Conservation ecology of *Frithia humilis*, an endangered succulent of sandstone outcrops in Mpumalanga, South Africa

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Declaration

I declare that the work presented in this Masters dissertation is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been acknowledged by complete reference.

Signature of the Student:……………………………………………………………

Signature of the Supervisor:…………………………………………………………

Signature of the Co-supervisor:J van den Berg……………………………………………………………

Abstract

Translocation involves the movement of organisms, by human intervention, from one area to other suitable (receptor) habitats. In a conservation context, translocation can be employed to support species preservation, population restoration and/or for ecological research. Despite decades of internationally published research, translocation remains a controversial endeavour. However, due to continual degradation and fragmentation of natural habitats in the face of human development, translocation is becoming a vital component of conservation efforts.

Prior to the development of an Exxaro coal mine in Mpumalanga, a population of an endangered Highveld succulent species, *Frithia humilis* Burgoyne (Aizoaceae/Mesembryanthemaceae), was saved from extirpation by means of translocation. Three receptor habitats were identified within the distribution range of the species. The largest part of the donor population was transplanted to sandstone outcrops of the Ecca Group (Karoo Supergroup), resulting in four subpopulations residing on geological substrates typical of the species' habitat. The remaining portion of the donor population was experimentally translocated to two habitats containing non-native geologies, namely sedimentary outcrops of the Wilge River Formation (Waterberg Group) and (igneous) felsite oucrops of the Rooiberg Group (Transvaal Supergroup). A control population was identified, occupying Ecca and Dwyka Group (Karoo Supergroup) sediments, as a measure to compare the response of translocated populations.

A monitoring programme, utilising a plant age classification system, was initiated in February of 2010 to elucidate demographic trends and to gauge the response of translocated populations to novel environments. Plant survival, plant growth, flowering, fruiting (representing reproductive response) and seedling emergence were chosen as indicators to measure translocation success over the short term. Furthermore, quantitative and qualitative entomological investigations into the identity of possible *F. humilis* pollinators, as well as the presence of pollinator species at receptor habitats, were made.

A repeatable methodology for post-translocation monitoring and scientifically sound baseline data for future comparative purposes were successfully established. Initial results showed that *F. humilis* subpopulations replanted on Ecca standstones had positive responses to translocation: Subpopulations survived and all but one increased in size. Individual plant growth increased, higher reproductive output was evident and seedling emergence was pervasive. Positive responses indicated that *F. humilis* populations translocated onto typical geologies had the potential to establish and persist over three years. Knowledge of this early success is of immense value to the conservation of the species, as a limited number of known natural populations remain. Coal mining, targeting coal seams underlying typical *F. humilis* habitats, is also likely to remain a threat.

The viability of translocating *F. humilis* populations to non-typical geological substrates has shown limited efficacy. Poor survival along with inferior reproductive response confirmed Wilge River Formation outcrops as poor receptor sites for translocated *F. humilis* populations. Rooiberg felsite outcrops also proved to be dubious receptor sites, primarily since there was a downward trend in seedling emergence over time, suggesting inferior germination conditions. Nevertheless, translocation to non-native geological substrates did not have disastrous short-term consequences for these populations, since flowering, fruit production and seedling emergence continued, albeit at reduced (or continually declining) rates.

Potential pollinator species of *F. humilis* were not revealed through quantitative surveys of insect diversity. Qualitative surveys proved more efficient and accurate at pinpointing insect pollinator species. This study provided the first evidence of Apidae, Megachilidae (Hymenoptera) and Bombyliidae (Diptera) insect species pollinating *F. humilis.* The generalist nature of the plant-pollinator relationship, as well as the presence of generalist pollinator species at some receptor habitats, probably contributed to the initial positive response of *F. humilis* flowering and fruiting after translocation.

Results from this study, however promising, should be viewed as initial indications of translocation success. The literature review revealed a plethora of literature recommending post-translocation monitoring programmes for five years to several decades. This study confirmed that successful establishment of *F. humilis* can be determined after three years, but that long-term monitoring is required to evaluate persistence.

Keywords: Plant translocation, endangered succulent species, population demographics, pollination ecology

Opsomming

Translokasie het betrekking op die verskuiwing van organismes, deur menslike ingrepe, van een area na ander, geskikte (ontvangs) habitatte. Translokasie kan in ´n bewaringskonteks geïmplementeer word om die bewaring van ´n spesie, bevolkingsrestorasie, en/of ekologiese navorsing te bevorder. Ondanks jarelange internasionaal-gepubliseerde navorsing, bly translokasie ´n kontroversiële taak. Deurlopende degradering en fragmentering van natuurlike habitatte, as gevolg van menslike aktiwiteite, noodsaak egter die gebruik van translokasie in natuurbewaringsaktiwiteite.

