permission to collect samples there, and for his enthusiastic encouragement of my research.

I would like to acknowledge the generous support I received from the California Sea Grant in the form of traineeship.

Personally, my sincere thanks go to John Reynolds for the conversations, by email or phone, which were not always (not even usually) about science. My mother Isabelle and step-father Dwight, and my father William and step-mother Angela, have provided unending support for which I am very grateful.

Finally, all my thanks and love to my wife, Olya. Her encouragement ("Ni puha, ni pera!") and selfless devotion to taking care of our daughter Victoria were essential in allowing me to finish. I dedicate this dissertation to them both.

ABSTRACT OF THE DISSERTATION

Nematode Ecological Study Of A Wetland Restoration-In-Progress At Bolsa Chica, California: Implications For Biomonitoring

by

Ian William King

Doctor of Philosophy, Graduate Program in
Ecology, Evolution and Organismal Biology
University of California, Riverside, December 2011
Dr. Paul De Ley, Chairperson

The meiofauna is abundant and diverse, occupying key roles in sediment food webs and nutrient cycling, and responds rapidly to environmental changes. Nematodes and copepods usually constitute the major components of the meiofauna. As well as being a large part of the meiofauna, nematodes are the most abundant and most diverse invertebrates in many sediments and soils. They occupy a wide variety of ecological roles in sediments, and their community structure provides important clues to the structure of benthic foodwebs, as well as to responses of sediment ecosystems to environmental changes such as pollution and changes in tidal range.

Despite the advantages of using nematodes for biomonitoring, they are used less frequently than other groups due to some major limitations, including the so-called “taxonomic impediment”. Some aspects of this impediment are illustrated via the description of new free-living nematode species.

One limitation is the scarcity of expert marine nematode taxonomists and the concentration of these experts mainly in Europe. The present study is the first to investigate Californian coastal nematodes to address environmental concerns in California, specifically the restoration-in-progress of the Bolsa Chica wetland.

In common practice, nematode studies usually refrain from analyzing species-level differences between sites and communities, settling instead for genus or family level identifications by way of more feasible compromise. This choice is made perforce by the challenges of working with nematodes, and meiofauna in general. Part of the present study examined whether this choice is justified in the case of free-living marine nematodes.

New molecular methods present opportunities to improve biomonitoring. Whole DNA extracts from a sediment core can be subjected to massively parallel pyrosequencing of taxonomically distinctive DNA loci, producing in one procedure a comprehensive list of the great majority of interstitial invertebrates living in that sediment across most animal phyla. Determining how the detailed and relatively robust phylogenies created with these new molecular tools can be incorporated in the study of biodiversity is an important research area, as biodiversity is the main facet of biomonitoring.

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CHAPTER 1: GENERAL INTRODUCTION

Biomonitoring, in its most liberal definition, is as old as humanity. Early humans would have been keenly aware of changes in animal and plant communities, especially those that were part of their prey and food supply, and they may have been able to make some tenuous connections between these changes and their changing environment. However, these early humans (and indeed many modern ones) would have been completely unaware of the changing populations of microscopic organisms under their feet, and what can be learned by scientific study of these sentinels.

The meiofauna is a varied group of organisms, defined based on size. In practice, it includes organisms that can pass through a sieve with 1 mm openings, but are retained on sieve with 45 μm openings (Higgins & Thiel 1988). The meiofauna is abundant and diverse, occupying key roles in sediment food webs and nutrient cycling (Baguley et al. 2008), exerting control over benthic microalgae and responding rapidly to for example pollutants (Carman et al. 1997, Danovaro 2000) or algal blooms (Garcia & Johnstone 2006). Nematodes and copepods usually constitute the major components of the meiofauna. As well as being a large part of the meiofauna, nematodes are the most abundant and most diverse invertebrates in many sediments and soils. They occupy a wide variety of ecological roles in sediments, feeding on many smaller organisms as well as being a major component

of the diet of benthic macrofauna and larval fish. Their community structure provides important clues to the structure of benthic foodwebs, as well as to impacts on sediment ecosystems of environmental changes such as pollution and changes in tidal range.

Although meiofaunal organisms in general, and free-living nematodes specifically, are not economically or esthetically valuable in their own right, they are nevertheless of great practical and scientific importance because their abundance and diversity are key to the functioning of aquatic ecosystems as a whole, and can be used to indicate changes that affect those organisms which are of direct economical or aesthetic value. Although meiofauna cannot be directly observed in the field and are typically taxonomically more challenging than macrofauna, some of the particular advantages of meiofaunal monitoring include the following .

- marine nematodes can be studied even in conditions of very extreme disturbances, because some species are much more resistant (for example to toxic pollutants) than all macrofauna and most other meiofauna,

- much smaller sediment samples are sufficient for analysis, minimizing physical disruption of the study site and allowing more easily for continued monitoring,

- the shorter generation times of meiofauna allow community structure to be observed even in short-term studies,

– meiofaunal assemblages are more stable on a seasonal or annual basis, reducing the incidence of potentially confounding fluctuations due to for example unusual weather conditions.

Despite the advantages of nematode diversity analyses for biomonitoring, meiofaunal analysis is conducted less frequently than macrofaunal monitoring. A major constraint is that marine nematologists are few in number, and as a result published community analyses of marine nematodes are largely restricted to those geographical regions with resident expertise. Nevertheless, over 150 studies were published worldwide in the past thirty years. The majority among these have focused on the northern Atlantic Ocean (especially the Baltic and North Sea), with more occasional studies in the Mediterranean, the Gulf of Mexico, the western and central Pacific and the Indian Ocean (for example Austen & Somerfield 1997; Garcia & Johnstone 2006; Gheskiere et al. 2005a,b; Gyedu-Ababio et al. 1999; Ingole et al. 2005; Lambshead et al. 2001; Lambshead et al. 2002; Lampadariou et al. 2005; Montagna & Harper 1996). By comparison, only a handful of studies have investigated the eastern Pacific (in the past decade only seven: Debenham et al. 2004; Gómez Noguera & Hendrickx 1997; Neira et al. 2001; Shanks & Walters 1997; Thistle et al., 1999; Vanreusel et al. 1997; Vopel & Thiel 2001). The relevance and versatility of nematode community analysis as an environmental monitoring technique has already been demonstrated in a wide range of oceanic and coastal management settings, including for example impact of heavy metals (Austen &

Somerfield, 1997; Gyedu-Ababio et al., 1999); oil drilling (Montagna & Harper, 1996); oil spills (reviewed in Danovaro, 2000); fish farming (Lampadariou et al., 2005); sewage (Hewitt & Mudge, 2004); tourism (Gheskiere et al., 2005a); benthic storms (Lambshead et al., 2001); deep-sea digging (Ingole et al., 2005) and coastal algal blooms (Garcia & Johnstone, 2006). None of these has investigated Californian coastal nematodes or addressed environmental concerns in California, such as the maintenance or restoration of the remaining wetlands (**Chapter 2**).

The identification of free-living marine nematodes by traditional microscopy methods is stymied by the lack of detailed monographs and species diagnoses, as well as the frequency with which new/undescribed species are encountered (**Chapter 5**). This novelty is part of the "taxonomic impediment" of working with nematodes. In addition to this is their sheer diversity (Lambshead 2004), the common occurrence of cryptic species (Rocha-Olivares et al. 2004, Derycke et al. 2007), as well as very limited numbers of experienced taxonomists and lack of comprehensive identification aids geared towards non-experts. Therefore, in practice, nematode studies usually refrain from analyzing species-level differences between sites and communities, settling instead for genus or family level identifications by way of more feasible compromise. This choice is imposed by the challenges of working with nematodes in particular, and meiofauna in general. Few studies have examined explicitly whether this choice is justified however, particularly in the case of free-living marine nematodes (**Chapter 3**).

New molecular methods now present the possibility of reducing the abovementioned limitations. Whole DNA extracts from a sediment core can be subjected to massively parallel pyrosequencing of taxonomically distinctive DNA loci, producing in one procedure a comprehensive list of the great majority of interstitial invertebrates living in that sediment across most animal phyla (Porazinska et al., 2009; Creer et al., 2010). Relatively comprehensive DNA phylogenies now exist for nematodes (van Megen et al., 2009). Although an inordinate number of species remain to be sequenced for molecular barcode data, the availability of detailed and relatively robust phylogenies allows us to pinpoint the approximate species relationships of almost any new nematode sequence obtained from metagenomic analysis, even if the majority of species in the sample were not previously represented in molecular databases (De Ley et al., 2005). Determining how the phylogenies created with these new molecular tools can be incorporated in the study of biodiversity is an important research area, as biodiversity is the main facet of biomonitoring (**Chapter 4**).

CHAPTER 2: BIOMONITORING OF THE BOLSA CHICA WETLAND RESTORATION-IN-PROGRESS BY USE OF FREE-LIVING NEMATODE ASSEMBLAGES

Introduction

The Bolsa Chica Wetlands, near Huntington Beach, California, are subject to the one of the largest wetland restoration projects in Californian history (Carlberg 2009). Like any restoration project, in addition to measuring physico-chemical aspects, clearly defined biological monitoring protocols are necessary to gauge the progress of the restoration. In the case of Bolsa Chica, these biological protocols (U.S. Fish & Wildlife Service 2001) focused on the important components of fish, birds and vegetation. The benthic sampling was designed such that it included only those macrobenthic and epifaunal organisms considered important food sources for birds and bottom-feeding fish (U.S. Fish & Wildlife Service 2001). The present study was undertaken, in part, to supplement this monitoring plan by studying free-living nematodes.

Free-living nematodes in general, and marine nematodes in particular, have many advantages as biomonitoring agents (Schratzberger et al. 2000a). Marine nematodes can be studied even in conditions of very extreme disturbances, because some species are much more resistant (for example to toxic pollutants) than all macrofauna and most other meiofauna. Since much smaller sediment samples are sufficient for analyses, physical disruption of the study site is minimized, allowing more easily for continued monitoring. Another advantage is that the shorter generation times of marine nematodes allow community structure to be observed

even in short-term (6-12 months) studies. As well, marine nematode assemblages are more stable on a seasonal or annual basis, reducing the incidence of potentially confounding fluctuations due to for example unusual weather conditions.

The relevance and versatility of nematode community analysis as an environmental monitoring technique has already been demonstrated in a wide range of oceanic and coastal management settings, including for example impact of heavy metals (Austen & Somerfield, 1997; Gyedu-Ababio et al., 1999); oil drilling (Montagna & Harper, 1996); oil spills (reviewed in Danovaro, 2000); fish farming (Lampadariou et al., 2005); sewage (Hewitt & Mudge, 2004); tourism (Gheskiere et al., 2005a); benthic storms (Lambshead et al., 2001); deep-sea digging (Ingole et al., 2005) and coastal algal blooms (Garcia & Johnstone, 2006).

Less common is the use of nematodes for monitoring environmental restoration. Most of the studies that have examined this have been in terrestrial settings (Biederman et al. 2008, Todd et al. 2006, Villenave et al. 2001). Ettema et al. (1998) looked specifically at bacterivorous nematodes in a restored freshwater riparian wetland. Studies published to date of nematodes from estuaries and wetland have not been concerned with restoration sites. Nevertheless, Schratzberger and colleagues have published a series of papers examining nematode response to various factors of direct relevance to estuarine and wetland restoration, including for example the effects of physical disturbance on nematode communities in sand and mud (Schratzberger & Warwick 1998), the effects of simulated deposition of dredged material on the structure of nematode

communities (Schratzberger et al. 2000b, 2000c), and the colonization of various types of sediment by estuarine nematodes (Schratzberger et al. 2004). Although these studies have all been laboratory and microcosm based, they have provided some insight in the practical application of using free-living nematode diversity analyses to biomonitor estuarine restoration.

The objective of this paper then is to apply the methods of biomonitoring using free-living nematodes to the restoration-in-progress of the Bolsa Chica Wetlands.

Material and Methods

Study areas

Bolsa Chica Ecological Reserve

The Bolsa Chica Wetlands (33°70′31″ N, 118°05′73″ W) is located in Huntington Beach, California, and originally covered about 2600 acres of tidal wetlands, mudflats, sand dunes, as well as salt and fresh water marshes (Figure 2-1). Its history in written archives started as part of a much larger land grant under Spanish ownership in 1784, followed by subdivisions into multiple Ranchos during Mexican rule in 1834, including two cattle ranches known as Rancho Las Bolsas and Rancho Bolsa Chica (both toponyms referring to local pockets of marshland). In 1895 Bolsa Chica was acquired by wealthy members of the local Gun Club, who implemented within the next few years various dam and levee construction works that resulted in separation of Inner Bolsa Bay from Outer Bolsa Bay, as well as

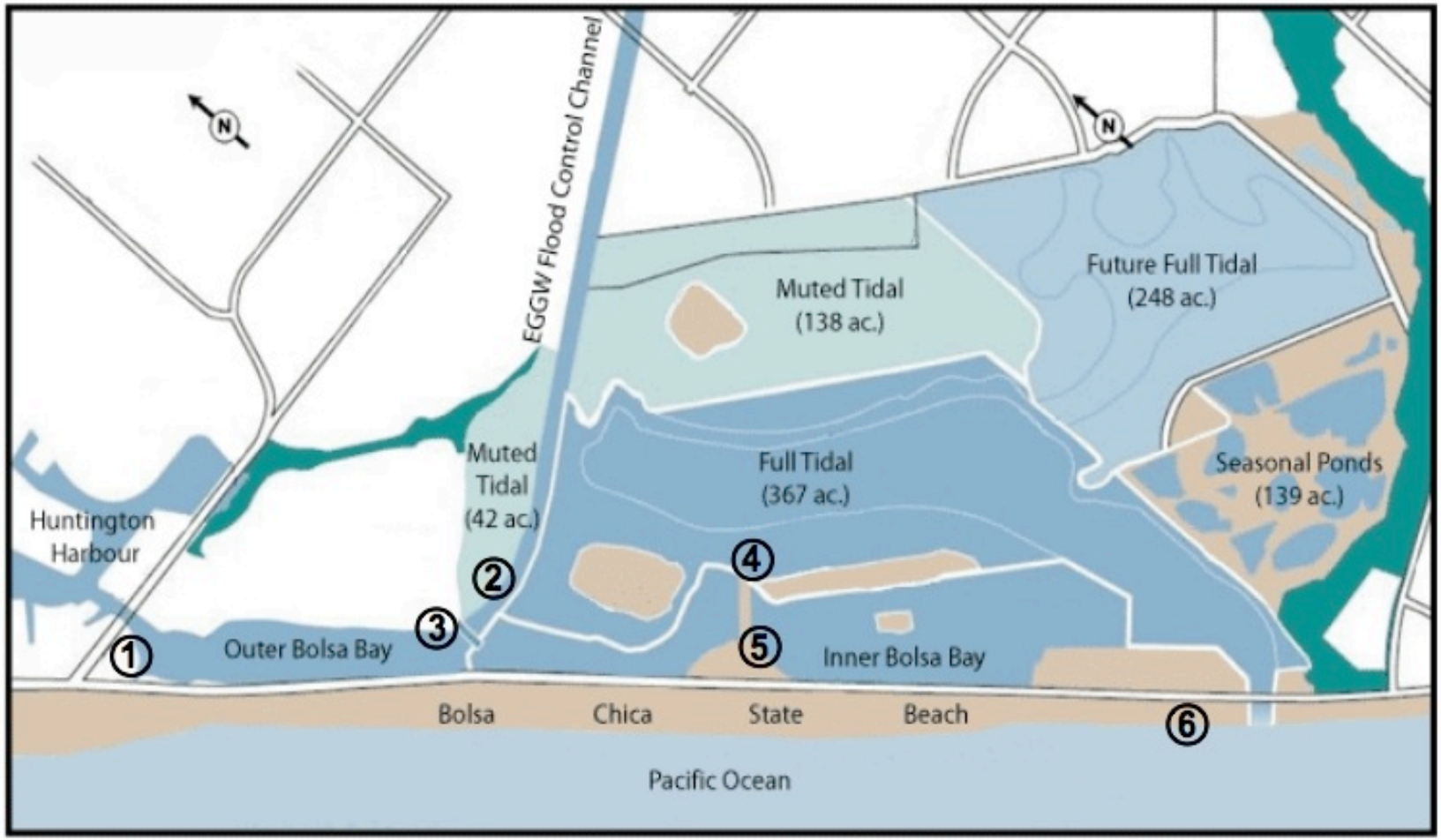

Figure 2-1: Map of Bolsa Chica indicating sampling sites (modified from figure at: http://epa.gov/region9/water/wetlands/bolsa-chica/projectDescMap.html).

closure of the main creek to access from the surrounding farmlands. From 1920 onwards, the wetlands were leased and then bought by oil companies, who constructed a grid of additional levees for access & maintenance roads. These successive developments led to significant losses of inshore salt marsh and greatly altered/diminished tidal flows, while the surrounding land was almost entirely converted from farmland to housing tracts and other real estate developments. In 1978, the California Department of Fish and Game began the first efforts to return the wetlands to more natural conditions by building two muted tidal wetlands. A large-scale restoration effort was started in 2004 as part of mitigation agreements to compensate for loss of habitats from expansion works at Long Beach harbor. The main features of this restoration plan at Bolsa Chica included the excavation of a new ocean inlet, several additional tidal basins, nesting habitat islands for birds, and a groundwater barrier. This restoration culminated with the opening of the new ocean tidal inlet in August, 2006 (Carlberg 2009). A major goal of this restoration was for the wetlands to regain substantially more of their previous ecological importance as a shelter, spawning ground and nursery for estuarine fish as well as for juvenile stages of oceanic fish. Despite the many dramatic reductions in habitat during much of the 20th century, Bolsa Chica continues to be an important flyway for migrating birds and for nesting populations of listed species of special concern, including the California least tern (*Sterna antillarum browni*), the western snowy plover (*Charadrius alexandrinus nivosus*), and Belding's savannah sparrow (*Passerculus sandwichensis beldingi*).

Carpinteria Salt Marsh

Carpinteria Salt Marsh (34°40′00″ N, 119°54′66″ W) is a 93 ha (230 acre) site, of which 49 ha (120 acres) is contained within a University of California Natural Reserve (Figure 2-2). As such, it is used in the present study as a reference site for comparisons with Bolsa Chica. The marsh is marine to hypersaline, and is composed of three basins, with freshwater input from the northeast (Callaway et al. 1990). This freshwater input is highly variable (Page 1997), and the marsh is subject to deposition of large quantities of sediment during catastrophic flood events (Callaway et al. 1990). Among the assortment of plants common to estuaries in California, *Salicornia virginica* is the predominant macrophyte (Sadro et al. 2007). Although immediately surrounded by real estate developments comparable to those of the Huntington Beach area surrounding Bolsa Chica, construction of dams and levees at Carpinteria was historically much more limited, never resulting in the cutting off of tidal water from entering or draining the wetlands. The salt marsh was never used for oil drilling or other similarly intrusive on-site industrial activities.

