Evaluation of Methods and Approaches for Surveying Savanna Invertebrates

By

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ABSTRACT

The savanna is an important biome, which is under threat from land transformation, and it is therefore a focus for conservation planning. Yet, the invertebrate fauna of this biome is poorly documented and hence there is a need to provide baseline data for this component of biodiversity. This project aimed to provide relevant information that can be used by conservation planners and ecologists, by recommending a sampling strategy for the collection of specific taxa for savanna invertebrate surveys.

The effectiveness and efficiency of a sampling strategy using passive and active sampling methods was assessed to provide recommendations for a multi-taxa approach to sampling invertebrates in a savanna ecosystem. In the collection of data, volunteers assisted and they were evaluated in comparison with experienced researchers to assess the effectiveness, efficiency and benefits of using volunteers to carry out multi-taxa invertebrate surveys. In addition, cross-taxon congruency and congruency across taxonomic levels were assessed between nine invertebrate taxa, to select potential surrogates to reduce biodiversity survey costs for conservation planning.

Fieldwork was carried out in the Mkhuze Game Reserve (27.67°S:32.27°E, 400km^2), Phinda Private Game Reserve (27.78°S:32.35°E, 140km^2) and False Bay Park (27.94°S:32.38°E, 25km^2) in north-eastern KwaZulu-Natal, South Africa. Forty-three different sites were sampled between November 2002 and March 2005 (summer months). Twenty of these sites were re-sampled across years and in different months during the summer season, giving 77 sampling events. Fifty-four volunteers recruited by the Earthwatch Institute assisted in the collection of data.

Lepidoptera, Hymenoptera (Apoidea), Diptera (Asilidae, Bombyliidae), Neuroptera, Odonata, Hemiptera (Cicadellidae), Coleoptera (Cetoniinae, Scarabaeinae), Orthoptera, Blattodea, Isoptera, Araneae (Araneidae, Thomisidae, Oxyopidae), Scorpionida, Myriapoda (Diplopoda, Chilopoda), Mollusca and Annelida were sampled using four active searching methods (transects, tree beating, leaf litter and sweep sampling) and two passive methods (pan traps and baited traps).

In its entirety, this project sampled 50 558 individuals from 797 invertebrate species and an extensive database consisting of 33 257 records now exists. A standardised sampling protocol is described for the effective sampling of multiple invertebrate taxa in a savanna biome and recommendations are made for improving the efficacy and completeness of invertebrate surveys based on the application of species accumulation models. Restrictive active searching methods (quadrats) were found to be more effective for sampling epigaeic invertebrates and should be used in conjunction with leaf litter samples. Flying and plant-dwelling invertebrates should be sampled using a range of sampling methods which include baited, malaise and pan traps, active searching along transects and vacuum sampling. I suggest over 75% of the

total estimated fauna to be a satisfactory and realistic level of inventory completeness for making valid comparisons between regions and across sites.

Volunteers sampled lower rates of species accumulation, species richness and unique species when using timed, active search methods. Nevertheless, volunteers and researchers were shown to perform equally well when using un-timed, active searching methods. Previous experience or knowledge of scientific method was beneficial when researchers assessed the perceived usefulness of volunteers to researchers for carrying out fieldwork. The project experience raised the volunteers' environmental awareness, knowledge about biodiversity, invertebrates and conservation research, and enabled volunteers to participate in or design locally relevant conservation based projects on their return home.

Cross-taxon congruencies were observed. However, relationships were weak and potential surrogates could not be selected. The use of higher taxonomic levels to represent species shows good potential as a surrogate but only in species-poor genera or families. The use of species density to determine congruency and select surrogates is likely to produce different results to those produced by community similarity. Furthermore, when selecting surrogates from congruency assessments an optimal ρ-value greater than 0.75 should be required. Below this value, the relationship is likely to be weak and if used as a surrogate misinterpretation may occur.

PREFACE

The research described in this dissertation was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, from November 2002 to April 2006, under the supervision of Professor Michelle Hamer and co-supervision of Professor Rob Slotow.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any university. Where use has been made of the work of others it is duly acknowledged in the text.

Chapters two, three and four are presented as papers for submission to international journals. Thus, each paper is independent and therefore some repetition is unavoidable. Figures and tables are placed in each chapter, and appendices and references are set out at the end of each chapter.

The thesis follows the style of the Journal 'Conservation Biology' with heading numbers added for readability.

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INTRODUCTION

The identification of biodiversity hotspot regions is useful for setting conservation priorities at a coarse spatial scale (Prendergast et al. 1993; Reid 1998; Myers et al. 2000). Extensive studies of biodiversity, endemism, rarity, congruency and surrogacy are useful in the selection of reserves at finer spatial scales (Howard et al. 1998; Wessels et al. 1999; Cowling & Heijnis 2001; Lombard et al. 2003; Oliver et al. 2004). However, in order to develop effective and holistic conservation plans it is important to understand the biodiversity patterns and assemblage patterns of the organisms inhabiting ecosystems.

1.1 INVERTEBRATES

Invertebrates are the most abundant and successful terrestrial animals. With about one million known species, invertebrates make up at least 95% of all species, the bulk of them being insects (Myers et al. 2000). Invertebrates occupy almost every terrestrial and freshwater habitat from the poles to the equator. They are important in all ecosystems in terms of species' numbers and biomass, and play vital roles in processes such as pollination, soil formation and fertility, plant productivity, organic decomposition and the regulation of populations of other organisms through predation and parasitism (Daily et al. 1997).

South Africa has an estimated 60 000 described invertebrate species, with an average level of endemicity of 70%, equalling 42 000 endemic species (Le Roux 2002). A critical problem facing conservation biology in southern Africa is the lack of taxonomic knowledge concerning insects and the lack of trained personnel to obtain that knowledge (Soulé 1991). Furthermore, data on the threat status of insects and other invertebrates are almost non-existent (Stork 1997).

1.2 SAVANNA

Tropical savannas are described by Solbrig (1996) as ecosystems formed by a continuous layer of graminoids (grasses and sedges) with a discontinuous layer of trees and shrubs. Many environmental and biotic factors correlate with the distribution of savannas, however, it is not always clear which of these factors cause the main features of savanna structure and function (Scholes & Walker 1993). The principal characteristic of tropical savannas is their diversity in climate, soil types, topography, vegetation, flora, fauna and land use; this diversity makes every savanna type unique and distinct (Solbrig 1996).

The African savannas lie between the lowland moist forests and Mediterranean-type vegetation in the 200 to 1800mm rain belt, on both sides of the equator from about 29°S to about 16°N (Johnson & Tothill 1985). Because of the long history of human settlement on the continent, it is now difficult to distinguish between natural and derived savannas and, consequently, to define the causative environmental conditions which result in natural savanna vegetation (Johnson & Tothill 1985). There are two broad classes of savanna in Africa: the broad-leafed and fine-leafed savannas, which tend to occur in nutrient

poor, high rainfall areas and nutrient rich, low rainfall areas, respectively (Scholes & Walker 1993). Within these two classes are many different vegetation types.

The savanna is an important ecosystem, representing a substantial terrestrial organic carbon pool, which could act as either a net source or a sink of atmospheric carbon dioxide in future decades (Scholes & Walker 1993). Savanna ecosystems occupy approximately 40% of the surface of the tropics, some 23 million km^2 (Cole 1986) and are distributed over the continents of Africa, South America, Asia and Australia. The savanna biome is the largest biome in southern Africa, occupying 46% of its area, and over one third of the area of South Africa (Low & Rebelo 1998). South African savannas are the basis of two major industries: cattle ranching and wildlife related tourism (Scholes & Walker 1993).

Biodiversity research into the savanna ecosystem is poorly represented. This was highlighted at the fifth meeting of the Conference of the Parties, Nairobi, Kenya, in 2000, where decisions were made to ensure the future assessment of the status, value and trends of biological diversity in savanna (Secretariat of the Convention on Biological Diversity 2001).

1.3 INVERTEBRATES AND CONSERVATION

Insects and their habitats are threatened in savanna areas by burgeoning human populations, outdated social engineering policies, social conflict and poor agricultural practices (Scholtz & Chown 1993). These problems are compounded by a lack of systematic and ecological information concerning insect faunas, lack of collation of available knowledge, and management practices aimed at temporal and spatial scales different to those at which insects operate (Scholtz & Chown 1993).

There is unanimous agreement among savanna ecologists that invertebrates play critical roles in the structure and function of tropical savannas throughout the world. Yet, despite the great functional importance of invertebrates, they have largely been ignored by savanna ecologists (Andersen & Lonsdale 1991). However, in recent years there has been significant development with some research groups focussing efforts on specific areas and taxa, examples include: Parr et al. (2004) assessment of ant responses to fire regimes; Samways and Kreuzinger's (2001), and Gebeyehu and Samways' (2003) studies of Orthoptera responses to grazing; van Rensburg et al. (1999) and McGeoch et al. (2002) studies on Scarabaeinae in the Maputaland region; and Stewart and Samways' (1998) study on Odonata in the Kruger National Park.

In Scholtz and Chown's (1993) commentary on insect conservation in South Africa it was discussed that virtually no attention had been given to savanna insect conservation at the habitat or landscape level, although it was noted that this was changing as more and more information on macro-insects became available, particularly in areas where conservation authorities were more aware of the importance on insects. However, since this commentary significant progress has been made with the databasing and evaluation of priority conservation areas for invertebrates in South Africa (e.g. van Jaarsveld et al. 1998;

van Rensburg et al. 1999; Samways 1999; Koch et al. 2000; Hamer 2000; Foord et al. 2002; Davis 2002; Herbert & Kilburn 2004; Parr et al. 2004). Despite this recent work, there are still inevitable lacunas in the data.

Several biodiversity conservation initiatives have been developed or implemented in South Africa. These include the Bioregional Approach to Protected Areas in South Africa; the Cape project (Cape Action Plan for the Environment); STEP (Subtropical Thicket Ecosystem Planning Project) by the Terrestrial Ecology Research Unit of the University of Port Elizabeth; the KwaZulu-Natal C-plan for land-use planning; the Biobase projects by Mpumalanga and the Limpopo Province as part of the Strategic Environmental Analysis commissioned by the Department of Water Affairs and Forestry; and the GAP analysis project of Gauteng Nature Conservation (Hamer & Slotow 2002). All of these initiatives were based on biological data, in particular data on areas of high endemism and diversity, and these projects used data mainly on plants and some vertebrate groups (Hamer & Slotow 2002).

Most terrestrial bio-assessment programmes are designed to assess the ecological integrity of ecosystems and these programmes generally focus on plants or vertebrate groups, therefore, the use of invertebrate groups in terrestrial monitoring programmes has progressed slowly (Jonas et al. 2002). Furthermore, the use of terrestrial invertebrates in conservation planning remains more a topic of scientific discourse than a part of land-management / conservation practice, largely because invertebrates' inordinate numbers, taxonomic challenges and general unfamiliarity make the study too intimidating for most land-management / conservation agencies (Andersen et al. 2002). Nevertheless, there are projects and conservation strategies that have incorporated invertebrates. These include the South African National Spatial Biodiversity Assessment 2004 (Rouget et al. 2005), National Grassland Biodiversity Program (Reyers et al. 2005) and the Maputaland Trans-national conservation project (Smith 2006) all of which incorporated data on threatened and endemic invertebrates species. Nevertheless, the current management of savanna reserves is focused on vegetation and large game communities and the needs of invertebrates are not yet directly catered for, as it is widely considered that the management of vegetation is sufficient to conserve invertebrates (Yen & Butcher 1997; Panzer & Schwartz 1998).

1.4 RESOURCES

Scientific knowledge of invertebrate species distributions, communities and the processes that influence these are poorly understood (Ward & Larivière 2004). Invertebrate surveys are time-consuming and research is generally poorly funded, meaning that resources and labour essential to fulfil research requirements are lacking. There is, however, an urgent need to characterise invertebrate biodiversity, which generally involves local or regional surveys. It is necessary to ensure that surveys produce comparable information (e.g. presence or absence of species, their geographical and ecological distribution) that can then be used both to establish measurements of rarity and diversity, and to provide an objective base for supporting priority-setting decisions in conservation (Yen & Butcher 1997).

1.5 MULTI-TAXA SURVEYS

Due to the sheer number of taxonomic groups, it is impossible to sample all invertebrates. To overcome this problem one can select specific focus taxa. It is recommended that a multi-taxa or 'shopping basket' approach, which does not focus sampling efforts on a single taxon, be used (Oliver & Beattie 1996). This study selected 17 taxa, which were sampled and identified to species level. The choice of taxa and rationale for inclusion is discussed in chapter 2.

The acceptance of invertebrates as being indispensable components of biodiversity has led to a rapid increase in broad-based surveys (i.e. a survey incorporating a wide range of invertebrate taxa) and greater pressure to provide information and guidelines for invertebrate conservation and monitoring (Ward & Larivière 2004). Multi-taxa surveys generally require a range of sampling methods. Sampling techniques must sample the chosen taxa across microhabitats. These techniques should be efficient, with each sampling a suite of species, although some species overlap across sampling techniques is inevitable (Oliver & Beattie 1996; Kotze & Samways 1999; Sauberer et al. 2004). An understanding of the effectiveness and efficiency of the methods for sampling the target taxa is important to provide some validity and rationale. While sampling methods are relatively uniform, the effect of a combination and the number of replicates required for different taxa in different habitats is not standardised. Several studies have used the multi-taxa approach to address a variety of ecological and conservation questions (e.g. Oliver & Beattie 1996; Lawton et al. 1998; Kotze & Samways 1999). However, the assessment of multi-taxa sampling strategies has been limited mainly to forest ecosystems (Lowman et al. 1996; Oliver & Beattie 1996; Lowman & Wittman 1996; e.g. Kotze & Samways 1999; Kitching et al. 2001). Furthermore, the assessment of multi-taxa sampling strategies in the savanna is poorly represented in the literature, with the exception of Druce et al. (2004) who assessed sampling methodologies for three epigaeic taxa in a South African savanna.

1.6 VOLUNTEERS

The use of volunteers is becoming increasingly common in biodiversity and conservation research globally (e.g. Coral Cay Conservation 2005; Earthwatch Institute (International) 2005; Global Vision International 2005). Many biodiversity studies around the world use volunteers for data collection, several of which have made a significant contribution to conservation biology (e.g. Karr 1990; Burgess et al. 1992; Lowman et al. 1996; Mumby & Harborne 1999). Volunteers provide a valuable workforce for fieldwork, a vital link for education within communities, and in many cases, they provide necessary funding for research. Nevertheless, there is some debate over the effectiveness and accuracy of unskilled and inexperienced volunteers in scientific work on the grounds that the information collected could be unreliable as a result of either insufficient training or a lack of consistency through the necessary use of a large number of different observers (Darwall & Dulvy 1996).

1.7 SURROGATES

Due to the large number of invertebrates, the lack of data and lack of resources available to carry out comprehensive invertebrate surveys, surrogates are used to represent invertebrate biodiversity in conservation planning, and for rapid biodiversity assessments. For example, taxonomic surrogates represent the biodiversity of other taxa (Sauberer et al. 2004), higher taxa can be used as surrogates for lower taxa (Balmford et al. 1996), and environmental variables can be used as surrogates for biodiversity (Sarkar et al. 2005), as can biotic and abiotic classifications such as vegetation types or geology (Cowling & Heijnis 2001). The selection of potential surrogates should be done carefully; there are no universally accepted surrogates, and suitable surrogates in one ecosystem or region may not be suitable in another (McGeoch 1998). Surrogates should be selected which represent the conservation or survey goals, are cost effective and logistically suitable and most importantly have good biological efficacy (McGeoch 1998).

Several studies have been published on the selection of surrogates; these studies have looked at a number of biodiversity features and measures at varying spatial scales using a variety of methods to measure congruency (Wessels et al. 1999; Lombard et al. 2003). Many studies on surrogacy have used biodiversity measures such as species richness, to identify congruent relations and select surrogates (Panzer & Schwartz 1998; Lawton et al. 1998). Nonetheless, other studies have shown that the selection of protected areas based on richness measures leads to area protecting overlapping species assemblages, thus duplicating conservation efforts (Williams et al. 1996; Wessels et al. 1999; Margules et al. 2002). Furthermore, studies have demonstrated that the use of surrogates to determine or prioritise protected areas may not be truly representative of patterns in all taxa (Prendergast et al. 1993; Bonn et al. 2002; Sauberer et al. 2004). This has highlighted the need for research on species assemblage patterns and the processes that influence them, and on testing the concepts of congruency, biodiversity indicators and surrogacy.

1.8 SURVEY BACKGROUND

This present survey was carried out in an important South African savanna ecosystem located in a biodiversity hotspot region that is threatened by land transformation. It was the first quantified multi-taxa invertebrate survey to be carried out in the region and provided much needed baseline data. Volunteers recruited by the Earthwatch Institute were used to assist experienced researchers to sample invertebrates.

1.9 STUDY REGION

Fieldwork for this study was carried out in the Mkhuze Game Reserve (27.67°S 32.27°E, 400km^2), Phinda Private Game Reserve (27.78°S 32.35°E, 140km^2) and False Bay Park (27.94°S 32.38°E 25km^2) in north-eastern KwaZulu-Natal, South Africa. The reserves are situated in the diverse region known as the Maputaland Centre. The Maputaland Centre extends from southern Mozambique into north-eastern

KwaZulu-Natal, and is bordered in the north by the Inkomati-Limpopo River, in the east by the Indian Ocean, in the west by the Lebombo mountains and in the south by the St Lucia estuary (van Wyk 1996). The Maputaland Centre consists of a mosaic of mainly extensive savanna communities arranged in complex patterns. It also contains forest, grassland and swamps, largely determined by local edaphic conditions (van Wyk 1996). The Maputaland Centre, for its size, is one of the most remarkable areas of biodiversity in the world; not only are the numbers of endemics high, but also they are spread over virtually the whole taxonomic spectrum (van Wyk 1996).

The study region has been broadly classified as Natal lowveld bushveld, which covers much of the lowveld of Zululand, lying between 150m and 450m altitude (Low & Rebelo 1998). At a finer scale, the study region covers nine different vegetation types classified by Mucina and Rutherford (2005) (Fig. 1.1).

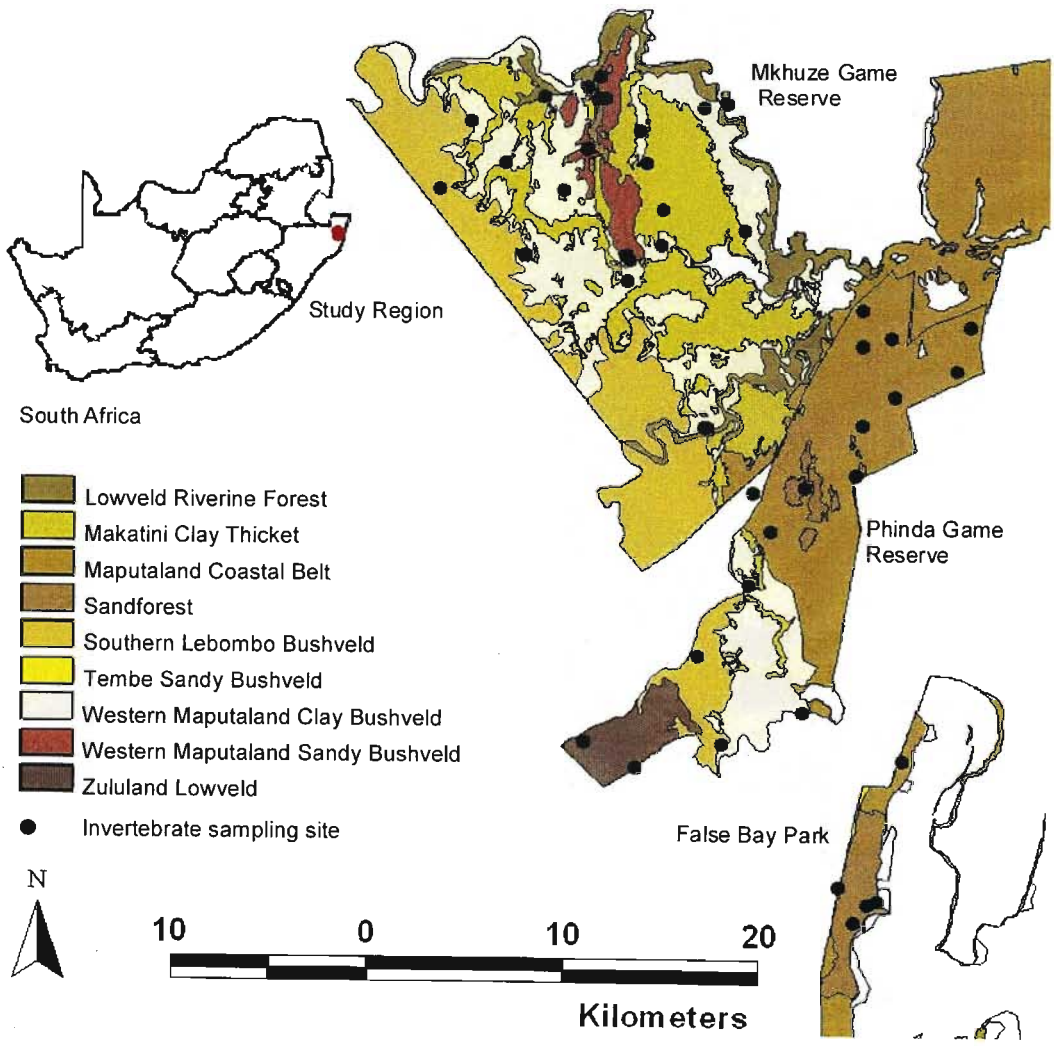

Figure 1.1 Location of the study sites within the three reserves and the associated vegetation types defined by Mucina and Rutherford (2005).

Bristow (1976) assessed the geology and geochemistry of the southern Lebombo and describes the following features of the region: the soils are either black clays, red structured clays or duplex soils derived from Ecca Group shale. Geologically Mkhuze Game Reserve consists of five easily recognisable components. In the west, volcanic rocks of the Jozini formation underlie the easterly sloping Lebombo Mountains. To the west of the Lebombo escarpment, in the Mkhuze flats, there are numerous basalt flows (Mfolozi River formation) and dolerite, similar both in age and in appearance to those found in the Drakensburg outcrop. To the east of the mountain range, there are Cretaceous sediments primarily of marine origin that underlie the flat-lying Zululand Coastal region, and overlying these Cretaceous beds are younger tertiary and recent sediments. Lastly, in the south of the reserve, rocks of the Bumbeni Volcanic complex outcrop are present.

Mkhuze Game Reserve has had a troubled history and there have been many battles to preserve it as a designated reserve. In 1939, the control of Mkhuze passed to the Department of Veterinary Services (Gush 2000). The Department of Veterinary Services was responsible for implementing the anti-Nagana campaign. This included the systematic eradication of all game and the aerial spraying of dichlorodiphenyltrichloroethane (DDT). After the conclusion of the anti-Nagana campaign, game capture operations were restarted in order to restock the reserve. The reserve survives today although it is suffering with the ongoing problems of poaching and land claims.

Mkhuze Game Reserve is situated on the coastal plain east of the Lebombo Mountains. The altitude varies from 30m above sea level in the south-east to 480m above sea level in the northwest. The reserve's topology is predominantly flat with undulating hills. Mkhuze has a variety of habitats including large Fevertree stands, Sandforest, dense thickets and open savanna. Wetland habitats include the Mkhuze River and Nsumo pan.

Phinda Game Reserve was established 1991 when 14 000 hectares were purchased. Phinda forms part of the Greater St Lucia Wetland Park. Seven distinct habitats exist at Phinda with the unique Sandforest (restricted to Maputaland and the extreme southern part of Mozambique) being of major significance (Butchart & Roche 2002). The Lebombo hills are located in the south-western corner of the reserve and wetland habitats include the Mzinene River.

False Bay Park is situated along the Western Shores of False Bay, which is part of Lake St. Lucia, a recently declared World Heritage Site. False Bay Park has a variety of habitats such as Sandforests, thornveld and open savanna.

1.10 AIMS AND OBJECTIVES

This project aimed to evaluate methods and approaches for the collection of invertebrate data in the savanna biome that would provide relevant information for conservation planners and ecologists

The project addressed the aims by assessing:

i) the effectiveness of a sampling strategy using a limited suite of invertebrate sampling methods, in order to provide recommendations for a multi-taxa approach to sampling invertebrates in a savanna ecosystem based on this assessment

ii) the use of volunteers for conducting invertebrate surveys by assessing the effectiveness, efficiency and benefits of using volunteers to carry out multi-taxa invertebrate surveys

iii) potential surrogates suitable for the representation of invertebrate biodiversity for use in conservation planning and rapid biodiversity assessments

Sampling for this study was carried out across reserves at locations where vegetation surveys had been carried out (Repton 2006) (Fig. 1.1). Sites were chosen from the vegetation data and geology maps to represent a range of vegetation and soil types. Sampling was conducted over three years, during the summer months (November to March), between 2002 and 2005. Forty-three different sites were sampled, 20 of these sites were re-sampled across the months and years, totalling 77 sampling events (Fig. 1.1). To address the aims subsets of the data were used for the analyses.

The rainfall recorded for this region over the three-year study period was 56% below average in the first sampling year, 68% below average in the second year and 95% below average in the third year (South African Weather Service 2006, unpublished data).

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ASSESSMENT OF A MULTI-TAXA SAMPLING STRATEGY FOR INVERTEBRATES IN A SAVANNA ECOSYSTEM

2.1 ABSTRACT

The documentation of invertebrate biodiversity in savanna is extremely poor; many species have yet to be discovered and few have had their distributions mapped at a spatial scale appropriate for conservation planning. This study assesses the effectiveness of a sampling strategy using a suite of invertebrate sampling methods developed to provide baseline data suitable for comparative studies and inventories within the savanna ecosystem, and assesses temporal variation to determine an optimal sampling period. The effectiveness of nine sampling methods including three different active ground searching methods, transects, tree beating, leaf litter and sweep sampling, and passive methods of colour pan traps and butterfly baited traps were tested to determine optimum sampling methods for: Lepidoptera (butterflies), Hymenoptera (Apoidea), Diptera (Asilidae, Bombyliidae), Neuroptera, Odonata, Hemiptera (Cicadellidae), Coleoptera (Cetoniinae, Scarabaeinae), Orthoptera, Blattodea, Isoptera, Araneae (Araneidae, Thomisidae, Oxyopidae), Scorpionida, Myriapoda (Diplopoda, Chilopoda), Mollusca and Annelida. A total of 49 961 individuals were recorded, representing 716 species. Species accumulation models were used to provide estimates of sampling completeness and analysis of similarity was used to determine methods that sample similar species assemblages. Assessments of species densities and time costs of methods were also made to determine sampling effectiveness. A standardised sampling protocol is described for the effective sampling of multiple taxa in a savanna biome. I make recommendations for improving the efficacy and completeness of invertebrate surveys based on the application of species accumulation models and I suggest over 75% of the total estimated fauna to be a satisfactory and realistic level of sampling completeness for making valid comparisons between regions and across sites. Inventory sampling should take place across all months and across multiple years in which larger cycles such as the El Niño – Southern Oscillation (ENSO) cycle are considered.

Keywords: Biodiversity assessments, inventory, comparative study, active sampling, passive sampling, temporal variation, species accumulation models, species estimation, species richness, species density, species assemblages, sampling protocol, conservation, completeness.

2.2 INTRODUCTION

Invertebrates are recognised as important components of biodiversity (Oliver & Beattie 1996; Kremen 2004). The maintenance of insect diversity is a pivotal part of the maintenance of ecosystem form and function (Samways 2005), this wider acceptance of invertebrates as indispensable components of biodiversity has led to a rapid increase in broad-based surveys (i.e. a survey incorporating a wide range of

invertebrate taxa) and greater pressure to provide information and guidelines for invertebrate conservation and monitoring (Ward & Larivière 2004).

Most entities of biodiversity, particularly at species and genetic levels, have not been discovered, let alone had their distributions mapped at a spatial scale appropriate for regional conservation planning (Ferrier et al. 2002). Furthermore, data on the threat status of insects and other invertebrates are almost non-existent (Stork 1997). Despite the efforts of several research groups (e.g. van Jaarsveld et al. 1998; van Rensburg et al. 1999; Samways 1999; Koch et al. 2000; Hamer 2000; Foord et al. 2002; Davis 2002; Herbert & Kilburn 2004; Parr et al. 2004), there are still inevitable lacunas in the data, and an urgent need to characterise invertebrate biodiversity, which generally involves local or regional surveys. It is necessary to ensure that such surveys produce comparable information (e.g. presence or absence of species, their geographical and ecological distribution) that can then be used for baseline monitoring and to provide an objective base for supporting priority-setting decisions in conservation (Yen & Butcher 1997).

Inventories provide a snapshot of the state of biodiversity and baseline data for the assessment of change (Stork et al. 1996). Complete inventories are useful to conservation planners because they provide the necessary information to help select fine-features required for conservation planning, such as species that are unique to a unit area or those with specific habitat requirements (Yen & Butcher 1997). Nonetheless, it is essential that inventories are quantified, unbiased and provide presence–absence data (Yen & Butcher 1997). Quantified inventories allow for comparative studies across regions or sites to be carried out. Comparative studies help conservation planners by providing the necessary information required to determine surrogates or features to act as coarse-filters in the conservation planning process (Yen & Butcher 1997).

Therefore, it is necessary that both inventories and comparative studies are used, and the level of sampling completeness is assessed. A method of assessing completeness and standardising data sets is through the use of species accumulation models (Soberón & Llorente 1993; Chazdon et al. 1998; Gotelli & Colwell 2001; Colwell et al. 2004). These models can be interpolated to standardise datasets, or extrapolated to estimate species richness. Extrapolation may be useful for the planning of field campaigns by estimating the effort required to add a given number or percentage of species, given a previous history (Soberón & Llorente 1993), or to statistically enlarge smaller sample sets for comparison with larger ones at a comparable level of sampling effort (Colwell et al. 2004).

Invertebrates are hyper-diverse and therefore it is impossible to sample all invertebrates. To overcome this problem the multi-taxa or 'shopping basket' approach can be used which does not focus sampling efforts on a single taxa (Oliver & Beattie 1996; Kotze & Samways 1999; Sauberer et al. 2004), as a single group may fail to serve as a biodiversity surrogate; to overcome this problem a set of taxa with different ecological requirements can be selected (Sauberer et al. 2004). Oliver et al. (1999) summarised several authors' proposed criteria for the selection of taxa suitable for characterisation of biodiversity. Taxa should be (i) functionally important in ecosystems, (ii) ubiquitous or with wide distributions on a

continental scale, (iii) represented in any one locality by a substantial but not excessive number of species, (iv) identifiable at species level, (v) easy to collect and sample and (vi) responsive to habitat variables at a convenient scale.

There are numerous sampling methods for the collection of invertebrates (New 1998; Southwood & Henderson 2000). Nevertheless, the challenge facing researchers is the development of a comprehensive and quantifiable invertebrate sampling strategy, which provides relevant information for conservation planners, and is appropriate relative to the available resources (e.g. person power, funds and time). The assessment of multi-taxa sampling strategies has generally been limited to forest ecosystems (Lowman et al. 1996; Oliver & Beattie 1996; e.g. Kotze & Samways 1999; Kitching et al. 2001), and there has been minimal assessment of invertebrate sampling techniques in savannas. One exception is the study by Druce et al. (2004) which assessed sampling strategies for Diplopoda, Chilopoda and Scorpionida in a South African savanna. Therefore, there is a need to develop and assess quantified sampling strategies in this biome.

Temporal variation is a factor that must be considered when designing a multi-taxa invertebrate survey. Most arthropod species are highly sensitive to environmental conditions, and use specific temperature, moisture or light conditions as triggers to initiate life history development (Didham & Springate 2003). Nevertheless, temporal variation is not limited to annual seasonal changes: there are regional atmospheric and oceanic circulation patterns (the gulf stream, El Niño, the Atlantic hurricane path) that enhance seasonal climatic variation (Didham & Springate 2003). Unfortunately, there is little research that investigates the effect of periodic cycling on terrestrial invertebrate assemblages.