Sample Collection

Restricted access rules apply during the spring and summer breeding season of Belding's Savannah Sparrow. Samples were therefore collected in September 2008, and January, September and December 2009 from the lower intertidal zone at five sites representing different basins and/or tidal regimes in the Bolsa Chica Ecological Reserve as well as one site on Bolsa Chica State Beach located just west of

Figure 2-2: Map of Carpinteria Salt Marsh indicating sampling sites (modified from figure at: http://carpinteria.ucnrs.org/images/restoration.jpg).

the new ocean inlet (Figure 2-1). Site 4 was in the Full Tidal Basin (FTB) and only became subject to tidal influence with the opening of the tidal inlet in August, 2006. At the same time, culverts were opened from Outer Bolsa Bay creating a muted tidal area in which Site 2 was located. Samples were collected at three sites at Carpinteria Salt Marsh Reserve (CP) in January and December, 2009 (Figure 2-2). In both study areas, 5 sample cores were taken at each sampling site. The first core was collected at the low-tide waterline. Continuing along the waterline at 0.1 and 1 m from this original core, two more cores were taken. The last two samples were taken 0.1 and 1 m from the original core, this time at a right angle to low-tide mark, away from the water (Figure 2-3). This nested hierarchical sampling design was adapted from Somerfield et al. (2007) and allowed for a study of the differences at different spatial scales. Each sediment core measured 15 cm long, with a cross-sectional area of 10 cm^2, and was taken at each replicate point with a plastic cylinder.

Lab methodology

All cores were cooled in an ice chest and taken to the laboratory for nematode extraction. Nematodes were extracted from each core by suspending them in artificial seawater and decanting the supernatant, including suspended nematodes, on a 500-mesh screen (25 μm diameter openings). This was repeated 5 times. Samples were "cleaned" (i.e. nematodes were separated from the fine sediment and debris carried over on the sieve) by a centrifugation/flotation technique (Hooper 1986). Samples were transferred to a centrifuge tube, artificial

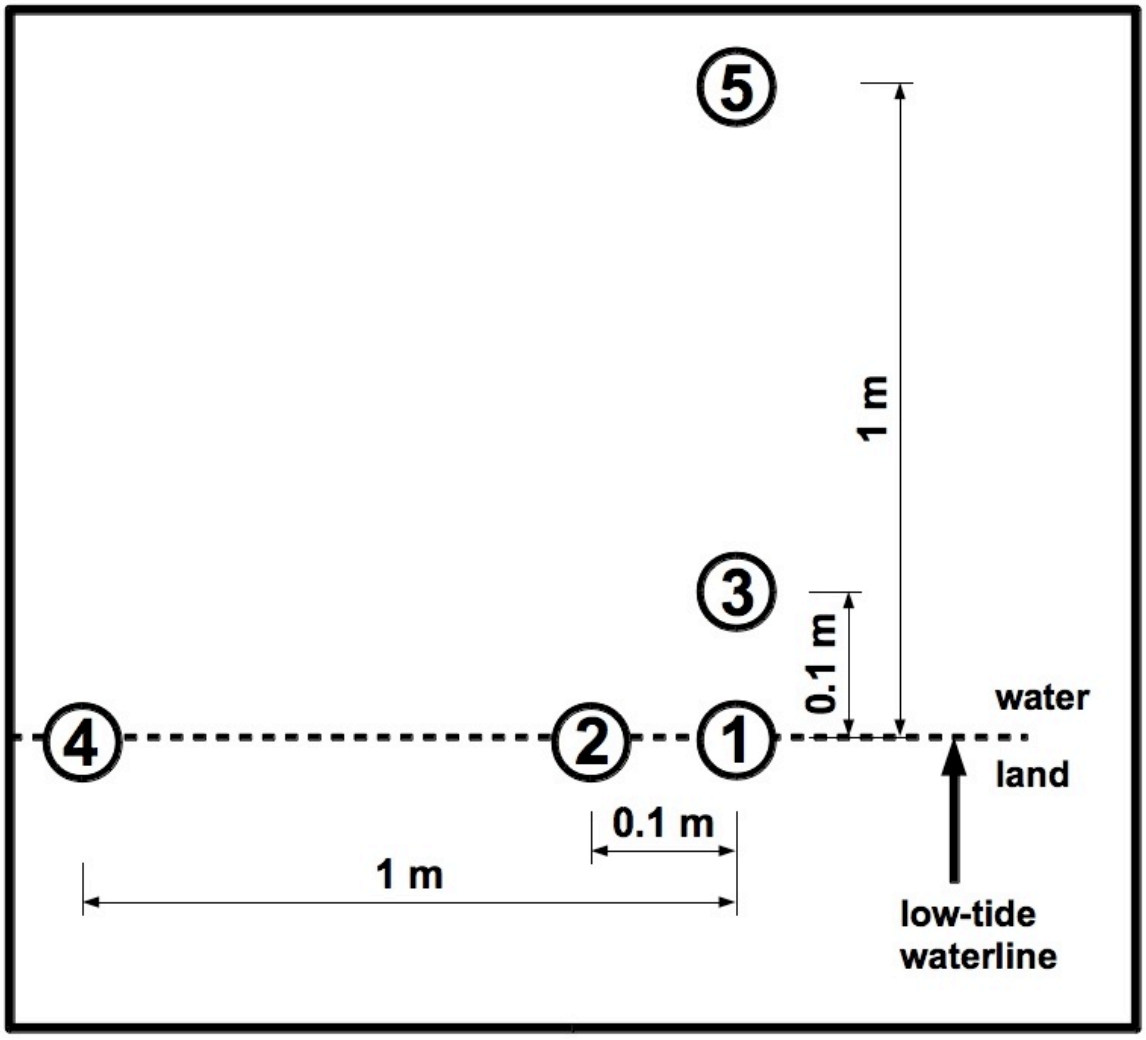

Figure 2-3: Schematic diagram showing the relative placement of replicate samples at each site in Bolsa Chica and Carpinteria Salt Marsh.

seawater was added, and then the tubes were centrifuged at 2300 rpm for 5 minutes. The supernatant was poured off and retained. The compacted sediment, containing the nematodes, was resuspended and mixed with a sucrose solution prepared to a specific gravity of 1.15. The tube was centrifuged at 1800 rpm for 2 minutes, after which the sucrose-solution supernatant was added to the supernatant from the first centrifugation. Artificial seawater was added to minimize osmotic shock, and the nematodes were sieved on a 500-mesh screen, then washed into a 250 ml beaker using DMSO/EDTA/saturate salt (DESS) solution to preserve morphology and extractable DNA (Yoder et al. 2006). For each sample, the nematodes were enumerated in counting dishes and 100 specimens were picked using randomization tables. These specimens were transferred to glycerin (Seinhorst, 1959) and mounted on permanent slides for identification to genus and tentative allocation to species or morphotype. Using an Olympus CX41 compound microscope, morphotypes were identified to the lowest possible taxonomic rank immediately identifiable, which was usually genus rank. De Ley & Blaxter (2002) was used as the phylogenetic classification scheme, and nematodes were identified using keys provided by Platt & Warwick (1983, 1988), and Warwick et al. (1998), sections relevant to estuarine nematodes in Eyualem et al. (2006), and the primary literature where necessary.

Statistical analyses

Statistical analyses were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA) and PRIMER v.6 (Clarke & Gorley 2006). The univariate measures of diversity calculated were: Margalef's species richness – d (Margalef 1958), Shannon index – H′ (Shannon 1948), rarefaction – ES(51) (Sanders 1968), and evenness – J′ (Pielou 1969). Differences in mean values of all these indices among sites and sampling times were tested by repeated measures 2-way ANOVA. Prior to ANOVA testing, graphical analyses of means and residuals were conducted, and fourth-root transformations were applied (Clarke & Gorley 2006) in those cases where the assumptions of normality required for ANOVA were not met. The multivariate relationship between species assemblages was analyzed by both cluster analysis, and ordination by non-metric multidimensional scaling (nMDS) using the Bray-Curtis similarity measure (Bray & Curtis 1957; Field et al. 1982). For the cluster analyses, group-average clustering was used as a compromise between single-link clustering, which tends to result in single samples being added sequentially to the first large group formed ("chaining"), and complete linkage, which tends initially to over-cluster many small groups (Clarke & Gorley 2006). To exam the effects of data transformation, the ordinations were carried out on untransformed and fourth-root transformed data. The significance of differences between nematode assemblages was tested using the analysis of similarity (ANOSIM) randomization/permutation procedure.

Results

Univariate

A total of 43 morphospecies were identified from the six sites at BC compared to 30 morphospecies from the three sites at CP. At BC Site 6 (Bolsa Chica State Beach) 18 morphospecies were identified, and 30 morphospecies from BC Sites 1 through 5. The respective intersections of these sets are shown in Figure 2-4. Densities ranged widely at both locations: 9 - 1092 individuals per $10cm^2$ at CP versus 6 - 2142 individuals per $10cm^2$ at BC. At BC, the highest mean density per site alternated between Site 6 in the 2 fall samples and Site 1 in the 2 winter samples (Table 2-1). Abundances for CP are shown in Table 2-2.

Bar graphs of the values of the univariate metrics are shown in Figures 2-5 to Figure 2-9. The 2-way ANOVA analyses of univariate metrics for both BC and CP (Table 2-3) revealed no significant differences between sampling sites or times for the univariate metrics at CP. At BC, H′ and ES(51) both showed significant differences between sites and times, although the interpretation of these results is confounded by a significant interaction factor (site x time) in both cases. The Margalef index was significantly different among sites, and again indicated a significant site x time interaction. A significant difference among sampling times occurred for Pielou's evenness.

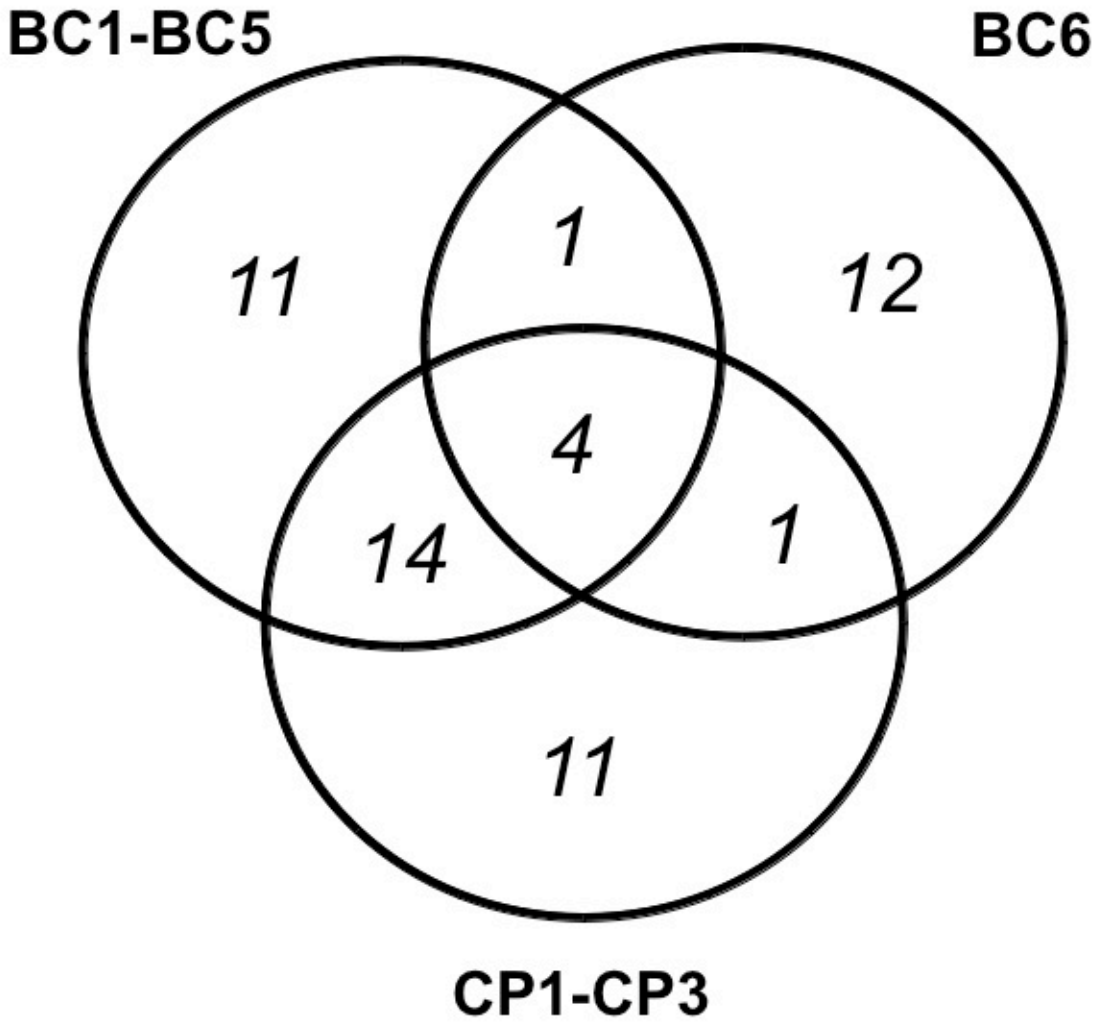

Figure 2-4: Venn diagram showing the intersection of sets of Bolsa Chica Sites 1-5 (BC1-BC5), Bolsa Chica Site 6 (BC6) and Carpinteria Sites 1-3 (CP1-CP3).

Table 2-1: Abundances of nematodes for each replicate at the 6 sampling sites for each of the 4 sampling times at Bolsa Chica.

Site	Replicate	Sept'08	Jan'09	Sept'09	Dec'09
1	1	323	334	10	432
	2	139	267	10	367
	3	418	1409	23	388
	4	299	595	6	407
	5	294	284	37	523
	Total	1473	2889	86	2117
2	1	11	199	44	122
	2	30	118	67	301
	3	29	61	89	191
	4	11	28	43	117
	5	7	104	70	134
	Total	88	510	313	865
3	1	30	148	23	109
	2	166	75	47	98
	3	94	174	52	74
	4	85	297	100	82
	5	206	35	21	92
	Total	581	729	243	455
4	1	34	61	38	40
	2	60	59	92	74
	3	17	24	61	71
	4	8	9	55	83
	5	76	102	65	55
	Total	195	255	311	323
5	1	110	182	112	133
	2	169	122	221	173
	3	256	343	189	189
	4	175	164	282	201
	5	326	231	104	121
	Total	1036	1042	908	817
6	1	907	245	289	277
	2	1328	349	166	341
	3	2142	417	84	299
	4	938	842	552	462
	5	1675	298	333	410
	Total	6990	2151	1424	1789

Table 2-2: Abundances of nematodes for each replicate at the 3 sampling sites for each of the 2 sampling times at Carpinteria Salt Marsh.

Site	Replicate	Jan'09	Dec'09
1	1	36	44
	2	48	250
	3	70	165
	4	95	153
	5	49	189
	Total	298	801
2	1	786	83
	2	245	92
	3	1092	168
	4	203	95
	5	455	97
	Total	2781	535
3	1	174	171
	2	157	19
	3	158	66
	4	135	9
	5	75	15
	Total	699	280

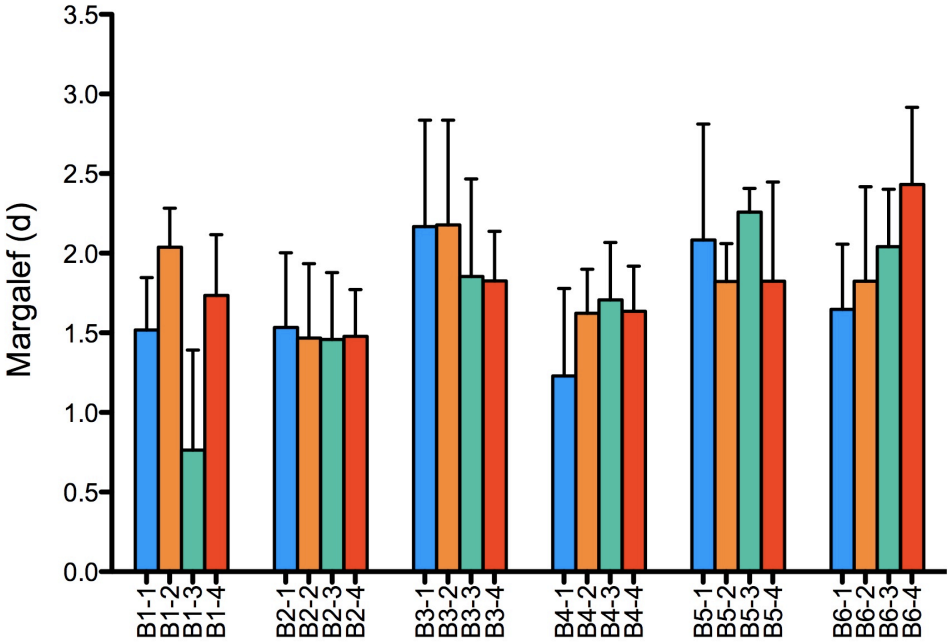

Figure 2-5: Mean values of Margalef's richness (d) from 5 replicates at 6 Bolsa Chica sites (1-6) over 4 sampling times (A-D).

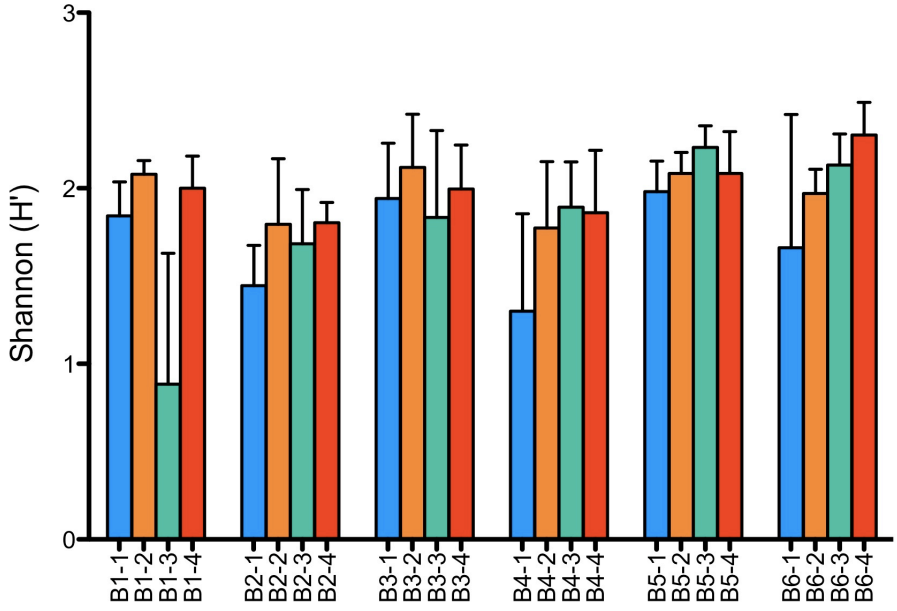

Figure 2-6: Mean values of Shannon index (H′) from 5 replicates at 6 Bolsa Chica sites (1-6) over 4 sampling times (A-D).

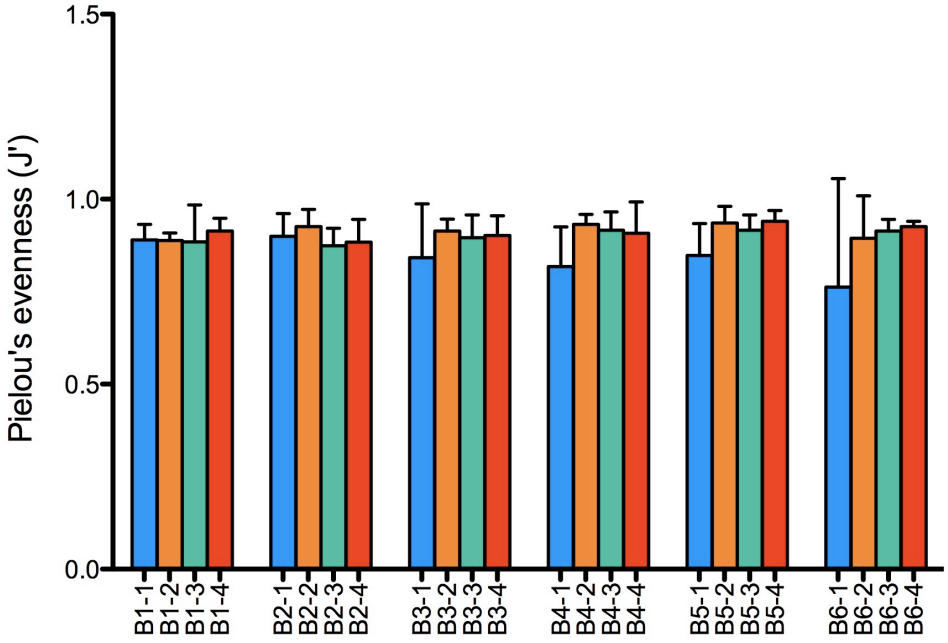

Figure 2-7: Mean values of Pielou's evenness (J′) from 5 replicates at 6 Bolsa Chica sites (1-6) over 4 sampling times (A-D).

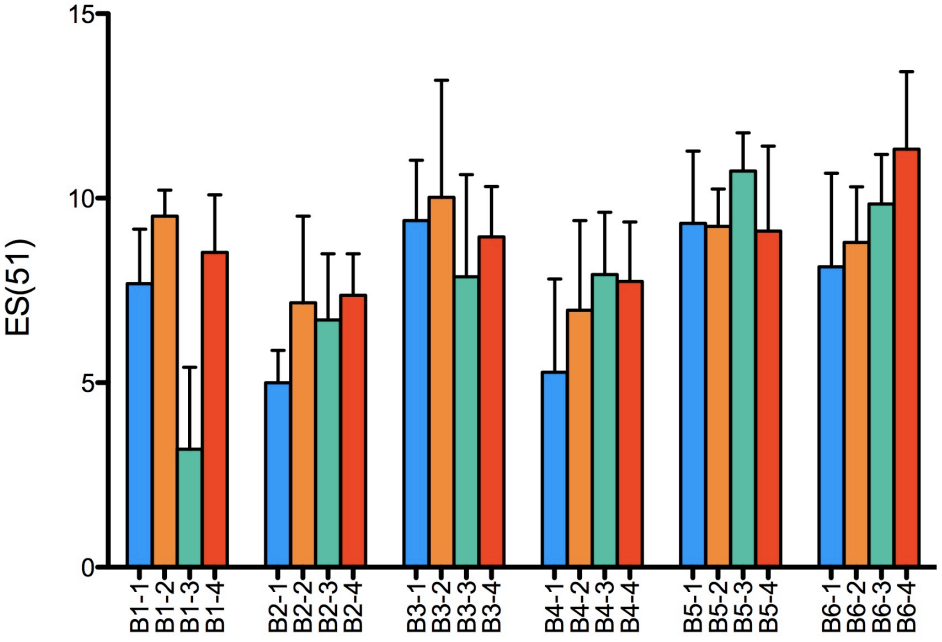

Figure 2-8: Mean values of rarefaction (ES(51)) from 5 replicates at 6 Bolsa Chica sites (1-6) over 4 sampling times (A-D).

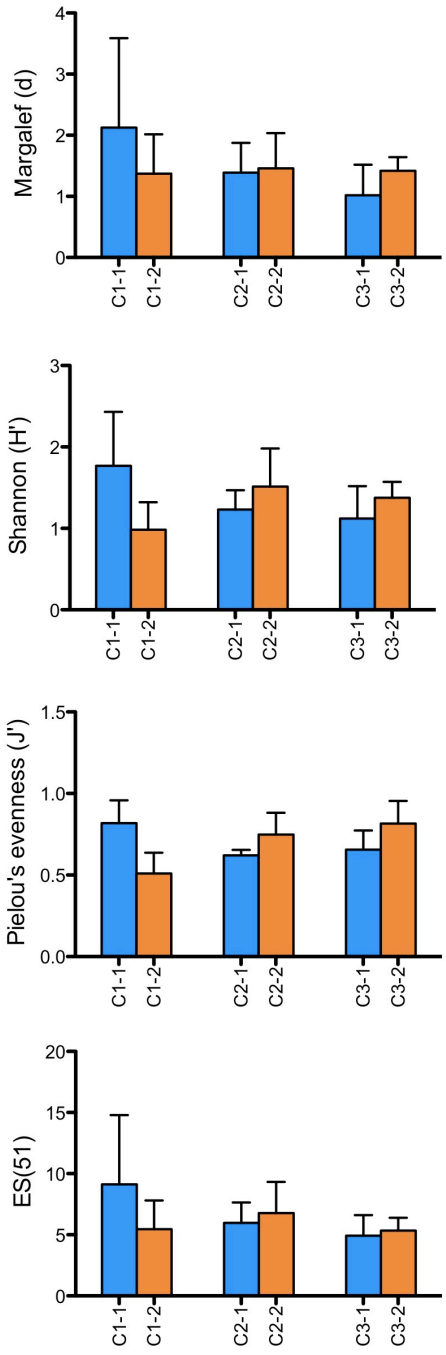

Figure 2-9: Mean values of various univariate metrics from 5 replicates from 3 Carpinteria Salt Marsh sites (1-3) over 2 sampling times (A-B).

Table 2-3: Results of repeated measures ANOVA on univariate metrics of nematode assemblages at Bolsa Chica (BC) and Carpinteria Salt Marsh (CP), including mean square values (MS). P-values in **bold** are significant at $p < 0.05$.

Metric	Factor	BC		CP	
		MS	p-value	MS	p-value
Margalef	Site	1.396	**< 0.0001**	0.712	0.1681
	Time	0.1837	0.2788	0.069	0.6697
	Site x Time	0.5033	**< 0.0001**	0.880	0.1144
Shannon	Site	0.6915	**< 0.0001**	0.0532	0.6238
	Time	0.6810	**< 0.0001**	0.0497	0.5089
	Site x Time	0.3735	**< 0.0001**	0.9288	**0.0017**
Evenness	Site	0.00870	0.5812	0.013	0.2740
	Time	0.03588	**0.0292**	0.0002	0.8896
	Site x Time	0.01366	0.2914	0.169	**<0.0001**
ES(51)	Site	32.26	**<0.0001**	11.84	0.1373
	Time	13.46	**0.0012**	5.012	0.3487
	Site x Time	11.04	**<0.0001**	15.35	0.0808

Multivariate

Ordination

The dendrogram resulting from the cluster analysis is presented in Figure 2-10. Site 6, the only true beach site, separated clearly from the cluster formed by the rest of the samples. Of particular interest is a difference in clustering found between BC and CP. The general pattern for BC was clustering based on site, where clusters were composed of the samples from the multiple sampling times for each single site. For Sites 4 and 5, all four sampling times for each site were clustered, while Sites 1 and 3 had clusters for three of the four sampling times (Figure 2-10). Conversely, CP samples were clustered based on sampling time – Sites 1, 2 and 3 from the first sampling comprised a cluster, while the three sites from the second sampling time formed a separate cluster.

Although largely reflecting these clustering results, the MDS (Figures 2-11 and 2-12) represents different aspects of the ordinations. Site 6 was so dissimilar from the others as to be placed very distant from all other sites in MDS-space. This led to a visually exaggerated appearance of clumping for all points of representing the other five sites (Figure 2-11). To clarify relationships among all the other sites, an MDS subset of those five sites was therefore plotted as well (Figure 2-12). The clustering of BC samples per site versus clustering of CP samples per year was also apparent from this analysis. What is easier to see here are the single sampling times within Sites 1, 2 and 4 that are more distant from the others in their respective

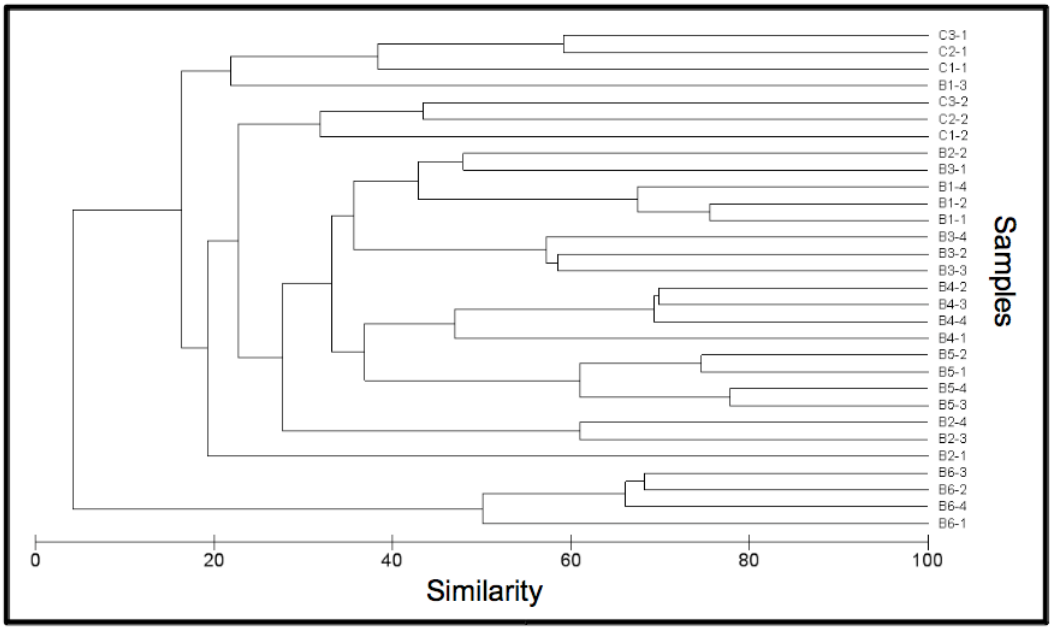

Figure 2-10: Dendrogram for hierarchical clustering of BC and CP sites using group-average linking of Bray-Curtis similarities of untransformed abundance data. Sample code: B (Bolsa Chica), C (Carpinteria), first number = site, second number = sampling time.

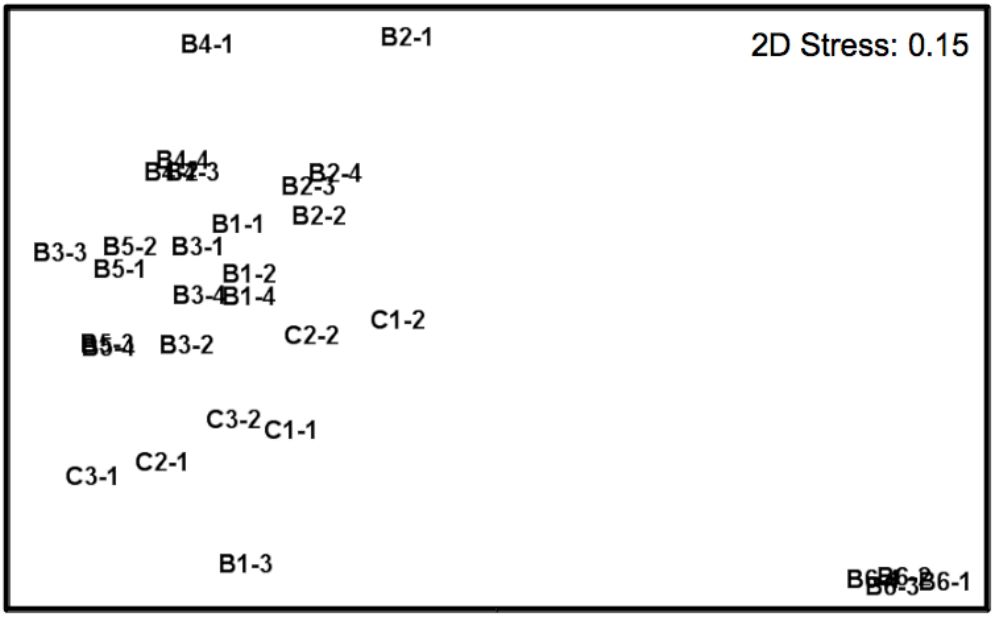

Figure 2-11: MDS ordination of all samples from BC and CP. Data were fourth-root transformed. Sample code same as in Figure 2-10.

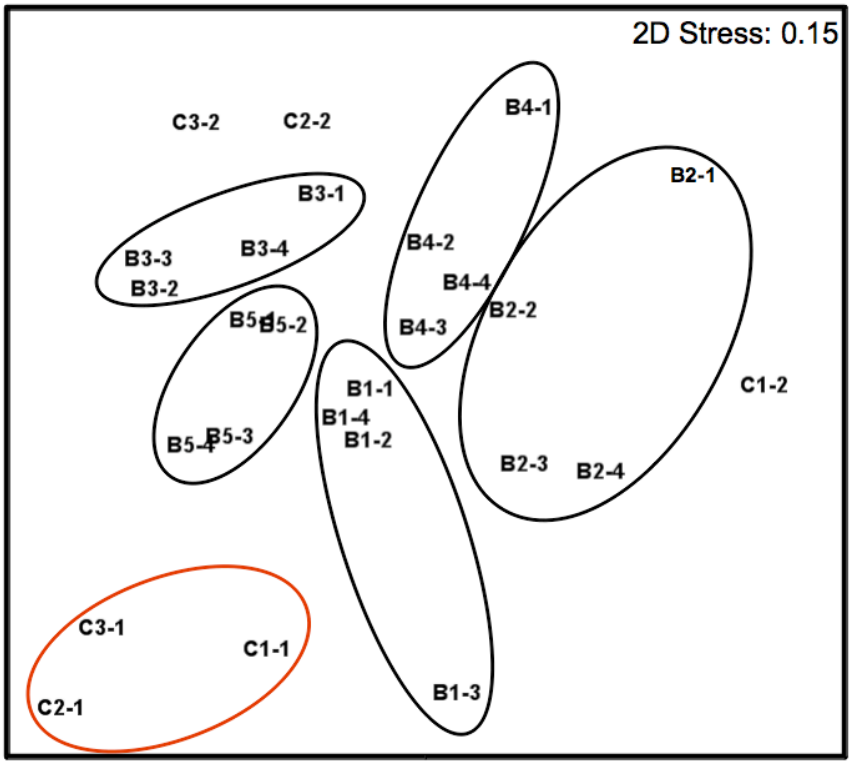

Figure 2-12: MDS subset of samples from BC and CP, excluding BC Site 6. Data were fourth-root transformed. Sample code same as in Figure 2-10.

clusters. Of note is that for both Sites 2 and 4, the first sampling time is the one that separates most from the others.

Almost identical results were found for untransformed and fourth-root transformed data (not shown), the only difference being that the degree of similarity tended to be higher within clusters for fourth-root transformed data.

ANOSIM

For BC and CP separately, the 2-way ANOSIM on fourth-root transformed data tests showed that differences were significant among "Sites" for BC (global $R=0.914$; $p<0.001$; 999 permutations), but not for CP (global $R=0.017$; $p>0.05$; 999 permutations). Differences were significant for "Time" at both BC and CP (global $R=0.459$ and global $R=0.717$, respectively; $p<0.001$; 999 permutations). In addition to the global R-values, the ANOSIM test furnishes pairwise comparisons of each pair of sites and each pair of sampling times. Every pairwise combination of "Times" for both locations, and "Sites" for BC, was significantly different ($p<0.001$), but two of the three pairwise combinations of "Sites" for CP were not significantly different (to wit: "Site 1 vs Site 2" and "Site 2 vs. Site 3", $p>0.05$). Only "Site 1 vs. Site 3", the two sites farthest apart, were significantly different.

Scale

The layout of the 5-replicate sampling design allowed comparisons of differences in spatial scales, with replicates 1, 2 and 3 serving as one set of

replicates at the scale of 0.1m and replicates 1, 4 and 5 serving as another set at the scale of 1m. As well, comparisons were made between the group of shoreline replicates (1, 2 and 4) and the replicates that were perpendicular to the shoreline (1, 3 and 5). Comparisons via ANOSIM of all these combinations of replicated from all sites and times at both BC and CP showed neither significant differences between scales of 0.1m and 1m (global $R=0.025$; $p>0.05$; 999 permutations), nor between shoreline and the replicate subset perpendicular to the shoreline (global $R=0.111$; $p>0.05$; 999 permutations).

Discussion

In broad terms, the similar order of magnitude for species counts and overlapping species compositions at the CP and BC sites suggest that nematode diversity at the latter location is close to what can be expected to occur in a coastal wetland surrounded by an urban environment in Southern California. With an identical species count as at CP, despite collections in the latter wetland from half as many sites and dates, BC nematode communities may still be in transition or under stress to some degree. In the absence of any autecological data on the 11 species exclusive to BC Ecological Reserve, as well as the 11 different species exclusive to CP, we cannot speculate whether some of these species might have (for example) dramatically higher tolerances of organic or inorganic toxicants and thus be potentially useful as indicator species for presence of such toxins. That being said,

the two subsets of 11 exclusive species may provide a logical starting point for future research seeking to develop a sentinel species approach, by establishing their specific tolerances for chemical and physical stressors. Previous studies have shown dominance of particularly tolerant nematode species in heavily polluted estuaries, to the extent that more polluted sediments harbor not just fewer species but also significantly different ones compared to less impacted sediments from the same area (Gyedu-Ababio et al 1999; Moreno et al 2009).

Some temporal changes that can be interpreted as the result of the restoration are evident from the ecological analyses of the nematode faunal assemblages. The restoration was facilitated chiefly by the creation of a new full tidal area (Site 4) and a new muted tidal area (Site 2). As Sites 2 and 4 were opened to tidal influence at the same time, it is here that the effects of the restoration are seen most clearly.

The nematode density and Shannon index were both significantly higher from the first to the last sampling times at Site 2. At Site 4, all univariate measures were higher in the last sampling series compared to the first, though none were significantly different. Differences are more clearly seen in the ordinations (Figures 2-10 to 2-12), which utilize all aspects of the multivariate "sample x species" dataset. This highlights the limitations of using only univariate metrics in biomonitoring – differences that become evident in multivariate space are missed due to loss of information resulting from the reduction to a univariate measure.

The first sampling times for both (B2-1 and B4-1, Figure 2-12) were separated from Sites 1, 3 and 5 in MDS space. Over the course of the sampling, samples from both sites showed matching trends as they moved closer in MDS space to the well-established Sites 1, 3 and 5, which themselves showed less change over time. This suggests that Site 2 and Site 4 became more similar to the other sites. This change could be the result of some combination of a number of factors. Firstly, the tidal waters reaching Sites 2 and 4 would pass the adjacent sampling sites. In doing so, the tidal flow of water could suspend and transport nematodes to nearby sites. Benthic nematodes have been shown to have resident time in the water column of up to 120 minutes after suspension (Ullberg & Olafsson 2003). Secondly, in addition to this tidal transport, Schratzberger et al. (2004) showed that estuarine nematodes could migrate laterally to non-native sediment on the scale of metres over a short period of time (weeks to months).

The full effect of the opening of the tidal inlet was gradually muted by tidal inflow and settling of extraneous drift sand on the order of 240 m^3/day since opening of the inlet, which led to the formation of a shoal (2008 Monitoring Report). This shoal may have reduced the changes in the nematode fauna that could have been expected at Site 4. Nematodes are very sensitive to changes in water depth (Heip et al. 1985), and as the shoal increased in size in 2008, the water depth at mean low tide decreased from 1.25m to 0.6m (2008 Monitoring Report). Comparisons of low tide in the full tidal basin (FTB) versus oceanic low tide, done since the opening of the inlet, have shown that a time lag developed between the

lowering of the tides in the FTB compared to the adjacent ocean, and that the FTB did not drain to the level of oceanic low tide. This tidal muting became more pronounced over the two years immediately succeeding the restoration (2008 Monitoring Report). This led to a dredging operation at the tidal inlet during January-April 2009 to restore the initial mean low tide water depth after completion of the inlet (this process was repeated in January-April 2011).