This study assesses the effectiveness of a sampling strategy using a limited suite of invertebrate sampling methods and provides recommendations for a multi-taxa approach to sampling invertebrates in a savanna ecosystem based on this assessment, by addressing the following objectives: (i) to examine species assemblages sampled by each method in order to identify redundant methods, (ii) to determine sampling effectiveness by assessing the extent to which the sampling methods contribute to the total estimated number of species as a measure of sampling completeness, (iii) to determine the sampling effort required at a site for a comparative survey, (iv) to determine the minimum number of sites required for an inventory and to assess inventory completeness, (v) to determine the optimum sampling period by assessing temporal variation in species richness and number of unique species sampled across summer months and across years and (vi) to make recommendations regarding a sampling strategy for selected taxa in the savanna and to comment on taxa which should be included in such a survey.

2.3 METHODS

2.3.1 Study Site

Fieldwork was carried out in the Mkhuze Game Reserve (27.67°S:32.27°E, 400km^2), Phinda Private Game Reserve (27.78°S:32.35°E, 140km^2) and False Bay Park (27.94°S:32.38°E, 25km^2) in north-eastern

KwaZulu-Natal, South Africa. The reserves are situated in the diverse region known as the Maputaland Centre, which consists of a mosaic of mainly extensive savanna communities arranged in complex patterns (van Wyk 1996).

Sampling was carried out across reserves at locations where detailed vegetation surveys and soil analysis had been carried out (Repton 2006). Sites were chosen from the vegetation data and geology maps to represent a range of vegetation and soil types. Sampling sites were 1ha plots of uniform vegetation types. Forty-three different sites were sampled between November 2002 and March 2005 (summer months), 20 of these sites were re-sampled in different months during the summer season and across years, giving a total of 77 sampling events.

Weather conditions, GPS location, site descriptions and sampling times were recorded at each sampling event. Photographs were also taken for future reference.

2.3.2 Sampling, Sample Processing and Species Identification

Fieldwork was carried out by teams of Earthwatch volunteers. Each team consisted of approximately 11 people divided into three groups; each group was supervised by an experienced research scientist and carried out different sampling methods.

Seventeen taxa were sampled and identified to address a variety of ecological and conservation questions. The taxa and rationale for inclusion are summarised in Table 2.1. In addition to these 17 taxa, Isopoda and Formicidae were also sampled, however, logistical and resource constraints prevented their identification to species level and they were therefore not assessed in this thesis.

Based on the location, available resources (time, finances) and the skill base of the volunteers a limited range of sampling methods were selected (Table 2.2), which, in total, would give a fairly wide window on the invertebrates of the savanna, such methodology is of course not exhaustive, Malaise traps were not considered suitable at the outset of the project due to the movement of large game within the reserves, D-vacing was not used due to equipment constraints and pitfall trapping was not assessed mainly due to time constraints.

Sampling was designed to obtain quantifiable data across multiple taxa and at different spatial scales. One site was sampled each day, and active sampling was completed by teams between 07h00 and 13h00 (Table 2.3).

Table 2.1 Classification of the target taxa and rationale for their selection. The characteristics outlined are generalisations of each taxon based on its adult stage. 'Complex life cycle' refers to metamorphic invertebrates and 'simple' refers to non-metamorphic; 'activity group' refers to where the majority of activity occurs; 'functional group' refers to the dominate function that the taxa perform in an ecosystem; 'body size' categorized into small (S) medium (M) or large (L); 'adult longevity' into those which mainly live for one season (seasonal) or taxa which live for more than a year (long). The 'number of species' refers to the number of species recorded in the Maputaland region (KZN Wildlife, unpublished data); 'abundance' refers to the abundance across the survey sites (all taxa are widely distributed across the savanna habitat); 'surrogate scheme' refers Andelman and Fagan (2000) classification into Flagship (F), Umbrella (U), biodiversity indicator (B) groups; the indicator / surrogate category refers to research demonstrating the taxa application as a biodiversity surrogate or environmental indicator; 'taxonomic research / future study' refers to the institutions interested in the study material. Information for Insecta taxa from Scholtz and Holm (1985), Mollusca from Herbert and Kilburn (2004), Myriapoda from Lawrence (1987), Araneae from Dippenaar-Schoeman and Jocque (1997), Scorpionida from Newlands (1978) and Annelida from Sims and Gerard (1985).

Order	Family	Referred name	Functional group	Activity group	Body size	Life cycle	Adult longevity	No. of species	Abundance	Surrogate scheme	Indicator / surrogate	Taxonomic Research interest / future study
Lepidoptera (butterflies)		Lepidoptera	Pollinators	Flying	M - L	Complex	Seasonal	370	Common	U, F, B	(Fleishman et al. 2005)	Lepidoptera Society
Hymenoptera	Apoidea*	Apoidea	Pollinators	Flying	S - M	Complex	Seasonal	22	Common	U, B	(Arnott 2006)	ARC
Orthoptera		Orthoptera	Herbivore	Plant / Flying	M - L	Simple	Seasonal	30	Common	U, B	(Samways 1997)	-
Blattodea		Blattodea	Detritivore	Epigaeic	M - L	Simple	Seasonal / Long	0	Common	U, B		UKZN
Odonata		Odonata	Predator	Flying	M - L	Complex	Seasonal	77	Sparse	U, F, B	(Stewart & Samways 1998)	SU
Hemiptera	Cicadellidae	Cicadellidae	Sap-suckers	Plant	S	Simple	Seasonal	36	Common	U, B	(Nickel & Hildebrandt 2003)	ARC
Isoptera		Isoptera	Detritivore	Epigaeic	S	Complex	Seasonal	12	Common	U, B	(Andersen 1990)	ARC
Coleoptera	Cetoniinae**	Cetoniinae	Scavengers	Flying	M - L	Complex	Seasonal / Long	31	Common	U, F		UKZN
	Scarabaeinae**	Scarabaeinae	Scavengers	Epigaeic	M - L	Complex	Seasonal / Long	78	Common	U, B	(McGeoch et al. 2002)	UP
Diptera	Bombyliidae	Diptera	Pollinators	Flying	S - M	Complex	Seasonal	24	Common	U, B	(Hughes et al. 2000)	NERC
	Asilidae		Predator	Flying	S - M	Complex	Seasonal	68	Common			NM
Neuroptera		Neuroptera	Predator	Flying	M - L	Complex	Seasonal	31	Common	U, B	(Stelzl & Devetak 1999)	ARC
Araneae	Oxyopidae	Araneae	Predator	Plant	S - M	Simple	Seasonal	14	Common	U, B	(Churchill & Ludwig 2004)	ARC
	Thomisidae		Predator	Plant	S - M	Simple	Seasonal	51	Common			ARC
	Araneidae		Predator	Plant	S - M	Simple	Seasonal	30	Common			ARC
Scorpionida		Scorpionida	Predator	Epigaeic	M - L	Simple	Long	14	Sparse	U		AMNH / ARC
Chilopoda		Chilopoda	Predator	Epigaeic	S - L	Simple	Seasonal / Long	15	Common	U, B	(Filzek et al. 2004)	UKZN / NM
Diplopoda		Diplopoda	Detritivore	Epigaeic	S - L	Simple	Long	25	Common	U, B	(Yi & Moldenke 2005)	UKZN / NM
Gastropoda		Mollusca	Herbivore	Epigaeic	S - L	Simple	Long	119	Common	U, B	(Filzek et al. 2004)	NM
Annelida		Annelida	Detritivore	Epigaeic	L	Simple	Long	13	Sparse	U, B	(Didden & Rombke 2001)	NM

* super-family, ** sub-family. Abbreviations: American Museum of Natural History (AMNH), Agricultural Research Council (ARC), National Environment Research Council Centre for Population Biology, UK (NERC) Natal Museum (NM), Stellenbosch University (SU), University of KwaZulu-Natal (UKZN), University of Pretoria (UP).

Table 2.2 Summary of the methods, sampling effort and target taxon.

Target taxa	Method	Replication	
		Minimum effort	Maximum effort
Blattodea Isoptera Scarabaeinae Scorpionida Chilopoda Diplopoda Mollusca Annelida	Plot	20m x 20m, 1hr search time (x2)	20m x 20m, 1hr search time (x2)
	Quadrat	2m x 10m search (x2)	2m x 10m search (x2)
	Random	N/A (method not used)	1hr search time (x2)
Micro-mollusca	Leaf litter	4 x 5l	8 x 5l
Lepidoptera Apoidea Orthoptera Odonata Diptera Neuroptera	Transects	2 x 50m	4 x 50m
Lepidoptera Apoidea Diptera Neuroptera Cetoniinae	Baited traps	x 10	x 20
Apoidea Cicadellidae Diptera Araneae	Pan traps	x 5 blue x 5 yellow	x 10 blue x 10 yellow
Cicadellidae Diptera Araneae	Sweep netting	x 10 sweeps (x2)	x 10 sweeps (x2)
Blattodea Cicadellidae Araneae Diplopoda	Tree beating	x 20 trees	x 20 trees

Table 2.3 Daily timetable for field data collection. The team was divided into three groups that carried out the activities listed below. In practice all times were influenced by weather conditions, habitat complexity and work rates of each group.

Time	Group 1	Group 2	Group 3
06:00	All groups travel to site from base camp		
07:00	Active searches	Active searches	
08:00	(plot, random, litter samples)	(quadrat)	
09:00	Tree beating		Transects and sweep netting
10:00	Set traps at site		
11:00	All groups travel to previous days site, clear traps, release duplicate invertebrates and return to base camp		
12:00			
13:00			
14:00	Break and organisation of field equipment and samples		
15:00			
16:00	Commence sorting of day's specimens		
17:00			
18:00			

Only representative invertebrate samples were kept for identification in order to minimise the effect of sampling on invertebrate populations. The remaining invertebrates were released at the collection site after being recorded. Reference specimens were frozen or placed in killing jars containing ethyl acetate, then preserved in 70% ethanol or pinned. Invertebrates were sorted into broad groups and recorded at the field station, then sorted to a lower taxonomic level in the laboratory. Expert taxonomists carried out species identification. In a few instances, species names could not be determined, and in these cases, species numbers were used. Morpho-species were used to identify Blattodea, because no taxonomic expert was available. Reference collections are currently housed in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa, for later transfer to an appropriate South African institution.

2.3.2.1 Epigaeic invertebrate sampling

Epigaeic (ground-dwelling) invertebrates were sampled using three different active searching methods referred to here as plot, quadrat and random searches. Active searching targeted Scarabaeinae, Blattodea, Isoptera, Scorpionida, Diplopoda, Chilopoda, Mollusca and Annelida. Leaf litter samples were taken for the sampling of micro-molluscs.

Three people, who each searched for 20 minutes, giving a total of one hour search time per plot, carried out sampling of a 20m x 20m plot. Measuring tapes were pegged out to act as plot boundaries. The plots were chosen to include a range of microhabitats within the vegetation type.

Quadrats sized 2m x 10m, divided into five 2m x 2m blocks, were thoroughly searched. One person searched one block using a trowel to sift through the leaf litter, soil and logs to a depth of approximately 15cm. No time limit was set for the completion of the search in each 2m x 2m block. The quadrats were sampled in different areas to include a range of microhabitats within the vegetation type.

Three people carried out a random search and each person searched for 20 minutes, giving a total time of one hour for each search. There was no measurable boundary to the search area. However, the area was limited to the 1ha sampling site.

To sample micro-molluscs, leaf litter samples of a standardised 5l volume were collected from each of the 20m x 20m plots and within each of the random searching areas. Samples were air dried at room temperature in the laboratory, and then sieved through mesh ranging in hole size from 3.5mm to 0.4mm. Material falling through 0.4mm hole size was discarded as Mollusca of this size are fragmented or juvenile, and cannot be identified. A dissecting microscope was used to search the sieved material for molluscs. This is a widely used and accepted method for sampling micro-molluscs (Herbert, 2005, personal communication).

In addition to the above sampling methods, tree beating was used to sample Mollusca and Diplopoda. The number of replicates used for each method is presented in Table 2.2.

2.3.2.2 Flying and plant-dwelling invertebrate sampling

Flying insects and plant-dwelling invertebrates namely Lepidoptera, Apoidea, Diptera, Neuroptera, Odonata, Cicadellidae, Cetoniinae, Orthoptera and Araneae, were sampled using transect walks, colour pan traps, baited butterfly traps, sweep netting and tree beating.

Transect walks were undertaken in different areas of the sampling site. A 50m tape was laid out in a straight line at a location representative of the vegetation type and covering a variety of microhabitats. Each transect walk was undertaken by three people observing five metres either side of the transect line walking parallel lines and keeping pace with each other. The distance along the transect that the target invertebrates were caught or observed was recorded. Orthoptera, Lepidoptera, Odonata, Neuroptera, Diptera and Apoidea were sampled.

Colour pan traps were set for 24 hours. An equal number of blue and yellow coloured pan traps with a diameter of 22cm, an internal diameter of 20cm, and a depth of 2cm, were set in two parallel lines 10m apart with each pan trap set at a 10m interval. Pan traps were filled with approximately 150ml of a water, detergent and glycerol mix to ensure a low surface tension and reduce evaporation. All invertebrates caught were retained. For the purpose of this investigation only Diptera, Apoidea, Cicadellidae and Araneae were included in the analysis. Yellow and blue pan traps were chosen as yellow was shown to best most effective across a broad spectrum of taxa, and for groups that yellow pan traps were poor attractors then blue pan traps were shown to be effective (Kirk 1984).

Fruit-baited butterfly traps with a uniform diameter of 30cm, length of 110cm and mesh hole size of 1mm, suspended at least 1m from the ground were set for 24 hours at each site. A fermented fruit bait consisting of a mix of fermented banana, pineapple, mango, apple and papaya was placed on the solid base of the trap. The traps were set in two parallel lines approximately 20m apart, with each trap set a minimum of 10m apart, but the location of the trees was the deciding factor on the placement of the traps. Cetoniinae, Diptera, Lepidoptera and Apoidea were recorded.

Low-level vegetation (grass, forbes, small shrubs) was sampled by sweeping, using nets of a 35cm diameter and 1mm size hole mesh. The contents of 10 full sweeps comprised one sample. Each sweep was made approximately 1m apart along a straight line. All invertebrates were collected but this method was aimed at collecting Araneae and Cicadellidae.

Tree beating was used to sample Cicadellidae, Blattodea, Araneae and Diplopoda. One tree was struck a total of 10 times with a single branch struck no more than five times. A 1m diameter tray was used under the branch to catch dislodged invertebrates and pooters were used to collect specimens from the tray. Invertebrates were placed straight into 70% ethanol, to prevent predation within the sample tube. The samples from each tree were kept separate and the tree species was recorded.

Table 2.2 presents the number of replicates of each method.

2.3.3 Data Analysis

2.3.3.1 Examination of species assemblages sampled by each sampling method

Bray-Curtis similarity measures based on presence–absence species data were used to construct similarity matrices across sampling events for each sampling method, these matrices are the basis for analysis of similarity (ANOSIM) and non-metric multi-dimensional scaling plots (MDS). PRIMER version 5.2.9 was used for calculating all the similarity matrices, ANOSIM and MDS. ANOSIM quantified the extent to which different methods sampled unique species assemblages or species assemblage overlaps. ANOSIM is a non-parametric permutation procedure, applied to the (rank) similarity matrix underlying the ordination or classification of samples (Clarke & Warwick 2001). ANOSIM calculates a test statistic R: the R-value gives an absolute measure of how separated two groups are, on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarities between groups). If $R>0.75$ groups are well separated, if $R>0.5$ groups are over-lapping but clearly different, if $R<0.25$ groups are barely separable (Clarke & Gorley 2001).

MDS was used as an ordination analysis to show the relationship between species assemblages and sampling methods. MDS constructs a 'map' or configuration of the samples, in a specified number of dimensions, which attempts to satisfy all the conditions imposed by the rank similarity matrix (Clarke & Warwick 2001). The statistical output from these analyses has the following interpretation: stress-value <0.05 gives excellent representation with no real prospect of a misleading interpretation, <0.1 corresponds to a good ordination with no real prospect of a misleading interpretation, <0.2 gives a potentially useful 2-dimensional picture, >0.3 indicates that points are close to being arbitrarily placed (Clarke & Warwick 2001).

Redundant methods were identified as methods where there was a complete overlap in the species assemblages sampled, as identified using ANOSIM and MDS plots. To determine the effect of removing a redundant method, species accumulation models were used to compare the level of sampling completeness.

2.3.3.2 Assessment of sampling completeness and effectiveness and determination of optimal Sampling effort at a site for a comparative survey

All sampling events were used in the analyses testing the effectiveness of sampling methods. To assess sampling completeness the observed and estimated species densities (S_d) were calculated at each sampling event. Species density is defined as being the number of species per specified collection area or unit (Magurran 2004). For this study 'species density' is defined as the number of species sampled during one sampling event using a defined sampling effort.

Species accumulation curves were constructed for each taxon at each sampling event for each of the significantly different sampling methods (determined by the ANOSIM and MDS) using the Species-Area

plot routine in PRIMER, in which the sample order was randomised using 999 permutations (Clarke & Warwick 2001). Where methods were not significantly different they were combined and a species density curve was calculated. A mean species accumulation curve was then calculated for each taxon using each method or combination of methods. This was then extrapolated to estimate the total mean species density at a sampling event, for each method, for each taxon.

For the purpose of this study, a single model was chosen for the extrapolation of species accumulation curves to estimate total species density. The two parameter hyperbolic function known as the Michaelis-Menton (MM) equation of enzyme kinetics is the most popular function used to model species accumulation curves for species estimation and is the most stable across all sample sizes (Palmer 1990; Colwell & Coddington 1994; Chazdon et al. 1998; Longino et al. 2002), and was used for this extrapolation. Other models for fitting curves and estimating species richness or density are available and deserve comparative analysis (Palmer 1990; Soberón & Llorente 1993; Chazdon et al. 1998). However, they are beyond the scope of this paper to be looked at in detail.

This paper assesses fieldwork methodologies to sample multiple invertebrate taxa. The analytical methods were used to assist in designing a sampling protocol but not to assess richness estimators, extrapolation methods or to test ecological theories. I am aware of the potential error in the extrapolation of datasets and that the prediction of the number of species by extrapolation is done under the condition that all properties remain the same as defined in the original data (He & Legendre 1996). Therefore, these estimates are valid only for the specific habitats sampled and across the same temporal window.

Effectiveness or completeness of the sampling at each sampling event was determined by calculating the observed species density as a percentage of the estimated species density. Percentages were calculated for comparisons because some methods were not repeated across all sampling events.

Where methods were shown to be sampling the same species assemblages, an assessment of the species densities sampled was carried out, to identify the most effective method sampling the highest number of species. The species densities sampled at each sampling event using each method were compared using the Friedman Test, as data were non-parametric, and each method was repeated at each sampling event and therefore variables were related. Where significant differences across the methods were observed, a paired analysis using the Wilcoxon Signed Ranks Test was used and the Bonferroni adjustment was applied to identify which methods produced significantly different results.

To assess further the effect of pan trap colour on sampling the number of unique species sampled by each colour was calculated for each taxon.

To determine the optimal sampling effort required for a comparative study the shapes of the species density curves were compared using the minimum and maximum sampling effort. In addition the levels of completeness were also compared.

2.3.3.3 Assessment of inventory completeness and determination of the minimum number of sampling events

Species richness (*S*) is defined as being the total number of species (Magurran 2004). For this study 'species richness' is defined as the total number of species sampled across all sampling events.

To assess inventory completeness for each taxon, species richness estimates were determined using data from all sampling events using the minimum sampling effort. The MM equation was fitted to the individual-based species accumulation curves for each taxon calculated using EstimateS 7.5 and the predicted asymptote determined the estimated richness of the region. The observed species richness as a percentage of the estimated species richness was used to assess completeness.

For comparative purposes the Chao 2 richness estimator, incidence-based coverage estimator (ICE) and the second-order Jackknife species richness estimator were also calculated. These were selected as they have previously been shown to be good estimators with least bias, while other estimators have been shown to be highly dependent on sampling effort and unreliable when species distributions are patchy (Gotelli & Colwell 2001). These species richness estimators provide only a minimum estimate of species richness. Species richness estimates using these estimators were calculated for each taxon using EstimateS.

To test our estimates of species richness, official records were obtained from Ezemvelo KZN Wildlife, thus enabling a comparison of these data with historical data for the region. Two comparisons were made namely, (i) the total number of recorded species and (ii) the species sampled during the months of November, January and March, which were the months in which the survey was carried out.

To determine the minimum number of sampling events, the MM equation was fitted to the individual-based species accumulation curve, and solved to estimate the number of individuals required to sample 80%, 90% and 95% of the total estimated species richness. The number of sampling events required to sample the invertebrate fauna of the area was calculated by applying equation 2.1.

$$\text{Estimated no. of sampling events} = \frac{\text{estimated no. of individuals}}{(\sum\text{individuals sampled} / \text{No. of sampling events sampled})}$$

Equation 2.1

2.3.3.4 Identification of the optimal temporal period to carryout survey

Sites that were sampled repeatedly in November, January and March were used to assess temporal variation across the summer season. Annual variation was assessed using sites that were sampled in the

same month across different years. Five sites were assessed for monthly variation and two sites for annual variation. Temporal variation was assessed by calculating the number of species sampled in the month or year and the percentage of unique species. ANOSIM was used to compare species assemblages sampled across months and years.

2.3.3.5 Criteria used to make recommendations for a sampling strategy and selection of taxa

The number of species sampled per hour using each method was calculated using the same combination of sites for each method. The following time allocations were used, based on the estimated time taken in the field to carryout each method: (i) two plot searches, two random searches, and 20 tree beats took an estimated two hours to complete each method, (ii) two 2m x 10m quadrat searches took an estimated 10 hours, (iii) four 5l leaf litter samples took an estimated 0.5 hour to sample, (iv) two 50m transects took an estimated one hour, (v) the set up and sampling duration for 10 baited and 10 pan traps took approximately 24.5 hours for each method and (vi) two sweep samples took an estimated 0.5 hour.

Recommendations for the methods to be included were made based on methods shown to be sampling different species assemblages, on methods sampling the highest number of species per hour, and on methods with the highest sampling completeness. Taxa selected were those shown to have been sampled with a high measure of completeness across all sampling events.

2.4 RESULTS

2.4.1 Survey Results

In the data presented here, 716 invertebrate species belonging to the focus taxa were recorded from 49 961 individuals. These figures can be broken down as follows: 39 856 individuals from 212 species sampled by plots, quadrats, random and little samples, 2 218 individuals from 221 species sampled along transects, 2 893 individuals from 86 species sampled by baited butterfly traps, 2 321 individuals from 260 species sampled using pan traps, 762 individuals from 78 species sampled by sweep netting and 1 483 individuals from 139 species sampled by tree beating. The number of individuals and number of species sampled in each taxon is summarised in Table 2.4.

Table 2.4 The total number of species and total number of individuals recorded during the survey for each focus taxon using the methods described.

Taxa	Sub-group	No. of species	No. of individuals
Lepidoptera		99	1 808
Apoidea		51	1 027
Orthoptera		46	1 331
Blattodea		27	4 349
Odonata		12	67
Cicadellidae		87	1 148
Isoptera		8	322
Cetoniinae		31	1 940
Scarabaeinae		43	314
Diptera	Bombyliidae	21	133
	Asilidae	46	248
Neuroptera		26	149
Araneae	Oxyopidae	33	714
	Thomisidae	49	751
	Araneidae	31	130
Scorpionida		6	140
Chilopoda		17	568
Diplopoda		26	14 519
Annelida		7	261
Mollusca		50	20 042
All invertebrates		716	49 961

2.4.2 Species Assemblages Sampled by each Sampling Method

There were clear species assemblages defined by the different sampling methods, (stress-value 0.1) (Fig. 2.1a). These differences may have been amplified due to the combination of methods sampling epigaeic invertebrates and methods sampling flying and plant-dwelling invertebrates. When the various methods for flying and plant-dwelling invertebrate were assessed, the methods sampled distinct species assemblages, (stress-value 0.08) (Fig. 2.1b). The assessment of plot, quadrat and random methods showed these methods were sampling the same assemblages, (stress-value 0.21) (Fig. 2.1c).

ANOSIM testing sampling methods using all invertebrate species produced an *R*-value of 0.71 ($P<0.01$), demonstrating that the different methods were generally sampling different species assemblages. However, the plot, quadrat and random methods sampled overlapping species assemblages (Fig. 2.1a). The pair-wise ANOSIM output for plot and quadrat ($R=0.105$, $P=0.002$) and quadrat and random ($R=0.122$, $P=0.003$), showed they were sampling similar species assemblages (Fig. 2.1c). A non-significant R-value was observed between plot and random ($R=-0.043$, $P=0.982$).

To identify an optimal sampling protocol, it is important that sampling efforts are not duplicated. The ANOSIM showed that the methods employed were sampling different species assemblages for most of the taxa. The analysis indicated that there was a species assemblage overlap when sampling Mollusca, Blattodea, Diplopoda, Chilopoda, Scorpionida, Scarabaeinae, Isoptera and Annelida using plot, random and quadrat sampling methods. Transects and baited traps sampled similar assemblages for Apoidea and

Neuroptera. Sweeps, pan traps and tree beats sampled similar Cicadellidae assemblages. Transects, baited traps, pan traps and sweeps sampled similar assemblages of Diptera. Sweeps and pan traps sampled similar assemblages of Araneae. For the detailed ANOSIM output see Appendix 2.1.

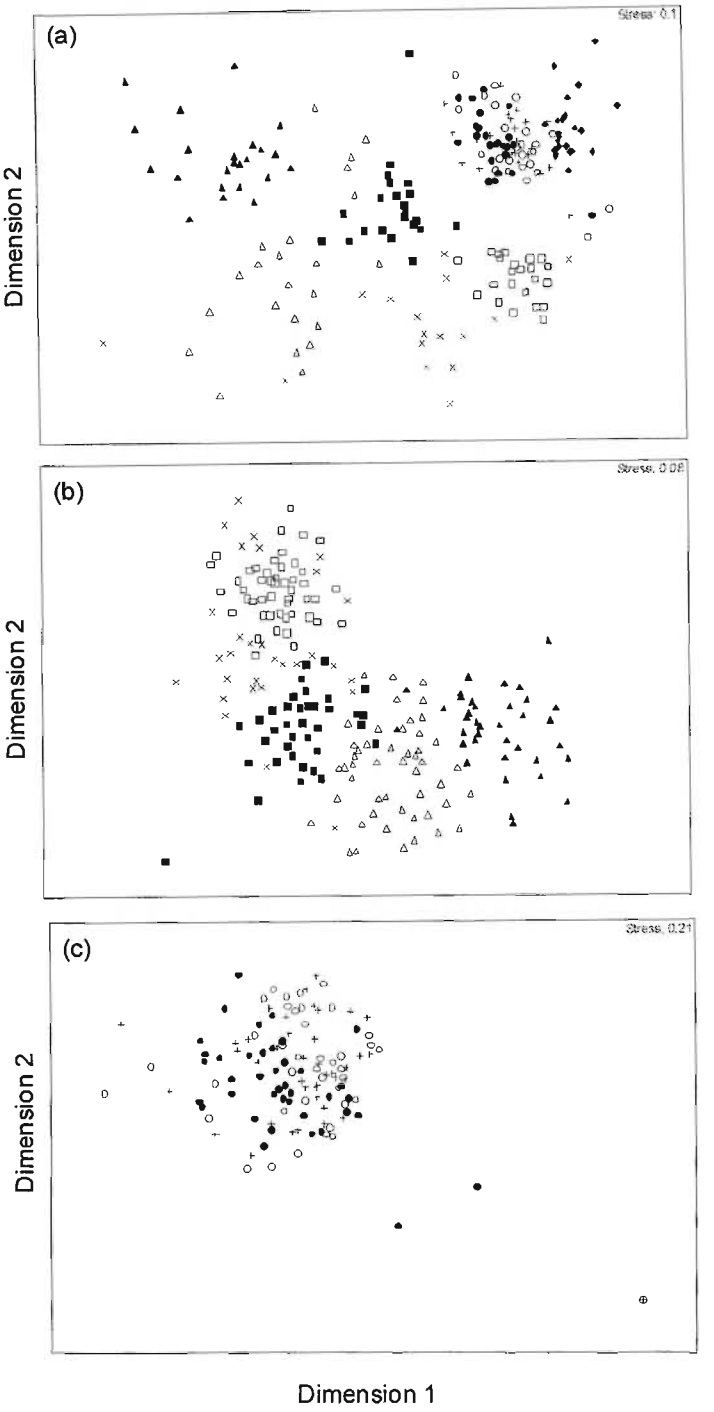

Figure 2.1 Assessment of invertebrate species assemblages sampled across all sampling events using the following methods: baited traps (▲), transect (Δ), pan traps (■), sweep (X), tree beat (□), quadrat (●), plot (○), random (+) and leaf litter (◆). A two-dimensional multi-dimensional scaling plot based on Bray-Curtis similarity matrix from presence–absence species data across sites. Multi-dimensional scaling plot for all invertebrates sampled using all methods **(a)**, invertebrates sampled using baited traps, transects, pan traps, sweeps and tree beats **(b)** and plots, quadrats and random sampling **(c)**.

2.4.3 Sampling Completeness and Sampling Effectiveness

2.4.3.1 Species assemblages and species density

The difference in the percentage of the estimated species density sampled (completeness) using the minimum and maximum sampling effort had no effect or resulted in a reduction in the level of completeness for Scarabaeinae, Lepidoptera, Diptera, Chilopoda, Annelida and Odonata (Table 2.5). An increase of 1 to 6% in the level of completeness was observed for Blattodea, Scorpionida, Diplopoda, Cicadellidae, Araneae and Neuroptera, and an increase of over 10% was observed for Mollusca, Apoidea, Isoptera and Orthoptera for the maximum sampling effort (Table 2.5).

Table 2.5 Comparison of the observed and estimated total species density using the minimum and maximum sampling effort which was calculated using the Michaelis-Menton equation. Figures in parentheses are the percentage sampled of the estimated species density.

	Observed mean species density at a site		Estimated mean species density using MM				% difference between max. and min. effort
	Min. effort	Max. effort	Min. effort		Max. effort		
Lepidoptera	6.9	7.9	15.1	(46)	18.5	(43)	-3
Apoidea	4.8	7.1	12.6	(38)	13.7	(52)	14
Orthoptera	5.2	7.9	9.1	(57)	10.5	(75)	18
Blattodea	5.6	4.7	8.9	(63)	7.4	(64)	1
Odonata	1.1	1.0	2.9	(38)	2.6	(38)	0
Cicadellidae	3.8	7.2	11	(35)	17.9	(40)	5
Isoptera	1.2	2.6	4.5	(27)	6.1	(43)	16
Cetoniinae	3.4	-	5.1	(67)	-	-	-
Scarabaeinae	2.1	2.5	7.6	(28)	12.9	(19)	-9
Diptera	2.4	3.8	8.4	(29)	14.8	(26)	-3
Neuroptera	1.7	1.9	6.7	(25)	6.2	(31)	6
Araneae	9.1	10.4	22.5	(40)	23.3	(45)	5
Scorpionida	1.4	1.7	4.9	(29)	5.4	(31)	2
Chilopoda	2.4	2.3	5.6	(43)	5.8	(40)	-3
Diplopoda	4.8	5.0	6.2	(77)	6.2	(81)	4
Mollusca	8.9	10.9	12.5	(71)	13	(84)	13
Annelida	1.3	1.4	1.6	(81)	1.8	(78)	-3

N.B. These figures are based on different datasets: in some instances, the percentage of estimated species density sampled using the maximum sampling effort is less than the minimum sampling effort because of the varying shape of the species density curves as a result of using data from different sites.

Mean species density sampled across all taxa was highest using quadrats (S_d=20.4), followed by random (S_d=15.0), transects (S_d=14.4), plot (S_d=14.3), pan traps (S_d=11. 9), baited (S_d=9.7), tree beat (S_d=8.9), litter (S_d=7.2) and sweep (S_d=2.8) (Table 2.6).

Table 2.6 Summary of the methods assessed and the mean species density of each taxon sampled using each method*. The un-shaded area represents the minimum sampling effort, and the shaded area represents the maximum effort used for the analysis of methods. Figures in parentheses show the mean species density of the plots and quadrats combined. By-catch (B) refers to specimens sampled unintentionally as method was not designed to target those species.

Method	Plot	Quadrat	Random	Leaf litter		Transect		Baited traps	Pan traps		Sweep	Tree beat
Number of replicates	20m x 20m 1hr search (x 2)	2m x 10m searches (x 2)	1hr search (x 2)	4 x 5l	8 x 5l	2 x 50m	4 x 50m	x 10	x 5 blue x 5 yellow	x 10 blue x 10 yellow	x 2	x 20 trees
Lepidoptera						3.8	4.4	4.2				
Apoidea						1.2	1.2	0.7	4.2	6.3		
Orthoptera						5.2	7.9					
Blattodea	2.0	4.3 (4.9)	1.7					[B] 0.06	[B] 0.7	[B] 2.8		0.9
Odonata						1.1	1.1					
Cicadellidae									3.7	6.6	0.6	0.4
Isoptera	0.6	1.1 (1.3)	1.1									
Cetoniinae								3.4				
Scarabaeinae	0.9	1.6 (2.1)	1.6						[B] 0.8	[B] 1.7		
Diptera						1.8	2.7	0.1	1.3	2.4	0.1	
Neuroptera						1.3	1.7	0.3 [B]				
Araneae									1.2	2.6	2.1	7.0
Scorpionida	0.5	1.3 (1.5)	0.7									
Chilopoda	0.9	1.8 (2.3)	0.6									
Diplopoda	2.8	3.7 (4.3)	2.9									0.6
Mollusca	5.9	5.1 (7.0)	6.1	7.2	8.9							
Annelida	0.7	1.5 (1.5)	0.3									

* Species density values for additional sampling effort for transects, pan traps and leaf litter are from data sets using a different combination of sites to the remaining methods.

2.4.3.2 Epigaeic invertebrates

The plot, quadrat and random methods sampled similar species assemblages (Fig. 2.1c, Appendix 2.1).

Significant differences in the species densities sampled using the three methods were identified for Blattodea (n=50, X^2_2=26.48, P<0.001), Mollusca (n=50, X^2_2=7.92, P=0.02), Scarabaeinae (n=17, X^2_2=6.88, P=0.032), Chilopoda (n=39, X^2_2=25.57, P<0.001), Scorpionida (n=22, X^2_2=16.71, P<0.001) and Annelida (n=6, X^2_2=8.38, P=0.015). No significant difference was detected across the methods for Diplopoda (n=49, X^2_2=5.26, P=0.072) or Isoptera (n=8, X^2_2=3.5, P=0.174).

A paired analysis (using Wilcoxon Signed Ranks Test and applying the Bonferroni adjustment) identified the significant differences to be between the following methods: Blattodea sampled using quadrats and plots (Z=-4.588, P<0.001) and quadrats and random (Z=-4.654, P<0.001); Chilopoda sampled using quadrats and plots (Z=-3.210, P=0.001) and quadrats and random (Z=-4.252, P=0.002); Scorpionida sampled using quadrats and plots (Z=-3.082, P=0.002) and quadrats and random (Z=-2.724, P=0.006).

To test whether the combination of plots and quadrats sampled significantly different species densities to other combination of methods a Wilcoxon Signed Ranks Tests were performed. In all cases, the combination of plots and quadrats sampled greater species densities than quadrats and random methods or plot and random methods (Table 2.6, Appendix 2.2). Significant differences were identified between the combination of plots and quadrats versus plots for Blattodea (Z=-5.309, P<0.001), Diplopoda (Z=-2.521, P=0.012), Chilopoda (Z=-3.749, P<0.001) and Scorpionida (Z=-3.044, P=0.002). The combination of plots and quadrats versus quadrats was significantly different for Chilopoda (Z=-2.348, P=0.019) and Mollusca (Z=-3.644, P<0.001). The combination of plots and quadrats versus random was significantly different for Blattodea (Z=-5.602, P<0.001) Diplopoda (Z=-2.412, P=0.016), Chilopoda (Z=-4.301, P<0.001), Scorpionida (Z=-2.850, P=0.004) and Mollusca (Z=-2.005, P=0.045).

2.4.3.3 Flying insects and plant dwelling invertebrates

ANOSIM between blue and yellow pan traps showed no significant difference in the species assemblages sampled for Blattodea (R=-0.007, P=0.531), Scarabaeinae (R=-0.041, P=0.828), Cicadellidae (R=0.011, P=0.231) and Araneae (R=-0.013, P=0.753). Significant R-values were detected for Apoidea (R=0.103, P=0.001) and Diptera (R=0.089, P=0.023). However, the R-values indicate that there are little differences in the assemblages and they are barely separable.

The MDS indicated that sweep and tree beats were sampling similar species assemblages (Fig. 2.1b). Although when assessing each taxon significant difference were only observed between pan traps and tree beats for Araneae (R=0.62, P=<0.001) see appendix 2.1 for detailed ANOSIM output for each taxon.

Friedman Test identified significant differences across the species densities sampled using sweep, pan traps and tree beats for Cicadellidae (n=40, X^2_2=78.71, P<0.001) and for Diptera species densities sampled using transects, baited traps, pan traps and sweeps methods (n=39, X^2_2=92.79, P<0.001).

The paired analysis (Wilcoxon Signed Ranks Test applying the Bonferroni adjustment) identified the differences in species density for Cicadellidae to be between tree beat and pan traps (Z=-5.401, P<0.001); sweep and pan traps (Z=-5.331, P<0.001) and sweep and tree beats (Z=-2.840, P=0.005). sweeps and pan traps sampled different Araneae species densities (Z=-3.1, P=0.002). Transects and baited traps methods sampled significant differences in the species densities for Apoidea (Z=-4.092, P<0.001) and Neuroptera (Z=-3.724, P<0.001). Lastly significant differences in the Diptera species densities sampled for: baited and pan traps (Z=-5.08, P<0.001), sweep and pan traps (Z=-5.02, P<0.001), transects and baited traps (Z=-5.56, P<0.001) and transects and sweeps (Z=-5.34, P<0.001).

Figure 2.2 shows the mean species densities sampled using blue and yellow pan traps. The Wilcoxon signed rank test detected no significant difference between the species densities sampled by blue or yellow pan traps for Apoidea (Z=-0.593, P=0.59), Blattodea (Z=-0.321, P=0.749) and Scarabaeinae (Z=-0.957, P=0.339). Significant differences between the species densities sampled by blue and yellow pan traps were identified for Cicadellidae (Z=-3.998, P<0.001), Diptera (Z=-4.156, P<0.001) and Araneae (Z=-2.138, P=0.033), with yellow pan traps sampling a greater number of species than blue.

Blue pan traps sampled 33 unique species, with Apoidea contributing 11 species, Diptera 4, Cicadellidae 6, Araneae 7, Blattodea 1 and Scarabaeinae 4. Yellow pan traps sampled 100 unique species, with Apoidea contributing 8 species, Diptera 18, Cicadellidae 49, Araneae 14, Blattodea 3 and Scarabaeinae 8.

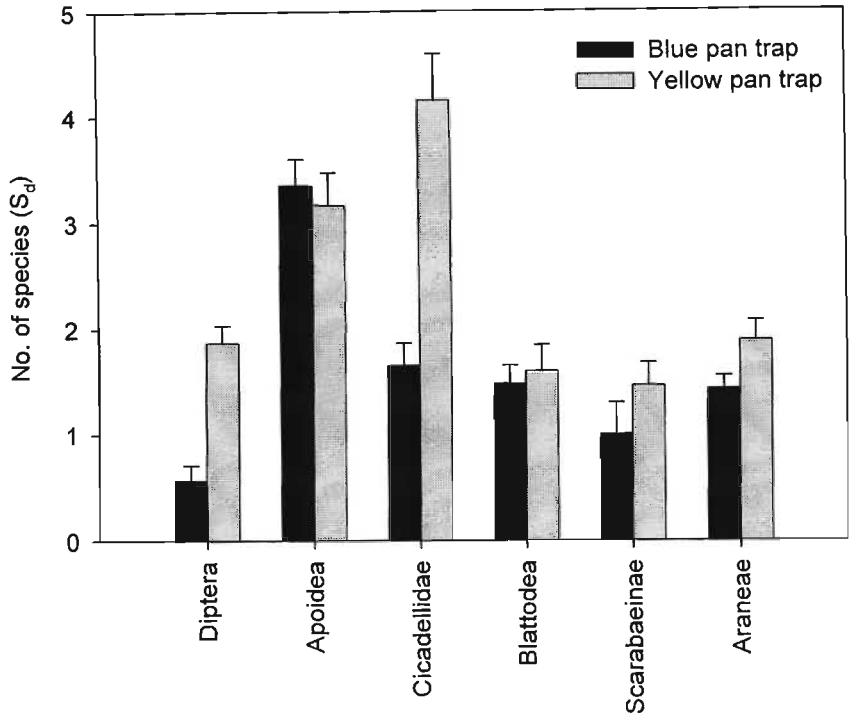

Figure 2.2 Comparison of mean species density of taxa sampled at a site using blue and yellow coloured pan traps. Error bars represent standard error. Diptera (n_{site}=30), Apoidea (n_{site}=55), Cicadellidae (n_{site}=40), Blattodea (n_{site}=25), Scarabaeinae (n_{site}=13) and Araneae (n_{site}=35).

2.4.3.4 Species richness

Table 2.7 summarises the observed species richness obtained from the minimum sampling effort across all sampling events (see Appendix 2.3 for species richness curves). Isoptera, Diptera, Scarabaeinae, Cicadellidae, Odonata and Neuroptera were particularly poorly sampled, with the minimum sampling effort capturing less than 67% of the estimated total species richness of the area. These groups also showed a wide range of estimates of species richness. Blattodea, Diplopoda, Mollusca and Chilopoda were sampled well, recording over 93% of the estimated species richness. Scorpionida, Apoidea, Cetoniinae and Orthoptera sampled over 83%. Lepidoptera, Annelida and Araneae sampled were estimated to be at 79%, 78% and 77% respectively, of the estimated species richness.

Data from Ezemvelo KZN Wildlife (unpublished) were not comprehensive and contained extensive records only for Mollusca, Lepidoptera, Odonata and Cetoniinae. In this study, the observed species richness for Mollusca and Cetoniinae exceeded the historical records and thus were non-comparable. The historical records for Lepidoptera and Odonata far exceeded the estimates for species richness for the region by approximately double (Table 2.7). Yet, when the historical records were filtered to include species sampled only during November, January and March, the historical records only marginally exceeded the estimates of maximum species richness.

Table 2.7 Comparison between the observed and estimated species richness which was calculated using the richness estimators: incidence-based coverage estimator (ICE), Chao 2, second-order Jackknife and the Michaelis-Menton (MM) equation, and the range of the percentage of the estimated total species richness all calculated using the minimum sampling effort. Comparison of the percentage of the estimated total species richness using the MM equation sampled using 77 sampling events and the estimated number of sites required to sample 75, 80, 90 and 95% of the estimated species richness. These estimates were calculated using individual based species accumulation curves in the MM equation which was fitted and solved. Comparison of the observed species richness and the historical records of the region from Ezemvelo KZN Wildlife (unpublished data).

	Observed species richness	**Estimated species richness**								Estimated no. of sites to reach the below % of the estimated species richness				Known species richness (Ezemvelo KZN Wildlife records)	
		MM equation		Jackknife 2		ICE		Chao 2		75%	80%	90%	95%	All records	Jan, March, Nov
Lepidoptera	76	96	(79)	114	(66)	103	(74)	106	(72)	58	77	172	361	217	117
Apoidea	41	48	(85)	56	(73)	51	(81)	48	(86)	39	51	116	248	2	1
Orthoptera	44	53	(83)	57	(77)	52	(85)	50	(89)	52	69	154	329	-	-
Blattodea	26	28	(93)	30	(87)	28	(92)	27	(96)	17	24	50	104	-	-
Odonata	10	18	(56)	14	(71)	16	(62)	11	(89)	189	253	578	1254	32	22
Cicadellidae	69	121	(57)	133	(52)	117	(59)	135	(51)	155	207	465	974	-	-
Isoptera	8	12	(67)	11	(73)	9	(91)	9	(94)	111	148	328	683	-	-
Cetoniinae	29	35	(83)	43	(68)	39	(74)	36	(80)	48	64	145	308	24	17
Scarabaeinae	27	48	(56)	55	(49)	54	(50)	57	(48)	162	217	488	1033	2	2
Diptera	51	93	(55)	100	(51)	97	(53)	95	(53)	170	227	511	1084	8	1
Neuroptera	23	46	(50)	50	(46)	61	(38)	49	(47)	204	272	612	1294	-	-
Araneae	97	126	(77)	155	(63)	144	(67)	138	(70)	67	90	202	428	-	-
Scorpionida	6	7	(86)	5	(120)	6	(100)	6	(100)	25	34	77	166	1	1
Chilopoda	17	18	(94)	22	(78)	19	(91)	19	(92)	23	31	70	146	-	-
Diplopoda	25	27	(93)	26	(96)	26	(96)	25	(100)	17	23	51	109	-	-
Mollusca	48	51	(94)	55	(87)	51	(95)	51	(95)	20	27	61	130	39	-
Annelida	7	9	(78)	11	(55)	10	(59)	8	(80)	111	148	326	662	-	-
All invertebrates	604	836		937		883		870		1468	1962	4406	9313	325	161

2.4.4 Optimal Sampling Effort for a Site and Number of Sites Required for an Inventory

The minimum set (refer to Table 2.2 for method summary, Appendix 2.2 for extrapolation details, Appendix 2.4 for species density curves) of pan traps (5 blue, 5 yellow) performed consistently poorly across all targeted taxa and sampled the following percentage of the estimated total species density (using only species sampled by the specific method): 44% of Apoidea, 26% of Araneae, 14% of Blattodea, 37% of Diptera, 38% of Cicadellidae. The minimum transect distance (2 x 50m) performed poorly, and sampled 40% of Lepidoptera, 57% of Orthoptera, 38% of Odonata, 40% of Neuroptera and 40% of Diptera. The minimum set of leaf litter samples sampled 67% of Mollusca species. The baited traps sampled 62 % of Lepidoptera, and 66% of Cetoniinae. Transects and baited traps did not sample different assemblages for the following taxa and their efforts combined sampled 21% of Apoidea, 25 % of Neuroptera and 29% of Diptera. Tree beating sampled 12% of Araneae, and 58% of Diplopoda. Plots, quadrats and random methods did not sample different species assemblages so were combined and sampled 61% of Blattodea, 20% of Scarabaeinae, 32% of Scorpionida, 40% of Chilopoda, 77% of Diplopoda, 85% of Mollusca, 79% of Annelida.

The doubling of the sampling effort does not produce mean species density curves that approach an asymptote for Apoidea and Araneae in pan traps, Orthoptera and Lepidoptera in transects, and Mollusca in leaf litter samples (Fig. 2.3). The increase in the sampling effort for pan traps and transects resulted in a less than 15% increase in the sampling effectiveness of Orthoptera, Neuroptera, Araneae, Scarabaeinae, Blattodea, a 6% increase in Cicadellidae and a 10% increase in Apoidea. Odonata, Lepidoptera and Diptera were unaffected by the increase in sampling effort.

Increasing the sampling effort provided over a 10% increase in the species density of Apoidea, Scarabaeinae, Diptera and Orthoptera, a 5 to 10% difference in the species density of Araneae, Blattodea, Diplopoda, Cicadellidae and Neuroptera, with a negligible (<5%) difference in the species density of Lepidoptera, Chilopoda, Diplopoda, Scorpionida, Isoptera and Annelida (Table 2.5).

The estimates for the number of sampling events required vary across the taxa and vary in magnitude, depending on the percentage of the estimated total species richness (Table 2.7). For the 17 taxa surveyed, the estimated number of sampling events to sample 75% of the estimated species richness varies from 23 to 272 events.

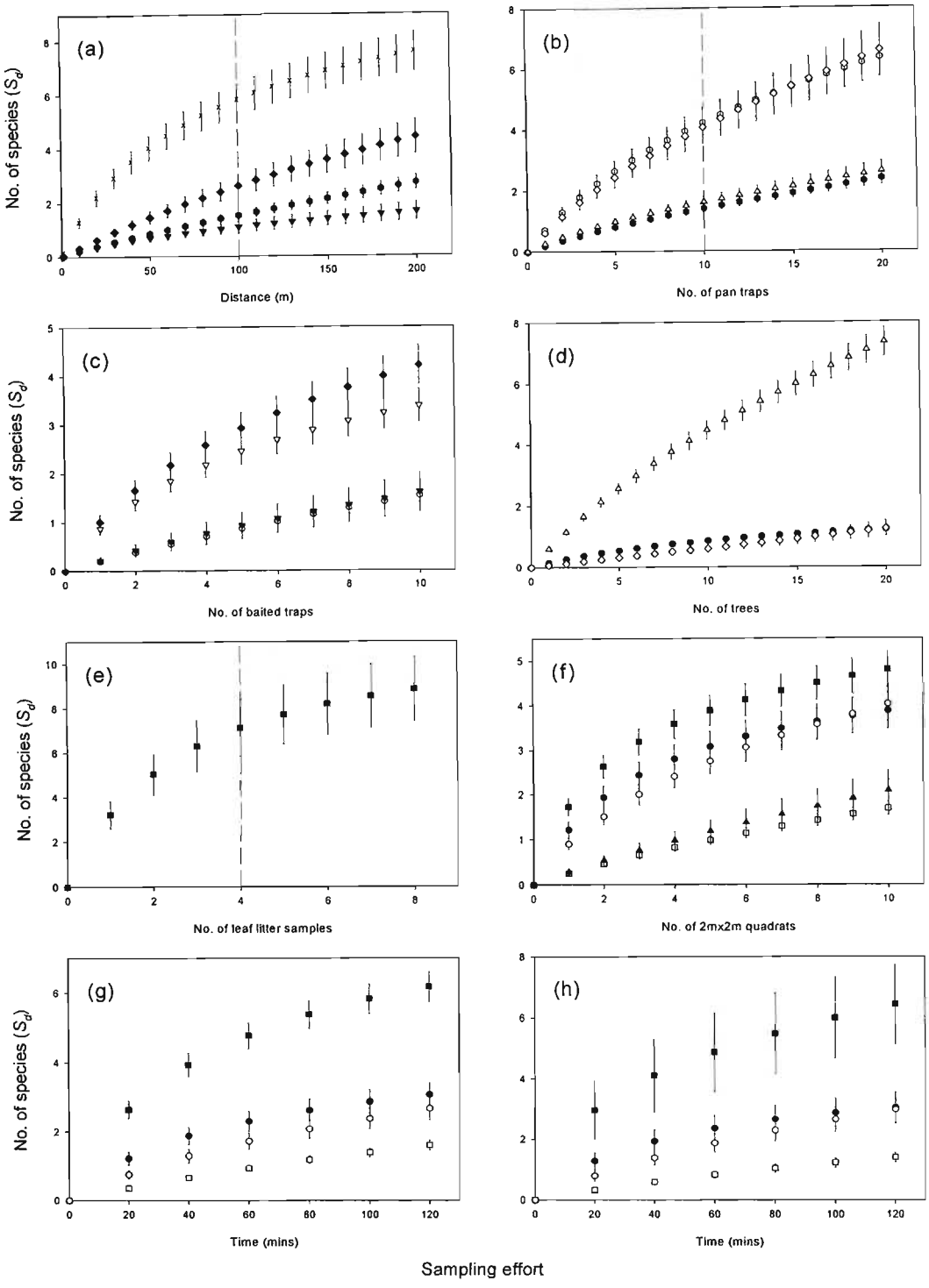

Figure 2.3 Species accumulation curves and the effect of increasing sampling effort on species density for different sampling methods for each taxon. **(a)** transects, **(b)** pan traps, **(c)** baited traps, **(d)** tree beats, **(e)** leaf litter, **(f)** quadrats, **(g)** plots and **(h)** random sampling for (♦) Lepidoptera, (r) Apoidea, (X) Orthoptera, (O) Blattodea, (◊) Cicadellidae, (▽) Cetoniinae, (▲) Scarabaeinae, (⬣) Diptera, (▼) Neuroptera, (△) Araneae, (□) Chilopoda, (•) Diplopoda and (■) Mollusca . Species accumulation curves were produced by randomising sampling order. Error bars represent standard error; horizontal dashed lines across the graphs represent the minimum sampling effort per site.

N.B. Insufficient numbers of species were recorded to generate meaningful curves for Odonata, Isoptera, Scorpionida and Annelida for each method.

2.4.5 Species Sampling Rate

The highest number of species per hour ($S hr^{-1}$) for all taxa combined was sampled using plots which provided a mean of 7.17 $S hr^{-1}$, followed by transects (6.74 $S hr^{-1}$), sweep netting (5.77 $S hr^{-1}$), tree beat (4.42 $S hr^{-1}$), litter (3.57 $S hr^{-1}$), quadrat (2.05 $S hr^{-1}$), pan traps (0.49 $S hr^{-1}$) and baited traps (0.22 $S hr^{-1}$).

2.4.6 Optimum Sampling Period

ANOSIM showed no significant differences or relations between the species assemblages over the temporal periods; however, this may be due to the small sample sizes ($n_{month}=5$, $n_{annual}=2$) and thus reduced statistical power. The mean species density and the mean number of unique species across all taxa were highest in March and in the third sampling year (Fig. 2.4, Appendix 2.5 provides details of monthly and annual changes in the number of unique species and the species density for each taxon).

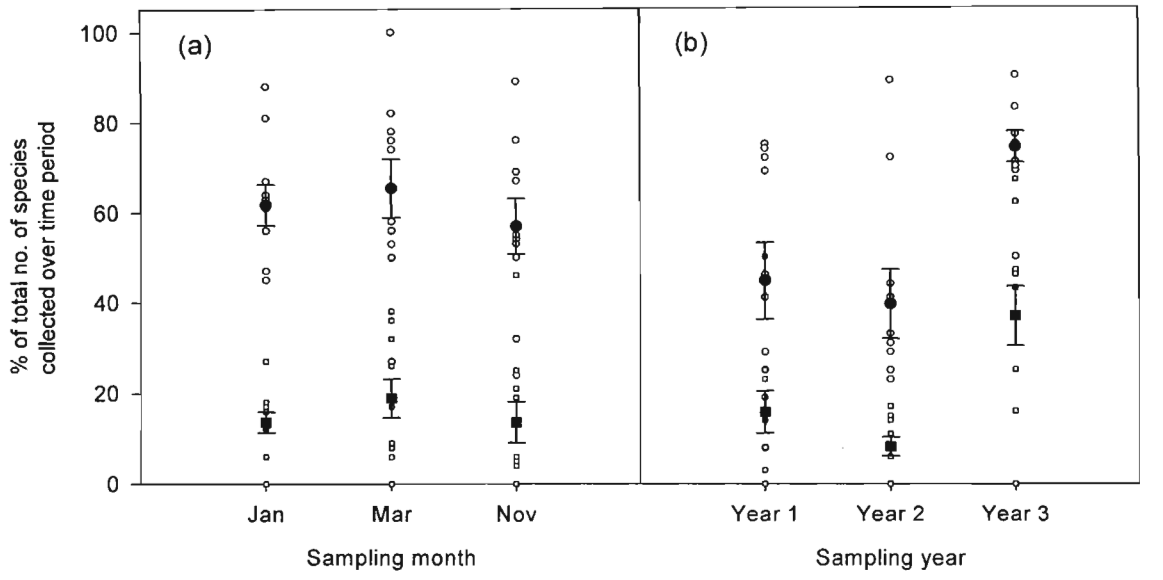

Figure 2.4 Comparison of the percentage of the total number of species recorded in each temporal window **(a)** summer months of January, March and November ($n_{month}=5$) and **(b)** the three-year sampling period ($n_{annual}=2$) in which sampling was repeated in the same month across years. • denotes the mean percentage of the total number of invertebrate species collected, ■ denotes the mean percentage of unique invertebrate species sampled only in that month. Error bars represent standard error. O represents the mean percentage of the total number of species sampled and □ represents the mean percentage of unique species sampled for each taxon group in the given period.

In November, the highest species density was sampled for Cetoniinae ($S_d=4$), Scarabaeinae ($S_d=5$) and Blattodea ($S_d=18$). January sampling yielded the greatest species densities for Apoidea ($S_d=16$), Mollusca ($S_d=22$) and Diplopoda ($S_d=13$). March sampling yielded the highest species densities out of the three

months for Lepidoptera (S_d=37), Orthoptera (S_d=18) and Chilopoda (S_d=11). The third sampling year recorded the greatest species densities for Lepidoptera (S_d=10), Apoidea (S_d=10), Cetoniinae (S_d=9), Orthoptera (S_d=4), Blattodea (S_d=12), Chilopoda (S_d=5), Araneae (S_d=19) and Mollusca (S_d=29). Scarabaeinae species were highest in the first year (S_d=3), and Diplopoda in the second year (S_d=17).

The unique species percentages follow a similar pattern, with the highest percentages for Cetoniinae (25%), Scarabaeinae (46%) and Diplopoda (19%) recorded in November. January sampling yielded 12% unique species for Mollusca. March sampling yielded the greatest unique species percentage for Lepidoptera (38%), Orthoptera (32%), Blattodea (19%), Araneae (26%) and Chilopoda (36%). Scarabaeinae (50%) and Diplopoda (11%) recorded the highest percentage in year one and two respectively. Year three sampling yielded the greatest percentages of unique species for Lepidoptera (67%), Apoidea (62%), Orthoptera (47%), Cetoniinae (46%), Araneae (37%), Chilopoda (43%) and Mollusca (16%).

2.5 DISCUSSION

Invertebrates, the bulk of terrestrial diversity, are often excluded from inventories of natural areas despite their importance in ecosystem functioning because, it is argued they (i) are protected by umbrella species, (ii) are too numerous to survey, (iii) lack appropriate methods for rapid assessment and (iv) are in such a state of taxonomic chaos that identification tools cannot be developed for use in biodiversity assessment (Fisher 1999). Nonetheless, this study agrees with the findings of Oliver and Beattie (1996), Fisher (1999) and Kotze and Samways (1999) that effective and practical methods to inventory hyper-diverse groups are possible, and can make important contributions to understanding invertebrate biodiversity patterns and to evaluating habitats for conservation.

A definitive goal for invertebrate conservation would be the wide use of a set of relatively standardised sampling methods, to collect and study a limited number of taxa (i.e. focal taxa) from many different habitats and ecosystems, with electronic access to a large amount of taxonomic and ecological data (Ward & Larivière 2004). This study provides a framework for the collection of standardised and comparable data on invertebrate species presence–absence, distribution patterns, habitat associations, diversity, rarity and abundance. These data are needed for effective conservation and monitoring of invertebrate species and their habitats (Ward & Larivière 2004).

2.5.1 Survey Design

This survey assessed 17 taxonomic groups which were selected according to the criteria of Oliver et al. (1999) for the selection of taxa suitable for characterisation of biodiversity. Methodologies were designed to be simple, and were focused on the target taxa, to enable the sampling of the target groups at a site in one day using unskilled volunteers, and to enable a comparative study to be carried out whilst providing critical baseline data for invertebrates in the region. The sampling strategy assessed was efficient in the

field, and post-fieldwork sorting was not excessive and could be completed between sampling trips. A large quantified dataset has been created, consisting of 33 000 records, which can be used to address a wide range of conservation and ecological questions. This is consistent with the findings of Oliver and Beattie (1996) in which the multi-taxa approach in sampling invertebrates was shown to have great potential for cost-effective assessment of invertebrate biodiversity in environmental monitoring, impact assessment and conservation evaluation.

The active search methods provided a good means of sampling the epigaeic invertebrates, with quadrats being the most effective method. One of the field researchers who led groups working on the plot and random methods is an expert malacologist, and this experience is reflected in the number of Mollusca species sampled by the random and plot methods. These methods enable the researcher greater freedom to sample microhabitats more likely to contain different species. This does, however illustrate that results provided by this type of active searching method are affected by the level of expertise of the sampler. Expert knowledge can improve results, but the sampling strategy should afford experts the freedom to sample likely habitats whist ensuring the methods are quantified.

Passive techniques such as pitfall trapping are commonly used for sampling epigaeic invertebrates, and although effective, these techniques do not sample less mobile species and sample very large numbers of target and non-target taxa, as well as large numbers of juveniles which cannot be identified (Slotow & Hamer 2000). This has an impact on biodiversity and raises ethical and conservation concerns (New 1999). Pitfall traps are commonly used for many different taxa, however, previous work has shown that pitfalls sample large numbers of long-lived species, in particular Diplopoda and Scorpionida (Druce et al. 2004) and therefore pitfall traps may be unsuitable for the sampling of these long-lived invertebrates such as Diplopda, Chilopoda and Scorpionida, as they potentially impact on species populations in an area. Furthermore, the time cost of setting, trap open time and collection time may not be a feasible option for short term surveys. Slotow and Hamer (2000) state that active methods can be repeatable and are most effective for less mobile species. Furthermore, using only one sampling technique, especially a passive one, may sample only one guild of a taxon, whereas a range of techniques can sample a much more representative spectrum of the total diversity within a higher taxon (Coddington et al. 1996). Furthermore, Mesibov (1995) found that active searching methods used to sample Diplopoda often yield a longer species list, since pitfall trapping is targeted at surface active invertebrates and will capture species from deep litter only occasionally. Therefore, active sampling methods are favoured for the sampling of long-lived epigaeic invertebrates.

Flying insects were less effectively sampled by active searching. Doubling the sampling effort increased the effectiveness for Orthoptera and Apoidea but effectiveness remained poor for the remaining taxa. Sweep netting was problematic due to the presence of defensive plants such as recruiting *Acacia* seedlings in the grass layer. To increase the effectiveness of sampling flying insects, Malaise traps should be used to bridge the gap in sampling species assemblages which were incompletely sampled by

transects, pans or butterfly baited traps. However, the effectiveness of Malaise traps is dependent on firstly, the setting location intercepting a flight path (New 1998), and secondly, on sufficient space being available to set the trap, which in some savanna habitats is restricted. Other problems may be created by the movement of large game species damaging expensive traps. However, our baited traps were largely left untouched by large game and therefore Malaise could be used, therefore, one must factor in the cost of traps being damaged or removed by large game. A vacuum sampling method, either using a vortis sampler or D-Vac may improve the efficiency of Araneae sampling (Samu et al. 1997) and Cicadellidae sampling (Kersting et al. 1997). Malaise and vacuum sampling do collect non-target invertebrates, however, the taxa are generally short-lived as adults and abundant which means that the impact of sampling is likely to be minimal. The setting of Malaise traps and the substitution of sweep netting with vacuum sampling would fit into the daily timetable without additional time costs.

2.5.2 Sampling Effort

In order for a comparative study to answer ecological questions, individual sites must be adequately sampled and equal sampling effort must be used across all sites. However, care must be taken to minimise the reduction in data quality when there is a reduction in sampling effort (Ward & Larivière 2004). Therefore, it is suggested that the suite of epigaeic invertebrates selected in this study be sampled using two 2m x 10m quadrats, two 20m x 20m plots and four 5l leaf litter samples. Quadrats and plots were chosen as they sampled a significantly greater number of species than the plots and random sampling combinations. The use of quadrats enabled focused and thorough sampling, whereas the plot sampling enabled the knowledgeable sampler greater freedom of habitat. Four leaf litter samples were chosen because there was some overlap in the species assemblages sampled by quadrats and because the time required to sort and extract the specimens from more than four samples was excessive. It must also be noted that this method samples post mortem Mollusca, the samples were air dried and therefore large mobile specimens were able to escape minimising by-catch.