While the shoal formation may have slowed down the transition of the FTB to a more "natural" full tidal regime, the extreme change in Site 4 after the first sampling is difficult to explain. The dredging had begun before the second sampling, but Site 2 showed a very similar trend in the absence of any dredging.

In addition to these anthropogenic effects, there are the natural seasonal differences in the tidal regime with concomitant differences of the tidal muting in the FTB. The highest levels of muting are in the mid-summer and in the winter months, when two of the Bolsa Chica sample series were collected, compared to the lowest levels in the fall, the time of the other two series. This seasonal difference in muting levels undoubtedly confounds the temporal analyses of nematode samples from Bolsa Chica. It is clear from the above analyses that a full biomonitoring picture for the restoration of Bolsa Chica requires a consideration of multivariate changes in the nematode fauna, as univariate can miss differences that are clear from the added information provided by multivariate analyses. Also clear is the fact that this restoration is "in progress" and is therefore not static. Although the opening of the tidal inlet was the most important action in the restoration plan, the

monitoring scheme must be sensitive to continuous changes, such as shoal formation by sand deposition and subsequent dredging. Biomonitoring using the free-living nematode assemblages has been shown to provide this.

CHAPTER 3: MULTIVARIATE DATA TRANSFORMATION AND TAXONOMIC AGGREGATION OF THE NEMATODE FAUNA OF THE BOLSA CHICA WETLAND RESERVE AFTER TIDAL RESTORATION: IMPLICATIONS FOR BIOMONITORING

Introduction

Despite the advantages (Kennedy & Jacoby 1999; Schratzberger et al. 2000) and proven usefulness of free-living nematodes as environmental indicators (Wilson & Kakouli-Duarte 2009), biomonitoring programs employing them are difficult to realize due to one major disadvantage – the so-called "taxonomic impediment" (Taylor 1983). Although nematodes are not the morphologically monotonous group thought by some, identification to species does require specialized knowledge. There is a number of challenges to anyone who wants to attempt nematode identification. For example, many keys to free-living species are regional (Platt & Warwick 1983, 1988; Warwick et al. 1998) or restricted to particular taxa. Original species descriptions are spread widely across the literature, with much of the early taxonomic work appearing as monographic treatments in a variety of languages. Even with ready access to so much literature online, anyone attempting a first foray into identification of all nematode species in a coastal sediment sample will find the task made even more difficult by the presence of many species that are new to science.

A potential way to overcome this impediment, common to most groups of organisms used for biomonitoring, would be to restrict specimen identification at

higher taxonomic levels only, something which tends to be substantially easier to identify. This idea spurred the development of the concept of Taxonomic Sufficiency (TS), which in its original conception by Ellis (1985) sought to balance the level of taxonomic identification with the requirement to know sufficiently the biology of the assemblage under study, including such things as diversity. Clearly, this balance will depend on a number of factors, such as the faunal group studied, habitats sampled, temporal and spatial scale of sampling, etc. Thus, TS applies only if there is a quantifiably significant relationship between species abundance/diversity and the abundance/diversity of higher taxa to which those species belong. One of the purposes of this chapter is to evaluate this relationship for data collected from Bolsa Chica Wetlands.

Besides taxonomic aggregation, another option for pre-analysis data treatment is the use of data transformation. These transformations represent a spectrum of severity - from least pronounced (no transformation), through intermediate (e.g. square root, fourth root), to most severe (e.g. presence/absence). Considered another way, this spectrum of transformations reflects the increasing weight given to the most common, through the intermediate, to the rarest species in the data set. Although from a study of the literature, these transformations are commonly applied in analyses (see literature reviewed in Olsgard et al. 1997), what are much less common are explicit justifications for the choice of type of transformation. Another purpose of the present study is therefore to elucidate the reasons for choosing one transformation method over others.

Most of the research examining the effects of transformation and/or aggregation has been done on macrofaunal organisms (e.g. Arvanitidis et al. 2009; Bacci et al 2009; Bertasi et al. 2009; Bevilacqua et al. 2009; De Biasi et al. 2003; Defeo & Lercari 2004; Dethier & Schoch 2006; Gomez Gestiera et al. 2003; Gray et al. 1988; Lasiak 2003; Magierowski & Johnson 2006; Mistri & Rossi 2001; Munari et al. 2008; Musco et al. 2009, 2011; Olsgard et al. 1998, 2003; Quijon & Snelgrove 2006; Sanchez-Moyano et al. 2006; Somerfield & Gage 2000; Vanderklift et al. 1996; Wlodarska-Kowalczuk & Kedra 2007). Of those studies that have looked at meiofauna (e.g. Lampadariou et al. 2005; Rubal et al. 2009), and nematodes in particular (De Troch et al. 2008; Heip et al. 1988; Herman & Heip 1988; Sajan et al. 2010), none have considered the combined effect of both transformation and aggregation. In these cases, it has been found that for nematodes, fidelity to the species-level results is robust up to the level of family, which has therefore been proposed as an acceptable level for use in biomonitoring studies.

The overall objective of this chapter is to examine how the choice of aggregation and transformation affects the results of multivariate analyses of the nematode fauna of the Bolsa Chica Wetland specifically, and what this means for the use of the nematode fauna in future biomonitoring.

Methods

Data consisted of abundances of identified nematodes from samples collected from a 5-replicate/site x 6-site x 4-time sampling scheme (see Chapter 2 for full description), resulting in a 120-sample x 43-species data matrix. All of the following described analyses were performed with PRIMER v6 software (Clarke & Gorley 2006). Nematode species abundances were pooled into families, suborders and feeding groups. For each group (species, family, suborder, feeding type), abundances were transformed by square root, fourth root, logarithmic and presence/absence transformation to test the effect of transformation on the results obtained from multivariate analyses. The Bray-Curtis similarity measure (Bray & Curtis 1957) was used to construct ranked matrices of similarities among samples for each combination of transformation and aggregation (5 levels of transformation x 4 levels of aggregation = 20 matrices). Multidimensional scaling (MDS) ordinations were plotted for each of these matrices. As one site (Bolsa Chica State Beach) was dissimilar to the others (and thus very distant from the other sites in MDS-space in each of the plots), it led to the clumping of the points of the other five sites. To address this, an MDS subset of those five sites was plotted instead.

For the purpose of examining correlations among various transformed datasets and among various aggregated datasets, pairwise Spearman rank correlations and their significances were calculated between corresponding elements in each pair of similarity matrices with the RELATE procedure in PRIMER. These pairwise correlations were then used to construct a matrix for use in the

plotting of a 2nd stage MDS plot (Somerfield & Clarke 1995). To examine the effects of transformation and aggregation on the result of testing for differences between sites and sampling times, the ANOSIM (analysis of similarities) test in PRIMER was applied to each combination of transformation and aggregation. This was done to see if the statistically significant differences found between sites and between sampling times using the original untransformed species abundances would be retained when abundances were transformed and aggregated.

Results

The number of sampling units and the number of taxa at each taxonomic level are shown in Table 3-1. Each genus was represented by only one species. An examination of the set of MDS ordination plots (Figure 3-1) provides a first indication of the effects of transformation and aggregation of nematode abundances. For the species and family levels, the general spatial relationship of the sites is retained through increasing severity of transformation. This is less apparent at the suborder aggregation, though interestingly, remaining similarities are more consistent in the plots of the fourth root, log and presence/absence transformations. The plots of the MDS for feeding groups show an increasing breakdown of the spatial relationship between groups with increased transformation severity.

Table 3-2 shows the pairwise Spearman rank correlations for various combinations of transformation and aggregation. The highest correlation is between

Table 3-1: Number of sampling units (replicates x sites x sampling times) and number of taxa at each level of taxonomic resolution

	Bolsa Chica
# Sampling units	5 x 6 x 4 = 120
# Taxonomic units	
- Species	43
- Genera	43
- Family	19
- Suborder	11
- Feeding groups	4

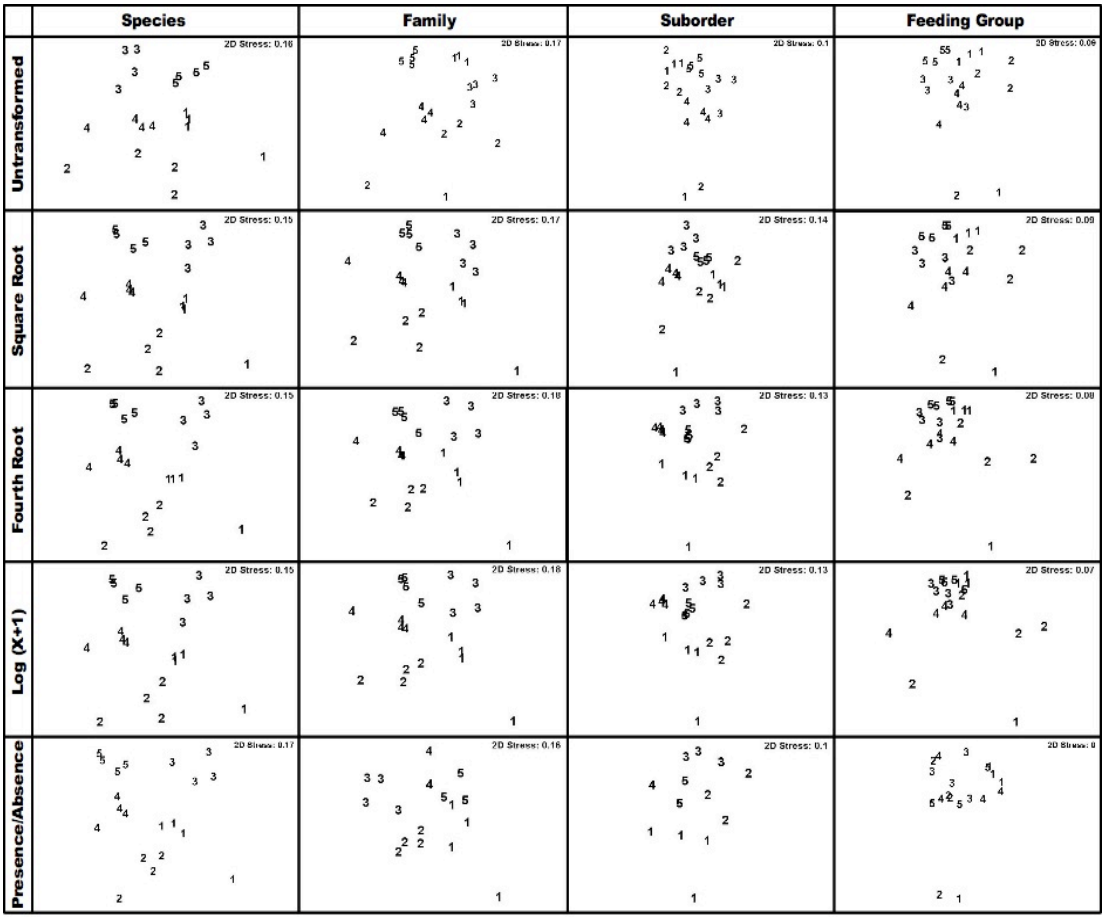

Figure 3-1: Multidimensional scaling (MDS), with stress values, of various combinations of transformations (left-hand side) and taxonomic aggregations (top) for the data from 4 sampling times for each of 5 sampling sites.

Table 3-2: Pairwise Spearman rank correlation values between similarity matrices derived from aggregated nematode abundance data, with various transformations. All correlations are significantly different from zero, by a permutation test, at least at $p<0.001$, unless otherwise indicated

	Species	Family	Suborder
UNTRANSFORMED			
Family	0.898		
Suborder	0.800	0.854	
Feeding	0.719	0.788	0.755
SQUARE ROOT			
Family	0.895		
Suborder	0.797	0.866	
Feeding	0.742	0.786	0.802
FOURTH ROOT			
Family	0.854		
Suborder	0.687	0.817	
Feeding	0.750	0.735	0.720
LOG			
Family	0.864		
Suborder	0.696	0.828	
Feeding	0.728	0.732	0.718
PRESENCE/ABSENCE			
Family	0.746		
Suborder	0.426	0.629	
Feeding	0.317*	0.364*	0.469*
	Species	Family	Suborder

* $p<0.05$

untransformed species and untransformed family abundances. The effect of transformation on the correlations between species and family, and species and suborder shows the same general pattern. The highest correlation is between untransformed abundances, versus weakening correlations for square root and fourth root transformations. The correlation using the log transformation is slightly higher than the fourth root transformation, while the presence/absence transformation gives the lowest correlation. For species and feeding groups, the highest correlation is found with the fourth root transformation, followed by square root, log, untransformed and presence/absence. For family and suborder, as well as suborder and feeding group, the highest correlation was found for square root transformed, and the second highest correlation for untransformed abundances. The lowest correlations were found consistently to be those for the presence/absence transformation. Only in the family and feeding group comparison was there a continuous decline in correlation values with increasing transformation severity, from untransformed abundances to presence/absence.

The second-stage MDS created from the similarity matrix for all correlations, including within-aggregation transformations (Table 3-3), is presented in Figure 3-2. Three observations are noteworthy: 1) the linear distance between plot points, from untransformed to presence/absence, increases with greater aggregation, from species to suborder; 2) the direction in the spread of each of these three groups changes from species to suborder to become almost completely perpendicular;

Table 3-3: R-values for all combinations of transformations and aggregations (FD – feeding type, FM – family, SO – suborder, SP – species 0 – no transformation, 1 – square root, 2 – fourth root, 3 – log, 4 – presence/absence).

	FD0	FD1	FD2	FD3	FD4	FM0	FM1	FM2	FM3	FM4	SO0	SO1	SO2	SO3	SO4	SP0	SP1	SP2	SP3
FD1	0.976																		
FD2	0.907	0.968																	
FD3	0.907	0.971	0.992																
FD4	0.429	0.506	0.633	0.594															
FM0	0.788	0.828	0.835	0.811	0.400														
FM1	0.738	0.786	0.805	0.781	0.428	0.957													
FM2	0.660	0.707	0.735	0.710	0.401	0.873	0.970												
FM3	0.687	0.733	0.754	0.732	0.389	0.897	0.983	0.994											
FM4	0.493	0.531	0.569	0.544	0.364	0.685	0.836	0.936	0.901										
SO0	0.775	0.800	0.803	0.782	0.331	0.854	0.813	0.754	0.766	0.608									
SO1	0.759	0.802	0.819	0.799	0.434	0.845	0.866	0.841	0.850	0.725	0.939								
SO2	0.634	0.684	0.720	0.702	0.483	0.722	0.795	0.817	0.815	0.772	0.780	0.931							
SO3	0.648	0.701	0.732	0.718	0.463	0.732	0.807	0.823	0.828	0.764	0.781	0.938	0.989						
SO4	0.341	0.364	0.402	0.387	0.469	0.399	0.492	0.565	0.540	0.629	0.416	0.605	0.816	0.754					
SP0	0.719	0.774	0.813	0.783	0.444	0.898	0.893	0.838	0.851	0.686	0.800	0.812	0.697	0.708	0.388				
SP1	0.690	0.742	0.786	0.752	0.421	0.875	0.895	0.856	0.868	0.720	0.771	0.797	0.696	0.707	0.399	0.984			
SP2	0.655	0.703	0.750	0.713	0.381	0.846	0.878	0.854	0.861	0.736	0.746	0.776	0.687	0.693	0.412	0.959	0.992		
SP3	0.667	0.717	0.763	0.728	0.392	0.854	0.884	0.854	0.864	0.727	0.752	0.782	0.687	0.696	0.400	0.968	0.996	0.998	
SP4	0.614	0.656	0.706	0.667	0.317	0.812	0.854	0.843	0.846	0.746	0.719	0.750	0.673	0.673	0.426	0.923	0.968	0.990	0.981

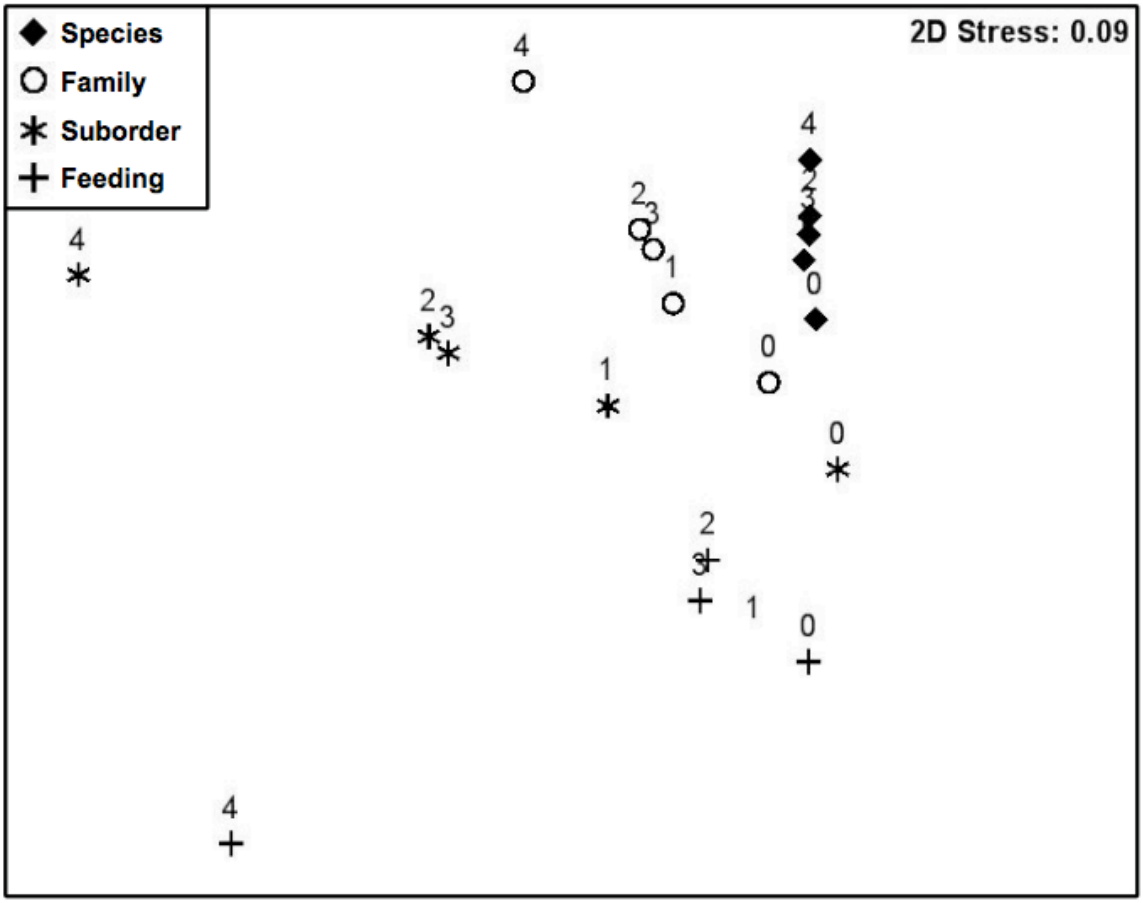

Figure 3-2: Second-stage multidimensional scale (MDS) of transformation-taxonomic aggregations. Key to transformations: 0 - none; 1 - square root; 2 - fourth root; 3 - log (X+1); 4 - presence/absence.