The sampling of flying and plant-dwelling invertebrates chosen for this study needs further investigation: the minimum effort did not effectively sample invertebrates, but even the maximum sampling effort did not show considerable increase in the sampling effectiveness, since the species accumulation curve continues to increase and does not approach an asymptote. This demonstrates that the additional sampling effort resulted in a greater number of estimated species so the level of completeness remained low. Therefore, to increase sampling effectiveness of these taxa it is suggested that additional methods be used. I suggest that four 50m transects, 20 tree beats, six 20 second vegetation vacuum sampling in a transect 10m apart (Arnold 1994), 10 fruit baited butterfly traps, 20 pan traps, four Malaise traps all set for 24 hours be used at each site (Table 2.8). Malaise traps are conventionally left open for minimum periods over 24 hours (New 1998, references therein), it is likely that four traps would yield interesting specimens if set for 24 hours, furthermore this fits in with the pan and baited trap sampling period. The use of four transects and 20 pan traps increases the effectiveness of the sampling by increasing the number of species

sampled. I suggest that an equal mix of blue and yellow traps be used, despite yellow traps sampling a higher species density as no significant differences were detected in the species assemblages sampled by the different colours, and significant differences were only detected in the species densities sampled of three of the six taxa sampled. In addition, blue traps were shown to sample unique species. Ten fruit baited traps sampled over 60% of the estimated total richness of Lepidoptera and Cetoniinae at a site. Tree beating was not effective as a stand-alone method; nevertheless, it did sample significantly different species assemblages to those sampled by other methods and therefore is recommended that it be included in the survey strategy.

There are several invertebrate sampling techniques which are summarised by New (1998) and Southwood and Henderson (2000). However, the present survey design was developed to enable effective sampling using a multi-taxa approach in savanna conditions using unskilled volunteers. Focus on different suite of taxa, or a single taxon would require a different sampling regime. Expert biologists may well have selected a different suite of methods.

This study used unskilled volunteers and therefore the use of visual sampling for taxa such as Orthoptera, Odonata and Lepidoptera was not suitable. However, these methods should be considered providing the sampler is skilled and the method can be quantified by applying a measure of effort, which would enable species accumulation curves to be constructed and estimates of species richness or measures of sampling completeness to be calculated.

Table 2.8 Recommended combination of methods and taxa for a multi-taxa survey of savanna invertebrates, indicating which taxa are suitable to be identified by a para-taxonomist (shaded).

Taxa		Taxa suitable to be identified by a Para-taxonomist	Method and Number of replicates								
			Plot 20m x 20m 1hr search (x 2)	Quadrat 2m x 10m searches (x 2)	Leaf litter 4 x 5l	Transect 4 x 50m	Baited traps (x 10)	Tree beat (x 20 trees)	Pan traps x 10 blue x 10 yellow	Vacuum sampling 6 x 20 seconds along a transect 10m apart	Malaise traps set for 24 hours (x 4)
Lepidoptera		✓				✓	✓				✓
Apoidea						✓	✓		✓		✓
Orthoptera		✓				✓					
Blattodea			✓	✓			✓	✓	✓		
Odonata		✓				✓					✓
Cicadellidae								✓	✓	✓	
Cetoniinae		✓					✓				
Diptera						✓	✓		✓		✓
Neuroptera		✓				✓	✓				✓
Araneae								✓	✓	✓	
Scorpionida		✓	✓	✓							
Chilopoda			✓	✓							
Diplopoda			✓	✓				✓			
Mollusca	Maco	✓	✓	✓							
	Micro				✓						

2.5.3 Taxa

This study shows that using the suggested methods the following taxa can be sampled effectively: Lepidoptera, Apoidea, Diptera, Neuroptera, Odonata, Cicadellidae, Cetoniinae, Orthoptera, Blattodea, Araneae, Scorpionida, Mollusca, Diplopoda and Chilopoda. With the inclusion of additional methods, it is likely over 75% of the estimated species richness of all of these taxa can be sampled. I suggest that Annelida, Isoptera and Scarabaeinae be removed from the survey unless additional sampling methods are used, for example dung baited traps as outlined by Larsen and Forsyth (2005), cellulose baited traps described in Dawes-Gromadzki (2003) and digging methods specifically designed to sampled earthworms (Plisko, 2006, personal communication). Although 78% of the predicted species of Annelida were sampled, there were large differences in the range of richness suggesting some error, which may have been caused by low species and individual numbers as a result of inadequate sampling methods focusing on leaf litter inhabiting species.

Ultimately, the choice of taxa is highly dependant on the questions a survey is aiming to address and the resources that are available. The following taxa can be identified by a para-taxonomist and the corresponding sampling methods are quick and effective (Table 2.8): Lepidoptera, Orthoptera, Odonata, Cetoniinae, Neuroptera (sampled using baited traps, transects and malaise traps), and Scorpionida and Mollusca (sampled by plots and quadrat active searches). These taxa and sampling methods are suitable for the rapid assessment of biodiversity in a unit area and give a fairly wide window on the invertebrates as a whole. While, these taxa can be identified without the input of expert taxonomists, it is recommended that specimens are checked against a reference collection identified by an expert taxonomist (i.e. museum collections). It must also be noted that one must not disregard the value of collecting data on specific, easily identifiable species belonging to other taxa (e.g. *Spinotarsus colosseus* (Diplopoda)).

2.5.4 Temporal Impacts on Survey

It is beyond the scope of this paper to address temporal variation in detail, because the topic is broad and there are many variables that need to be taken into account to quantify temporal variation. Invertebrates are highly variable and many have complex life histories that are poorly documented in terms of temporal scales. It is evident from the descriptive analysis that there are differences in the species densities and number of unique species sampled across the summer months, but no correlation regarding monthly variation was observed across all taxa. There are no clear trends observed across years, but the third year of sampling showed a dramatic increase in species richness and the number of unique species, which corresponds to the annual rainfall returning to a near average level from drought conditions in previous years. My results indicate that in order to carry out a comprehensive survey, sites must be sampled across months and across years. The need to sample across seasons is further highlighted when one looks at the historical records for Lepidoptera, where 217 species are recorded in this area. However, when one looks at records from November, January and March only 117 are recorded. Studies in pollination biology have

revealed differences among pollinator taxa of plant species across years and that conclusions drawn from a single sampling season are potentially suspect (Fishbein & Venable 1996).

It is of interest to note that the present study was carried out in a drought cycle which has been attributed to the ENSO cycle, which is one of the major influences on inter-annual rainfall variability over southern Africa. It is reported that the ENSO frequency is variable and observed intervals of about three to 10 years may be considered typical (Mason 1997). Usually, although not always, La Niña and El Niño events are associated with the occurrence of wet and dry years, respectively (Mason 1997). Therefore, in order to carry out an effective inventory or comparative study, sampling should take place across all months and across multiple years in which larger cycles such as the ENSO cycle must be considered. Furthermore it must be remembered that inventories are a temporal snapshot of biodiversity at any one time, and form the baseline for measuring change (Samways 2005).

2.5.5 Sampling Completeness

For both an inventory and a comparative study, there is a need to have an understanding of the completeness of the survey. When sites are poorly surveyed, considerable error occurs in any analysis. Furthermore, many species richness estimators are not stable when a low percentage of the total species richness is sampled (Chazdon et al. 1998) and samples biased either temporally or spatially will yield extrapolations valid only for the spatial and temporal conditions sampled (Soberón & Llorente 1993). For comparative studies, it is essential that equal sampling effort is used to compare unit areas. However, the taxa must be sampled with enough completeness to capture the main body of the species assemblage present. I suggest that a minimum level of 75% of the total estimated fauna is a satisfactory and realistic level of survey completeness for the purpose of making valid comparisons. This level of completeness was selected as it was likely that data collected would provide valid information about the majority of the invertebrates. However, rare species, defined by Gaston (1994) as the rare quartile (25%) species, are likely to be excluded from the data.

Invertebrate inventories need to be compiled differently to comparative studies. The invertebrate inventory must span seasons, climatic cycles, habitats, and use a suitable suite of taxa. By definition, reaching 100% of richness would require an infinite effort, and the rate of species recorded per effort invested decreases markedly as the curve approaches the asymptote (Soberón & Llorente 1993). The minimum number of sites required for sampling varies across taxa. I estimate the required effort to reach a level of 75% completeness to be 60 sampling events (see Table 2.5) using the suggested sampling protocol and taxa (Table 2.8). However, one must remember that these estimates are based on data collected only in the summer months and if sampling is to continue across all seasons, more than 60 sampling events are likely to be required, but this will vary depending on the spatial area being surveyed. It must be considered that the estimation of the number of sites required is merely a guideline indicating the volume of work required to carry out a survey, and as reported by Willot (2001), the guideline does not distinguish between genuine differences in species richness among sites.

2.5.6 Conclusion

The Maputaland Centre consists of a mosaic of mainly extensive savanna plant communities arranged in complex patterns and for its size is one of the most remarkable areas of biodiversity in the world (van Wyk 1996), and this region has a high level of heterogeneity. The invertebrate fauna of this heterogeneous landscape is poorly studied and it is not known whether invertebrate assemblages in the region are similarly heterogeneous (van Rensburg et al. 1999). Therefore, the recommendations made may not be directly applicable to other savanna habitats. It is likely that invertebrate surveys in more homogeneous savannas will require less overall effort. There is a need for further research into the spatial heterogeneity of invertebrates in this region and the relation of the invertebrate assemblages to biotic and abiotic features at varying spatial scales.

Particular methods are more suitable than others for sampling invertebrates. Future sampling in the savanna should use a combination of active searching of quadrats, transects, leaf litter sampling, tree beats and passive techniques of fruit baited butterfly traps, pan traps (blue and yellow), vegetation vacuum sampling and Malaise traps. I recommend that Lepidoptera, Apoidea, Diptera, Neuroptera, Odonata, Cicadellidae, Cetoniinae, Orthoptera, Blattodea, Araneae, Scorpionida, Mollusca, Diplopoda and Chilopoda be included in savanna invertebrate surveys. Using this sampling strategy, I suggest that a minimum level of 75% of the total estimated fauna is a satisfactory and realistic level of survey completeness for making valid comparisons. Sampling should take place across all months and across multiple years in which larger cycles such as the ENSO cycle are considered.

My findings are concurrent with those of Moreno and Halffter (2000) and show practical applications for species accumulation models, including the assessment of inventory and within inventory completeness, and the estimation of the minimum sampling effort required to complete an inventory or comparative study, which can lead to important improvements in sampling design, ensuring money, time and effort are invested efficiently to maximise species capture as a function of cost (Moreno & Halffter 2000). Therefore, it is important for abundance data to be collected, to enable individual-based species accumulation curves to be produced so extrapolation can be carried out in order for completeness to be assessed.

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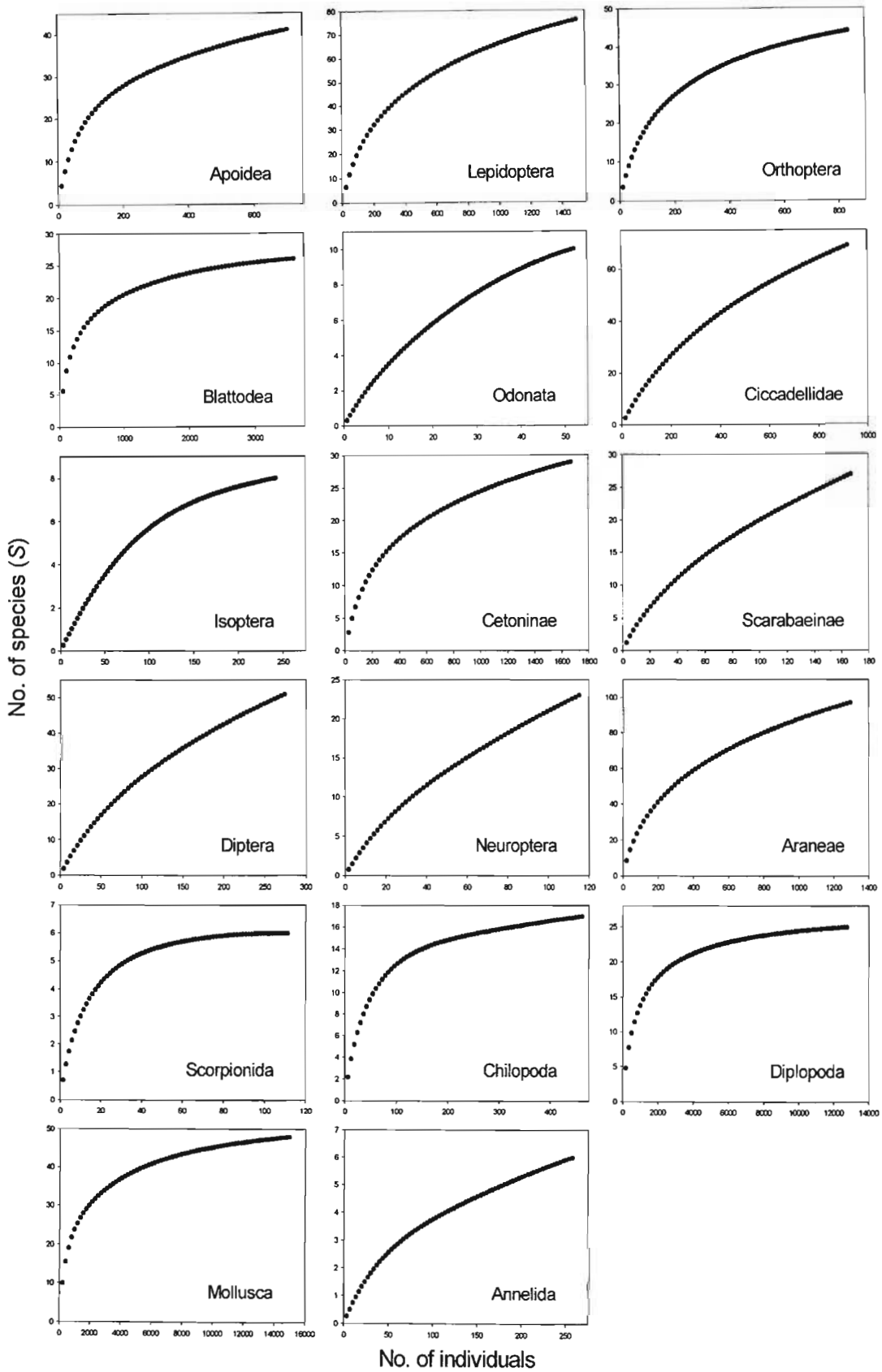
Appendix 2.1 Analysis of similarity between sampling methods used for each taxon and the species assemblages sampled. Un-shaded areas show the pair-wise analysis of sampling methods in which the Bonnferoni adjustment was used to determine significance. *R*-value gives an absolute measure of how separated two groups are, on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarities between groups), if $R>0.75$ groups are well separated, $R>0.5$ groups are overlapping but clearly different, $R<0.25$ groups are barely separable (Clarke & Gorley 2001). *P*-value refers to significance level.

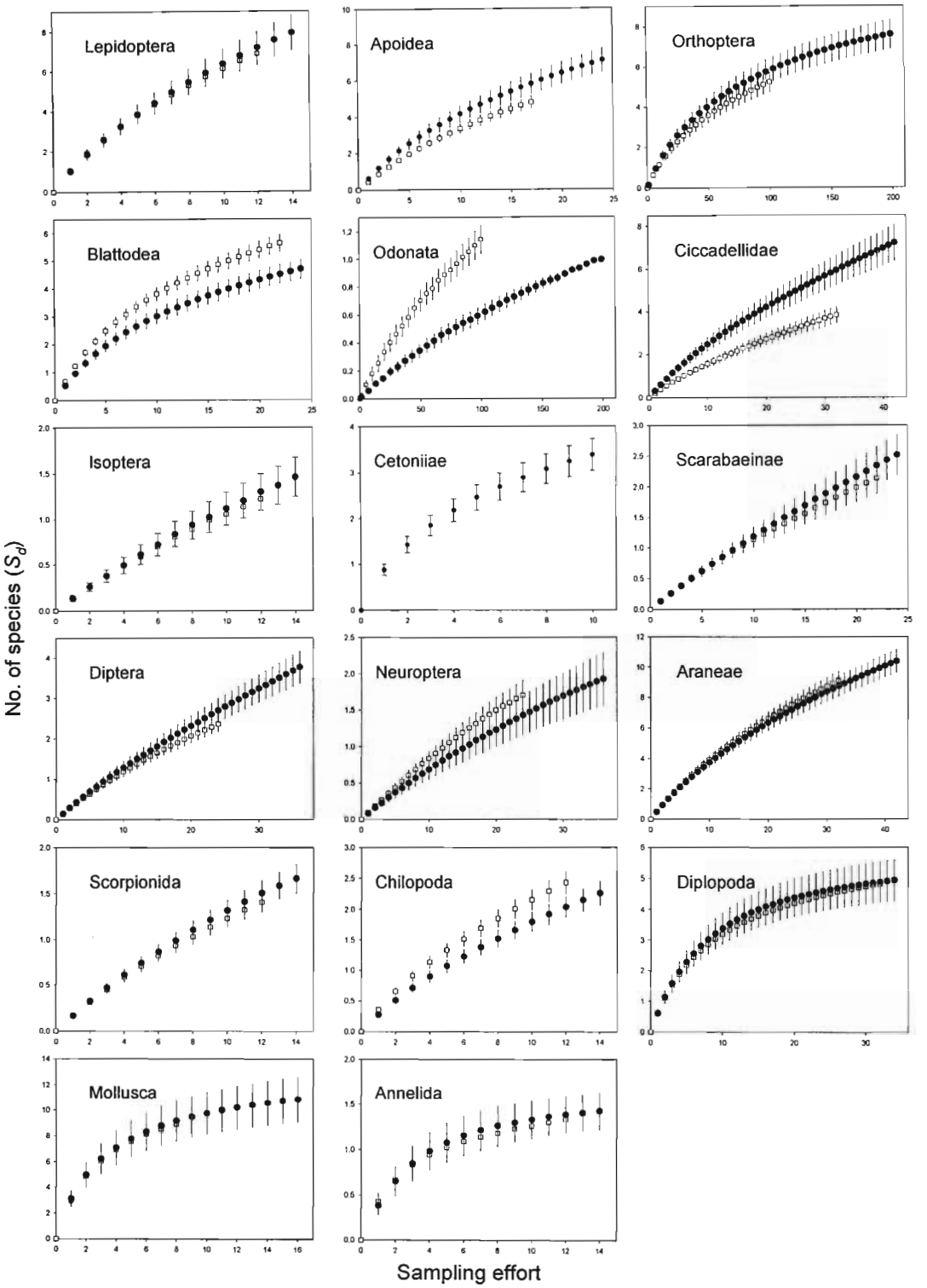
Taxa	Methods	*R*-value	*P*-value
Lepidoptera	Transects vs Baited traps	0.43	0.001
Apoidea	All methods	0.40	0.001
	Pan traps vs Baited traps	0.41	0.001
	Pan traps vs Transects	0.45	0.001
	Baited traps vs Transects	0.21	0.058
Orthoptera	Transects	Single sampling method	
Blattodea	All methods	0.13	0.001
	Plot vs Quadrats	0.11	0.001
	Plot vs Pan traps	0.14	0.002
	Plot vs Random	-0.03	0.982
	Quadrats vs Pan traps	0.41	0.001
	Quadrats vs Random	0.12	0.001
	Pan traps vs Random	0.18	0.001
Odonata	Transects	Single sampling method	
Cicadellidae	All methods	0.27	0.001
	Sweep vs Pan traps	0.19	0.001
	Pan vs Tree beat	0.36	0.001
	Sweep vs Tree beat	0.13	0.061
Isoptera	All methods	-0.06	0.84
Cetoniinae	Baited traps	Single sampling method	
Scarabaeinae	All methods	0.09	0.014
	Pan traps vs Quadrats	0.32	0.003
	Pan traps vs Plot	0.16	0.014
	Pan traps vs Random	0.30	0.002
	Quadrats vs Plot	0.02	0.221
	Quadrats vs Random	-0.01	0.462
	Plot vs Random	-0.02	0.523
Diptera	All methods	0.01	0.171
Neuroptera	All methods	0.04	0.164
Araneae	All methods	0.43	0.001
	Pan traps vs Sweep	0.19	0.001
	Pan traps vs Tree beat	0.62	0.001
	Sweep vs Tree beat	0.40	0.001
Scorpionida	All methods	0.28	0.001
	Random vs Quadrats	0.37	0.001
	Random vs Plot	0.03	0.215
	Quadrats vs Plot	0.34	0.002
Chilopoda	All methods	0.01	0.387
Diplopoda	All methods	0.20	0.001
	Plot vs Quadrats	0.01	0.303
	Plot vs Random	-0.02	0.924
	Plot vs Tree beat	0.50	0.001
	Quadrats vs Random	0.02	0.104
	Quadrats vs Tree beat	0.61	0.001
	random vs Tree beat	0.51	0.001
Mollusca	All methods	0.09	0.001
	Litter vs Plot	0.17	0.001
	Litter vs Quadrats	0.22	0.001
	Litter vs Random	0.20	0.001
	Plot vs Quadrats	-0.01	0.746
	Plot vs Random	-0.02	0.956
	Quadrats vs Random	0.00	0.452
Annelida	All methods	-0.07	0.602

Appendix 2.2 Regression statistics from fitting and solving the Michaelis-Menton equation for each method or combination of methods which were shown to sample different species assemblages. a_{max} corresponds to the estimated species richness or asymptote of the species accumulation curve, all regression statistics were significant at 99%.

Taxon	Method	Observed mean statistics		Regression statistics		
		Mean species density	% of a_{max}	R^2	a_{max}	b
Lepidoptera	Baited traps	4.2	61.6	0.998	6.8	6.5
	Transects (100m)	3.8	40.3	0.999	9.4	149.4
	Transects (200m)	4.4	32.1	0.999	13.7	420.4
	Min sampling effort	6.9	45.8	0.999	15.1	14.6
	Max sampling effort	7.9	42.6	0.999	18.5	19.0
Apoidea	Pan traps 10	4.2	43.8	0.999	9.6	13.0
	Pan traps 20	6.3	53.6	0.999	11.8	17.6
	Baited traps	1.5	23.9	0.999	6.4	31.9
	Transects (100m)	1.2	5.2	0.999	23.4	1826.9
	Transects (200m)	1.2	12.0	0.999	9.8	1464.7
	Transects (100m) & baited traps	1.6	21.0	0.999	7.6	43.6
	Transects (200m) & baited traps	2.1	20.9	0.999	10.0	53.9
	Min sampling effort	4.8	38.2	0.999	12.6	27.4
	Max sampling effort	7.1	51.9	0.999	13.7	22.7
Orthoptera	Transects (100m)	5.2	57.2	0.999	9.1	75.9
	Transects (200m)	7.9	74.9	0.999	10.6	80.2
Blattodea	Pan traps 10	1.7	13.6	0.999	12.5	63.8
	Pan traps 20	2.8	28.1	0.999	10.0	52.6
	Quadrat	4.0	58.5	0.999	6.9	7.4
	Plot	2.9	42.6	0.999	6.8	164.0
	Plot & quadrat	5.4	65.3	0.998	8.3	6.8
	Plot, random, quadrat	5.9	60.9	0.998	9.7	9.2
	Min sampling effort	5.6	62.7	0.999	8.9	13.2
	Max sampling effort	4.7	63.5	0.999	7.4	14.3
Odonata	Transects (100m)	1.1	38.2	0.999	2.9	155.8
	Transects (200m)	1.0	38.2	0.999	2.6	326.6
Cicadellidae	Pan traps 10	3.9	37.9	0.999	10.3	16.7
	Pan traps 20	6.6	43.6	0.999	15.1	26.8
	Min sampling effort	3.8	34.6	0.999	11.0	60.3
	Max sampling effort	7.2	40.2	0.998	17.9	64.1
Isoptera	Min sampling effort	1.2	26.7	0.999	4.5	32.4
	Max sampling effort	2.6	42.5	0.999	6.1	44.7
Cetoniinae	Baited traps	3.4	66.4	0.998	5.1	1.0
Scarabaeinae	Pan traps 10	1.5	23.3	0.999	6.5	34.4
	Pan traps 20	1.7	44.6	0.998	3.8	26.4
	Quadrat	2.1	26.0	0.999	8.0	28.7
	Plot, random, quad	2.1	19.9	0.999	10.5	58.0
	Min sampling effort	2.1	27.5	0.999	7.6	57.3
	Max sampling effort	2.5	19.6	0.999	12.9	98.9
Diptera	Pan 10	1.6	37.4	0.999	4.3	16.6
	Pan 20	2.4	34.2	0.999	7.0	39.8
	Transects (100m)	1.8	39.8	0.999	4.5	152.7
	Transects (200m)	2.7	25.1	0.999	10.7	596.7
	Min sampling effort	2.4	28.5	0.999	8.4	59.7
	Max sampling effort	3.8	25.7	0.999	14.8	106.7
Neuroptera	Transects (100m)	1.3	39.8	0.999	3.3	144.7
	Transects (200m)	1.7	55.6	0.998	3.1	176.3
	Min sampling effort	1.7	25.2	0.999	6.7	70.4
	Max sampling effort	1.9	30.5	0.999	6.2	80.5
Araneae	Tree beat	7.3	38.2	0.999	19.2	32.8
	Pan traps 10	1.6	26.0	0.999	6.3	28.7
	Pan traps 20	2.6	44.0	0.999	5.9	26.3
	Min sampling effort	9.1	40.4	0.999	22.5	47.9
	Max sampling effort	10.4	44.6	0.999	23.3	53.0
Scorpionida	Min sampling effort	1.4	28.9	0.999	4.9	29.3
	Max sampling effort	1.7	31.5	0.999	5.4	31.0
Chilopoda	Quadrat	1.7	31.8	0.999	5.3	21.7
	Plot	1.4	32.1	0.999	4.4	253.9
	Min sampling effort	2.4	43.2	0.999	5.6	15.7
	Max sampling effort	2.3	40.0	0.999	5.8	21.8
Diplopoda	Tree beat	1.2	56.3	0.999	2.1	14.6
	Quadrat	3.9	75.2	0.999	5.1	3.3
	Plot	3.1	69.0	0.999	4.5	54.9
	Plot & quadrat	5.2	79.3	0.999	6.6	3.0
	Plot, random, quad	5.2	77.2	0.999	6.2	2.7
	Min sampling effort	4.8	77.4	0.999	6.2	9.4
	Max sampling effort	5.0	80.5	0.999	6.2	8.4
Mollusca	Litter 4	7.2	67.3	0.999	10.7	1.9
	Litter 8	8.9	75.2	0.999	11.8	2.7
	Quadrat	4.8	80.6	0.998	5.9	2.6
	Plot	6.2	73.0	0.999	8.5	45.2
	Plot & quadrat	7.3	80.5	0.998	9.1	3.2
	Plot, random, quadrat	7.8	84.8	0.998	9.2	2.7
	Min sampling effort	8.9	71.3	0.999	12.5	3.2
	Max sampling effort	10.9	83.6	0.999	13.0	3.31
Annelida	Min sampling effort	1.3	79.8	0.998	1.6	2.9
	Max sampling effort	1.4	78.7	0.998	1.8	3.3



Appendix 2.3 Individual-based species richness accumulation curves for each taxon sampled using data from all sites, using the minimum sampling effort.



Appendix 2.4 Mean species density curves for each taxonomic group using the minimum (□) and maximum (●) sampling effort used to sample each taxon across sites. Error bars represent standard error, curves are randomised using 999 permutations.

N.B. Sampling sites for minimum and maximum sampling effort versus species density values for additional sampling effort for transects, pan traps and leaf litter are from data sets using a different combination of sites than the remaining methods.

Appendix 2.5 Comparison of the species density and unique species sampled in each temporal period. Sites sampled repeatedly across months (n_{site}=5) and in the same month across different years (n_{site}=2) were assessed.

Taxa		January	March	November	Combined months	Year 1	Year 2	Year 3	Combined years
Lepidoptera	Species density	28	37	12	50	3	1	10	12
	No. & % of unique species	9 (18%)	19 (38%)	3 (6%)	-	1 (8 %)	1 (8%)	8 (67%)	-
Apoidea	Species density	16	14	12	24	1	4	10	13
	No. & % of unique species	4 (17%)	4 (17%)	1 (4%)	-	1 (7.7%)	2 (15%)	8 (62%)	-
Orthoptera	Species density	14	18	7	22	1	4	10	12
	No. & % of unique species	3 (14%)	7 (32%)	1 (5%)	-	1 (8%)	2 (17%)	7 (47%)	-
Blattodea	Species density	11	14	18	16	11	7	12	16
	No. & % of unique species	1 (6%)	3 (19%)	1 (6%)	-	4 (25%)	0 (0%)	4 (25%)	-
Cetoniinae	Species density	10	8	11	16	6	3	9	13
	No. & % of unique species	2 (13%)	1 (6%)	4 (25%)	-	3 (23%)	0 (0%)	6 (46%)	-
Scarabaeinae	Species density	5	3	6	11	3	1	2	4
	No. & % of unique species	3 (27%)	1 (9%)	5 (46%)	-	2 (50%)	0 (0%)	1 (25%)	-
Araneae	Species density	20	23	23	43	11	11	19	27
	No. & % of unique species	7 (16%)	11 (26%)	9 (21%)	-	5 (19%)	3 (11%)	10 (37%)	-
Chilopoda	Species density	5	11	6	11	2	2	5	7
	No. & % of unique species	0 (0%)	4 (36%)	0 (0%)	-	1 (14%)	1 (14%)	3 (43%)	-
Diplopoda	Species density	13	9	11	16	14	17	14	19
	No. & % of unique species	2 (13%	0 (0%)	3 (19%)	-	0 (0%)	2 (11%)	0 (0%)	-
Mollusca	Species density	22	19	19	25	23	23	29	32
	No. & % of unique species	3 (12%)	2 (8%)	1 (4%)	-	1 (3%)	2 (6%0	5 (16%)	-

N.B. There was insufficient species data to assess temporal variation for Diptera, Cicadellidae, Odonata, Scorpionida, Annelida, Neuroptera and Isoptera.

ASSESSMENT OF THE USE OF VOLUNTEERS FOR INVERTEBRATE BIODIVERSITY SURVEYS

3.1 ABSTRACT

It is widely recognised that global biodiversity is threatened, yet current knowledge of species' distributions, assemblage patterns and the processes that influence these are poorly understood for many taxa. Therefore, there is an urgent need to obtain data to fill knowledge gaps but this requires resources and manpower. Globally the use of volunteers to collect data is becoming increasingly common in biodiversity research. This study assesses the effectiveness and efficiency of volunteers sampling invertebrates in comparison to experienced researchers, and examines the potential contribution of volunteers to surveys of invertebrates. Fifty-four volunteers, recruited by the Earthwatch Institute, were evaluated during their participation in a project surveying invertebrate biodiversity in a savanna ecosystem. Individual volunteers, when compared to researchers, sampled significantly lower rates of species accumulation, species richness and unique species when they sampled using timed active search methods. Yet, when the combined efforts of two volunteers were assessed, these differences were no longer present. Volunteers and researchers were shown to perform equally when using un-timed active searching methods. Volunteers were shown to be effective at sampling invertebrates in conjunction with experienced researchers. Previous experience or knowledge of scientific method was beneficial when researchers assessed the perceived usefulness of volunteers to researchers when carrying out fieldwork. The project experience raised the volunteers' environmental awareness, knowledge about biodiversity, invertebrates, and conservation research, and enabled volunteers to participate in or design locally relevant conservation based projects on their return home. The benefits of using volunteers are discussed and guidelines are provided to assist in the inclusion of volunteers in surveys of invertebrates. This survey has shown that the use of volunteers in surveys of invertebrates enables researchers to carryout comprehensive surveys in a short period to provide much need data for conservation.

Keywords: Unskilled workers, conservation, environmental education, sampling efficiency, sampling effectiveness, guidelines, active searching, species assemblage patterns, savanna, survey design.

3.2 INTRODUCTION

The current knowledge of invertebrate distributions, species assemblage patterns and the processes that influence these are poorly understood (Ward & Larivière 2004). Within the scientific world, it is widely accepted that more biodiversity research is required if conservation efforts are to effectively conserve biodiversity (Brooks et al. 2004). Invertebrates are the main component of biodiversity at the species level, and are critical for ecosystem functioning. However, surveys of invertebrates are time-consuming and research is generally poorly funded, resulting in a lack of resources and labour that are essential to

fulfil research requirements.