3) feeding group does not show the same approximate linearity of transformation points within an aggregation seen in the other three groups.

Tables 3-4 and 3-5 show the global R-values for the 1-way and 2-way ANOSIM tests. Differences between sites were significant for all combinations of transformation and aggregation. This was true for both the 1-way ANOSIM test of "Site" and for the 2-way ANOSIM test with "Site" and "Time" as the two factors. There was no clear pattern in the R-values for the various transformations within each aggregation (columns). For "Sites", looking at each transformation, the R-values decreased with increasing aggregation in every case (rows). All combinations of transformation and aggregation for "Time" (tested separately as a 1-way ANOSIM) were significantly different, except for presence/absence at both the family and suborder levels. When tested as one of two factors, with "Site" being the other, the differences were significant in all cases. In addition to the global R-values, the ANOSIM test furnishes pairwise comparisons of each pair of sites and each pair of times. The number of these pairwise comparisons that were not significantly different in each case (even though the overall difference is significant) is given in Tables 3-4 and 3-5. Whether tested by 1-way or 2-way ANOSIM, the only cases of pairwise "Site" comparisons not being significantly different is for the feeding types-presence/absence combination. For "Time", all pairwise comparisons were significantly different when "Time" was analysed as part of a 2-way ANOSIM. When "Time" was tested separately via a 1-way ANOSIM, there were many instances of

Table 3-4: Global R-values for 1-way ANOSIM with "Site" and "Time" tested separately. Number of pairwise comparisons that were not significantly different given in bracket.

		Species	Family	Suborder	Feeding
Site	Untransformed	0.733	0.638	0.468	0.395
	Square root	0.786	0.696	0.593	0.390
	Fourth root	0.802	0.716	0.656	0.347
	Log (X+1)	0.786	0.687	0.614	0.346
	Presence/absence	0.803	0.708	0.646	0.188 (4)
Time	Untransformed	0.038 (3)	0.047 (3)	0.060 (2)	0.039 (4)
	Square root	0.034 (3)	0.041 (4)	0.060 (2)	0.045 (3)
	Fourth root	0.030 (4)	0.033 (4)	0.050 (4)	0.046 (3)
	Log (X+1)	0.033 (4)	0.041 (4)	0.060 (2)	0.050 (2)
	Presence/absence	0.025 (4)	0.022*(5)	0.023*(4)	0.024 (4)

Table 3-5: Global R-values for 2-way ANOSIM with "Site" and "Time" as factors. Number of pairwise comparisons that were not significantly different given in bracket.

		Species	Family	Suborder	Feeding
Site	Untransformed	0.897	0.829	0.683	0.591
	Square root	0.919	0.859	0.783	0.561
	Fourth root	0.914	0.850	0.804	0.491
	Log (X+1)	0.913	0.847	0.790	0.495
	Presence/absence	0.895	0.816	0.738	0.268 (2)
Time	Untransformed	0.488	0.361	0.325	0.234
	Square root	0.482	0.370	0.360	0.236
	Fourth root	0.459	0.349	0.334	0.222
	Log (X+1)	0.470	0.363	0.354	0.218
	Presence/absence	0.417	0.295	0.221	0.122

multiple pairwise comparisons not being significantly different, while the global differences were significant.

Discussion

In the present study of the nematode fauna of the Bolsa Chica Wetlands, the observed differences between sites and sampling times were statistically very robust to both data transformation and aggregation. The results support the conclusion that for biomonitoring purposes, with our data the best combination of processability and informativeness is given by an aggregation to family level, combined with a moderate transformation (i.e. square root or fourth root).

The rank correlation values between similarity matrices tend to decrease with increasing aggregation and increasing transformation severity. Olsgard et al. (1997) found the same pattern in their 2nd stage MDS, which they termed a "fan pattern". There was a separation along one axis based on transformation and another separation along the perpendicular axis based on aggregation. This indicates that the effects of aggregation and transformation are different and, to a large extent, unrelated. As they also reported, the effect of transformation increased with increasing taxonomic aggregation, as evidenced by the greater spread in the points for each aggregation.

Transformation affects the relative emphasis of common, intermediate or rare species to intersample similarities (Clarke 1993). For biomonitoring, the choice of transformation should be informed by the nature of the biomonitoring, namely

which aspects of the community are to be studied (e.g. whether selected indicator species or whole-assemblage compositional change is considered most important). When looking just at presence/absence, we essentially examine compositional changes only in the assemblage, which is in fundamental contrast to a change in relative abundance (Anderson et al. 2005). Indicator species, identified *a priori*, may be common enough across samples to warrant a particular transformation to allow more-powerful parametric analysis to be done (Clarke & Warwick 2001). If a species appears at least once in each sample, and data are converted to presence/absence, this ubiquity means that it this very common species will be of no use for discriminating between samples (Clarke & Warwick 2001). The influence of common species in general will be diminished when the presence/absence transformation is utilized. In effect, this leads to the inclusion of more species in the determination of the ordination and, as a result, usually leads to an increase in the stress values seen in MDS plots. This was the case in this study only at the species level, where the presence/absence transformation showed a higher stress value than the other transformations. Even here, the increase was slight, suggesting a reduced influence of disproportionately abundant and common species.

Within the spectrum of transformation severity, Clarke & Warwick (2001) argued that intermediate transformations, such as square root or fourth root, give a balance between no transformation, where results are influenced by the high abundances of a few dominant species, and severe transformation, such as presence/absence, which includes the influence of all species, regardless of how

rare they might be. They saw these ends of the spectrum as representing a narrow versus a wide view of the community. The results of the current study agree with this assessment. In almost all cases for the 2-way ANOSIM test for significant differences between sites and times, the untransformed and presence/absence R-values were the 2 lowest of the 5 transformation treatments.

Previous studies of meiofaunal assemblages in general, and nematode assemblages in particular, have shown that much of the information from species-level analyses is retained up to the level of family, which in some cases is even better for discriminating between sites than species-level identification. It has been suggested that higher-taxa aggregation helps offset the natural fluctuations in species abundances. Reducing the influence of this natural variability results in increasing statistical power for detecting differences in assemblages (Olsgard et al. 1997). Warwick (1988) suggested that observed changes in fauna in response to natural gradient variables are more likely to be the result of species replacement rather than changes in the proportions of higher taxa. This "dampening" of natural variability in faunal patterns improves the detection of small changes by subsequent analyses (Ferraro & Cole 1990, 1995; Frost et al. 1992). The sufficiency of family-level aggregation may be the result of assemblages being family rich, but with few species per family (Dethier & Schoch 2006). If there were few species per family, then any assemblage differences seen at the species level (or any changes in species) would be reflected as strongly at the family level as differences or changes.

Although similar cost analyses have not been done for nematodes, Ferraro & Cole (1995) found that it cost 95, 80, 55 and 23% less to identify macrofauna to the phylum, order, family and genus levels, respectively, as compared to species-level identification. De Biasi et al. (2003) corroborated the family-level cost at 50%.

These results are important for future studies, especially for continued biomonitoring research by non-specialists, as it can be seen that the taxonomic impediment of identifying nematodes to the level of species or genus may not be necessary to get a sufficiently resolved biomonitoring picture. If previous baseline studies exist, or at the very least faunal lists have been compiled for an area, then species identities could be useful in choosing sentinel species as ecological indicators. In cases where particular species are easily identifiable and known to be particularly sensitive to or tolerant of the environmental parameters of greatest concern, greater efforts in identification to species level may be warranted (see for example Brinke et al., 2011; Derycke et al., 2007). Such sentinel species are unfortunately not available for coastlines and basins outside the Northern Atlantic, including the very poorly studied intertidal meiofauna of the Eastern Pacific.

Because of the patchy distributions commonly seen in nematode assemblages, adequate representation of the assemblage requires intense sample replication. The time and resources saved by identifying specimens to a coarser taxonomic level only will allow for the collection of more replicates at different temporal and spatial scales. Where biomonitoring is the main goal of a study, this will be more useful than identifying all specimens to species.

CHAPTER 4: THE NEED FOR THE INCORPORATION OF PHYLOGENY IN THE MEASUREMENT OF BIOLOGICAL DIVERSITY, WITH SPECIAL REFERENCE TO ECOSYSTEM FUNCTIONING RESEARCH.

(published as: King, I. 2009. The need for the incorporation of phylogeny in the measurement of biological diversity, with special reference to ecosystem functioning research. *BioEssays* **31(1)**: 107-116.)

Introduction

No biological term has been embraced more by the media, culture and nonscientific layperson as has been 'biodiversity'. This ubiquitous usage belies the fact that no clear definition of the term is to be found in the scientific literature, or more to the point, that there exist numerous competing definitions. To many, the word seems to be immediately understandable but more difficult to define. To give an approximate idea of the increasing use of the term in the scientific literature, Figure 4-1 shows the number of references found when 'biodiversity' was entered as the keyword in the 'Topic' search box in the ISI Web of Science® database for each of the years 1988 through 2006.

Of greater interest to ecologists and evolutionary biologists are the important scientific questions to which measures of biodiversity can be applied. From an academic research point of view, biodiversity measures are only as useful

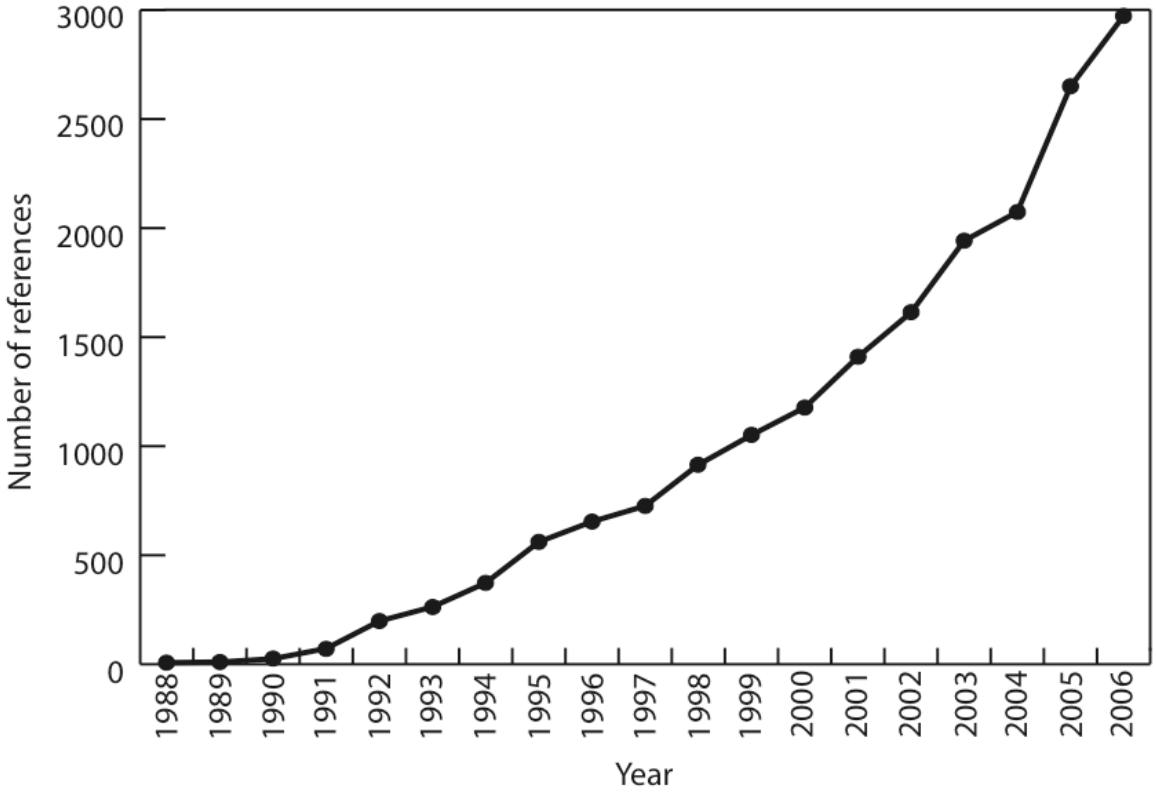

Figure 4-1: Number of references found using the keyword "biodiversity" in ISI® Web of Science for the years 1988 through 2006.

as the questions they raise and can be used to address, and insofar as the hypotheses they generate are subject to rigorous empirical inquiry. Of more practical importance, any means of improving the measurement of biodiversity will have the tangible benefit of aiding those charged with making management and conservation decisions. These decisions are crucial in their influence on the preservation of biodiversity. Besides the intrinsic and aesthetic values that many believe are reason enough to preserve biodiversity, there are numerous ways in which a diverse biota is important to humans, such as in providing sources of food, medicines and industrial products. More importantly, biodiversity is the source of many non-market services, such as in environmental modulation, ecosystem functioning and filling ecological roles (Kunin & Lawton 1996). Although management decisions include myriad other considerations besides biological ones (most notably economic and political), providing policy- and decision-makers with the best possible science is a duty of biodiversity researchers.

The first part of this paper will deal with biodiversity - its definitions and measurements, and how phylogenetic considerations have been incorporated into these. This provides the necessary underpinnings for the second part, which addresses future applications of phylogenetically informed biodiversity measures in ecological research, particularly in ecosystem functioning studies.

Definitions of 'Biodiversity'

Harper and Hawksworth (1994) trace the term 'biodiversity' from its antecedent 'biological diversity' as used by Lovejoy (1980) and Norse and McManus (1980) The former used 'biological diversity' to refer to the number of species, though not explicitly. The latter referred to the number of species as 'ecological diversity' and also referred to 'genetic diversity'; these were considered the two elements of 'biological diversity'. Gaston (1996) makes a useful distinction between 'biodiversity' as a concept, a measurable entity and a social/political construct. As a concept, he lists a number of definitions proposed by various authors. Two of the most often cited are:

> *"Biological diversity refers to the variety and variability among living organisms and the ecological complexes in which they occur. Diversity can be defined as the number of different items and their relative frequency. For biological diversity, these items are organized at many levels, ranging from complete ecosystems to the chemical structures that are the molecular basis of heredity. Thus, the term encompasses different ecosystems, species, genes, and their relative abundance."*
>
> OTA - Office of Technology Assessment 1987

> *"'Biological diversity' means the variability among living organisms from all sources including,* inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems."*
>
> Johnson 1993

There have been numerous other definitions proposed from the very specific to the all-encompassing (Gaston 1996). The primary cause of ambiguity in definitions of 'biodiversity' is the blurring of the distinction between biodiversity as a concept and operational measures of it. This general problem of confusion

between concept and application occurs repeatedly in many of the issues addressed in this paper and its implication should be borne in mind throughout. Some of the conceptualizations of 'biodiversity' are so general or vague as to make them useless in informing any hypothesis-driven, biodiversity research programme.

Considerations of scale in definitions of biodiversity

Biodiversity is defined often as occurring at different scales. As exemplified in the OTA (1987) definition of biodiversity above, this distinction manifests itself most commonly as definitions at the genetic, species and ecosystems levels (Gaston & Spicer 1998; Heywood 1994; May 1994; McAllister 1991). At a more-resolved scale and in the context of conservation, Soulé (1991) considered biodiversity at five of what he termed 'biospatial scales': genes, populations, species, assemblages and whole systems at the landscape or ecosystem level. Recognizing in many definitions a lack of consideration of the processes that maintain biodiversity, Noss (1990) used the three attributes of biodiversity (compositional, structural and functional) proposed by Franklin et al. (1981) to create a nested hierarchy. In it, he identified how the three attributes manifest themselves on four levels of organization (genetic, population-species, community-ecosystem, regional landscape). This definition is one example of an important advancement in the conceptual framework of ecological biodiversity studies: a shift away from thinking about biodiversity in terms of elements and towards considering biodiversity in terms of processes.

As important as this advancement is, the next step to be taken is to include an explicit phylogenetic component to the definition and measurement of biodiversity, the need for which is the main argument of this paper. Arguing for the incorporation of phylogeny is essentially an argument for the consideration of a dynamic temporal component to biodiversity. This is important enough to be considered a fourth attribute of biodiversity, and is illustrated in Figure 4-2 (after Noss 1990). The fruitful avenues of inquiry concerning the relationship between biodiversity and ecosystem functioning opened up by this change in thinking will be discussed later in this paper. Before that, it will be informative to examine how biodiversity has been measured traditionally to understand fully the results of this change in focus.

Measuring Biodiversity

The above section is not just an example of the etymology of a biological term. Ideally, the proper conceptualization of 'biodiversity' informs, and is informed by, how it is measured. The confusion caused by a lack of consensus on a definition of 'biodiversity' is compounded in the various ways in which it has been measured.

Species richness and evenness

Despite Hurlbert's (1971) warning that 'species diversity' was at risk of becoming a "meaningless concept" and that the term should be abandoned, it is still

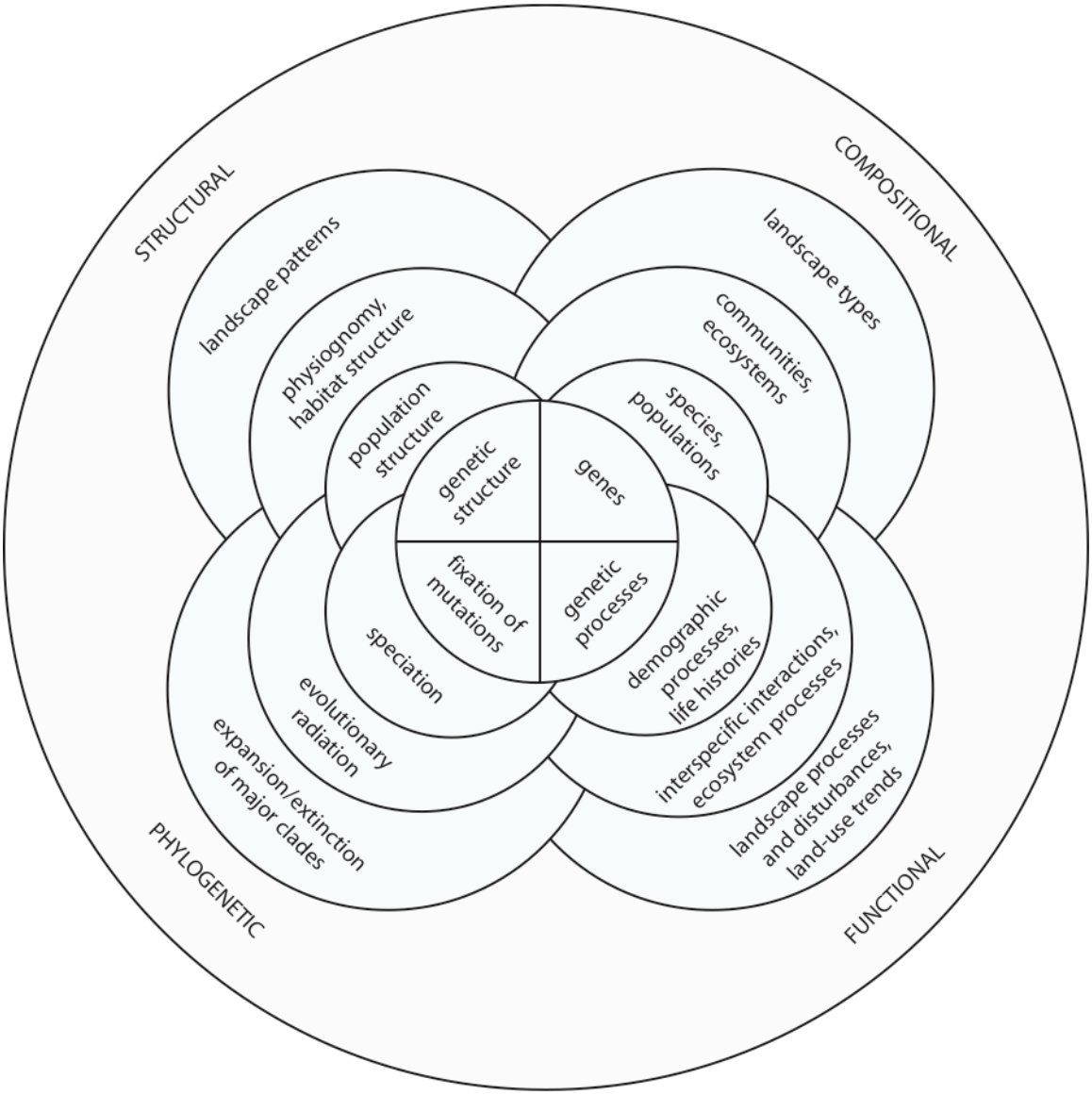

Figure 4-2: Nested hierarchy of four attributes of biodiversity (periphery) at each of four levels of organization (after Figure 1 from Noss 1990).

one of the most often used biodiversity measures. Gaston (1996) gives four reasons why species richness is the most widely applied measure:

1) species richness is thought by many to capture much of the essence of biodiversity

2) the meaning of species richness is widely understood and there is no need to derive complex indices to express it

3) species richness is considered to be a measurable parameter in practice

4) much data on species richness already exists

The first two are conceptual and the second two are operational, but all seem vague and insufficient reasons to employ species richness. Recognizing this, he gives six empirically supported reasons why species richness may be a useful indicator of biodiversity. These include correlations between species richness and measures of a) ecological diversity, b) higher taxonomic units, c) phylogenetic disparity, d) structures of food webs and e) topographic diversity.