The use of volunteers is becoming increasingly common in biodiversity and conservation research globally (e.g. Coral Cay Conservation 2005; Earthwatch Institute (International) 2005; Frontier 2005; Global Vision International 2005). Since 1971, the Earthwatch Institute has recruited over 65 000 volunteers in support of 2 800 field research projects in 118 countries, contributing over 10 million hours and £35 million to essential fieldwork (Earthwatch Institute (Europe) 2004). Volunteers provide a valuable workforce for fieldwork, a vital link for education within communities, and in many cases the necessary funding for research. Nevertheless, there is some debate over the effectiveness of unskilled volunteers in scientific work on the grounds that the information collected will be unreliable as a result of either insufficient training or a lack of consistency through the necessary use of a large number of different observers (Darwall & Dulvy 1996).

Many biodiversity studies around the world use volunteers for data collection, and there are several which have made a significant contribution to conservation biology. These include Karr's (1990) work on birds in Panama, the assessment of Burgess et al. (1992) of coastal forests in Tanzania, the work of Lowman et al. (1996) on arthropods in Australian rainforests and Mumby and Harborne's (1999) development of a classification scheme for marine habitats. However, the use of volunteers is rarely documented in journals, and the validation of volunteers' work is even rarer. Exceptions are Foster-Smith and Evans (2003), Darwall and Dulvy (1996) and Goffredo et al. (2004) who all examined marine ecological data collected by volunteers, and Newman et al. (2003) who assessed volunteers' monitoring of mammals.

No studies have been undertaken to assess effectiveness of volunteers in surveys of invertebrates. Although many of the techniques used in surveys of invertebrates are simple to implement, the scientific complexity lies in the recognition and capture of the target taxa. Many of the target species are cryptic, small or inconspicuous making them difficult to find, and other species are fast moving and difficult to catch. Therefore, there is a need to investigate the sampling techniques that can be used successfully by unskilled volunteers in an invertebrate survey.

Conservation efforts need to be supported by the general public. Negative perceptions of invertebrates make conservation strategies aimed at invertebrates difficult to implement. Among the general public most invertebrates are perceived with attitudes of fear, antipathy and aversion (Kellert 1993). In order to address these perceptions of invertebrates it is necessary for the general public to understand the importance of invertebrates and to appreciate their diversity. Interaction between researchers in this field and non-scientists is one mechanism to achieve this.

This study aimed to contribute to invertebrate conservation by investigating the use of volunteers for surveys of invertebrates by addressing the following objectives: (i) to assess the efficiency of volunteers in sampling invertebrates relative to researchers by comparing the mean rates of species accumulation, (ii) to examine time benefits associated with the use of volunteer teams to carry out comprehensive

invertebrate surveys, (iii) to assess how effective volunteers were in sampling invertebrates by comparing the number of species, unique species and species assemblages sampled by volunteers and researchers, (iv) to identify whether volunteers were only able to sample common, abundant or obvious species and therefore did not sample rare species, (v) to identify what qualities make volunteers valuable to field researchers, (vi) to investigate changes in perceptions among volunteers as a result of their participation in the expedition by assessing changes in perceptions regarding invertebrates and assessing post expedition activities and (vii) to provide guidelines for the use of volunteers for invertebrate surveys.

3.3 METHODS

3.3.1 Study Site

Fieldwork was carried out in the Mkhuze Game Reserve (27.67°S 32.27°E), Phinda Private Game Reserve (27.78°S 32.35°E) and False Bay Park (27.94°S 32.38°E) in north-eastern KwaZulu-Natal, South Africa. These reserves are situated in the diverse region known as the Maputaland Centre, which consists of a mosaic of mainly extensive savanna communities arranged in complex patterns.

Sampling sites were 1ha plots of uniform vegetation types. Forty-three different 1ha plots were sampled between November 2002 and March 2005 (summer months). Twenty of these sites were re-sampled in different months during the summer season and across years, giving a total of 77 sampling events. For this volunteer study, data from 46 sampling events, sampled between November 2003 and March 2005, were assessed.

3.3.2 Volunteers

Earthwatch Institute recruited all volunteers to participate in the research. A total of 54 volunteers participated in seven field trips. There was an uneven gender split (Fig. 3.1) consisting of 39 men and 24 women across seven age groups. Sixteen volunteers were funded through Earthwatch as part of the African Fellows programme. This programme consisted of individuals who work in conservation and / or have a tertiary education in a field directly related to the project. Two African fellow teams had a sound understanding of the scientific method, and five of these volunteers were experienced field researchers of invertebrates. Rio Tinto funded seven volunteers. These volunteers were bird guides from the region and all were placed on the project to help raise their awareness of invertebrates and scientific research. Twenty-one volunteers were funded by corporate businesses as part of employee development schemes, Earthwatch Institute placed them on the expedition, they therefore did not choose an invertebrate sampling expedition in their application. Nine volunteers were independent and selected this expedition. One volunteer was an Earthwatch representative. Each volunteer contributed funds of approximately US $200 per day towards Earthwatch administration, research costs, food and accommodation.

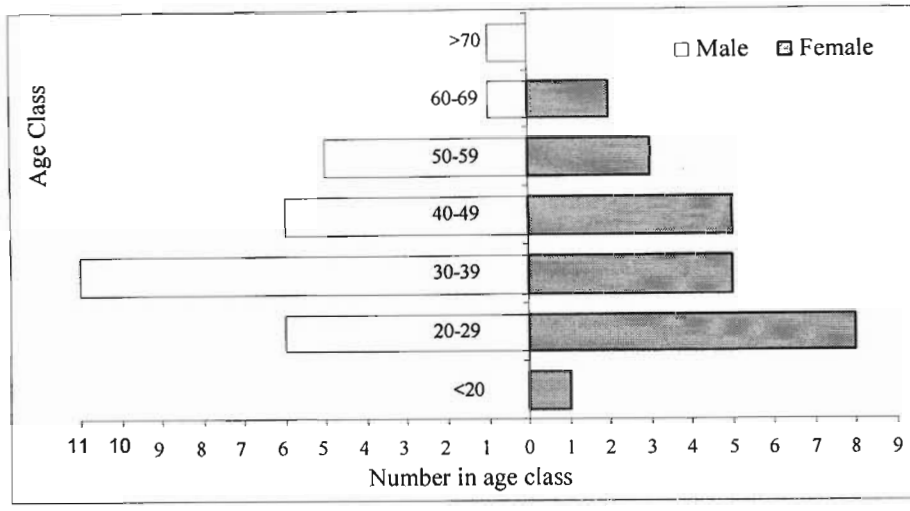


Figure 3.1 Volunteer age and gender profiles.

Volunteers spent 10 nights on the project, enabling one day of training, eight sampling days and one day off. The volunteers' primary focus was the sampling of invertebrates, but volunteers also participated in a range of activities in the field and at base camp including GPS and off-road navigation, data entry, photography and cooking.

3.3.3 Pre-sampling Tasks

Volunteers were asked to independently complete simple questionnaires by giving scored answers to the following questions, prior to fieldwork, to gauge their understanding of biodiversity, environmental awareness and attitudes towards invertebrates.

i) Please assess the extent to which you feel a responsibility towards protecting the environment.
ii) When you go into the countryside, do you / will you investigate the invertebrates living here?
iii) Do you think you are likely to join another ecological research survey in the future?
iv) In your opinion which of the following best fits the statement that volunteers can make a significant contribution to ecological studies.
v) How well do you understand the term biodiversity?
vi) List five scientific journals in which research on biodiversity might be published.
vii) Do you actively conserve invertebrates at home?
viii) What is your perception of invertebrates?
ix) On your return, do you think you will share you experiences and knowledge with family and friends?
x) How would you rate the importance of invertebrates on a global scale?

The volunteers were given a presentation on the aims and objectives of the project and its relevance to ecology, conservation and reserve management. Volunteers were given opportunities to ask questions throughout the presentation.

Full training in the sampling techniques was given, together with basic invertebrate identification focusing on the taxa to be sampled. Simple field guides to invertebrates were provided and basic method cards were made available at all times in the field (Appendix 3.1 and 3.2). All volunteers practised sampling techniques in the training session, and guidance was provided on how to recognise and catch target invertebrates and place them into bottles.

During the expedition, a small library was available which provided field guides on invertebrates and a range of research papers on conservation, biodiversity and invertebrates.

3.3.4 Sampling

Volunteers participated in all sampling methods under the supervision of at least one experienced researcher. A range of replicated sampling methods were carried out to sample the following 17 taxa: Lepidoptera (butterflies), Hymenoptera (Apoidea), Diptera (Asilidae, Bombyliidae), Neuroptera, Odonata, Hemiptera (Cicadellidae), Coleoptera (Cetoniinae, Scarabaeinae), Orthoptera, Blattodea, Isoptera, Araneae (Araneidae, Thomisidae, Oxyopidae), Scorpionida, Myriapoda (separated into Diplopoda, Chilopoda classes), Mollusca and Annelida.

One site was sampled each day, with active sampling completed by teams during the morning. Only representative invertebrate samples were kept for identification in order to minimise the effect of sampling on the invertebrate populations. The supervising scientist determined these representative samples. These retained invertebrates were frozen or placed in killing jars containing ethyl acetate before being preserved in 70% ethanol or pinned. Invertebrates were sorted and recorded according to broad groupings at the field station by the volunteers together with researchers. Samples were then further sorted in the laboratory into the relevant taxon. Expert taxonomists did the species identification, and reference collections were developed for future use.

3.3.4.1 Epigaeic invertebrates

Epigaeic (ground-dwelling) invertebrates were sampled using three different active searching methods: plot, quadrat and random searches. Active searching targeted Scarabaeinae, Blattodea, Isoptera, Scorpionida, Mollusca, Diplopoda, Chilopoda and Annelida.

One hour timed searches of a 20m x 20m plot were carried out by three people (two volunteers and one experienced researcher), each person searching for 20 minutes. Measuring tapes were pegged out to act as plot boundaries. The plots were chosen to include a range of microhabitats within the vegetation type.

Two 2m x 10m quadrats divided into five 2m x 2m blocks were set up to cover a range of microhabitats such as rotting logs, leaf litter, the base of trees and more open areas. One individual (either a volunteer or researcher) thoroughly searched each block and no time limit was set.

3.3.4.2 Flying and plant-dwelling invertebrates

Flying and plant-dwelling invertebrates from the following taxa: Lepidoptera, Apoidea, Diptera, Neuroptera, Odonata and Orthoptera were sampled using transect walks. Two 50m transect walks were performed in different areas of the sampling site. A 50m tape was laid out in a straight line at a location that was representative of the vegetation type and covered a variety of microhabitats. Each transect walk was carried out by three people observing five metres either side of the transect line, walking parallel lines and keeping pace with each other. The distance along the transect at which the target invertebrates were observed was recorded. One researcher and two volunteers sampled each transect.

The volunteers also assisted in the setting and collection of fruit baited traps, colour pan traps, sweep netting and collection of leaf litter to sample the remaining taxonomic groups. Passive sampling however, is not assessed in this study, as, unlike active sampling, it is not influenced by the sampler.

3.3.5 Post-sampling Tasks

The same pre-sampling questionnaire was completed at the end of the expedition to assess changes in volunteers' responses.

Each supervising researcher assessed each volunteer at the end of the sampling trip. The assessments were based on the principles described by Newman et al. (2003). A subjective scale of 0 to 5 was used to score each of the volunteers on the following (on this scale an experienced, professional researcher working with this method would score 5/5): (i) ability of the volunteer to understand principles of the task, (ii) execution of the task correctly and efficiently, (iii) ability to work reliably without supervision, (iv) attention to information and directions given by the supervisor, (v) fitness (scored from 0=lacking the physical stamina to carry out five days of light fieldwork, to 5=comfortably able) and (vi) enthusiasm to complete the task.

Volunteers were informed beforehand of the general principles of the study, but they were not aware of the scoring criteria of the assessment.

3.3.6 Analysis

SPSS version 13.0, Primer version 5.2 and EstimateS version 7.5.0 were used for the analyses. All assumptions of the analyses were tested and the Bonferroni adjustment was used to avoid Type 1 error where applicable.

3.3.6.1 Volunteer efficiency

Researchers and volunteers were directly comparable as two volunteers worked simultaneously with an experienced researcher on two 20m x 20m plots for a total of 40 minutes each, and for each 50m transect. Chilopoda, Diplopoda, Blattodea and Mollusca were examined separately and as a combined group were termed 'epigaeic invertebrates'.

Species accumulation rates were calculated for each individual at each sampling event for plots. This rate was calculated by using the total number of species sampled across the two plots and then dividing by 40 minutes to give a rate. Graphs were then constructed using minutes as the measure on the x-axis and species density on the y-axis for (i) each volunteer (ii) the two volunteers working on each plot for the 'epigaeic invertebrate' group only (iii) the researcher. The efforts of the two volunteers working on each plot were combined to identify if any differences were present when two volunteers were used as opposed to one, as the number of volunteers available is greater than that of researchers.

Quadrats were also assessed, although quadrats covered a variety of microhabitats, the repetition across microhabitats avoided variation. Species accumulation rates for each 2m x 2m quadrat sampled were calculated by using the total number of species in the 2m x 2m quadrat and then dividing by four to give an accumulation rate per unit area for each volunteer and each researcher.

Transects were used to compare volunteers' and researchers' sampling of flying insects. Diptera, Lepidoptera, and Orthoptera were assessed individually and combined (termed 'flying insects'). Rates of species accumulation for transects were calculated by using metres as the measure on the x-axis. The species accumulation rate was calculated by using Primer, and permutating 999 times the number of species sampled over 100m for (i) each volunteer (ii) the two volunteers working on each plot for the epigaeic invertebrate group only (iii) the researcher.

The mean rate of species accumulation was calculated for each method to enable a comparison to be made between volunteers and researchers, and the efforts of two volunteers and a researcher (plot and transects only).

Analysis of Variance (ANOVA) was used to compare rates of species accumulation between volunteers and researchers to determine whether volunteers were as efficient as researchers. The mean species accumulation rate of two volunteers who worked on the same plot was compared to that of the researcher to ascertain whether two volunteers were as efficient as a single researcher.

3.3.6.2 Examination of time benefits

To determine the extent to which the use of volunteers increases the overall efficiency of invertebrate surveys time benefits were examined by calculating the number of hours, days and years required to complete one sampling site using a team of three people, the minimum requirement to complete the

sampling protocol. To ensure the study was comparable, it was necessary to complete the sampling between 06h00 and 13h00, and therefore the number of hours to complete the site was divided by seven hours to give the number of sampling days required to sample one site. This was then multiplied by the number of sampling events to give an estimate of the number of sampling days required to complete this survey, and then the number of years in which the survey would need to span to ensure the same temporal (month) period was assessed.

3.3.6.3 Volunteer effectiveness

To assess whether the survey as a whole successfully sampled the target taxa, an individual-based species accumulation curve was calculated in EstimateS Version 7.5.0 for all invertebrates using all data from the survey.

To assess the effectiveness of volunteers compared with researchers, the species density and number of unique species sampled in each plot and transect were calculated for each individual. Species density is defined as being the number of species per specified collection area or unit (Magurran 2004). For this study species density is defined as the number of species sampled at one sampling event using a defined sampling effort. Unique species were defined as being species which were sampled by only one person at each site. The species density and unique species values across all sites for volunteers and researchers were compared using the Mann-Whitney U analysis because data were non-parametric.

Data from quadrats were also analysed, in which the species density sampled by each quadrat by each individual was calculated. Differences between the species densities sampled were assessed using the Mann-Whitney U analysis.

3.3.6.4 Species assemblage comparison

In order to determine whether volunteers sample different species assemblages in comparison to trained researchers, similarity matrices were constructed using Bray-Curtis similarity measures based on presence–absence, which has the effect of giving potentially equal weights to all species, whether rare or abundant (Clarke & Warwick 2001). These similarity matrices were used to carry out an analysis of similarity (ANOSIM) to compare differences in the species assemblages sampled by volunteers and trained researchers. ANOSIM is a non-parametric permutation procedure, applied to the (rank) similarity matrix underlying the ordination or classification of samples (Clarke & Warwick 2001). The *R*-value gives an absolute measure of how separated the groups are: $R>0.75$ groups are well separated, $R>0.5$ groups are overlapping but clearly different and $R<0.25$ groups are barely separable (Clarke & Gorley 2001). Species which were sampled at a site by all three individuals using the method were removed, this removal emphasised the differences in the species assemblages. Transect and plot data were used for this study because the researcher and volunteers worked simultaneously in a set area, and therefore data were directly comparable.

3.3.6.5 Rare species assessment

Rare species were defined as being species occurring in the first quartile of the frequency distribution of species abundances (Gaston 1994). Rare species were identified using only plot and transect data. To calculate the number of species comprising a quartile the total number of species sampled for each taxon was divided by four. The species abundances were then ranked and the species with the lowest abundances comprising the first quartile, were selected as rare. The number of rare species sampled by each individual at each site was calculated and the two groups (volunteers and researchers) were analysed using the non-parametric Mann-Whitney U test.

3.3.6.6 Volunteer assessment

To assess factors which influence the volunteers sampling performance and their usefulness to researchers in the field a number of volunteer variables were examined.

Spearman Rank correlations (data were non-parametric) were used to assess correlations between predictor variables and i) the researchers' perceived usefulness of the volunteers in the field and ii) the effectiveness of volunteers in sampling epigaeic invertebrates using plot and quadrats and sampling flying insects using transects. A mean score of the researchers' assessment of the volunteers' determined usefulness, and the mean number of species sampled at a site using each method determined the effectiveness of volunteers.

The predictor variables used were: age (7 categories), experience (3 categories), physical fitness determined by a self assessment on the Earthwatch application form (5 categories), and enthusiasm (8 categories) determined by the cumulative score of pre-sampling questions numbers 2, 7 and 8. The experience profile was constructed using a rating system of 0 to 2, based on knowledge of scientific methods, ecological assessments and invertebrate sampling experience. A score of 0 identified an individual with no previous experience or knowledge, 1 identified an individual with either a sound or working knowledge of scientific method determined by current employment or education history, a score of 2 identified a trained researcher of invertebrates.

The Kruskal-Wallis test was then applied to explore whether the predictor variables had a significant effect on the researchers' perception of the usefulness of volunteers in the field, and the sampling effectiveness of volunteers for each method.

3.3.6.7 Changes in perception

Qualitative analysis of the questionnaires was made to identify positive aspects of using volunteers in conservation biology work and to assess changes in the perceptions volunteers had of invertebrates, and the role this change plays in conservation. Data were obtained from Earthwatch regarding the post-expedition projects in which the corporate-funded volunteers participated in. These data were qualitatively assessed.

3.4 RESULTS

In its entirety, this project sampled 50 558 individuals from 797 invertebrate species. The cumulative time the survey covered was 11 weeks, and an extensive database consisting of 33 257 records now exists. Plots sampled 3 987 individuals from 88 species, quadrats sampled 11 695 individuals from 119 species and transects sampled 572 individuals from 92 species.

3.4.1 Efficiency of Volunteers Sampling Invertebrates

Significant differences in the rate of species accumulation (using the Bonferroni adjustment P=0.01) were identified between researchers (n_{res}) and volunteers (n_{vol}) in the sampling of Diplopoda (n_{res}=1880, n_{vol}=2800, $F_{1, 4679}$=79.79, P<0.001), Blattodea (n_{res}=1880, n_{vol}=2800, $F_{1, 4679}$=309.89, P<0.001), Mollusca (n_{res}=1880, n_{vol}=2800, $F_{1, 4679}$=253.73, P=0.01), epigaeic invertebrates (n_{res}=1880, n_{vol}=2800, $F_{1, 4679}$=325.97, P<0.001) and for the combined efforts of two volunteers (n_{2xvol}) in the sampling of epigaeic invertebrates (n_{res}=1900, n_{2xvol}=1900, $F_{1, 3879}$=68.97, P<0.001) for plot sampling (Fig. 3.2). For each taxon, the rate of accumulation was greater for the researchers (Fig. 3.2). There was no significant difference between researchers and volunteers in the rate of species accumulation for Chilopoda.

Significant differences in the rate of species accumulation (using the Bonferroni adjustment P=0.02) were identified between researchers and volunteers in the sampling of Diptera (n_{res}=1600, n_{vol}=3200, $F_{1, 4679}$=43.51, P<0.001), Lepidoptera (n_{res}=1600, n_{vol}=3200, $F_{1, 4679}$=39.81, P<0.001) and flying insects (n_{res}=1600, n_{vol}=3200, $F_{1, 4679}$=28.39, P<0.001) in transect sampling (Fig. 3.3). For each taxon, the rate of accumulation was greater for the researchers (Fig. 3.3). The assessment of the combined efforts of two volunteers (n_{2xvol}) in the sampling of flying insects showed a significant difference from the researchers (n_{res}=1600, n_{vol}=1600, $F_{1, 3199}$=68.97, P<0.001), in which the rate of accumulation was greater for the volunteers (Fig. 3.3). There was no significant difference between researchers and volunteers in the rate of species accumulation for Orthoptera. There were no significant differences between the rate of species accumulation between research and volunteers for taxa sampled using quadrats (Fig. 3.4a).

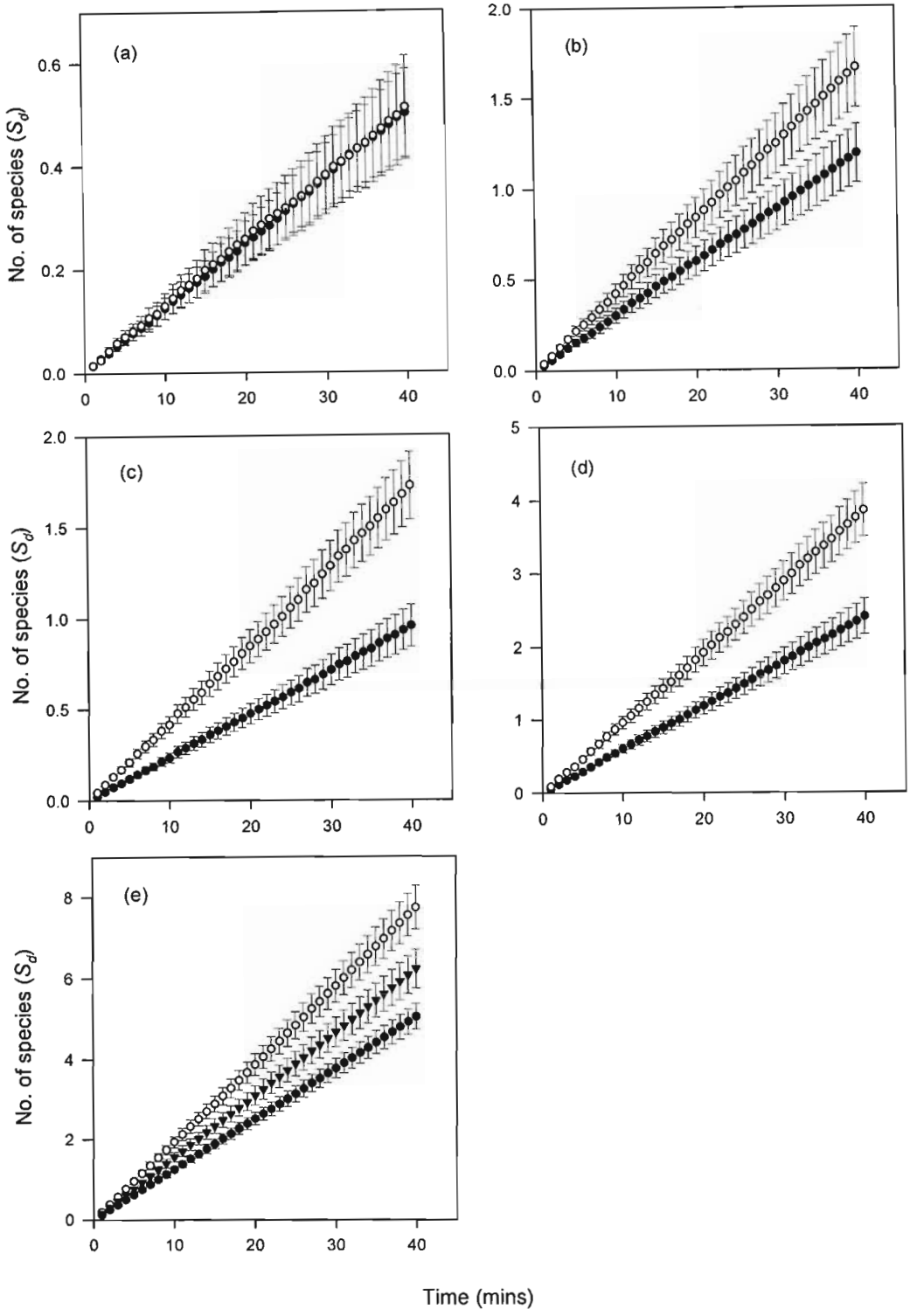


Figure 3.2 Comparison of rates of species accumulation using the plot method for researchers (O),volunteers (●) and the combined efforts of two volunteers (▼) for Chilopoda **(a)**, Diplopoda **(b)**, Blattodea **(c)**, Mollusca **(d)** and Epigaeic invertebrates **(e)**. Error bars represent standard error.

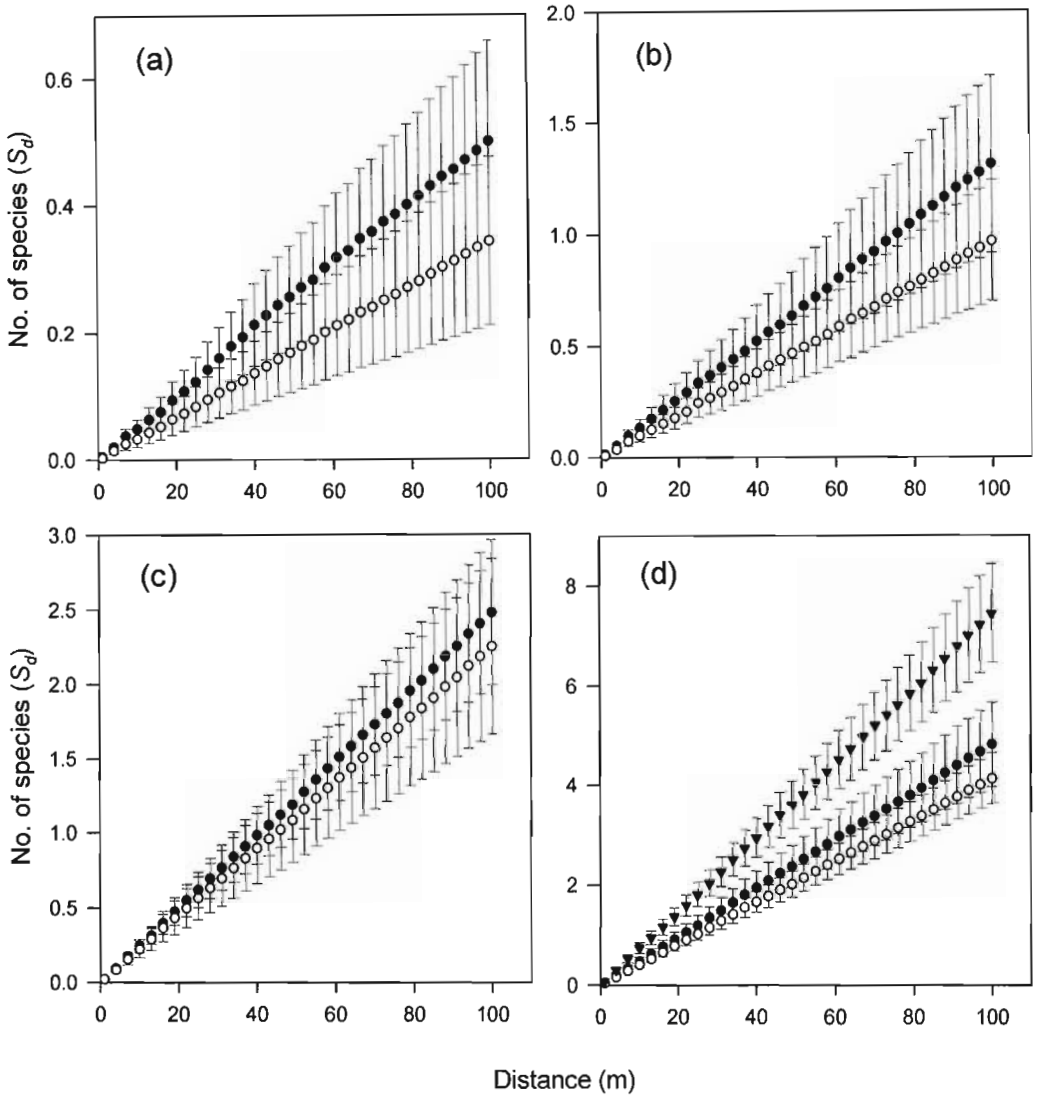


Figure 3.3 Comparison of rates of species accumulation using the transect method for researchers (o),volunteers (•) and the combined efforts of two volunteers (▾) for Diptera **(a)**, Lepidoptera **(b)**, Orthoptera **(c)** and Flying insects **(d)**. Error bars represent standard error.

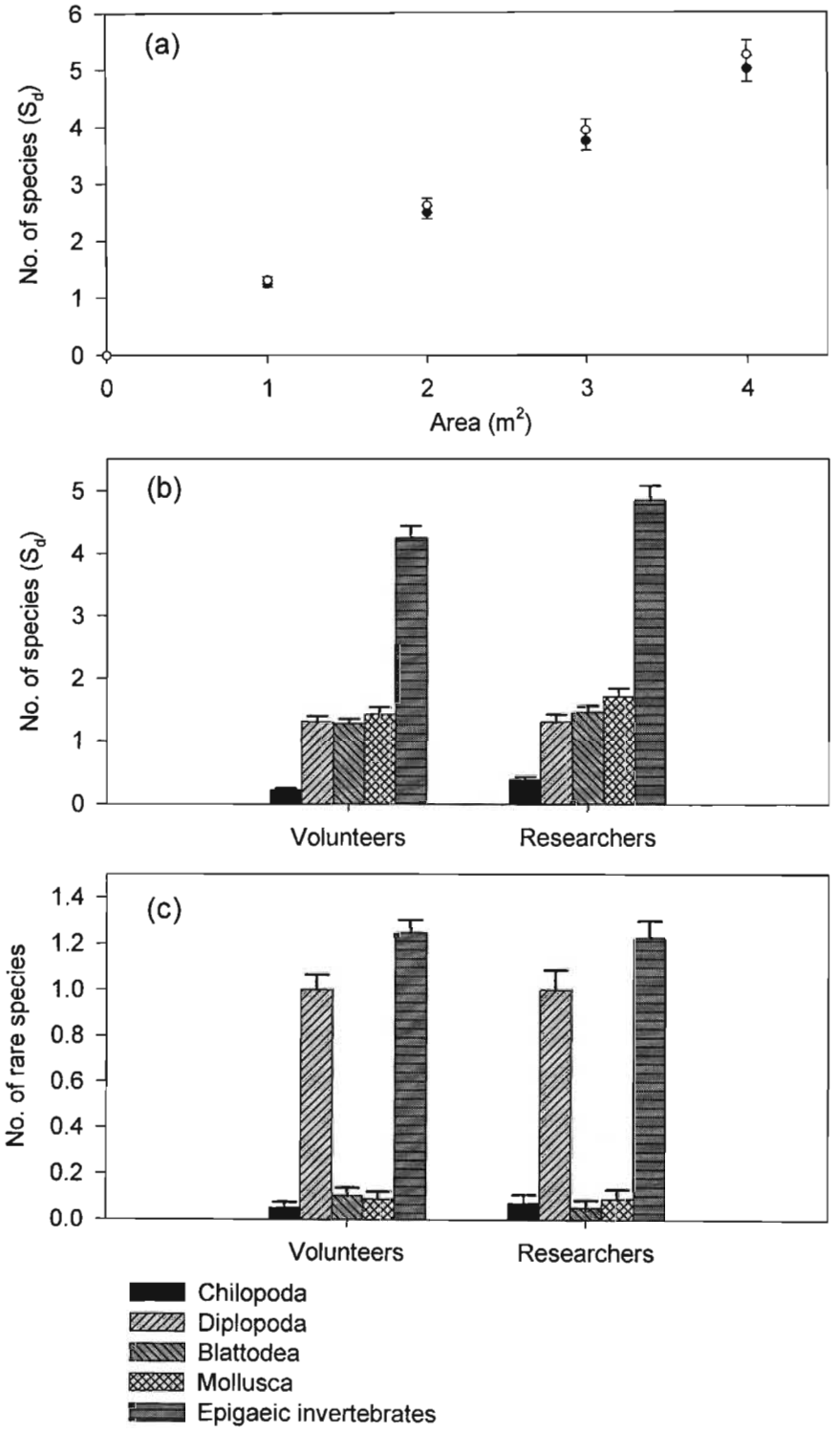


Figure 3.4 Comparison of sampling efficiency and effectiveness between volunteers and researchers using the quadrat method. Comparison of sampling rates of epigaeic invertebrates for researchers (O), volunteers (●) **(a)**, mean species density **(b)** and the mean number of rare species sampled **(c)**. Error bars represent standard error.

3.4.2 Examination of Time Costs

Eleven people working simultaneously in the field, required approximately seven hours to complete a site. This study completed 77 sampling events over a three-year period, sampling only for two weeks each in November, January and March, which totals of 77 sampling days. A research team of three people (minimum requirement for sampling protocol) would have to spend 282 days in the field to complete an equivalent survey, and if carried out in the same temporal window, the survey would have to be carried out over 11 years.

3.4.3 Effectiveness of Volunteer Sampling of Invertebrates

The individual-based species accumulation curve generated from all data collected on the project shows the curve approaching an asymptote (Fig. 3.5). This demonstrates that using volunteers to sample invertebrates resulted in a high level of sampling completeness for the target taxa.

There were no significant differences in the species densities sampled using quadrats between researchers (n_{res}) and volunteers (n_{vol}) for all taxa sampled (Fig. 3.4b). There was a significant difference in the species density sampled between researchers and volunteer groups for Blattodea (Z=-3.789, n_{res}=49, n_{vol}=74, P<0.001), Mollusca (Z=-3.255, n_{res}=49, n_{vol}=74, P=0.01) and epigaeic invertebrates (Z=-4265, n_{res}=49, n_{vol}=74, P<0.001) sampled using plots (Fig. 3.6a). There were no significant differences in the mean number of species sampled between the two groups for any of the taxa sampled using the transect method. There was no significant difference between the researchers and the efforts of two volunteers for any of the taxa sampled by plots. However, there were significant differences between the researchers (n_{res}) and the efforts of two volunteers (n_{2xvol}) for Diptera (Z=-2.374, n_{res}=16, n_{2xvol}=16, p=0.018), Lepidoptera (Z=-3.620, n_{res}=16, n_{2xvol}=16, P<0.001), Orthoptera (Z=-4.963, n_{res}=16, n_{2xvol}=16, P<0.001) and flying insects (Z=-5.554, n_{res}=16, n_{2xvol}=16, p<0.001) sampled using transects (Fig. 3.6b).

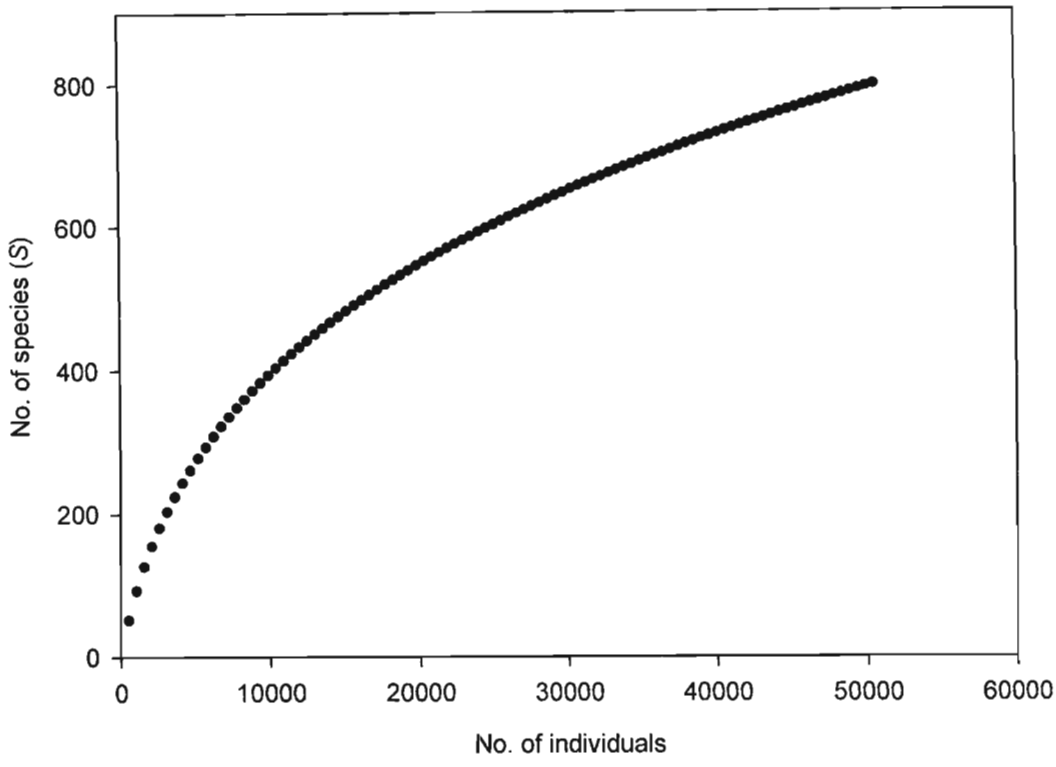


Figure 3.5 Individual-based species accumulation curves computed from all data obtained from the project.

3.4.4 Unique Species Assessment

The analysis of the mean number of unique species of Diplopoda (Z=-3.694, n_{res}=49, n_{vol}=74, P<0.001), Blattodea (Z=-3.816, n_{res}=49, n_{vol}=74, P<0.001), Mollusca (Z=-5.906, n_{res}=49, n_{vol}=74, P<0.001) and epigaeic invertebrates (Z=-6.514, n_{res}=49, n_{vol}=74, P<0.001) (Fig. 3.6c) showed significant differences between the researchers and volunteers. However, there were no significant differences between the researchers and volunteers for any of the taxa sampled by transects. There were no significant differences in the number of unique species between the researchers and the efforts of two volunteers for taxa sampled by plots (Fig. 3.6d). The analysis for the transect method showed significant differences in the number of unique species between the researchers and the efforts of two volunteers for Orthoptera (Z=-2.368, n_{res}=16, n_{2xvol}=16, P=0.018) and flying insects (Z=-2.257, n_{res}=16, n_{2xvol}=16, P=0.024) (Fig. 3.6d).

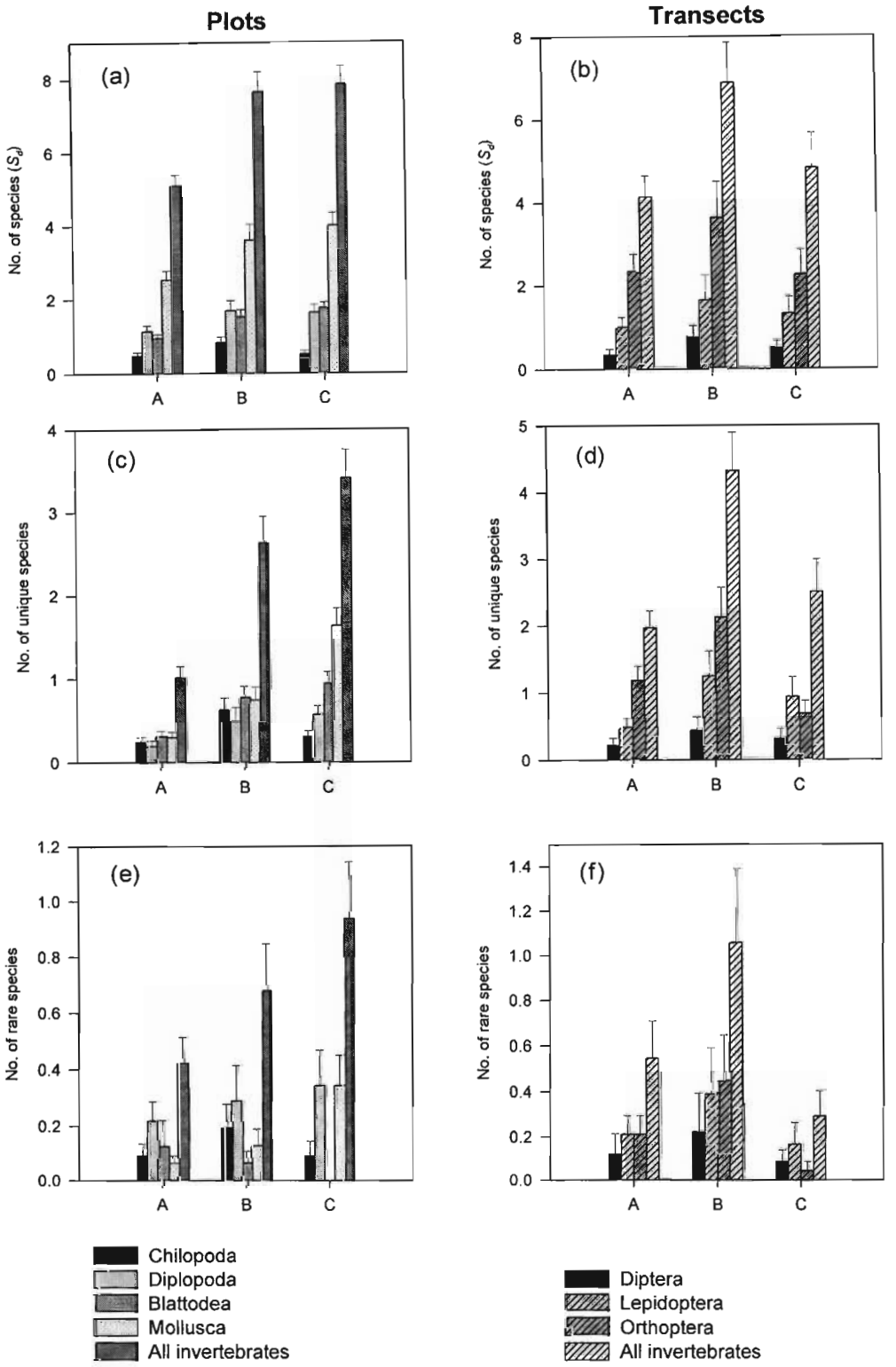


Figure 3.6 Comparison of sampling effectiveness of groups using the plot and transect methods. Mean species density **(a, b)**, mean number of unique species **(c, d)** and mean number of rare species **(e, f)** for each sampling group: volunteers (A), two volunteers (B) and researchers (C). Error bars represent standard error.

3.4.5 Rare Species Assessment

No significant differences were observed in the number of rare species sampled by researchers and volunteers using the quadrat method (Fig 3.4c). There was a significant difference in the mean number of rare species sampled by volunteers and researchers using the plot method for Mollusca (Z=-2.986, n_{res}=32, n_{vol}=64, P=0.003) and epigaeic invertebrates (Z=-2.315, n_{res}=32, n_{vol}=64, P=0.021), with researchers sampling a greater number of Mollusca and epigaeic invertebrates (Fig. 3.6e) than did volunteers. There was no significant difference between the two groups for taxa sampled using the transect method (Fig. 3.6f), and no significant differences in the number of rare species sampled by researchers and the combined efforts of two volunteers using the transect method or plots (Fig. 3.6e, f).

3.4.6 Species Assemblage Assessment

The ANOSIM produced significant R-values for the sampling of three taxa by researchers and volunteers, Diplopoda (R=0.1, P=0.007) and Blattodea (R=0.078, P=0.008) using plots and Diptera (R=0.095, P=0.046) using transects. These values indicated that species sampled by the volunteers and by the researchers were barely separable. No other significant R-values were identified between the species assemblages sampled for the remaining taxa by the two groups.

3.4.7 Volunteer Assessment

Correlations were identified between the perceived usefulness score given by the researchers and experience of volunteers (ρ=0.332, n=54, P=0.014), and between the effectiveness of volunteers using the transect method and their experience (ρ=0.617, n=24, P=0.004).

The analysis of the factors affecting the perceived usefulness of volunteers showed that experience (n=63, X^2_2=8.277, P=0.016) was a significant factor. Furthermore, experience was shown to have a significant effect on the volunteers' effectiveness in sampling using the transect method (n=20, X^2_2=7.434, P=0.024). Experienced people were perceived by the researchers to be more useful in the field, and perceived to be more effective at sampling flying insects (Fig. 3.7).

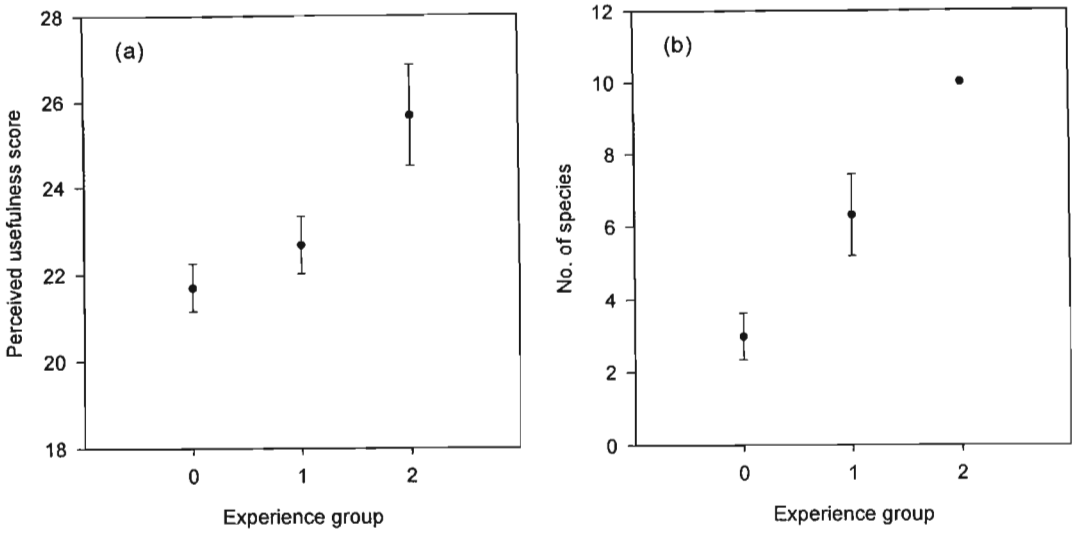


Figure 3.7 Relation between experience and perceived usefulness **(a)**, and transect effectiveness and experience **(b)**.

3.4.8 Changes in Volunteer Perceptions

3.4.8.1 Analysis of questionnaires

Most of the participants in the project were already environmentally aware, with all 54 volunteers having, before the sampling, a strong sense of responsibility towards protecting the environment (Table 3.1). All volunteers both pre- and post-sampling would like to participate in other ecological research surveys, although logistical restraints restricted the possibility of this (Table 3.1). Participants agreed that volunteers made a significant contribution to ecological studies, with the strength of this agreement increasing post-sampling (Table 3.1). The understanding of the term 'biodiversity' and perhaps an understanding of its complexity increased over the duration of the sampling trip; by the end of the expedition all volunteers had a complete, or almost complete, understanding of the term (Table 3.1). The changes observed in the answers to question six demonstrated that volunteers gained knowledge as to where scientific research is published (Table 3.1).

The changes in the answers to questions numbers two, seven and eight demonstrated that post-sampling all volunteers had gained an active interest in invertebrates and all would investigate invertebrates and actively conserve them in the future (Table 3.1). All volunteers changed their perception of invertebrates, with 48 volunteers stating that they had positive perceptions and six that they had raised their perceptions from negative to tolerant (Table 3.1). All volunteers, both pre- and post-sampling, rated invertebrates as being highly important at a global scale (Table 3.1). Most importantly in terms of conservation, all volunteers stated that they would share their experiences and knowledge with friends and family on their return home (Table 3.1).

Table 3.1 The questionnaire which volunteers were asked to complete before and after the expedition. The mean volunteer score for each question is shown; parentheses show the percentage of the maximum score.

	Question	Total Score	Mean pre-sampling score	Mean post-sampling score	% change in score
Q 1	Please assess the extent to which you feel a responsibility towards protecting the environment *Very strongly (4), strongly (3), to a limited extent (2), hardly at all (1), not at all (0)*	4	3.6 (90)	3.8 (95)	5
Q 2	When you go into the countryside, do you / will you investigate the invertebrates living here? *Always (3), sometimes (2), occasionally (1), never (0)*	3	1.9 (63)	2.8 (94)	31
Q 3	Do you think you are likely to join another ecological research survey in the future? *Certainly (3), probably (2), unlikely (1), certainly not (0)*	3	2.6 (87)	2.8 (92)	5
Q 4	In your opinion which of the following best fits the statement that volunteers can make a significant contribution to ecological studies. *Strongly agree (3), agree (2), disagree (1), strongly disagree (0)*	3	2.5 (83)	2.8 (92)	9
Q 5	How well do you understand the term biodiversity? *Completely (4), almost completely (3), partly (2), vaguely (1), not at all (0)*	4	2.7 (68)	3.3 (82)	14
Q 6	List five scientific journals in which research on biodiversity might be published *(score 1 for each journal named)*	5	1.9 (38)	2.4 (47)	9
Q 7	Do you actively conserve invertebrates at home? *Always (2), occasionally (1), never (0)*	2	1 (50)	1.6 (81)	31
Q 8	What is your perception of invertebrates? *Positive (2), tolerate (1), negative (0)*	2	1.5 (75)	1.9 (94)	19
Q 9	On your return do you think you will share you experiences and knowledge with family and friends? *Certainly (3), Probably (2), Unlikely (1), Certainly not (0)*	3	2.9 (97)	3 (99)	2
Q 10	How would you rate the importance of invertebrates on a global scale? *0 being of no importance,5 being of critical importance (score corresponded to value stated)*	5	4.5 (90)	4.8 (95)	5

3.4.9 Post-expedition Activities

Twenty-one volunteers were funded by corporate business. These volunteers were required by their funding companies to set up a small conservation project on their return. Examples of projects included:

i) The designing of 'communication kits' to relay scientific research on local threatened species to raise awareness of the general public about the research and conservation issues
ii) Assisting with the development of museum displays to educate the public about invertebrates living in local tree species
iii) Assisting with the development of a bird hide in a local nature reserve
iv) Developing a restoration project of a land patch invaded by alien plants
v) Developing environmental awareness in the work place and planting schemes to benefit biodiversity

3.5 DISCUSSION

Surveys of invertebrates are rarely considered to be cost effective (Oliver & Beattie 1996), but even limited sampling of invertebrates can yield an enormous number of specimens and an immense array of species (Ward & Larivière 2004). Time costs associated with surveys of invertebrates are major constraints for research. Costs can be reduced and substantial amounts of data can be collected in relatively short periods of time by involving volunteers in invertebrate surveys, which enables extensive surveys of invertebrates to be carried out within a temporal window. Volunteers significantly reduce time costs associated with biodiversity surveys; this is consistent with the findings of all assessments of volunteers mentioned in this paper.

However, volunteers were not as efficient as researchers. This may be explained by the time it takes for a volunteer to physically catch and bottle specimens. Volunteers who were less experienced struggled with the removal of a specimen from the net and the capture of epigaeic invertebrates. This reduced volunteers' searching time and therefore reduced their species accumulation rate for plots. Nevertheless, species accumulation rates were not significantly different for the transect method, in which, the three people walking the transects had to walk together in a line, and had to wait for each other before walking forward, making it likely that each individual was actively sampling for the same period of time regardless of difficulties in removing specimens from the net. This was not the case in the plot sampling where individuals worked independently and time spent pursuing and capturing a specimen would reduce their total searching time.

It is important to be able to identify whether data collected by inexperienced volunteers are comparable to those collected by experienced researchers. Differences between researchers and volunteers in the mean number of species and unique species sampled using the plot method were negligible when the efforts of two volunteers were combined. This demonstrates that for certain methods volunteers are as effective at

sampling invertebrates as experienced researchers and some methods are unsuitable or need to be adjusted to suit the volunteers' experience.

Volunteers and researchers were sampling the same species assemblages. Furthermore, the analysis of the species density and number of unique species indicated that they were sampling invertebrates from the same assemblage and not a subset of the population sampled by the researchers. The assessment of rare species demonstrated that volunteers were not biased towards common, abundant or obvious species, with the exception of Mollusca. This is likely to be explained by the presence of an expert malacologist who led groups working on the plots. This method enables the researcher greater freedom to sample microhabitats likely to contain different species, and his experience is reflected in the number of rare Mollusca species sampled.

The differences observed between researchers and volunteers sampling of rare species along transects is likely to be a result sampling incompleteness, furthermore the sample size of this group was low. Nevertheless, this highlights the potential problem that specialist scientists may focus their efforts on a particular taxa or species and therefore sampling may be biased. Volunteers lack this bias and therefore sampling may be more balanced.

In order to carry out a comprehensive invertebrate survey, it is necessary for all members of the survey group to work together as a team to complete the required tasks. An ability of volunteers to understand concepts of the scientific method such as the necessity for repeatability when carrying out a sampling method, makes it easier for the volunteer to perform a task effectively, and therefore increases their perceived usefulness to the researcher directing the team. Furthermore, previous experience increases the effectiveness of the volunteer for certain methods that require technical skill e.g. the use of sweep nets to catch flying or fast moving invertebrates.

The collection of scientific data is by no means the sole reason for engaging volunteers in research (Foster-Smith & Evans 2003). Other benefits include broadening their horizons through meeting different people and experiencing different ecosystems, and providing opportunities for them to help solve environmental problems (Gilmour & Saunders 1995). This survey of invertebrate biodiversity provided a unique educational experience for the participating volunteers. Volunteers, whilst carrying out a survey alongside experienced researchers, gained knowledge and understanding about the complexity of biodiversity and the issues associated with effective conservation. It is shown that volunteers' enthusiasm for investigating biodiversity increases because of their participation in the project, and all parties felt that they would share their experiences with family, friends and local communities. The relaying of experiences is one of the most influential methods of spreading an appreciation and understanding of the importance of biodiversity and the complexity of the issues associated with conservation. The majority of volunteers participating in this survey changed their attitudes towards invertebrates from negative to positive, and all volunteers were keen to relay their experiences to family, friends and their communities. This is further evidence of the benefits of using volunteers for invertebrate surveys, because it raises

awareness of invertebrates, and spreads a conservation message.

Newman et al. (2003) reported that after volunteering a minimum of 30% of volunteers have joined conservation groups. Thus it seems that, as well as providing volunteers with skills necessary to contribute to wildlife conservation, it is also possible to foster their enthusiasm and encourage them to put these skills to good use (Newman et al. 2003). As Foster-Smith and Evans (2003) indicated there are major educational and social benefits to be derived from the involvement of volunteers in scientific projects. This is demonstrated by some of the post-expedition projects which have been developed as a result of the volunteers' experience on this project.

3.5.1 Recommendations and Guidelines

The experience volunteers gain in the field is extremely important. It is imperative to remember that a volunteer (dictionary definition: a person who freely offers to do something), is participating in the survey of his or her own free will. It is important to design those studies involving volunteers so that the tasks are realistic and achievable (Foster-Smith & Evans 2003), and to provide encouragement and offer gratitude to the volunteers for the work they do. It is essential that the volunteers know that guidance is available at all times. Frequently volunteers required reassurance about the species and abundance they were collecting, because volunteers became easily disheartened when a species-poor area was being surveyed. The use of the identification (Appendix 3.1) and methodology cards (Appendix 3.2) in the field gave the volunteers a brief reminder of the target taxa and their characteristics. Each group of volunteers was supervised at all times by experienced field researchers and taxonomic experts. Researchers were available to assist and answer questions regarding the research, and for background information about the project, study region and invertebrates in general.

The sampling methods or tasks that volunteers are asked to complete must be easily understood. Language barriers must be considered when developing a task, and often a compromise must be reached between strict scientific protocol and productive sampling using volunteers. In this survey volunteer groups were sub-divided in the field into three smaller groups. In order to maintain good group dynamics, individuals were rotated in the groups, and the tasks that each group performed were rotated, to ensure each volunteer participated in all tasks and interacted with different members of the group in each task. This ensured that groups benefited from the experience of the different researchers and did not become bored with the tasks or individuals.

The following summarises the key points that must be considered when working with volunteers to carry out surveys of invertebrate biodiversity:

i) Design a sampling protocol which is simple to implement and which considers the difficulties inexperienced volunteers encounter when sampling
ii) Avoid using methods with time restrictions, but if this cannot be avoided then consider the efforts

of two volunteers to be equal to that of a researcher

iii) Provide information on the aims and objectives of the survey

iv) Provide information outlining the principles of scientific method

v) Provide training and focus on the catching and bottling of different invertebrates

vi) Provide cards which the volunteers can use to remind them of the sampling protocol and the key characteristics of the target taxa

vii) Ensure an experienced researcher is working alongside the volunteers who can thus be assisted and provided with information

viii) Maintain good group dynamics and rotate the tasks that volunteers perform

ix) Ensure that the volunteers feel their work is valued and appreciated

3.5.2 Conclusion

This invertebrate study has been successful, and volunteers have assisted in the collection of an extensive data set that has applications in conservation biology, ecology, and systematics research. These findings are concurrent with Mumby et al. (1995), Darwall and Dulvy (1996), Fore et al. (2001), Newman et al. (2003) and Goffredo et al. (2004) who have demonstrated that volunteers can collect data sets that are accurate and reliable. This study shows that volunteers collect valid data, sample invertebrates as effectively as a trained researcher, samples collected are non-biased and there are enormous time benefits to this approach. Volunteers provide a valuable resource to researchers carrying out biodiversity surveys and raises environmental awareness and an appreciation of biodiversity.

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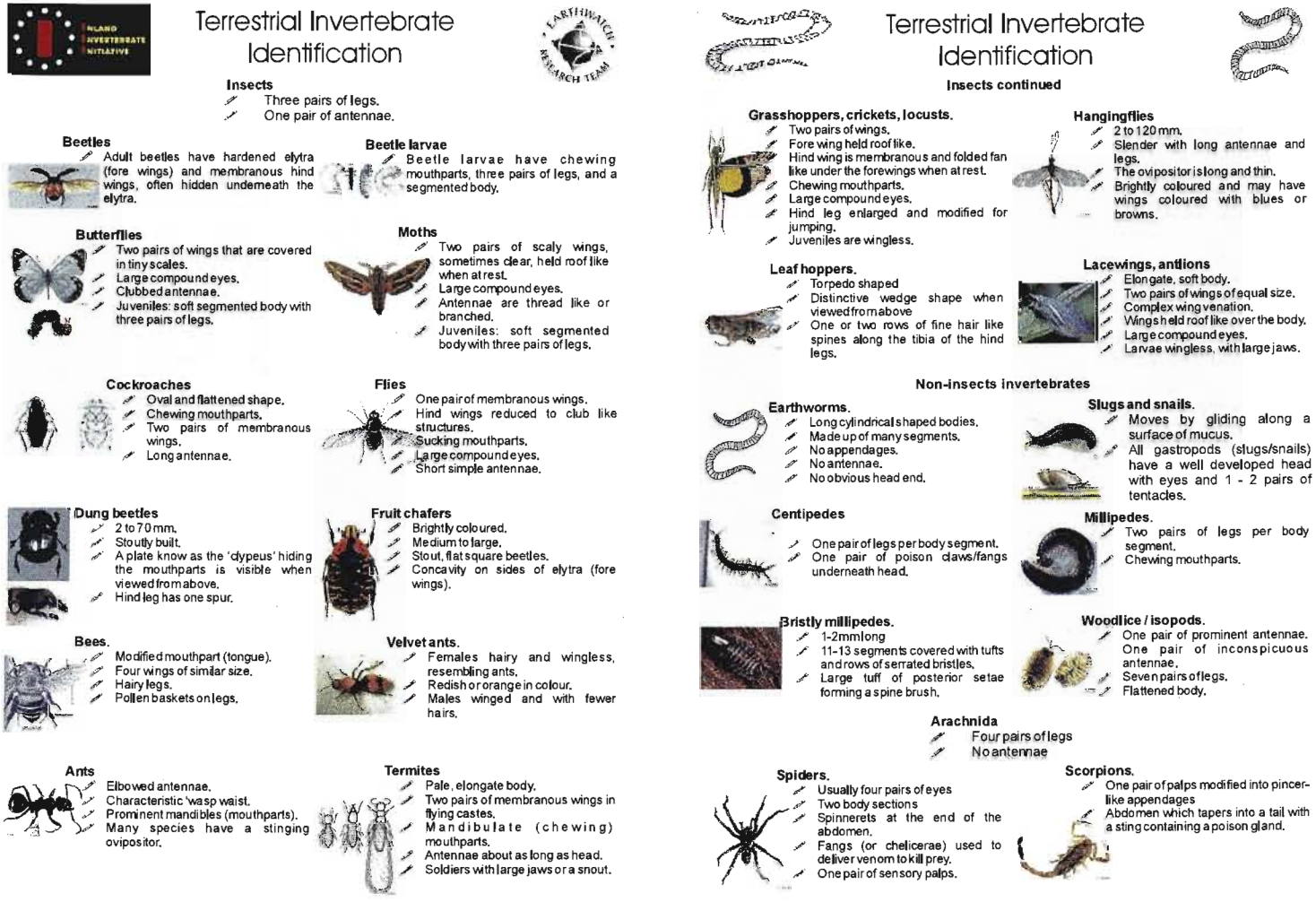


Appendix 3.1 Invertebrate identification guide, available to the volunteers at all times in the field.

Transect Walks

Method

- Two transect walks will be carried out, recording the invertebrates below.
- Each transect is 50 m long, invertebrates will be recorded 5 m either side of the transect line.
- A minium of two people are needed to perform the transect walk.
- The distance on the transect line the invertebrates are observed must be recorded along with the initials of the observer.

Invertebrates to be collected

Hand Collecting Quadrats

Method

- Two 10m x 2m quadrats divided into 5 plots will be throughly searched
- Habitat details for each quadrate plot will be recorded.
- One person will search each plot and samples collected must be labelled with the collectors initials.
- The time taken on each plot must be recorded.
- Each sample must be labelled with site details, date, quadrate number and plot number (Q1.5).

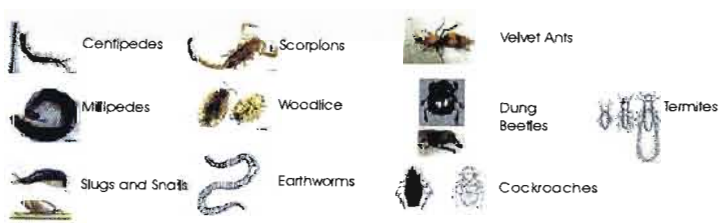
Invertebrates to be collected

Hand Collecting Plots 20m x 20m

Method

- Two 20 x 20 plots will be searched for one hour. Three people will collect for 20 minutes each.
- Each persons samples must be kept separate and labelled with the collectors initials.

Invertebrates to be collected



Tree Beats

Method

- Two sets of tree beats will be carried out at each sampling site. A 1m x 1m tray will be used to collect the invertebrates. This will be placed under the tree / branch which is beaten.
- The tree / branch will be struck 10 times, this will be repeated on 10 different trees, this will make one set. However invertebrates from each tree need to be kept separate.
- Samples taken need to be labelled with the site detail, date, tree beat set number and tree number (TB1.5), the team number and the initials of the beater.

Invertebrates to be collected

Appendix 3.2 Examples of the sampling method guides available to volunteers at all times in the field.