A simple count of the number of species present in an area is of little use for comparisons between areas or times without some idea of relative abundance of the species present. Indices have been created that address this by including an element of 'evenness' in addition to species richness. The downside of this approach is that there is some loss of information when the two dimensions of species richness and evenness are reduced to the single dimension of an index value (Purvis & Hector 2000). Magurran (2004) provides a concise review of some of the issues related to using species diversity as a measure of biodiversity.

Arguments against using species richness as a measure of biodiversity include the possible inconsistency in species discrimination, either in definition or in observation - the so-called 'recorder effect' (Gaston 2000). Also, differences in sampling strategies and effort will produce differing results. In general, a greater number of species are recorded with an increase in the area sampled or duration of a study. The issue of the scale-dependence of the results is the most serious problem in using species richness in biodiversity studies and will be revisited later.

Species diversity at different scales

Whittaker (1965) distinguished between two types of measures of species diversity: the first based on the number of species in the sampling units (e.g. Margalef index), the second based on quantitative relations of species (e.g. Simpson index). He later suggested that three measures were required to encompass species diversity in its entirety (Whittaker 1972). The first, termed *alpha diversity*, is the measure of species richness at a sampling point. The differentiation of species richness between communities along habitat gradients he called *beta diversity*. The diversity for a geographic area is the *gamma diversity*, and is the product of the alpha diversity of its communities and the degree of beta differentiation amongst them.

Species concepts as related to measurements of biodiversity

Mayden (1997) suggested that we had reached "the denouement in the saga of the species problem" whereas Sites and Marshall (2003) claimed that the species concept was a "Renaissance issue in systematic biology". Species are often seen as the 'unit of currency' of biodiversity (Agapow et al. 2004, 2005; Claridge et al. 1997; Wilson 1992). As such, differences in defining species will have an effect on measures of biodiversity based on species richness. Also, this should mean that the debate about a single, widely applicable species concept should be a focus of biodiversity research (Cracraft 1992).

The debate as to whether species are real, biological entities or human constructs with no meaning in reality falls without the scope of this review. For the purposes of the following discussion, it is taken that species are meaningful entities and can be distinguished as such. The question then becomes how to define and differentiate species. As in definitions of biodiversity, this highlights the importance of distinguishing between conceptual (define) and operational (differentiate) definitions of species. Some indication of how difficult this has proven is shown by the fact that Mayden (1997) lists 22 different species concepts! Coyne and Orr (2004) list nine species concepts that they divide into four categories. The first category is based on interbreeding and includes the Biological Species Concept (BSC)(Mayr 1942, 1963). The second category includes three species concepts: the Genotype Cluster Species Concept (Mallet 1995), Recognition Species Concept (Patterson 1985) and the Cohesion Species Concept (Templeton 1989), which are

based on genetic or phenotypic cohesion. Those concepts based on evolutionary cohesion - the Ecological Species Concept (Van Valen 1976) and the Evolutionary Species Concept (Wiley 1978) - make up the third category. Finally, evolutionary history serves as the basis of three different Phylogenetic Species Concepts (Baum & Donoghue 1995; Cracraft 1989; de Queiroz & Donoghue 1988). An examination of the basis of this separation is useful as it leads to the realization of the greater applicability of the Phylogenetic Species Concepts, based as they are on evolutionary history. A logical extension of the argument for the incorporation of phylogeny into biodiversity measurement is to propose the use of a species concept that is itself based on phylogeny. Phylogeny then, will included at another level of the measurement of biodiversity - that of the definition of the unit of measurement. Ideally, if biodiversity studies are to be comparable, then the species used as the unit of biodiversity should be of the same currency. For more detailed discussions of various species concepts see Wheeler and Meier (2000) and Coyne and Orr (2004).

Alternatives to species diversity as measure of biodiversity

Alternative measures of biodiversity often mirror the differing views on the definition of biodiversity. Besides the arguments against using species richness mentioned above, there is the assertion that species diversity is not synonymous with biodiversity, but is just a measure of a single facet of it (Gaston 1996). If species are considered the currency of biodiversity, then as in monetary currency there are differently valued denominations - all species are not of equal value. Using species

diversity assumes that all species are equal as they are at the same taxonomic level (Vane-Wright et al. 1991). Part of the impetus to add information from phylogenies to biodiversity studies was the recognition that species are not equal, especially with respect to conservation. One can follow the progression of ideas in the literature of biodiversity studies from equal weighting of species, through attempts to weigh species differently based on classification, to the unequal weighting that is inherent in methods that incorporate phylogenies (Faith 1992; Vane-Wright et al. 1991).

i) Higher taxon diversity

This has been proposed as a more easily measured indicator of biodiversity (Gaston 1994; Harper & Hawksworth 1994; Ray 1988). This is predicated on the assumption that differences in numbers of higher taxa reflect proportional differences in the number of species contained in these higher taxa. This may not be the case when comparing widely disparate groups at higher taxonomic levels. For example, the close relationship between numbers of families and numbers of species in those families has been found to occur in some cases (Williams & Humphries 1994), but not in others (Prance 1994). The correspondence is less likely to hold the higher the taxonomic level considered.

Another criticism of this approach concerns the problem introduced by the histories of the classifications of the organisms studied. Higher taxa boundaries vary widely in different groups, so measures utilizing higher taxa will be sensitive to

classifications (Nixon & Wheeler 1992). As Harper and Hawksworth (1994) wrote, these measures may be "better indicators of taxonomic traditions than of the scale of natural biological diversity."

Some argue that until well-resolved phylogenies are available for most taxonomic groups, basal taxa (i.e. species in most cases) should assume a key role when phylogenies are considered and that higher-level taxa are of limited use (Stiassny & Pinna 1994). This argument closes particular avenues of research unnecessarily. As I will go on to demonstrate, diversity measures employing Linnnean hierarchical classifications have proved very useful and the robustness of their results counters arguments such as the one above. Also, well-resolved phylogenies are scarcely available for even relatively small subsets of some of the groups most useful in diversity studies (such as nematodes, insects and bacteria). Waiting for phylogenies to catch up with diversity research will retard potentially rewarding research.

ii) Molecular measures

Mallet (1996) and Crozier (1992, 1997) suggested that as the nature of diversity is fundamentally genetic, measures of genetic diversity should be the focus of biodiversity and conservation studies. 'Species', when viewed as 'non-integrating genotypic clusters in local areas', provide a testable pattern of genotype frequencies (Mallet 1996). This avoids the problems associated with including evolutionary hypotheses into definitions of the very same objects of these hypotheses.

iii) Ecological measures

These often take the form of considering 'functional' (e.g. trophic) groups or 'guilds' (Gaston 1994). Martinez (1996) provides a good overview to this different approach, including quantitative measurements of ecosystem diversity and hypothesis testing.

iv) Indicator groups or surrogate groups

One 'shortcut' for assessing biodiversity involves the use of indicator groups or surrogate taxa, whose species richness is considered to be representative of total site species richness (Eiswerth & Haney 2001; Fleishman et al. 2005; Rempel et al. 2004; Williams & Gaston 1998; Williams et al. 2006). Specific examples of using surrogate taxa as proxies of biodiversity include the use of macroinvertebrates in ponds (Briers & Biggs 2003; Bilton et al. 2006), butterflies and skippers (Kerr et al. 2000), insects in general (McGeoch 1998) and polychaetes for marine biodiversity (Olsgard et al. 2003). Often, however, the congruence between the indicator taxa and the overall biodiversity has not been tested (Bilton et al. 2006).

It is important to appreciate the difference between 'indicators' of disturbance and 'indicators' of diversity. The indicator taxa chosen for disturbance or ecological integrity monitoring will not necessarily be the same as those chosen to indicate overall species diversity. McGeoch (1998) divides bioindicators into environmental indicators, ecological indicators and biodiversity indicators, and stresses the importance of researchers defining clearly the purposes of their studies.

There is debate in the literature as to whether the diversity of indicator taxa is representative of the diversity of closely related taxa (Fleishman et al. 2005), let alone that of more distantly related groups.

Incorporating Phylogeny into Biodiversity Studies

Recognizing the contribution of phylogeny to biodiversity, Pielou (1975) asserted that diversity would be higher in a community in which species are divided amongst many genera as opposed to one where the majority of species belong to the same genus. In reviewing research that had attempted to add information from phylogenies to biodiversity studies, May (1990) referred to this work as "…the beginnings of what might be called the calculus of biodiversity." It was from these beginnings that the following ideas arose.

Taxic Diversity (Vane-Wright et al.) and Phylogenetic Diversity (sensu Faith, sensu Nixon and Wheeler)

Vane-Wright et al. (1991) proposed a 'taxic measure' of diversity incorporating information from the classifications of the organisms studied. Expanding on that, Faith (1992) defined 'phylogenetic diversity' (PD) and showed how it also incorporated phylogenetic considerations, but gave different priorities for conservation than those based on the 'taxic measure'. In the elaboration of this concept of phylogenetic diversity (Faith 1994a, 1994b, 1996, 2002; Faith et al. 2004), a common thread is that 'features' of species, and not species themselves, are the units of biodiversity. This idea will be revisited in the second part of this paper

with respect to the relationship between biodiversity and ecosystem functioning. Faith linked his idea of conserving 'features' of species to the 'option value' idea of McNeely et al. (1990). Biological diversity was seen as the reservoir available to groups of organisms to deal with changing environmental conditions. Faith considered his 'features' of species as a measure of this option value. It follows logically that taxonomically distinct groups, by providing more distinctly different features, will contribute more to 'option value'.

Separately, Nixon and Wheeler (1992) formulated two measures of 'phylogenetic diversity', which they defined as "the relative species diversity of clades." One measure was termed an 'unweighted binary' phylogenetic diversity index and the other a 'weighted' phylogenetic diversity index. These measures ranked species based on their 'phylogenetic uniqueness' (i.e. species diversity of a monophyletic group relative to its sister group). These, it was argued, could be used as the basis of conservation decisions.

The taxic measure of Vane-Wright et al. (1991) was calculated based on hierarchical classifications in which equivalent ranks are treated as equal. Concerns about this method included whether or not equivalent ranks are comparable between very different groups. Both forms of phylogenetic diversity, being based on cladistically inferred phylogenies, are open to the usual criticisms of any method that includes phylogenies – they are only as good as the phylogenies upon which they are based.

Taxonomic Diversity and Taxonomic Distinctness

Warwick and Clarke (1995, 1998; Clarke & Warwick 1998, 2001) proposed two diversity measures that include elements of phylogenetics – 'taxonomic diversity' and 'taxonomic distinctness'.

Taxonomic diversity (Δ) is calculated as:

$$\frac{\sum\sum_{i<j} w_{ij} x_i x_j + \sum_i 0, x_i (x_i\text{-}1)/2}{\sum\sum_{i<j} x_i x_j + \sum_i x_i (x_i\text{-}1)/2}$$

where x_i is the abundance of the ith species (i = 1,..., S) and w_{ij} is the 'distinctness weight' given to the path length linking species i and j in the hierarchical classification. This measure can be thought of as the average (weighted) path length between every pair of individuals. In its earliest manifestation, the 'distinctness weight' was set to w_1 = 1 for species in the same genus, with a linear scale for higher levels, up to species in different phyla (w_6 = 6) (Figure 4-3). This simple assumption of constant increments gave results as highly robust as any of the alternatives examined (Clarke & Warwick 1999).

Taxonomic distinctness (Δ^*) is defined qualitatively as the taxonomic diversity (Δ) divided by the value it would take in the simplest case of all species in a sample belonging to the same genus. This is equivalent to ignoring the path lengths between individuals of the same species (Warwick & Clarke 1995).

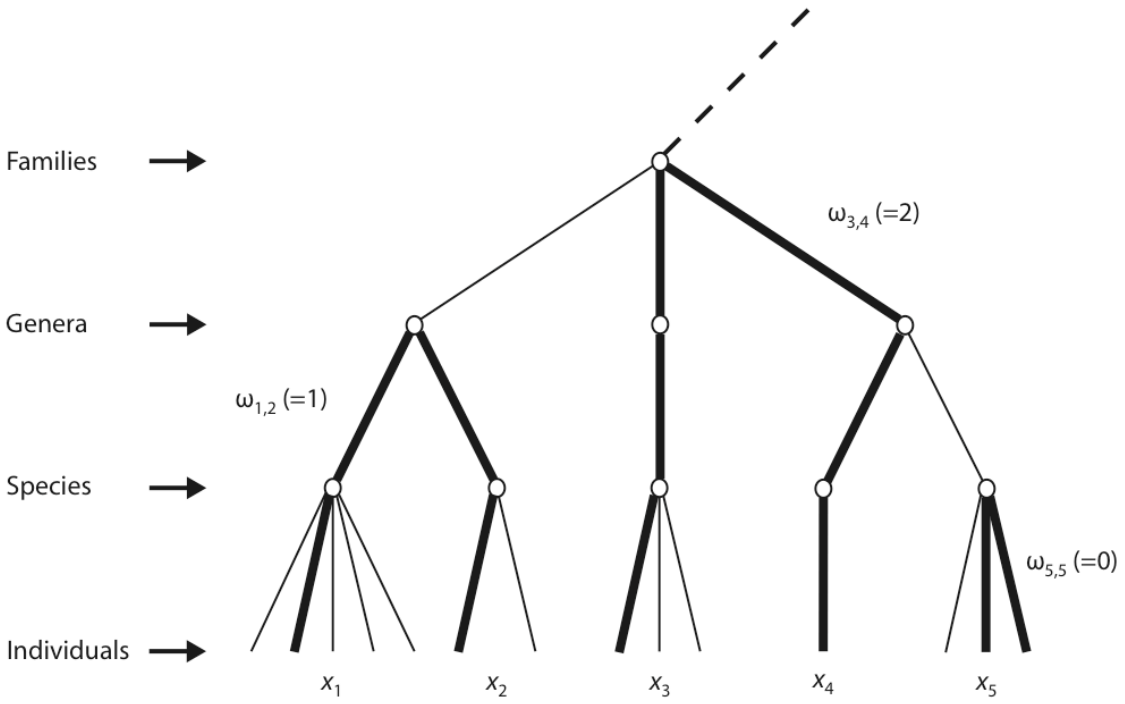

Figure 4-3: Example of part of a taxonomic classification. Taxonomic diversity and distinctness measures are defined using path length weights ($\omega_{i,j}$)(after Figure 1 from Clarke & Warwick 1998).

Quantitatively, taxonomic distinctness (Δ^*) is calculated as:

$$\frac{\sum\sum_{i<j} w_{ij} x_i x_j}{\sum\sum_{i<j} x_i x_j}$$

Both of these indices can be reduced to a third index, "averaged taxonomic distinctness" (Δ^+) (Clarke & Warwick 1998) calculated as:

$$\frac{\sum\sum_{i<j} w_{ij}}{s(s-1)/2}$$

There are a number of major advantages of these indices over all previous ones. Firstly, they are more sensitive to environmental changes. In situations along gradients of increasing environmental disturbance, both have been shown to decrease while other indices have remained constant (Warwick & Clarke 1995). Secondly, recognizing the sampling-effort dependency of many standard diversity estimates, these measures include higher taxa richness and evenness concepts while being formulated to be insensitive to sampling effort (Warwick & Clarke 1995). This is a tremendous advantage as it removes the most serious known bias of all the other indices. This means that they can be used for measures other than species counts, such as biomass and presence/absence data.

Novel inquiries can emerge from thinking about these ideas in different ways. For example, instead of incorporating phylogeny into measures of diversity to study disturbance, one could look at how disturbance affects phylogenetic structure on different scales. Those interested in biological effects monitoring can still use properties of this structure as measures of biodiversity, but more importantly, this

will allow research biologists to generate hypotheses on the causes of these changes. Even though controlled experiments have greater strength of inference than comparative studies (Yoccoz et al. 2001), a project examining phylogenetic changes in response to environmental degradation would benefit from comparisons to other research. Studies driven by hypotheses of change in phylogenetic structure resulting from environmental disturbance would be helped tremendously by the independence from sampling effort affording by taxonomic diversity and distinctness indices of Warwick and Clarke.

Implications for Biomonitoring

> *"Monitoring of populations is politically attractive but ecologically banal unless it is coupled with experimental work to understand the mechanisms behind system changes."*
>
> Krebs 1991

The main limitation of most biomonitoring programmes is that they have suffered from a lack of well-articulated objectives and have not been hypothesis driven. For the research scientist, biomonitoring should not involve just measuring differences in biodiversity between places and times. Even in biomonitoring programmes designed for making management decisions, objectives should be stated explicitly.

The importance of scale is obvious when biodiversity is the focus of biomonitoring. If biodiversity is seen as a manifestation of processes occurring on large time scales, then the degree to which this can inform a monitoring programme, the focus of which is such a short time scale, must be addressed.

Biodiversity and ecosystem functioning

This second part of this review deals with specific application of the above issues to studies of ecosystem functioning. The impact of biodiversity loss on the functioning of ecosystems and their ability to provide ecological services has become a central issue in ecology (Loreau & Hector 2001). Biodiversity studies can be useful in and of themselves, but their scientific utility is only as good as the clarity with which their objectives and hypotheses are delineated. Measures of biodiversity will be more useful when used as tools for other studies in ecology and evolution.

Background

> "*…the more diversified in habitats and structures the descendants…become, the more places they will be enabled to occupy…it has been experimentally proved if a plot of ground be sown with one species of grass, and a similar plot of ground be sown will several distinct genera of grasses, a greater number of plants and greater weight of dry herbage can be raised in the latter than the former case."*
>
> Darwin 1859

As is often the case for other issues in ecology and evolution, we can find reference to this issue in Darwin's writings, in this case to the relationship between biodiversity and productivity. Elton (1958) expressed a similar sentiment when he wrote that a decrease in diversity led to decrease in ecological stability and functioning.

Hawksworth and Harper (1994) provided examples of related questions from which hypotheses can be generated. They include:

1) does biodiversity confer stability?
2) does biodiversity confer productivity?

3) does biodiversity reflect sustainability?
4) does biodiversity reflect the evolutionary time elapsed without major disturbances?

Before considering how to address these questions, we must ask if they can be addressed at all. In other words, an important question to ask is whether or not there is a causative relationship between diversity and ecosystem properties and processes. If there is, then what is the nature of the relationship? Loreau et al. (2001) describe two main schools of thought with regard to diversity-function issues. In the first, the relationships between diversity and ecosystem functioning are seen to be clear and causative. According to the second, it is the main functional traits or attributes of the dominant species that drive ecosystem properties, and not species diversity, *per se* (recall the ideas of Faith on 'features' and 'option value').

Ecosystem functioning hypotheses

Some of the hypotheses that have been proposed concerning the relationship between biodiversity and ecosystem functioning are represented graphically in Figure 4-4 (from Naeem et al. 2002). The central question addressed in Figure 4-4 is: what are the possible forms the graph can take between the points of zero diversity and 'natural levels' of diversity? Schläpfer and Schmid (1999) have categorized biodiversity-functioning hypotheses into three classes.

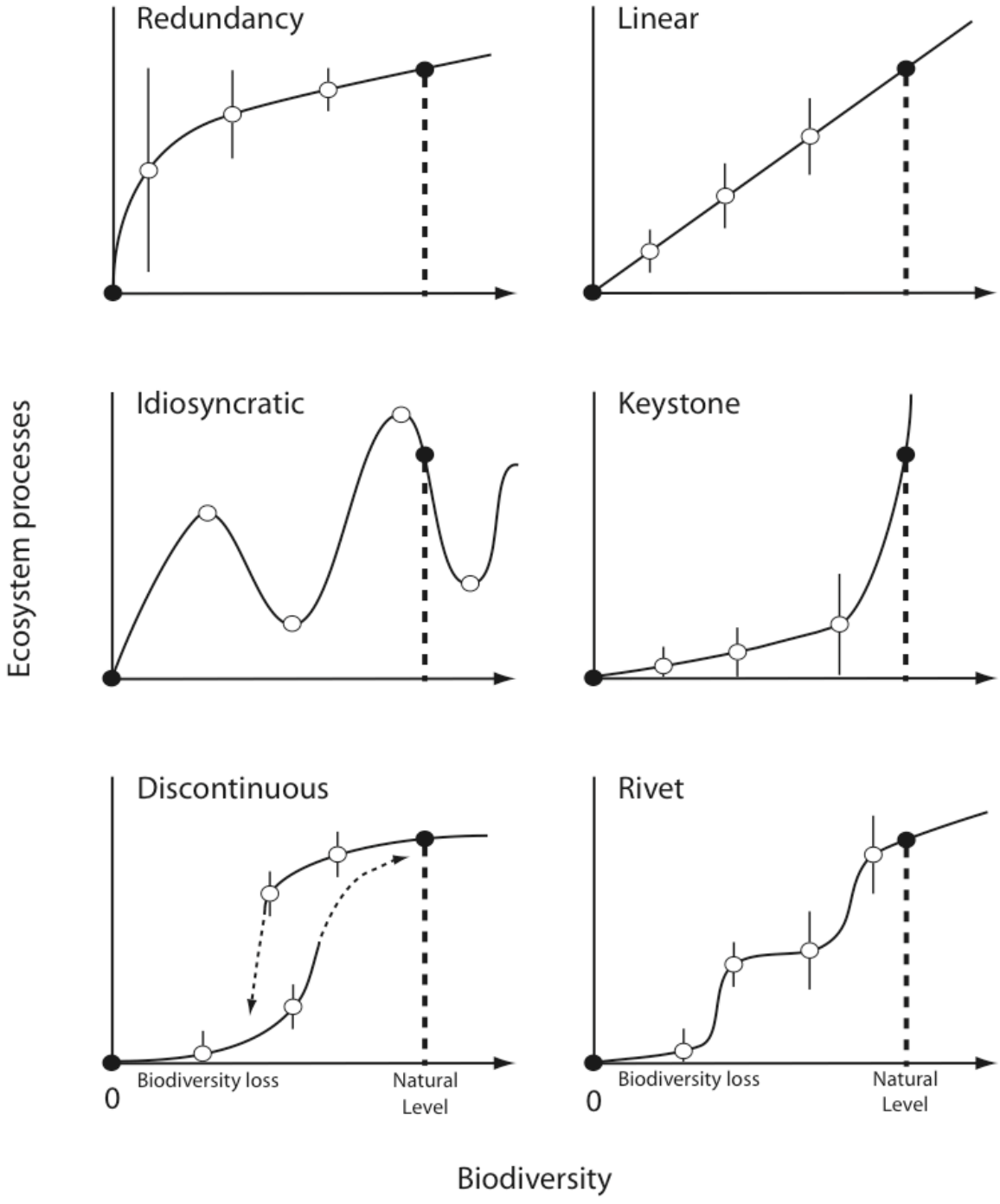

Figure 4-4: Graphical representation of hypothetical relationships between biodiversity and ecosystem functioning (after Figure 1.2 from Naeem et al. 2002).

They are ones in which:

1) species are primarily redundant (e.g. redundancy hypothesis)
2) species are primarily singular (e.g. keystone hypothesis)
3) species impacts are context-dependent and therefore unpredictable (e.g. idiosyncratic hypothesis)

In the remainder of this section I will outline two representative hypotheses, and in the final section I will show how aspects of biodiversity measurement influence this research.

Diversity-Stability Hypothesis

This is actually an umbrella term for a number of hypotheses that relate diversity and ecological stability (e.g. 'rivet hypothesis'). These hypotheses all hold that increased diversity leads to increased stability. The significance of the results of tests of this hypothesis has been the subject of much, sometimes acrimonious, debate. If this relationship is causative, then what is the mechanism? Species differ in their traits, and communities with more species have a greater probability of containing higher phenotypic trait diversity. More diverse ecosystems are more likely to contain species with greater 'complementarity' of traits (Loreau et al 2001).

Species-Redundancy Hypothesis

Another hypothesis is that many species are so similar that ecosystem functioning is independent of diversity if major functional groups are present. Above a critical diversity, most species are functionally redundant so species lost

from a system can be compensated for by species that fill the functional role of the lost species without affecting the functioning of the ecosystem (Naeem et al. 2002).

Advancing our understanding

We now return to some of the ideas presented in the first part of this paper. What is the impact of different measurements of biodiversity on these ecosystem functioning hypotheses, and how does incorporating phylogeny affect the results?

Much of the debate on the above hypotheses depends on the unit of biodiversity measured (species versus functional group). For the diversity-stability hypothesis, an understanding of the measurement of biodiversity is essential to its testing. 'Ecological selection' of species with particular traits ("selection effect") and complementarity among species with different traits ("complementarity effect") are two ways by which phenotypic diversity maps onto ecosystem processes (Loreau & Hector 2001). These two mechanisms, however, may be viewed as two poles on a continuum from pure dominance (where species redundancy is high) to pure complementarity among particular sets of species or functional groups. Insomuch as phylogenies represent maps of the ways in which various traits differ, separating these two effects can be aided by phylogenetic considerations. In this need to be able to measure trait diversity, considering phylogeny is important as not including it will give results that are at best ambiguous, and at worst erroneous.

Related to the species-redundancy hypothesis, Loreau et al. (2001) suggest that if dominant species control ecosystem processes, then mostly rare (in the sense

of abundance) species will go extinct. Referring back to the work of Warwick and Clarke, it would be interesting to see if those rare species are more likely to be from taxa with few representatives. This is another example where phylogenetic considerations can inform areas of biodiversity-ecological functioning studies.

Most studies involving biodiversity and ecosystem functioning have focused only on changes in the number and variety of species and/or functional groups on local scales. By virtue of their sampling effort independence, taxonomic diversity and distinctness measures would allow questions of biodiversity-ecosystem functioning relationships to be examined at larger scales.

Conclusion

General

There are practical and logistic reasons why phylogenetic measures of biodiversity are advantageous over species-based measures. In some cases, biodiversity research is thwarted by the difficulties in compiling species inventories, such as in taxonomically challenging groups like nematodes and bacteria where species identification is often prohibitively time-consuming and undescribed species are encountered frequently. Future development of sequencing techniques, such as 454 sequencing, may allow all the organisms from one phylum occurring in entire, bulk samples to be selectively sequenced. Using these sequences to construct a phylogeny, and using a measure of biodiversity based on this phylogeny, would

allow studies of biodiversity to continue in the face of the difficulties experienced when working with species.

Biomonitoring

Do phylogenetic considerations have more to offer to biomonitoring than simply providing the classifications from which indicator taxa are chosen or upon which biological measures of disturbance are based? One area of research that has not received any attention is to consider how phylogenies can be incorporated into the selection of bioindicators or in their assessment during biomonitoring. It is important to remember that these 'indicators' will not necessarily be the same as those used as a surrogate of biodiversity. A number of factors, such as degree of susceptibility or tolerance and life-history strategies, go into the selection of disturbance indicators. Phylogeny should be another of these factors.

Before the work of Warwick and Clarke, much research dealt with incorporating degrees of relatedness in diversity measurement to help choose taxa for conservation. They differed with their emphasis on including relatedness as it applied to environmental degradation or remediation. A potentially rewarding avenue of inquiry is to be found in building on the emphasis of this work. It is clear how their research was improved greatly by incorporating phylogeny.

Conservation

The 'biodiversity crisis' (Eldredge 1992) has driven much research in this area and led to the development of new techniques and methods. For the purposes of conservation, including a phylogenetic component allows the prioritization of populations, species and habitats, the loss of which would represent the greatest loss of phylogenetic diversity (Nixon & Wheeler 1992). Regardless of the facet of biodiversity under consideration (species, functional group, genetic diversity, 'option value', 'feature diversity'), it is more likely to be preserved with greater preservation of phylogenetic diversity and uniqueness.

Ecosystem functioning

As important as the conservation issue is, the potential of biodiversity studies to provide insights into many processes in ecology and evolution, including ecosystem functioning, will drive much future research.

Biodiversity measurements are the straw from which the bricks of ecosystem functioning hypotheses can be made. As such, a clear understanding of the limitations and biases of these measures is essential for the results of ecological studies to be meaningful. Incorporation of phylogeny has been shown to be an informative addition to these measures as it helps overcome some of these limitations and biases. Studying species outside of a phylogenetic context makes it more difficult to tease out their roles in ecosystem functioning systems. Studying the impact of the addition of phylogeny on biodiversity measures can be done

simultaneously with research on ecosystem functioning, as one will illuminate the other.

CHAPTER 5: DESCRIPTION OF A FREE-LIVING NEMATODE SPECIES AS AN EXAMPLE OF ASPECTS OF THE TAXONOMIC IMPEDIMENT.

(published as: King, I., M. Mundo-Ocampo and P. De Ley. *Xyala finneyae* sp. n. (Nematoda: Xyalidae), from the intertidal region of the eastern Gulf of California, Mexico. *Nematology* **12(5)**: 673-680.)

Introduction

As mentioned previously (Chapter 3), the "taxonomic impediment" (Taylor 1983) is a serious limitation to using nematodes at the species level for biomonitoring. One aspect of the taxonomic impediment is the occurrence of species new to science in many benthic and estuarine samples. As has been seen, identification to species may not be necessary for biomonitoring. However, there are many other cases of questions in nematode and benthic ecology where identification to species is essential. The first step in identifying a species is to determine if, in fact, it is a new species. The degree of difficulty of determining this will depend partly on the taxonomic history of the group to which the species under consideration belongs (e.g. synonymizations, history of taxonomic "clumping" vs. "splitting"). Also, decisions have to made about the nature of "species".

As an example of some of the difficulties faced in nematode studies at the species level, a new species from the genus *Xyala* Cobb, 1920, is described. A species belonging to this genus was found in samples from Bolsa Chica. This is not surprising as species belonging to this genus occur in intertidal sandy sediments

and are amongst the most commonly found nematodes in this type of habitat (Stewart & Nicholas 1994). The systematics of species in this genus are impaired by the lack of detail given in the original description of the type species, *X. striata* Cobb, 1920. This problem has been compounded by some of the subsequent descriptions of new species in this genus and by the potentially erroneous identification of specimens as *X. striata* in the literature. Nichols (1979) states: "Those reports that include a species description indicate that the *X. striata* phenotype is quite variable." Alternatively, a compilation of measurements of morphological structures and calculated ratios leads to such a large range of values as to suggest that multiple species have been lumped together under the name *X. striata*. Vincx and Furstenberg (1988) described specimens from the North Sea in greater detail than previous descriptions of *X. striata* and we accept their description as the best record of the species currently available, although it should be noted that the type locality of *X. striata* is not located in the North Sea but rather at Woods Hole, USA, in the north-western Atlantic.

The species described in this paper, while being most similar to *X. striata* in measurements, is sufficiently different in other aspects of its morphology to be recognised as new based on the morphological species concept.

Materials and Methods

Sampling site

Samples were collected from the same intertidal location at Santa Clara, Sonora, Mexico, in November, 2004 (SC1) and April, 2006 (SC2). The Gulf of Santa Clara is located at the eastern edge of the Colorado River Delta on the north-western coast of the state of Sonora (31°41' N; 114°30' W). The site was characterised by sediments ranging from fine sand near the low tide water line to silt 60 m inland from the low tide water line with a few rocky outcrops surrounded by pebbles. The specific collection site included sand with deposits of limestone-clay, while silt-clay predominated in the substrata (see Mundo-Ocampo et al. 2007).

Sample processing

Field samples from the first collecting trip were fixed with a 5.0% buffered (pH 7.0) formalin solution. Fixed nematodes were isolated from the samples by decanting with a large volume of water and retaining the supernatant, including suspended nematodes, on a 45 μm mesh screen. Nematodes were washed into a 250 ml beaker and then mixed with Ludox™ solution prepared to a specific gravity of 1.15 (two parts Ludox™ to three parts water). After suspension with Ludox™, each sample was washed on to a 45 μm mesh sieve six times. *Xyala* specimens were concentrated in a 25 ml glass vial of distilled water and submerged in a container of hot water (65°C) to heat-relax the nematodes. After one minute, the vial was removed and filled with a 4.0% formalin solution. Nematodes in the resulting

formalin dilution were left for a minimum of 24 h (up to several days) before being processed to anhydrous glycerin.

Field samples from the second collecting trip were fixed on-site with DESS (Yoder et al. 2006). Each sediment sample was collected in a bucket; seawater was added and the contents were stirred. Live nematodes were isolated from the samples by decanting, retaining the supernatant, including suspended nematodes, on a 45 µm mesh sieve. This process was repeated two times more. DESS was poured directly onto the sieve containing the nematodes and the nematodes were washed into vials using DESS. The nematodes were transferred to glycerin (Seinhorst 1959, as modified by De Grisse 1969).

SEM preparation

After primary fixation in formalin or DESS, nematodes were first rinsed with several changes of deionised water followed by several changes of 0.1 M phosphate buffer (pH 7.0). Adult females and males were hand picked and transferred to a BEEM® capsule (Ted Pella, Redding, CA, USA). Distortion and loss of rigidity to fragile structures on the head were limited by post-fixation for 2-4 h (or overnight) in a 4.0% OsO_4 solution. Post-fixed specimens were first rinsed for 15 min with several changes of cold (4°C) 0.1 M phosphate buffer, and then dehydrated through a series of absolute ethanol aqueous dilutions ranging from 20% through 100% absolute ethanol. Dehydrated specimens were critical point dried in a Balzers Union® - 815 critical point drier. Dried specimens were mounted in several positions on top of double sticking copper tape attached to aluminium stubs. Stubs

with mounted nematodes were coated for 3 min with a 25 nm layer of gold palladium in a Cressington 108 Auto sputter coater. Specimens were observed using a Philips® XL30-FEG Scanning Electron Microscope (SEM) operated at 10 kV.

Morphological observations

Measurements and illustrations were prepared from *camera lucida* drawings on an Olympus® CX41 microscope. Line drawings were prepared using Adobe® Illustrator® CS 11.0.0 software.

Selected formalin- and DESS-preserved nematodes were mounted temporarily under a cover glass on ringed fluorescence slides in a drop of deionised water and multi-focal images were recorded as described in De Ley and Bert (2002) via a Matrox RTMac on a Macintosh G4 PowerPC. The resulting images were edited in Final Cut Pro 3 (Apple, San Jose, CA, USA), archived in Quick-Time™ version 7.1.3 format and submitted to NemATOL (http://nematol.unh.edu/). Virtual specimens of the holotype and paratype in permanent glycerin mounts were created in a similar fashion (http://faculty.ucr.edu/~pdeley/vce/Xyala/SpeciesB/thumbsB.html)

Results

Xyala finneyae* **sp. n.**

(Figures 5-1 to 5-3)

Measurements

See Table 1.

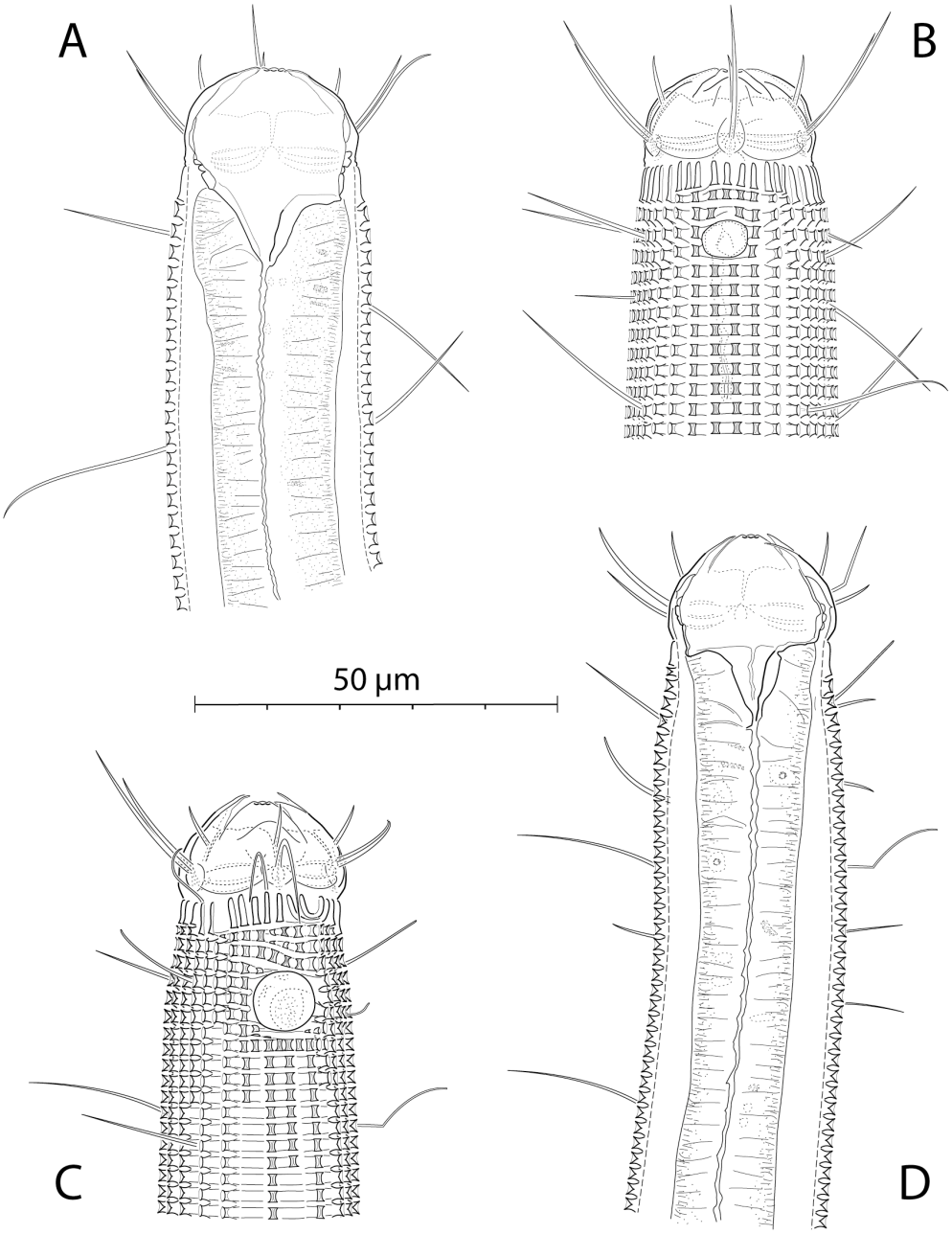

Figure 5-1: *Xyala finneyae* sp. n. A-B: female; C-D: male. A: Anterior end, median section; B: Anterior end, surface view; C: Anterior end, surface view; D: Anterior end, median section.