ASSESSMENT OF CONGRUENCY ACROSS INVERTEBRATE TAXA AND TAXONOMIC LEVELS TO IDENTIFY POTENTIAL SURROGATES

4.1 ABSTRACT

Owing to the huge number of invertebrate species, the lack of available data and lack of accessible resources to carry out comprehensive surveys of invertebrates, it is necessary for surrogates to be used to represent invertebrate biodiversity in conservation planning, and to make rapid biodiversity assessments. The selection of surrogates is a popular topic in the literature, but there is no consensus regarding the criteria for the selection of appropriate surrogates. This study investigates the use of species density and species assemblage patterns to identify potential coarse-filter surrogates at a local scale, to assess cross-taxon congruency and congruency across taxonomic levels and activity groups using nine invertebrate taxa: Lepidoptera (butterflies), Hymenoptera (Apoidea), Coleoptera (Cetoniinae), Orthoptera, Blattodea, Araneae (Araneidae, Thomisidae, Oxyopidae), Myriapoda (Diplopoda, Chilopoda) and Mollusca. Furthermore, differences in the observed congruency when using species density and species assemblage patterns were assessed. Although cross-taxon congruencies were observed, the relations were weak and therefore surrogates could not be selected. The use of higher taxa to represent lower taxa shows good potential as a surrogate, but only in species-poor genera or families and only in regions where the biodiversity is well documented. The use of species density and species assemblage patterns to determine congruency and select surrogates produced different results. Furthermore, when selecting surrogates, a ρ-value of greater than 0.75 should be used as an optimal level of congruency; below this value the relation is likely to be weak, and if used as a surrogate, misinterpretation may occur. The lack of congruency between invertebrate taxa supports the use of a multi-taxa approach for the incorporation of invertebrates into conservation plans. When data permits, species assemblage patterns should be used in conjunction with measures of species density for conservation planning, in particular, when an ecosystem consists of diverse habitats and species turnover occurs.

Keywords: Biodiversity survey, conservation planning, rapid biodiversity assessment, species density, species assemblage patterns, coarse-filter surrogates, higher-taxon, cross-taxon, local scale.

4.2 INTRODUCTION

Invertebrates make up at least 95% of all species (Myers et al. 2000), and they occupy almost every terrestrial and freshwater habitat from the poles to the equator. Invertebrates play vital roles in processes such as pollination, soil formation and fertility, plant productivity, organic decomposition and the regulation of populations of other organisms through predation and parasitism (Daily et al. 1997).

Current scientific knowledge of invertebrate species distributions, species assemblage patterns and the processes that influence these are poorly understood (Ward & Larivière 2004). Even in relatively data-rich regions, most entities, particularly at the species and genetic levels, are yet to be discovered, let alone have their distributions surveyed and mapped (Ferrier et al. 2002).

The wide acceptance of invertebrates as being indispensable components of biodiversity has led to a rapid increase in broad-based surveys (i.e. a survey incorporating a wide range of invertebrate taxa) and greater pressure to provide information and guidelines for invertebrate conservation and monitoring (Ward & Larivière 2004). Within the scientific world, it is widely accepted that more biodiversity research is required if conservation efforts are to effectively conserve biodiversity (Brooks et al. 2004). Nevertheless, comprehensive surveys of invertebrates are time-consuming and research is generally poorly funded and lacks the necessary resources and labour. Comparative surveys across areas, sites and taxa help conservation planners by providing the necessary information required to determine surrogates, or features to act as coarse-filters in the conservation planning process. Owing to the huge number of invertebrate species, surrogates must be used to represent invertebrate biodiversity in conservation planning and to make rapid biodiversity assessments.

Surrogacy is a relation between a surrogate or indicator variable and a target variable (Sarkar & Margules 2002). Therefore, a surrogate variable represents the target variable. Taxon surrogates, (e.g. birds or butterflies) can be used represent the biodiversity of other taxa, higher taxa (families or genera) can be used as surrogates for lower taxa (species), also environmental variables (e.g. land classes or soil structure) can be used as surrogates to represent biodiversity at different taxonomic levels (Ferrier 2002). The selection of potential surrogates should be done carefully, there are no universally accepted surrogates, and suitable surrogates in one ecosystem or region may not be suitable in another. One should select surrogates which represent the conservation or survey goals, which are cost-effective, logistically suitable and, most importantly, have good biological efficacy (McGeoch 1998). Once a potential surrogate has been selected, the relation between the surrogate and the target variable needs to be characterised to determine whether a significant congruent relation exists.

Several studies have been published on the selection of surrogates. These studies have looked at a number of biodiversity features and measures at varying spatial scales, using a variety of methods to measure congruency (Wessels et al. 1999; Lombard et al. 2003). Nevertheless, the topic of surrogacy is a topic of scientific debate and research continues to be published on the topic of cross-taxon surrogacy (Lund & Rahbek 2002; Ricketts et al. 2002; Sauberer et al. 2004; Bilton et al. 2006) and higher taxon as surrogates (Baldi 2003; Villasenor et al. 2005; Bertrand et al. 2006; Goldberg et al. 2006).

Many studies on surrogacy have used biodiversity measures such as species richness to identify congruent relations (Panzer & Schwartz 1998; Lawton et al. 1998). Yet, the reduction of data sets to a single value ultimately results in a loss of information, and these values may be irrelevant or misleading with respect to conservation planning (Goldstein 1999). Wessels et al. (1999), Margules et al. (2002) and Williams et

al. (1996) showed that the selection of protected areas based on richness measures leads to overlaps in species assemblages, thus duplicating conservation efforts. However, the use of species assemblages in systematic conservation planning for the prioritisation of protected areas minimises the duplication of conservation efforts (Reyers et al. 2002). Furthermore, many studies related to the selection of surrogates for conservation planning have been on large or coarse scales, for example the assessment by Prendergast et al. (1993) of diversity hotspots across Britain, the investigation by Pearson and Carroll (1999) of species richness patterns of butterflies and birds across western North America, and the assessment by Warman et al. (2004) of cross-taxon distribution patterns across Canada. These coarse scale studies identified such large areas as priorities for conservation that they did not provide sufficiently detailed guidance at the local level, where most conservation decisions are made (Prendergast et al. 1993; Ricketts et al. 2002). Furthermore, a surrogate may show excellent representation at a local scale and yet demonstrate no correlation at a regional or landscape level, or vice versa suggesting that spatial scale influences the efficacy of surrogates (Reyers et al. 2002; Lombard et al. 2003). Therefore, the application at a local scale of surrogates that are determined at a coarse scale is likely to be inappropriate.

Crucial conservation decisions, such as establishing reserves within a local area or identifying critical habitats within established reserves are made at scales much smaller than typically investigated in habitat-prioritisation studies (Ricketts et al. 2002). There is little support for concordance among indicator taxa at local scales (Ricketts et al. 2002), and it remains to be seen whether this short cut for identification of high priority areas is a useful tool for local conservation planning (Grand et al. 2004). Furthermore, studies have demonstrated that the use of surrogates to determine or prioritise protected areas may not be truly representative of patterns in all taxa (Prendergast et al. 1993; Bonn et al. 2002; Sauberer et al. 2004). Furthermore, the correlation of species richness between pairs of taxa has been found to be highly variable both taxonomically and geographically, and indeed better assessments of surrogate taxa may be possible through examination of cross-taxon congruence in community similarity (Su et al. 2004). This has highlighted the need for research at a local scale on species assemblage patterns and on testing the concepts of congruency, indicators and surrogacy.

The present study aimed to identify potential surrogates that represent invertebrate diversity patterns for use in conservation planning and for making rapid biodiversity assessments at a local scale by addressing the following objectives: (i) identify congruency in species density across taxonomic groups, including flying and epigaeic invertebrate groups and taxonomic levels to identify potential surrogates for conservation planning and for surveys of invertebrates, (ii) identify congruency in species assemblage patterns across taxonomic groups, habitat groups and taxonomic levels to identify potential surrogates for conservation planning and for surveys of invertebrates, (iii) compare the potential surrogates identified, using species density and species assemblage patterns to identify differences between the approaches and (iv) test the effect of the higher (family, genus) to lower (species) taxon ratio on the strength of the congruency across the taxonomic levels to determine a maximum number of species to higher taxon ratio for surrogates to be effective.

4.3 METHODS

4.3.1 Study Site

Fieldwork was carried out in the Mkhuze Game Reserve (27.67°S 32.27°E), Phinda Private Game Reserve (27.78°S 32.35°E) and False Bay Park (27.94°S 32.38°E) in north-eastern KwaZulu-Natal, South Africa. These reserves are situated in the diverse region known as the Maputaland Centre, which consists of a mosaic of mainly extensive savanna communities arranged in complex patterns.

Sites were chosen from vegetation data and geology maps to represent a range of vegetation and soil types. Sampling sites were 1ha plots of uniform vegetation types. For this study, 39 different sites were sampled between November 2002 and March 2005 (summer months), 16 of these were re-sampled in different months and over different years during the summer season, giving a total of 66 sampling events.

4.3.2 Sampling, Sample Processing and Species Identification

A range of replicated sampling methods was carried out to sample the following nine invertebrate taxonomic groups: Lepidoptera (butterflies), Hymenoptera (Apoidea), Coleoptera (Cetoniinae), Orthoptera, Blattodea, Araneae (Araneidae, Thomisidae, Oxyopidae), Myriapoda (Diplopoda, Chilopoda classes) and Mollusca. Only representative invertebrate samples were kept for identification in order to minimise the effect of sampling on invertebrate populations. Expert taxonomists carried out species identifications, and in a few instances, where species names could not be determined, species numbers were used. Blattodea were identified to morpho-species, as no taxonomic expert was available. Reference collections are currently housed in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Two activity groups of taxa were assessed, those defined as epigaeic (ground-dwelling) and as flying insects. Epigaeic invertebrates consisted of Blattodea, Mollusca, Chilopoda and Diplopoda. Flying insects consisted of Apoidea, Lepidoptera and Cetoniinae. Two taxonomic levels were used, 'higher taxa' is defined as being genus or family level and 'lower taxa' as species level.

4.3.2.1 Epigaeic invertebrates

Active searching for epigaeic invertebrates targeted Mollusca, Blattodea, Diplopoda and Chilopoda. Leaf litter samples were taken to sample micro-molluscs.

Three people who each searched for 20 minutes, giving a total of one hour search time per plot, carried out searches of a 20m x 20m plot. The plots were chosen to include a range of microhabitats within the vegetation type. Two 20m x 20m plots were sampled, each in a separate region of the 1ha sampling site.

A 10m x 2m quadrat, divided into five 2m x 2m plots, was thoroughly searched. An individual searched one plot using a trowel to sift through the leaf litter, soil and logs to a depth of approximately 15cm. No

time limit was set for the completion of the search in each 2m x 2m plot. The quadrats were chosen to include a range of microhabitats within the vegetation type. Two quadrats were sampled in different areas, covering a range of microhabitats within the sampling site.

Four leaf litter samples of a standardised 5-litre volume were collected from each site to sample micro-molluscs. Leaf litter was taken from a range of microhabitats within the areas being searched.

In addition to the above sampling methods, Mollusca and Diplopoda were sampled by tree beating.

4.3.2.2 Flying and plant-dwelling invertebrates

Flying and plant-dwelling invertebrates Araneae, Orthoptera, Cetoniinae, Lepidoptera and Apoidea were sampled using transect walks, colour pan traps, baited traps, sweep netting and tree beating.

Two 50m transect walks were undertaken in different areas of the sampling site. A 50m tape was laid out in a straight line at a location that was representative of the vegetation type and covered a variety of microhabitats. Each transect walk was undertaken by three people observing five metres either side of the transect line. The distance at which the target invertebrates were observed was recorded. Orthoptera, Lepidoptera and Apoidea were collected and recorded.

Colour pan traps were set for a period of 24 hours. Five blue, and five yellow coloured pan traps with a diameter of 22cm, an internal diameter of 20cm, and a depth of 2cm, were set in two parallel lines 10m apart with each pan trap set at a 10m interval. The pan traps were filled with approximately 150ml of a water, detergent and glycerol mix to ensure a low surface tension and reduce evaporation. All invertebrates caught were collected. For the purpose of this investigation, only Apoidea and Araneae were included in the analysis.

Ten baited traps with a uniform diameter of 30cm, length of 110cm and mesh hole size of 1mm, suspended at least 1m from the ground were set for 24 hours at each invertebrate sampling site. Fermented fruit bait was placed on the solid base of the trap. The baited traps were set in two parallel lines approximately 20m apart. Cetoniinae, Lepidoptera and Apoidea were recorded.

Sweeping using nets of a 35cm diameter and 1mm hole mesh sampled low-level vegetation. The contents of 10 full sweeps comprised one sample and two samples were taken. All invertebrates were collected, but only Araneae were extracted for this study.

Tree beating was used to sample Araneae, Diplopoda and Mollusca. A total of 20 trees were beaten at each sampling site. One tree was struck a total of 10 times; each branch was struck no more than five times. A 1m diameter tray was placed under the branch to collect falling invertebrates. Invertebrates were placed straight into 70% ethanol, to prevent predation within the sample tube.

4.3.3 Data Analysis

4.3.3.1 Congruency in species density across taxonomic groups and taxonomic levels

Sampling effort was equal at each site, and it is unlikely that species richness was completely sampled. In this study, the term 'species density' (S_d) is defined as being the number of species per specified collection area or unit (Magurran 2004).

Species (S_d), genera (G_d) and family (F_d) density were calculated as the total number sampled at each site for each taxonomic group. Spearman rank correlations were used to test correlations using species density. These were performed using SPSS version 13.0.

4.3.3.2 Congruency in species assemblage patterns across taxonomic groups and taxonomic levels

Statistical analyses of species assemblage patterns were performed using PRIMER version 5.2.9. Similarity was calculated between every pair of sampling events in terms of the biological species they contained, using Bray-Curtis similarity measures based on $\sqrt{}$ transformed species abundance data and a similarity matrix was constructed. The $\sqrt{}$ transformation has the effect of down weighting the importance of the highly abundant species, so that similarities depend not only on their values but also on those of less common species (Clarke & Warwick 2001). At sites where no species were recorded for a taxon, a single pseudo-species, genus or family was added to prevent Type 1 error that would result if sites without representatives were excluded from the species assemblage similarity analysis. Therefore, site similarity matrices were constructed for each taxon, each taxonomic level and activity group.

For every pair of similarity matrices, it is easy to define how closely the patterns match (congruency) by using Mantel tests, using the Spearman Rank Correlation method with 999 permutations. The Mantel analysis tests the null hypothesis of no relation between multivariate patterns from two sets of samples, in this instance species assemblages (Clarke & Gorley 2001). The null hypothesis of no difference in species assemblages is rejected at a significance level of $P<0.05$. The ρ-value indicates the strength of the observed relation with 0 denoting no relation, 1 denoting a very strong relation.

Mantel tests were used to test congruency in species assemblages sampled across taxa, across taxonomic levels and activity groups.

When assessing congruency between a taxon and combined group of taxa (i.e. 'all invertebrates'), the data for that taxon were removed to prevent Type 1 error as a result of finding significance due to the duplication of species data in the 'all invertebrate' group.

4.3.3.3 Comparison of potential surrogates identified using species density and species assemblage patterns

A qualitative analysis of the significant congruent relations using the species density and the species assemblage similarity methods based on the ρ-values and the interpretation of the graphs was used to assess the differences in congruency result from using species density and species assemblage patterns.

Cluster analysis can be used to define species assemblages, i.e. groups of species that tend to co-occur in a parallel manner across sites (Clarke & Warwick 2001). Cluster analysis attempts to group samples into discrete clusters, and not to display their inter-relations on a continuous scale; the latter is the province of ordination (Clarke & Warwick 2001). Therefore, cluster analysis is often best used in conjunction with ordination (Clarke & Warwick 2001). Super-imposition of the clusters (at various levels of similarity) on an ordination plot will allow any relation between the groups to be informatively displayed, and agreement between the two representations strengthens belief in the adequacy of both (Clarke & Warwick 2001).

Several similarity matrices can be compared by assessing the agreement of the site similarity matrices across each taxonomic group, and by generating a matrix of ρ-values between each pair of taxa (rank correlation matrix), which is then used to produce a second-stage Multi-Dimensional Scaling (MDS) plot showing the relatedness of each taxon according to the species assemblage similarity across sampling events.

To identify how the taxa relate to each other in terms of species density across sampling events, an average linkage hierarchal cluster analysis using Euclidean distance measures was used. This clustered the taxa based on species density at each sampling event. A MDS analysis was then performed using Euclidean distance. The results from the cluster analysis enabled the taxa plotted on the MDS to be grouped according to similarity clustering.

To assess how taxa relate to each other in terms of their species assemblage patterns across sampling events, a second-stage MDS plot and cluster analysis was performed. The second-stage MDS plot was generated from a rank correlation matrix derived from the Bray-Curtis similarity matrices for the species assemblage similarity for each taxon. A cluster analysis using average linkage was performed on the rank correlation matrix to enable the taxa to be grouped on the second-stage MDS plot.

The two MDS plots were then compared, to assess the patterns produced by the two approaches and to investigate the impact of using different approaches on the result obtained.

Epigaeic, flying and 'all invertebrate' groups were not included in ordinations or cluster analyses as their placement was biased towards the dominant taxon in each group (i.e. epigaeic invertebrates were biased towards Mollusca and flying insects towards Lepidoptera).

4.3.3.4 The effect of the lower (species) to higher (family, genus) taxon ratio on the strength of congruency across taxonomic levels

The maximum number of species to higher taxon required for there to be sufficient congruency between lower and higher taxa was calculated for the species density and species assemblage pattern approaches. The ratio of lower (species) to higher (genus or family) taxon was calculated for each taxon, using the total number of species, genera and families sampled across all sites. The relation between the lower to higher taxon ratio and the strength of the relation (ρ-value) between lower and higher taxa were assessed using a nonlinear regression analysis in SigmaPlot version 8. The curve model with the highest R^2 value was selected as the appropriate model for each regression. The ratios of lower to higher taxon were used as the independent variable and the ρ-value as the dependent variable; ratios and ρ-values for all taxa were included in the regression. The ρ-value from the species assemblage similarity method and the species density method were assessed separately. Furthermore, regressions were performed using reduced datasets having removed outliers located in the 10^{th} and 90^{th} percentiles. All assumptions were tested prior to analyses.

4.4 RESULTS

From the 66 sampling events, 36 524 individuals from 414 invertebrate species were recorded, comprising: Lepidoptera (1 543 individuals, 82 species, 43 genera, 10 families), Apoidea (711 individuals, 41 species, 19 genera, 4 families), Cetoniinae (1700 individuals, 30 species, 26 genera), Orthoptera (746 individuals, 45 species, 34 genera, 3 families), Blattodea (3717 individuals, 27 morpho-species), Araneae (1335 individuals, 99 species, 37 genera, 3 families), Diplopoda (12 055 individuals, 26 species, 12 genera, 8 families), Chilopoda (383 individuals, 16 species, 6 genera, 3 families) and Mollusca (14 333 individuals, 47 species, 30 genera, 17 families).

4.4.1 Congruency in Species Density across Taxonomic Groups, Habitat Groups and Taxonomic Levels

The analysis of cross-taxon congruency using species density identified several significant congruent relations (Table 4.1). However, the strength of these was weak in all instances due to low ρ-values. Figure 4.1 shows the strongest cross-taxon relations using species density.

The congruency analyses of density values between higher (genus and family) and lower (species) taxa yielded significant results (Table 4.2, Fig. 4.1). Congruency was observed between species and family level across all taxonomic groups, but the strength of the relation varied considerably across taxa.

Significant congruency was identified between Diplopoda and all other epigaeic invertebrates (Table 4.1, Fig 4.2b), and between Lepidoptera, Apoidea, Cetoniinae, Orthoptera and all other flying insects (Table 4.1).

Table 4.1 Matrix showing the strength of the cross-taxon congruency using species density which was determined using Spearman Rank Correlations **(shaded area)**, and cross-taxon congruency using community similarity which was determined using Mantel tests **(un-shaded area)**. The strength of the cross-taxon relation is determined by the ρ-value indicating the strength of the observed relationship, 0 denoting no relationship, 1 denoting a very strong relationship. Parentheses show the *P*-value, values in **bold** represent a significance of less than 0.05. $n = 66$ for each taxon.

Taxon	Lepidoptera	Apoidea	Cetoniinae	Orthoptera	Blattodea	Araneae	Mollusca	Diplopoda	Chilopoda	Epigacic	Flying insects	All invertebrates
Lepidoptera	-	0.441 (<0.001)	0.225 (0.07)	0.164 (0.187)	-0.002 (0.99)	0.222 (0.074)	-0.113 (0.365)	-0.125 (0.318)	0.223 (0.072)	-0.064 (0.612)	0.372 (0.002)	0.201 (0.106)
Apoidea	0.183 (0.001)	-	0.363 (0.003)	0.323 (0.008)	0 (0.998)	0.265 (0.031)	0.098 (0.433)	0.018 (0.883)	0.158 (0.206)	0.078 (0.535)	0.489 (<0.001)	0.441 (<0.001)
Cetoniinae	0.133 (0.009)	0.132 (0.003)	-	0.402 (<0.001)	0.136 (0.276)	-0.074 (0.553)	0.154 (0.217)	0.165 (0.187)	0.042 (0.737)	0.197 (0.113)	0.329 (0.007)	0.368 (0.002)
Orthoptera	0.11 (0.027)	0.119 (0.002)	0.15 (0.002)	-	0.464 (<0.001)	-0.12 (0.338)	-0.125 (0.315)	0.054 (0.665)	0.335 (0.006)	0.147 (0.239)	0.402 (0.001)	0.264 (0.032)
Blattodea	0.074 (0.125)	0.109 (0.025)	0.128 (0.01)	0.171 (0.002)	-	-0.047 (0.706)	-0.116 (0.352)	0.115 (0.359)	0.377 (0.002)	0.044 (0.727)	0.094 (0.455)	0.175 (0.158)
Araneae	0.192 (0.003)	0.106 (0.024)	0.095 (0.051)	0.016 (0.391)	0.005 (0.465)	-	0.205 (0.099)	0.178 (0.153)	0.116 (0.355)	0.164 (0.184)	0.179 (0.150)	0.164 (0.188)
Mollusca	0.144 (0.007)	0.101 (0.026)	0.124 (0.012)	0.077 (0.079)	0.103 (0.057)	0.077 (0.126)	-	0.613 (<0.001)	-0.154 (0.216)	(0.208 (0.094)	0.007 (0.957)	0.087 (0.489)
Diplopoda	0.088 (0.063)	0.091 (0.029)	0.093 (0.037)	0.054 (0.121)	0.163 (0.003)	0.049 (0.186)	0.273 (0.001)	-	-0.075 (0.547)	0.552 (<0.001)	-0.020 (0.871)	0.275 (0.032)
Chilopoda	0.122 (0.019)	0.057 (0.11)	-0.014 (0.602)	0.063 (0.103)	0.089 (0.06)	0.038 (0.256)	0.144 (0.007)	0.122 (0.006)	-	0.025 (0.839)	0.255 (0.069)	0.277 (0.024)
Epigaeic	0.086 (0.076)	0.129 (0.009)	0.127 (0.011)	0.119 (0.006)	0.158 (0.006)	0.071 (0.126)	0.277 (0.001)	0.294 (0.001)	0.180 (0.001)	-	0.068 (0.589)	-
Flying	0.193 (0.001)	0.193 (0.001)	0.176 (0.002)	0.202 (0.001)	0.177 (0.003)	0.19 (0.001)	0.129 (0.011)	0.142 (0.006)	0.088 (0.054)	0.184 (0.003)	-	-
All invertebrates	0.16 (0.004)	0.167 (0.003)	0.18 (0.001)	0.148 (0.004)	0.167 (0.001)	0.149 (0.012)	0.274 (0.001)	0.293 (0.001)	0.159 (0.002)	-	-	-

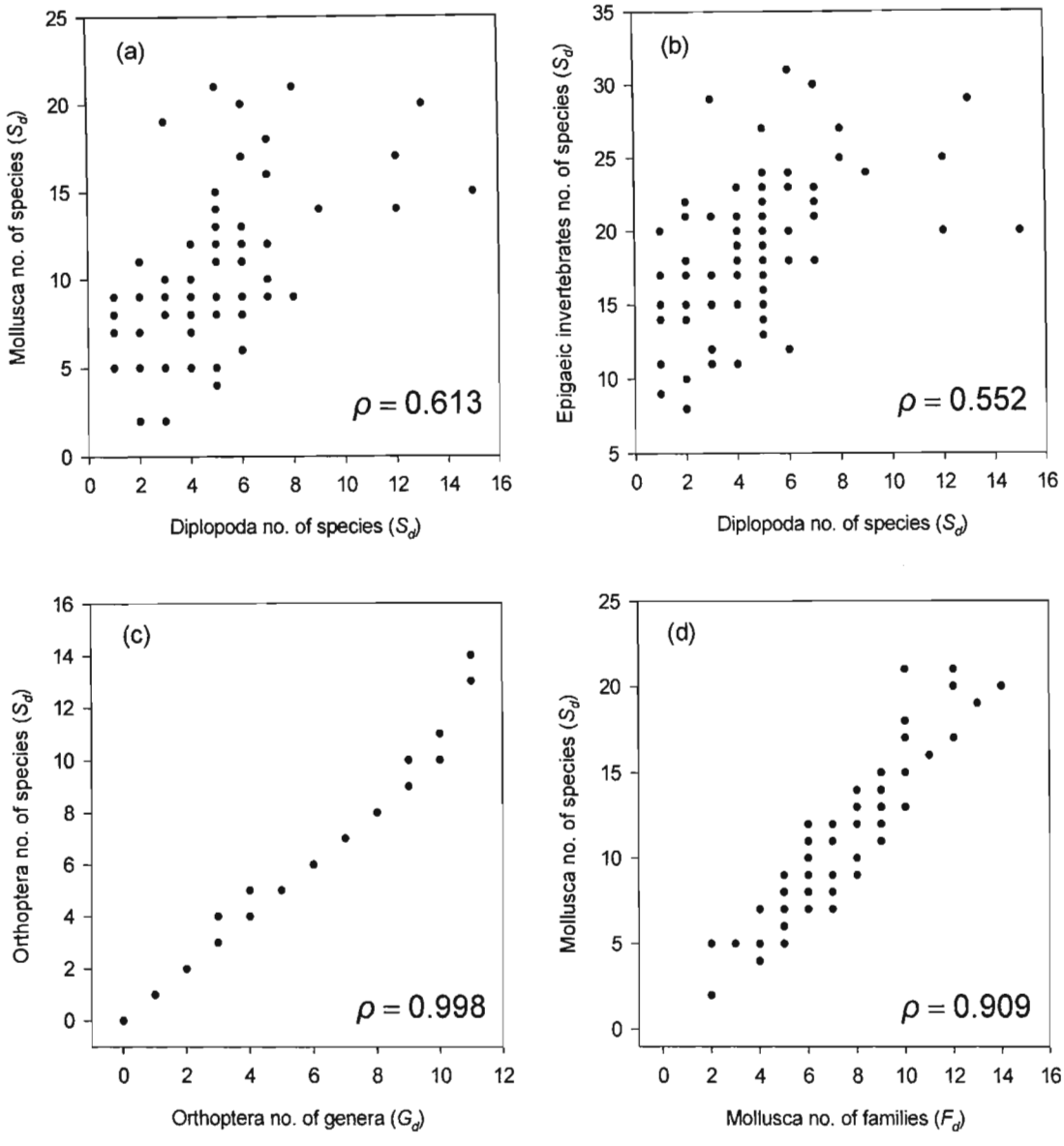


Figure 4.1. Congruency using density measures between **(a)** Diplopoda and Mollusca species density, **(b)** Diplopoda and epigaeic invertebrates (excluding Diplopoda) species density, **(c)** Orthoptera genus and species density and **(d)** Mollusca family and species density. ρ statistic indicates the strength of the observed relationship, 0 denoting no relationship, 1 denoting a very strong relationship. All correlations are significant at 99% ($P<0.001$).

Table 4.2. Strength of the congruency between lower and higher taxa, determined using Spearman rank correlations for density values **(a)** and higher lower taxon congruency determined using Mantel tests for species assemblage similarity **(b)**. All relationships are significant at 99% ($P<0.001$).

Taxon	Higher taxonomic level	Species to higher taxon ratio	ρ-value (a)	ρ-value (b)
Lepidoptera	Genus	1.9 : 1	0.944	0.728
	Family	8.2 : 1	0.875	0.618
Apoidea	Genus	2.2 : 1	0.924	0.759
	Family	10.3 : 1	0.735	0.570
Cetoniinae	Genus	1.2 : 1	0.996	0.988
Orthoptera	Genus	1.3 : 1	0.998	0.988
	Family	15.0 : 1	0.761	0.673
Araneae	Genus	2.7 : 1	0.898	0.734
	Family	33.0 : 1	0.561	0.533
Mollusca	Genus	1.6 : 1	0.974	0.931
	Family	2.8 : 1	0.909	0.881
Chilopoda	Genus	2.7 : 1	0.944	0.770
	Family	5.3 : 1	0.710	0.454
Diplopoda	Genus	2.2 : 1	0.950	0.693
	Family	3.3 : 1	0.888	0.683

4.4.2 Congruency in Species Assemblage Patterns across Taxonomic Groups, Habitat Groups and Taxonomic Levels

Significant cross-taxon congruency was identified between the species assemblage patterns of a range of invertebrate taxa (Table 4.1). However, the strength of the significant congruent relations was weak in all instances as indicated by the low ρ-values. Figure 4.2 shows the strongest cross-taxon relations using species assemblage similarity.

The congruency analyses of species assemblage similarity values between higher (genus and family) and lower (species) taxa yielded significant results and showed strong congruency across all taxonomic groups between species and genus levels (Table 4.2a). Congruency was observed between species and family level across all taxonomic groups, but the strength of the relation varied considerably across taxa. Figure 4.2 demonstrates the relations showing the highest cross-taxon congruency between higher and lower taxa for species assemblage similarity.

Significant cross-taxon congruency was identified between Apoidea, Cetoniinae, Orthoptera, Blattodea, Mollusca, Diplopoda, Chilopoda and epigaeic invertebrates, but the relations were all very weak (Table 4.1). Significant congruency was identified between all taxa except Chilopoda and epigaeic invertebrates, however, the relations were again very weak (Table 4.1).

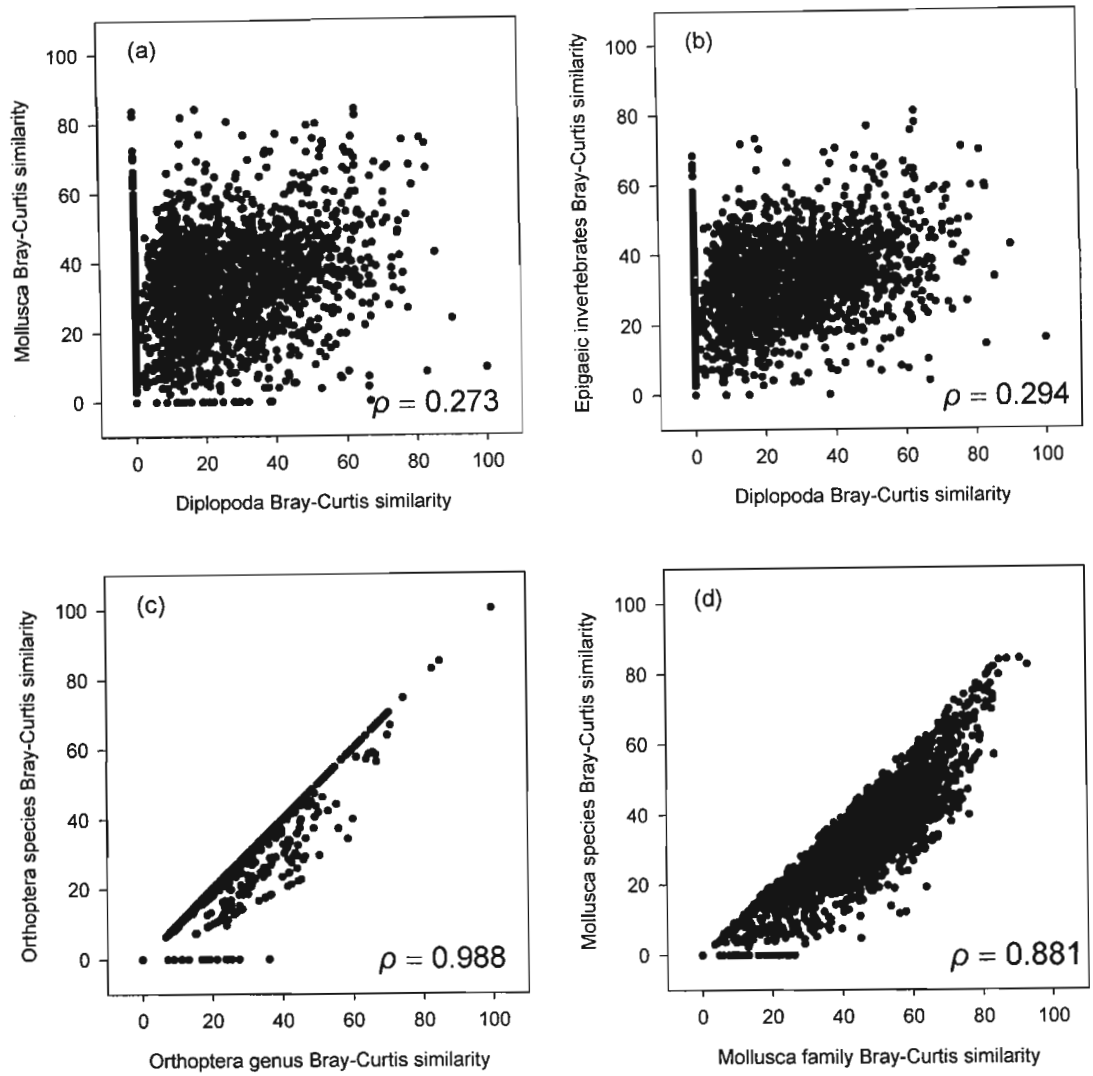


Figure 4.2. Congruency across groups and the taxonomic levels using species assemblage similarity between Diplopoda and Mollusca **(a)**, Diplopoda and epigaeic invertebrates (excluding Diplopoda) **(b)**, Orthoptera genus and species **(c)** and Mollusca family and species **(d)**. ρ statistic indicates the strength of the observed relationship, 0 being no relationship, 1 denoting a very strong relationship, all correlations are significant at 99% ($P<0.001$).