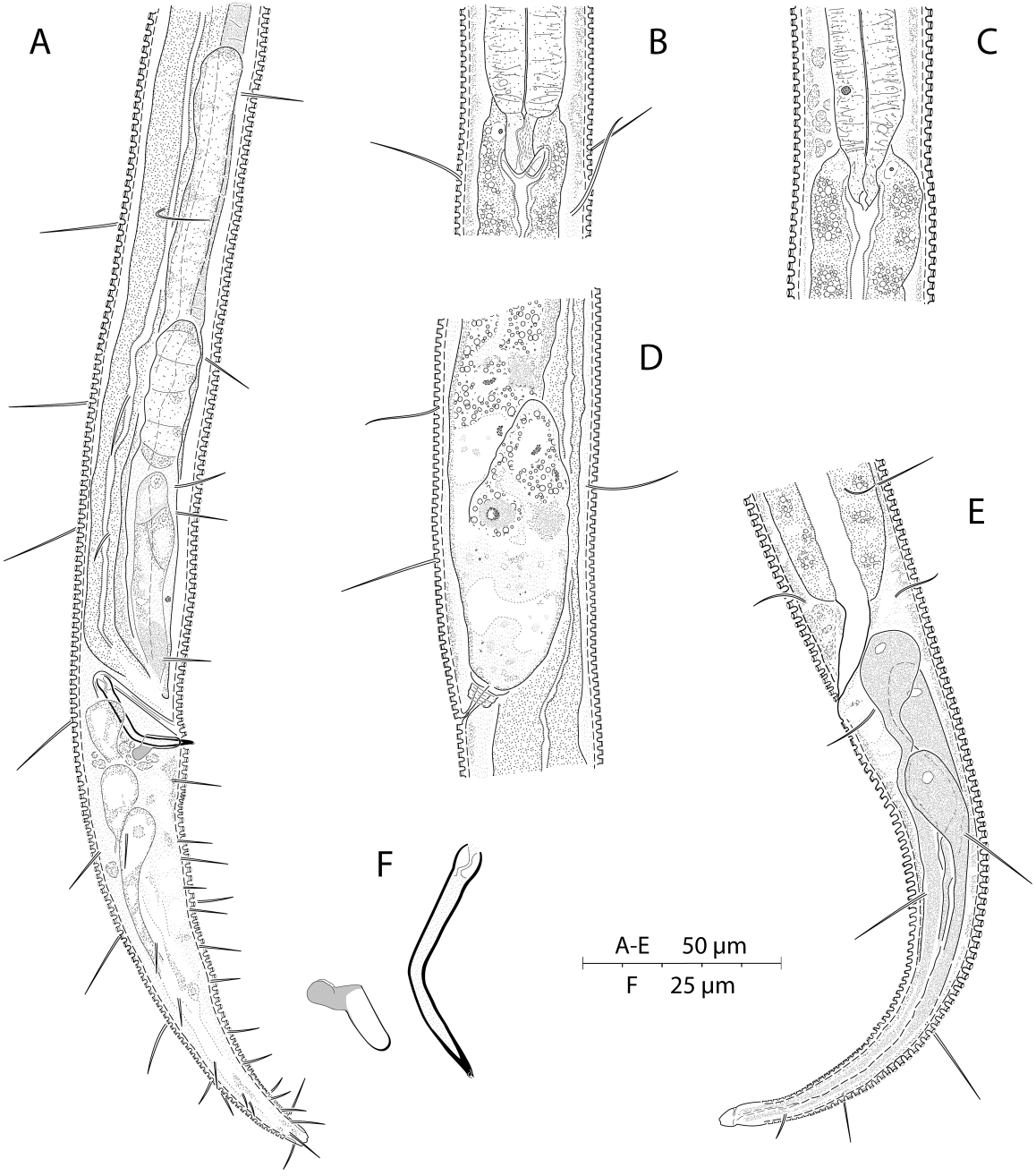

Figure 5-2: *Xyala finneyae* sp. n. A-B: male; C-E: female. A: Posterior region; B: Cardia,; C: Cardia; D: Vulval region showing spermatheca; E: Anal region and tail.

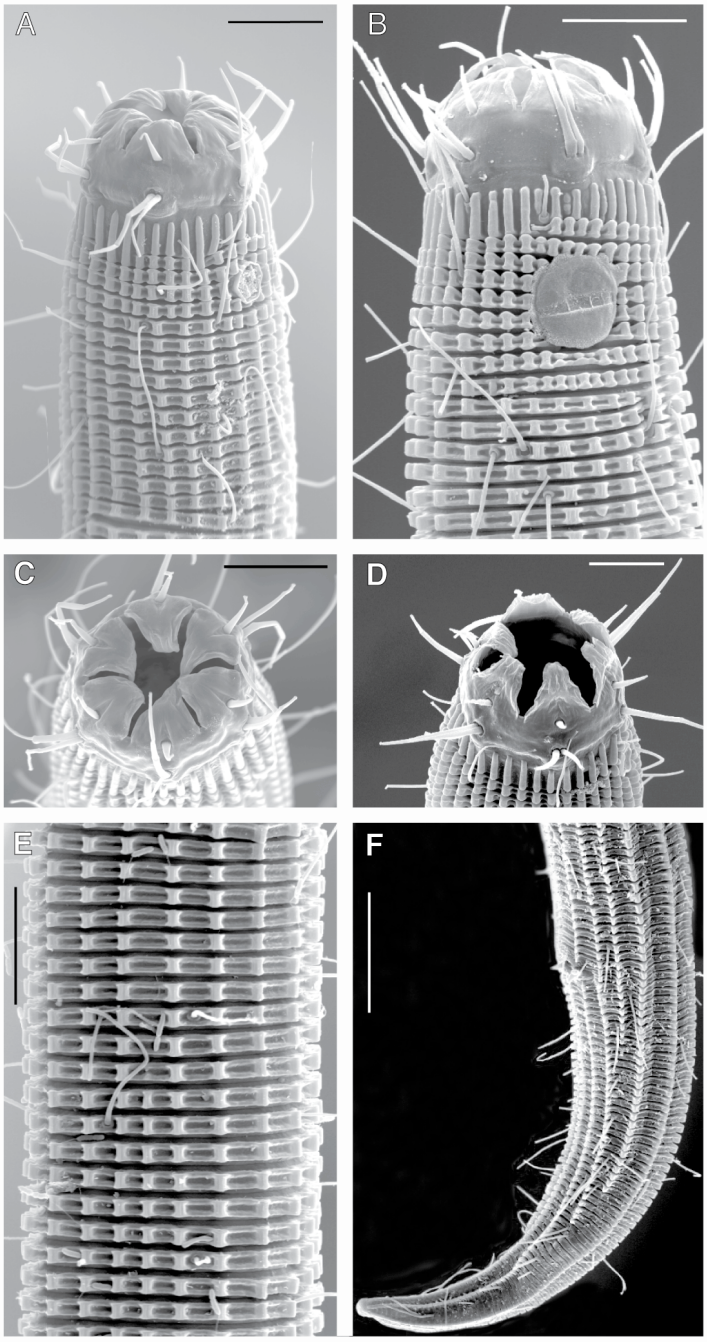

Figure 5-3: Scanning electron micrographs of *Xyala finneyae* sp. n. A, C, D: female; B, E, F: male. A: Sublateral view of anterior; B: Lateral view of anterior end; C, D: Oblique face views; E: Cuticle near mid-body; F: Tail. (Scale bars: A-E = 10 μm; F = 20 μm.)

Table 5-1. Morphometrics of *Xyala finneyae* sp. n. from the type locality on two dates. Measurements are in µm and in the extended format: mean ± s.d. (range).

Character	November, 2004			April, 2006	
	Holotype	Paratypes	Paratypes	Paratypes	
	Male		Female	Male	Female
n	1	2	6	3	6
L	1455	1450; 1548	1625 ± 107.7 (1485-1725)	1350-1482	1499 ± 157.3 (1299-1704)
a	45.5	43.9; 48.4	39.7 ± 4.5 (36.2-44.8)	40.2-42.6	33.5 ± 4.4 (26.4-38.9)
b	4.5	4.6; 4.5	4.6 ± 0.2 (4.3-5.0)	4.7-4.9	4.7 ± 0.2 (4.4-5.0)
c	10.4	12.2; 11.3	11.2 ± 0.8 (10.0-12.4)	11.2-11.9	11.6 ± 0.8 (10.9-12.8)
c'	4.1	3.5; 4.0	4.0 ± 0.3 (3.7-4.5)	3.6-4.4	4.2 ± 0.5 (3.5-4.9)
V	-	-	73.8 ± 2.0 (70-76)	-	74.6 ± 0.9 (73-76)
Max. body diam.	32	33; 32	42 ± 3.3 (38.5-45)	32-36	45 ± 6.9 (40-58)
Neck	310	298; 324	351 ± 19.9 (317-372)	279-291	321 ± 34.4 (264-351)
Tail	140	119: 137	145.4 ± 9.7 (135-156)	114-124.5	131 ± 20.7 (104-152)
Anal body diam.	34	34: 34	36.5 ± 0.9 (34.8-37.2)	28.2-31.8	31.3 ± 2.38 (28.8-34.2)
Anterior end to vulva	-	-	1200 ± 92.3 (1075-1288)	-	1118 ± 107 (978-1262)
Spicule (arc)	30	28; 29	-	28.8-31.2	-
Spicule (chord)	24.9	24; 25.2	-	25.2-28.2	-
Gubernaculum	12.5	12; 13.2	-	12.6-13.8	-
Apophyses	4.1	4.1; 4.2	-	4.2-4.3	-
Amphid fovea diam.	8.5	8.5; 9.5	5.8 ± 0.5 (5.3-6.5)	7.8-9	5.6 ± 0.6 (4.8-6.6)
Anterior end to anterior edge of amphid	24	19; 25	21.2 ± 0.9 (20-22.5)	19.8-22.8	21 ± 3.3 (15.6-25.8)
Buccal cavity					
depth	14.8	14.9; 15.2	14.2 ± 0.5 (13.7-14.8)	14.4-15	14.1 ± 0.7 (13.8-15.6)
diam.	17.5	17.6; 17.9	18.5 ± 0.7 (17.7-19.5)	17.4-18	18.1 ± 1.6 (16.8-20.4)
Cardia length	14.2	14; 13.9	14.4 ± 0.3 (14.1-14.8)	13.5-13.9	14.0 ± 0.2 (13.8-14.2)

Description

Male

Body cylindrical, of relatively uniform diam. from anterior end to cloacal opening. Cuticle strongly annulated. Portions of annular cuticle raised at regular intervals around circumference of each annule and projecting to form longitudinal crests. Crests aligned on consecutive annules but not longitudinally connected, although lower magnifications produce superficial appearance of continuous longitudinal striae. Crest length per annule = 1.8 μm, consistent along length of nematode, except for first ring of crests which are 4-6 μm long and located at base of head region anterior to first body annule proper. Anterior crests 1.2 μm apart on consecutive annules, crests 0.3 μm apart from *ca* mid-pharynx to tail-tip. Crests twice as wide in mid-body compared to cervical and caudal regions (1.2 *vs* 0.6 μm, respectively). Number of crests per annule varying from anterior to posterior: 32 in anterior region immediately posterior to amphidial fovea reducing to 25 within six annules posterior to that, 13 at mid-body, ten at level of cloaca and seven by mid-tail. Lips triangular, thin, each with one inner labial sensilla at centre of base. Lip region offset from first ring of crests by shallow depression. Six distinct, semi-circular, lobes at posterior end of lip region, bearing six outer labial setae (15-18 μm) and four cephalic setae (8-9 μm; two subdorsal and two subventral) in one circle. Somatic setae common in anterior quarter of body and in tail region (no terminal setae), sparse in mid-body region. Amphid fovea circular, 25-29% of

corresponding body diam., anterior margin located at 0.75-1 corresponding body diam. from anterior end of nematode. Amphid fovea interrupting annules 3-5. Buccal cavity cylindrical, anteriorly broad, lined by two or three contiguous, granular to comma-shaped, sclerotisations, funnel-shaped posteriorly. Pharynx surrounding conical part of buccal cavity, cupping base of cylindrical part just posterior to sclerotisations. Pharynx cylindrical, muscular. Nerve ring inconspicuous, observed in a single specimen at 33% of pharynx length. Cardia conical in lateral view, but actually wedge-shaped with two lateral triangular projections embedded in intestine on left and right side. Anterior testis situated to left, posterior testis to right of intestine. Posterior end of *vas deferens* accompanied by two lateral series of *ca* eight glandular cells plus a pair of dorsosublateral gland cells anterior to lateral series (only visible relatively clearly in three males). Spicules equal in size, sharply curved, L-shaped, with well developed, stirrup-shaped, capitulum and bifid distal tip. Gubernaculum tubular, with two broad, proximal apophyses, which are 4.2 μm long and project obliquely towards the dorsal contour of the body. Tail conical, tapering from cloacal aperture. Three caudal glands present, their outlet(s) unclear in all observed specimens.

Female

Resembling male with respect to most characters but differing as follows. Longer than male. Pronounced sexual dimorphism of amphid fovea (smaller in

female). Amphid fovea interrupting annules two to three. As in male, crest length per annule = 1.8 μm, consistent along length of nematode, with first ring of crests 5-6 μm long. Anterior crests 1.2 μm apart on consecutive annules, crests 0.9 μm apart from *ca* mid-pharynx to mid-body, with distance reduced to 0.3 μm from mid-body to tail-tip. Number of crests per annule 34 in anterior region immediately posterior to amphid opening, reducing to 30 within four annules posterior to that and continuing to mid-body, then 24 at vulval level (with three striae ending within 15 annules posterior to vulva), reducing to 15 by mid-tail. Spermatheca 3.5-4 times as long as corresponding vulval body diam., visible as protruding outgrowth of uterus. Single ovary outstretched, to left of intestine. Vagina short, proximally surrounded by constrictor muscle.

Type habitat and locality

Type specimens were collected from intertidal sandy sediment at Santa Clara, Sonora State, Mexico (N: 31°41'; W: 114°30').

Type material

Holotype male (slide #30572) deposited in the UCR Nematode Collection, CA, USA, along with three female and two male paratypes (slides #30573-30577, inclusive). Two female and one male paratypes deposited in the Zoology Museum, Ghent University, Belgium (slides UGMD 104142 - UGMD104144, inclusive).

Diagnosis and relationships

Xyala finneyae sp. n. is characterised particularly by the crests at the base of the lip region being twice as long as the subsequent crests on the body annules, sexual dimorphism in amphid fovea size (7.8-9.5 in males *vs* 4.8-6.6 µm in females), vulva located at three-quarters of the body length and spicules measuring 29-31 µm.

Xyala finneyae sp. n. is most similar to the type species, *X. striata*, and *X. oxybiotica* Jensen, 1986. It differs from the former species in having the first ring of crests markedly longer than all subsequent ones, in having shorter outer labial and cephalic setae on the lip region (8-9 and 15-18 *vs* 12 and 22 µm, respectively), in a more anterior position of the vulva (V = 70-76 *vs* 79-81 in *X. striata* according to Vincx and Furstenberg, 1988) and in the shorter spicules (29-31 *vs* 44-47 µm).

Compared to *X. oxybiotica*, the new species has a more spherical lip region with thinner cuticle on the lips (*vs* lips more protruding forward and with thicker cuticle in optical section), shorter outer labial and cephalic setae on the lip region (8-9 and 15-18 *vs* 13 and 23 µm, respectively), a more anterior vulva (V = 70-76 *vs* 81-89) and shorter spicules (29-31 *vs* 35 µm). Jensen (1986) reported the presence of lateral guiding pieces in *X. oxybiotica*, a feature that might constitute another difference but which could also be an erroneous interpretation of the bifid spicule tips seen in *X. finneyae* sp. n..

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

General Conclusions

Based on previous studies and on differences in sediment type, it was expected that nematode diversity would be highest at Site 6, the Bolsa Chica State Beach site, and that abundance would be higher in the finer sediments found in the Bolsa Chica Wetlands. Diversity (as represented by the Margalef, Shannon-Wiener and ES(51) indices) increased over the four sampling times for Site 6 with the values for the fourth sampling time being the highest of all samples at all sites. Contrary to expectations, nematode abundances were significantly higher at Site 6 for the first and third sampling dates (both September sampling times). Site 1 had the highest abundances for sampling times 2 and 4, but they were not significantly higher than abundances at Site 6. This may suggest that seasonal differences play a greater role in community structure than sediment characteristics, though longer-term studies would be necessary to see if the season trend continues.

The multivariate data analyses showed that the nematode communities differed among sites at Bolsa Chica. Of greatest interest, results of the MDS ordination suggested that the assemblages at Sites 2 and 4, newly opened to tidal influence, became more similar to Sites 1, 3 and 5. The immediate impacts of the restoration could not be detected since sampling was started perforce well after the inlet/culverts were opened; nevertheless the parallel trends in BC Site 2 and BC Site 4 suggest that both sites may have started out as being even more different from BC

Sites 1, 3 and 5.

An examination of data transformation and taxonomic aggregation showed that the effect of transformation increased with increasing aggregation. This result was made evident by the application of a 2nd Stage MDS ordination. The significant differences between sites and between sampling times observed previously were confirmed, and were found to be statistically robust to both transformation and aggregation.

The increasing availability of molecular data of whole-sample sequences produced by next-generation technology means that one of the products of these data, namely phylogenies, can be employed in the service of biomonitoring. Including these phylogenies in biomonitoring will require new ways of thinking, and considerations of how to adapt current biomonitoring approaches. This approach will help to overcome the "taxonomic impediment" that has frustrated attempts to use free-living nematodes in biomonitoring.

Conclusions and Implications for Biomonitoring/Management at Bolsa Chica

This work represents the first attempt at using free-living nematodes for biomonitoring in western North America. As such, the diversity of the identified species can be used as a baseline for continued monitoring. These data have direct applicability for consultants and conservation management personnel as once baselines are established, routine nematode analysis can be performed much more

easily than the original baseline establishment. Routine identification of nematode families from anatomical characters is much easier than genus/species identification. As a follow-up, a simple pictorial key to the species found at Bolsa Chica will be created that will simplify even the process of species/genus identification. As well, videos of these species will be included in the NemaScope online key to nematode identification (Yoder et al. 2005).

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