4.4.3 Comparison of Potential Surrogates Identified using Species Density and Species Assemblage Patterns

The strength of the relation between higher and lower taxa was stronger for all taxa when using values of species density as opposed to species assemblage patterns (Table 4.1). When the relations are represented graphically, the spread of data points away from a hypothetical trend-line can be observed easily as the ρ-value decreases (Fig. 4.1 and 4.2).

Cross-taxon congruency using species density values and species assemblage similarity values produced a different number of significant relations across taxa (Table 4.1). The significant ρ-values using species

assemblage similarity varied from 0.11 to 0.294 with the highest valued between Diplopoda and Epigaeic invertebrates. The significant ρ-values using species density values varied from 0.265 to 0.613 in which the highest value was between Diplopoda and Mollusca. Significant cross-taxon species density relations were observed between Apoidea and Araneae; Chilopoda and Blattodea; and Chilopoda and Orthoptera. Nevertheless, these relations were not significant when using species assemblage similarity values.

Two of the 19 cross-taxon species density relationships were not observed when species assemblage similarity was assessed; these were between Chilopoda and Orthoptera; and Chilopoda and Blattodea. Twenty-eight of the 44 cross-taxon species assemblage pattern relationships were not observed when species density was assessed (Table 4.1).

When cross-taxon congruency is represented in an ordination (Fig. 4.3), the two approaches group taxa according to similarity in very different ways. When grouped using species assemblage similarity, the epigaeic invertebrates Diplopoda, Mollusca and Chilopoda are in a coarse cluster, the pollinators and plant-dwelling groups Apoidea, Lepidoptera and Araneae are in a second group, and the remainder are grouped together. When grouped using species density, taxa do not appear to cluster according to functional group, habitat preference or life history (Fig. 4.3).

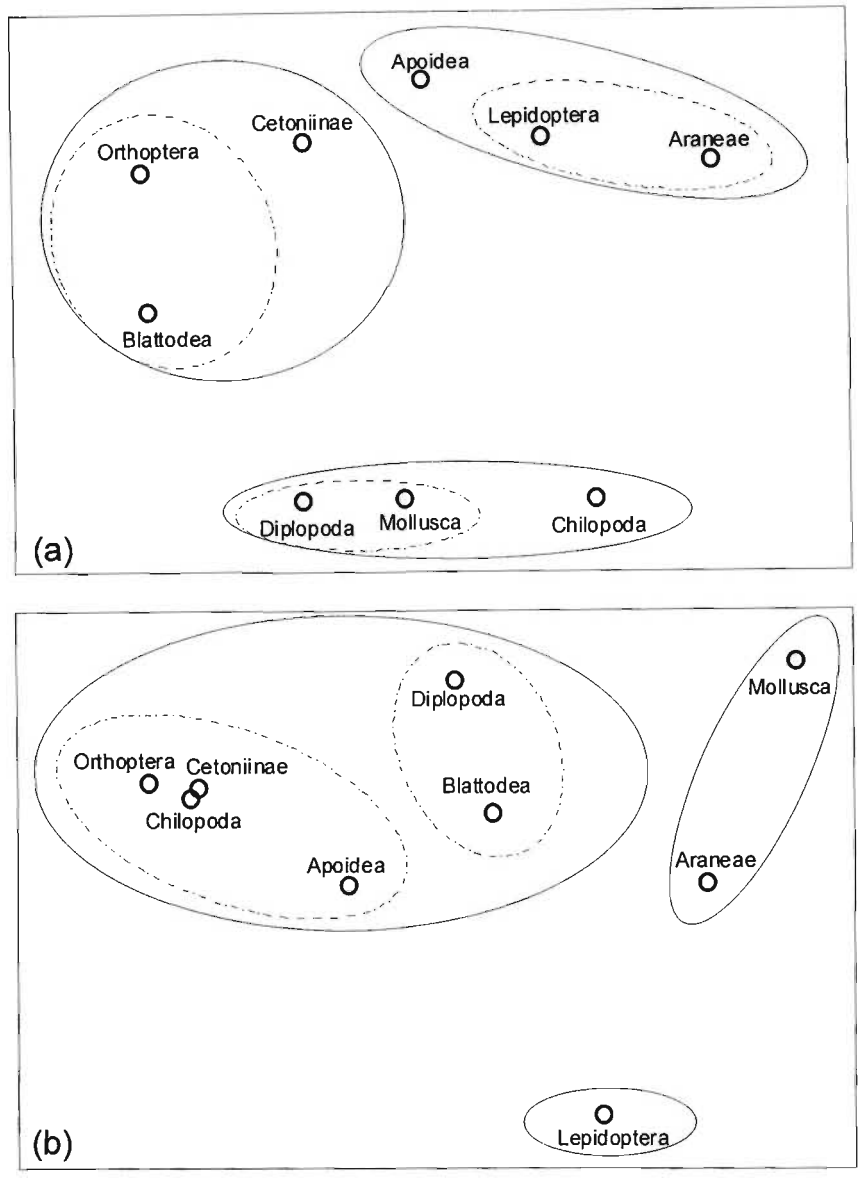


Figure 4.3. Comparison of the clustering of taxa using species assemblage similarity and species density. Second-stage multi-dimensional scaling (MDS) plot showing the relatedness of each taxonomic group to each other which was determined using Bray-Curtis similarity measures; taxa are grouped in coarse clusters, below a fusion level of 55% similarity (—) and fine clusters, above a fusion level of 60% similarity (- - -), MDS stress value = 0.11 **(a)**. MDS plot of the relatedness of the species density of each taxonomic group to each which was determined using taxonomic Euclidean distances, two levels of dendrogram clusters are superimposed: coarse clusters below a fusion level of 55% (—) and fine (clusters above a fusion level of 65% similarity (- - -), MDS stress-value = 0.03 **(b)**.

4.4.4 The Effect of Lower (Species) to Higher (Family, Genus) Taxon Ratio on the Strength of Congruency across the Taxonomic Levels

There is a reduction in the strength of the relation between lower and higher taxa as the ration number of species to higher taxa increases (Fig. 4.4). Removing outliers had little effect on the shape of the curve or the corresponding R^2 value (Fig. 4.4).

The maximum ratio of species to higher taxa in which good congruency is likely to be observed, using a minimum ρ-value of 0.75, when using species density is 10.21. When using species assemblage patterns, the ratio is reduced to 2.41. The removal of the outliers changes these figures to 12.46 for species density and 2.61 for species assemblage patterns.

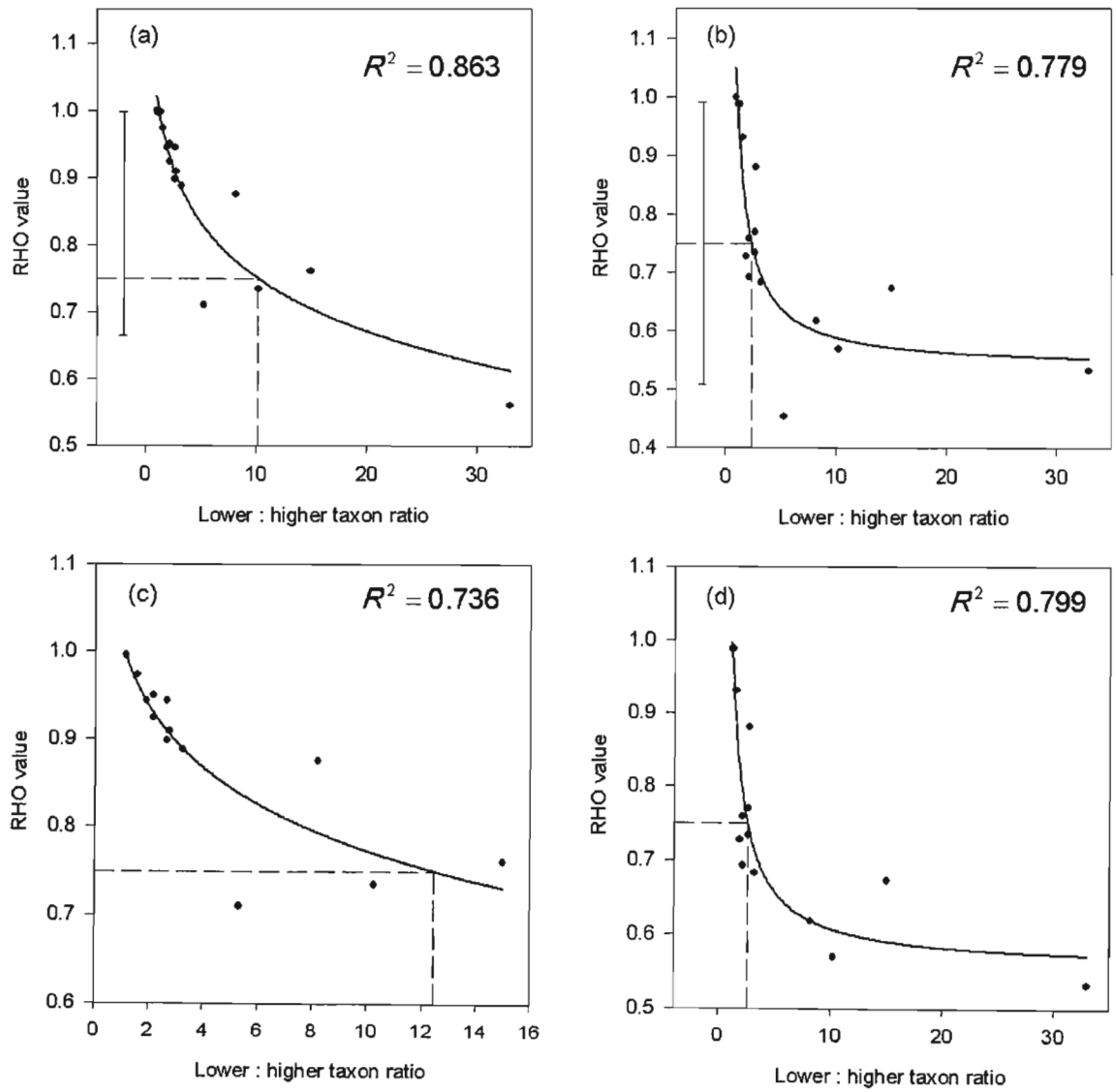


Figure 4.4. The relation between the ratio of lower to higher taxon and the ρ-value demonstrating the strength of the higher / lower taxa congruency using species density **(a)**, species assemblage similarity **(b)**, species density with outliers removed **(c)** and species assemblage similarity with outliers removed **(d)**. Error bars show the 10^{th} and 90^{th} percentile range, data points lying outside the error bars were removed. The regression line is shown (—) and the reference lines (– –) show the maximum lower to higher species ratio required to obtain a strong congruent relation with a ρ-value of > 0.75.

4.5 DISCUSSION

Ecological communities may be dominated by weak trophic interactions; if this is true, then the removal or addition of species can lead to pronounced changes in community composition and structure (McCann 2000). Therefore, there is a need for invertebrates to be incorporated into conservation plans. If they are not, key invertebrate species or communities may be lost. Brooks et al. (2004) call for species data to remain central to conservation planning, and for conservation planners not to rely on remote-sensing data and computer models without the incorporating of the available species data. However, invertebrate data are incomplete and are spatially and taxonomically biased (Ferrier 2002). Surrogates are needed because it is impossible to measure all biodiversity (Margules et al. 2002). These surrogates can be used to reduce costs in the survey process or to represent taxa in the conservation planning process. Nonetheless, there is much debate regarding the selection and use of surrogates, and many conflicting research findings.

The cross-taxon relations assessed in this study were shown to be weak and not suitable as effective surrogates, which agrees with the findings of Negi and Gadgil (2002), Ricketts et al. (2002) and Grand et al. (2004). These findings have shown that a single taxon surrogate will not adequately represent invertebrate taxa. Invertebrates are highly diverse in terms of life history, body size and ecological role, and it is therefore necessary to select a range of invertebrate taxa that are representative of these traits and that can be used as surrogates for all invertebrate diversity. The lack of cross-taxon congruency supports the hypothesis of Ricketts et al. (2002) that species-richness patterns of different taxa are unlikely to correspond at local scales if taxa do not share similar patterns in habitat specificity, and my findings extend this hypothesis to include species assemblage similarity patterns.

The lack of cross-taxa congruency of different taxa at a local scale supports the use of the multi-taxa approach. I agree with Grand et al. (2004) and Sauberer et al. (2004) who suggest that conservation planners use multi-taxa, multi-scale assessments to develop more comprehensive conservation strategies.

Good congruency between higher and lower taxa was identified using both species density and species assemblage similarity. These findings concur with those of Negi and Gadgil (2002), Cardoso et al. (2004) and Villasenor et al. (2005) where higher-level identifications were found to be effective surrogates for species level identifications when determining trends across sites and for site prioritisation. Nevertheless, higher-level taxonomic identification can be used as a surrogate but only in species-poor genera or families. I suggest that a maximum ratio of 10.21 species to the higher taxon is used as a surrogate when assessing species density. However, when assessing species assemblage patterns, the maximum species to higher taxa ratio is reduced to 2.61 (figures based on regression curve with highest R^2 value). It is therefore necessary to have an accurate inventory of the region in order to assess whether the taxon, prior to its selection as a surrogate, meets this criterion. This can be done through a desktop study of the literature to determine the likely species to higher taxa ratio using broad distribution maps. However, the results should be interpreted with caution if the taxonomic diversity of the study region is poorly

documented and confidence in the number of species to higher taxa ratio is poor. In reality, these findings have little application because high priority survey regions generally do not have any available data, and broad distribution maps do not exist. Nevertheless, these ratio values could be used as a basis for further studies in this study region, but further work is required to identify if these ratios are applicable in other ecosystems, and at different spatial scales.

It is argued that high quality biodiversity inventories for the selection of representative reserve systems are cost efficient in the long run (Balmford & Gaston 1999). Nevertheless, the resources and time required to conduct these biodiversity inventories are usually unavailable (Reyers & van Jaarsveld 2000). Therefore, it is necessary to address this problem by attempting to reduce research costs by using surrogates. The use of higher taxa as a surrogate would reduce resource costs because professional taxonomists would not be required to identify specimens to a higher taxonomic level for many taxa and identification could be completed by para-taxonomists. Para-taxonomists can provide high quality biological specimens and ecological information, ensuring database growth will be rapid and results will be published in a timely manner (Basset et al. 2004). Furthermore, Balmford et al. (1996) showed that fieldwork that targets genera and families rather than species reduced the survey costs (financial) by a minimum of 60% and 80% respectively. These considerable savings are unlikely to be directly applicable to the fieldwork stage of surveys of invertebrates because fieldwork techniques do not distinguish between taxonomic levels, although the sampling effort required to reach an asymptote will be less. However, once material is in the laboratory, identification to higher taxonomic levels will be rapid and will considerably reduce costs post-fieldwork. In terms of conservation planning, the use of higher taxa as surrogates would only be useful for identifying species rich areas for prioritisation.

The relations observed using species densities measures indicate that moderate cross-taxon relations exist; when species assemblage similarity was assessed, these relations were weak. However, Su et al. (2004) found good cross-taxon congruence using community structure between plants, birds and butterflies, and poor congruence between the taxa when assessing species richness, in a survey at a local scale in a relatively homogenous ecosystem. Due to the heterogeneity of the savanna region assessed in this study, it is likely that weak cross-taxon congruence using species assemblage patterns was a result of species turnover responding to different biotic and abiotic variables. Furthermore, the congruency patterns across taxa varied depending on whether species density or species assemblage similarity was assessed.

These results indicate that the use of species density measures to assess congruent relationships may lead to the misinterpretation of data, even at a local scale, in a heterogeneous ecosystem. The reduction of species abundance data to a single value is likely to lead to false interpretation where sites may have equal species densities but have different species assemblages. Thus, the use of species density measures to prioritise sites for local conservation planning may be ineffective (Grand et al. 2004). I agree with Howard et al. (1998) and Su et al. (2004) that measures of species assemblage similarity and not simple

species density or richness should be used in future studies of coarse-filter surrogates and for conservation planning, in particular, when an ecosystem consists of diverse habitats.

Surrogates should be used in surveys as an initial approach, and be utilised only when resources are limited or species identification is not possible in the timescale required to produce results, as the real value of any biodiversity dataset is at the species level when the species are scientifically named (Samways 2005). In terms of conservation planning, the lack of congruency between invertebrate taxa supports the use of a multi-taxa approach for the incorporation of invertebrates in conservation plans. Furthermore, when data permits, species assemblage patterns should be assessed in conjunction with species density measures.

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SUMMARY

It is now widely accepted that biodiversity needs to be conserved for future generations. However, the documentation of biodiversity, in particular invertebrate biodiversity is poor. Many species have yet to be discovered and few have had their distributions mapped at a spatial scale appropriate for conservation planning (Ferrier et al. 2002). The lack of information about invertebrates has made it difficult for biologists to come up with effective conservation guidelines for this component of the fauna, consequently, invertebrates have been largely ignored in conservation (Yen & Butcher 1997), nevertheless, more recently invertebrates have been included, but gaps remain in the data. In order for invertebrates to be extensively incorporated into conservation plans, further data that provides relevant information in an appropriate time-period must be obtained, and the recent efforts of research groups must be supported.

The savanna biome is an important biome, which is under threat from land transformation, and therefore it is a focus for conservation planning. Nevertheless, the invertebrate fauna of this biome is poorly documented; hence, there is a need to provide baseline data on invertebrates. Invertebrate biodiversity of this biome is rich and this makes surveys of invertebrates costly. Furthermore, sampling requires much labour to complete.

Owing to the huge number of invertebrate species, it is impossible to sample all taxa and species. By definition, reaching 100% of richness would require an infinite effort (Soberón & Llorente 1993). It is therefore necessary to use surrogates, which represent invertebrate biodiversity for conservation planning and aid in the rapid assessment of biodiversity.

Cross-taxon congruencies were observed, but relationships were weak and potential surrogates could not be selected. The lack of cross-taxon congruency supports the hypothesis of Ricketts et al. (2002) that species-richness patterns of different taxa are unlikely to correspond at local scales if taxa do not share similar patterns in habitat specificity. My findings extend this hypothesis to include species assemblage patterns, which further supports the use of the multi-taxa approach. I agree with the suggestions of Grand et al. (2004) and Sauberer et al. (2004) that conservation planners use multi-taxa, multi-scale assessments to develop more comprehensive conservation strategies.

Other local scale congruency studies have found good cross-taxon congruence when using species assemblage patterns and poor congruence between the taxa when assessing species richness, in a survey of plants, birds and butterflies at a local scale in a relatively homogenous ecosystem (Su et al. 2004). These findings are opposed to my findings, which may be due to the heterogeneity of the Maputaland savanna region. It is likely that weak cross-taxon congruence using species assemblage patterns was a result of species turnover within each taxon responding to different biotic and abiotic variables.

The use of species density measures to assess congruent relations leads to a different interpretation of data

compared to the use of species assemblage similarity, even at a local scale, in a heterogeneous ecosystem. The reduction of data on species abundance to a single value is likely to lead to false interpretation where sites may have equal species densities but have different species assemblages. Thus, the use of species density to prioritise sites for local conservation planning may be ineffective (Grand et al. 2004), in particular when an ecosystem consists of diverse habitats; however, this depends on the goals of the conservation strategy being addressed.

The use of a higher taxon to represent lower taxon shows good potential as a surrogate but only in species-poor genera or families. These surrogates only really have an application in areas where the invertebrate diversity and distributions are well documented and further comparative studies are required to answer new questions.

When selecting surrogates from congruency assessments an optimal ρ-value greater than 0.75 is required, below this value, the relation is likely to be weak and if used as a surrogate, misinterpretation may occur. However, surrogates should be used in surveys as an initial approach, and be utilised only when resources are limited or species identification is not possible in the timescale required to produce results. Furthermore, when data permit, species assemblages should be assessed in conjunction with species density measures.

In terms of conservation planning, the lack of congruency between invertebrate taxa supports the use of a multi-taxa approach for the incorporation of invertebrates in conservation plans. Nonetheless, using a multi-taxa approach makes the assumption that congruency occurs between the selected taxa (surrogates) and the biodiversity they represent.

Effective surrogates need to reflect general bio-geographical patterns and the evolutionary processes that have given rise to these, and their efficiency is likely to be influenced by several factors, including the spatial scale of species turnover and the overall congruence of the bio-geographical history (Moritz et al. 2004). Therefore, further investigation is required to identify surrogates, or a suite of surrogates, that accurately predict invertebrate biodiversity and distributions, by investigating the turnover rate of invertebrate species at a local scale in conjunction with the assessment of the use of land classes, vegetation data and other biotic and abiotic features as surrogates. Furthermore, congruency across different evolutionary and bio-geographical histories needs to be assessed and incorporated in surrogacy studies. Although data regarding the evolution and biogeography of many invertebrate species are non-existent, it is likely, with the rapid development of conservation genetics, that sufficient data will be available in the near future to carry out such studies.

Multi-taxa surveys of invertebrates require resources and labour which are often limited. To overcome this problem, volunteers can be used to assist in the collection of data. Volunteers with previous experience or knowledge of scientific method increased the perceived usefulness of volunteers to researchers when carrying out fieldwork, and increased their sampling effectiveness.

It was shown that volunteers sampled significantly lower rates of species accumulation (efficiency), species richness and unique species when sampling using timed active search methods. Conversely, it was shown that volunteers and researchers performed equally when using un-timed active searching methods. It was shown that volunteers were effective at sampling invertebrates in conjunction with experienced researchers and provided a valuable resource to researchers carrying out biodiversity surveys. Furthermore, volunteers sampled invertebrates as effectively as trained researchers and samples collected were non-biased. Valid data can be collected providing adequate supervision is provided and surveys are designed to suit volunteers' capabilities.

When using volunteers in invertebrate surveys, the sampling methods that volunteers complete must be simple, and often a compromise must be reached between strict scientific protocol and productive sampling that is possible using volunteers. I recommend the use of un-timed methods that restrict the volunteers to a specific search area. Furthermore, expert supervision must be available at all times to ensure that scientific protocol is being followed, and to answer questions and assist volunteers when necessary.

Surveys of invertebrates require groups to work together to set up sampling areas, assist with packing and unpacking equipment. Therefore, it is essential that volunteers are encouraged and feel appreciated to ensure that morale is kept high and good team dynamics are maintained. Furthermore, due to the repetitive nature of many sampling techniques, it is necessary to ensure that the tasks are rotated, in conjunction with rotating the members of each team.

The project experience raised the volunteers' environmental awareness, increased their knowledge about biodiversity, invertebrates and conservation research, and enabled volunteers to participate or design locally relevant conservation based projects on their return home. Therefore, the use of volunteers in local invertebrate surveys has far-reaching implications that are beneficial to conservation.

Therefore, to complete an effective multi-taxa survey of invertebrates in a Maputaland savanna that uses unskilled volunteers to assist in sampling, a standardised sampling protocol with a suite of quantified sampling methods needs to be used. Restrictive active searching methods (2m x 10m quadrats) are more effective for sampling epigaeic (ground-dwelling) invertebrates when using a multi-taxa approach. This method forces samplers to thoroughly search a unit area, focusing efforts and reducing bias towards particular microhabitats, or taxa.

The sampling of flying and plant-dwelling invertebrates requires a pragmatic approach due to the high diversity of life histories, body size and behaviours. Therefore, a range of sampling methods needs to used, for example, active searching along a transect, and passive trapping methods using baited traps, flight interception and pan traps.

To improve the efficacy and completeness of surveys of invertebrates, based on the application of species accumulation models, I suggest over 75% of the total estimated fauna as being a satisfactory and realistic

level of inventory completeness for making valid comparisons between regions and across sites. Nevertheless, it is likely that rare species will not be sampled using this level of completeness.

Furthermore, inventory sampling should take place across months and across multiple years in which larger cycles such as the El Niño – Southern Oscillation cycle are considered. In addition, I believe that it is important for taxonomists to be involved from the planning stage of the survey, because expert knowledge can provide invaluable insights into the sampling and processing requirements. This ensures that a suite of methods is tailor-made, which would fulfil the sampling requirements of the target taxa and the study region. It is crucial that all aspects of the sampling procedure are covered in the planning process, in particular the logistics of the sampling protocol. I have suggested that the use of vacuum sampling would be suitable for this survey; but one must note that for this study it was possible to take a vehicle within 200m of all the survey sites, this may not always be possible and the logistics of carrying the equipment to the site must be considered. Therefore, the choice of sampling methods will be dependent on the ability to transport equipment to and from the study site.

My findings have shown that the application of species accumulation models to assess inventory and within inventory completeness, and to estimate the minimum sampling effort required to complete an inventory or comparative study, can lead to important improvements in sampling design. This ensures resources are invested efficiently and effectively to maximise species capture, and this agrees with the findings of Moreno and Halffter (2000). Therefore, species accumulation models should be used to assess sampling techniques in pilot studies to ensure that sampling protocols are effective and efficient and that resources are being utilised with maximum efficiency. In addition, it is important for abundance data to be collected, to enable individual based species accumulation curves to be produced so extrapolation can be carried out in order for completeness to be assessed.

The Maputaland Centre consists of a mosaic of mainly extensive savanna plant communities arranged in complex patterns and for its size is one of the most remarkable areas of biodiversity in the world (van Wyk 1996). Therefore, this region has a high level of heterogeneity. The invertebrate fauna of this heterogeneous landscape is poorly studied and it is not known whether invertebrate assemblages in the region are similarly heterogeneous or to what extent the habitat types are characterised by endemic or specialist faunas (van Rensburg et al. 1999). Therefore, these recommendations may not be directly applicable to other savanna habitats. It is likely that surveys of invertebrates in more homogeneous savannas will require less overall sampling effort; however, there is a need for further research into the heterogeneity of invertebrates in this region and the relation of the invertebrate species to biotic and abiotic features at varying spatial scales.

Slotow and Hamer (2000) state that before embarking on taxonomic surveys, or general surveys of biodiversity, careful consideration should be given to the potential use of the data for conservation. This present study could not have identified the material to species level in a timely manner without the assistance of taxonomists across the world. Furthermore, I agree with Brooks et al. (2004) that when

resources permit, species level identification should be carried out because species data are a precondition of conservation and one cannot understand the relations between the components of biodiversity without knowing what those components are. Therefore, surveys should strive to obtain species level identifications. However when the taxonomic diversity of a region is well known then higher taxa can be used as a surrogate if the ratio of lower : higher taxa is below 10.21 when assessing species density or 2.61 when assessing species assemblage patterns.

It is essential that taxonomists work in conjunction with conservation planners, ecologists and surveyors to identify lacunas in biodiversity data and to select appropriate target taxa and survey methods. Furthermore it is important that suitable sites are selected, where appropriate algorithms can be applied to determine priority sites requiring biodiversity surveys to enable effective conservation planning, for example Chown and Freitag-Ronaldson (2002) 'record absence gradient selection' which selects grid cells for surveys in poorly sampled or completely unsurveyed areas.

This project contributes information for use by conservation planners and ecologists by providing preliminary methodological frameworks for the collection of data on invertebrates. This project also provides solutions to resource issues frequently associated with the completion of comprehensive surveys of invertebrates. In addition, this study has contributed towards increasing knowledge of invertebrate biodiversity in the Maputaland savanna ecosystem. A comprehensive database has been compiled which contains new distribution records and new species to science as well as much needed baseline data for the region.

However, it must be remembered that the term 'invertebrates' is a highly artificial grouping of many different lineages of vastly different levels of complexity (Yen & Butcher 1997). It is clear that invertebrate biodiversity cannot be represented by a single invertebrate taxon. To maximise the effectiveness of strategies for invertebrate conservation, it is necessary for conservation planners to use a multi-taxa approach and not to base strategies on a single taxon. In addition, due to the importance of invertebrates, there is a need to investigate further, potential surrogates for conservation planning at a local scale, particularly in data deficient regions, where decisions regarding land use or management need to be made prior to survey completion.

5.1 FURTHER STUDY

Surrogates are needed to represent invertebrates as they account for 95% of biodiversity (Myers et al. 2000), they are hyper-diverse, and data regarding diversity and distributions are poor (Ferrier 2002). Many conservation initiatives use land classes, in particular vegetation data, as surrogates for invertebrate data (Panzer & Schwartz 1998). However, there is confounding evidence in the literature that land classes represent invertebrate communities. Furthermore, many land classes which are used in conservation planning are classified at coarse spatial scales (e.g. KZN Wildlife C-plan), and areas prioritised for conservation as a result are at such coarse scales they are rarely applicable to local conservation planning

(Prendergast et al. 1993; Ricketts et al. 2002). In terms of invertebrate conservation, there is a need to test the theories of using land classes as surrogates for invertebrates, and to assess the spatial scale that is most appropriate for invertebrate conservation and local conservation planning.

Due to the heterogeneity of the vegetation in the study region and the comprehensive invertebrate data obtained in this study the dataset is ideally suited to investigate further the concept of surrogacy in conservation planning by assessing vegetation classification and other biotic and abiotic features are surrogates for invertebrate biodiversity at a fine spatial scale, by assessing the following topics:

i) the use of land classes at different spatial scales as surrogates of invertebrate communities, by testing the theory that invertebrate species turnover correlates with vegetation turnover, therefore assessing whether invertebrate communities are heterogeneous across vegetation types and homogenous within a vegetation type.

ii) the effect of spatial scale on invertebrate species turnover by assessing the effect of distance (latitudinal and longitudinal) within a land class on invertebrate communities, by addressing the question of species turnover in relation to spatial scale within land classes, therefore developing a measure of whether land classes need to be zoned to an appropriate scale to cater for species turnover.

iii) the biotic and abiotic features that contribute to areas of high species richness and taxonomic uniqueness. I propose to analyse the effect of a variety of abiotic features such as soil characteristics, topography and biotic features such as tree communities and individual invertebrate taxon communities have on the invertebrate community structure, to assess key features that influence invertebrate communities and diversity. This has application in predictive modelling for sites or areas not surveyed.

This work would provide crucial information for the inclusion of invertebrates into the conservation planning process and will help to develop conservation planning at a local level (within the savanna ecosystem) by aiding the development of locally relevant surrogates which account for invertebrates. This study has compiled a comprehensive database and therefore no additional fieldwork needs to be carried out, furthermore, vegetation and abiotic data at a fine spatial resolution are available for analysis.

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