´n Bevolking van die bedreigde Hoëveld vetplantspesie, *Frithia humilis* Burgoyne (Aizoaceae/Mesembryanthemaceae), is, met behulp van translokasie, voor die ontwikkeling van ´n Exxaro steenkoolmyn in Mpumalanga, van uitwissing gered. Drie ontvangshabitatte is binne die verspreidingsgebied van die spesie geïdentifiseer. Die grootste deel van die skenkerbevolking is na sandsteendagsome van die Ecca Groep (Karoo Supergroep) getranslokeer. Vier subbevolkings is sodoende na geologiese substraat tipies vir die spesie herplant. Die oorblywende plante in die skenkerbevolking is eksperimenteel na twee nie-tipiese geologiese habitatte verplant, naamlik sedimentêre dagsome van die Wilge Rivier Formasie (Waterberg Groep) en felsitiese stollingsgesteentes van die Rooiberg Groep (Transvaal Supergroep). ´n Kontrolebevolking, wat Ecca- en Dwyka Groep (Karoo Supergroep) sedimentêre gesteentes beset, is uitgeken. Die reaksie van die getranslokeerde bevolkings kon dus in vergelyking met die kontrole gemeet word.

´n Moniteringsprogram is in Februarie 2010 van stapel gestuur. Daar is van ´n ouderdomsklassifikasie-stelsel gebruik gemaak om demografiese tendense, sowel as die reaksies van getranslokeerde bevolkings op hul nuwe omgewings, aan te toon. Die reaksies is gemeet aan die hand van die volgende aanduiders van translokasiesukses oor die korttermyn: oorlewing en groei van plante, blom- en vrugproduksie (verteenwoordigend van voortplantingsrespons) en saailingopkoms. Kwantitatiewe- en kwalitatiewe entomologiese ondersoeke is verder ingespan om moontlike *F. humilis* bestuiwers te identifiseer, asook om die teenwoordigheid van bestuiwerspesies in die ontvangshabitatte te bevestig.

´n Herhaalbare metodologie vir na-translokasie-monitering en betroubare grondslagdata vir toekomstige vergelykende studies, is suksesvol gevestig. Aanvanklike resultate het aangetoon dat *F. humilis* plante wat na Ecca sandstene getranslokeer is, positief op translokasie gereageer het: Die subbevolkings het oorleef en almal (met een uitsondering), het in getalle toegeneem. Individuele plante het groei aangetoon, voortplantingsuitsette is gelewer en die ontkieming van saailinge was algemeen. Hierdie positiewe reaksies het aangetoon dat *F. humilis* bevolkings wat na tipiese geologieë herplant is die potensiaal om te vestig en oor drie jaar te oorleef, het. Kennis van hierdie vroeë sukses is van onmeetbare waarde vir die bewaring van die spesie, aangesien daar slegs ´n beperkte aantal natuurlike bevolkings bestaan. Mynboubedrywighede, wat steenkool onder tipiese *F. humilis* habitatte teiken, sal ook waarskynlik ´n blywende bedreiging wees.

Die poging om *F. humilis* bevolkings na nie-tipiese geologiese substrate te translokeer, het beperkte effektiwiteit getoon. Swakker oorlewings- en voortplantingsresponse op Wilge Rivier Formasie dagsome het hierdie geologie as ongepaste ontvangshabitatte vir getranslokeerde *F. humilis* bevolkings bevestig. Rooiberg felsiet dagsome is ook as twyfelagtige ontvangshabitatte aangetoon, hoofsaaklik omdat daar `n afwaartse neiging in die getal saailinge, aanduidend van minder gunstige ontkiemingtoestande, oor tyd was. Translokasie na nie-tipiese geologieë het egter nie rampspoedige korttermyn gevolge vir die bevolkings gehad nie, omdat blom- en vrugproduksie, sowel as ontkieming, hoewel teen verlaagde (en dalende) koerste, tog teenwoordig was.

Moontlike bestuiwerspesies van *F. humilis* is nie deur kwantitatiewe opnames van insekdiversiteit uitgewys nie. Insekbestuiwers is meer effektief en akkuraat deur kwalitatiewe opnames geïdentifiseer. Hierdie studie het vir die eerste keer aangedui dat Apidae, Megachilidae (Hymenoptera) en Bombyliidae (Diptera) insekspesies verantwoordelik is vir die bestuiwing van *F. humilis*. Die generalistiese aard van die plant-bestuiwerverhouding, sowel as die teenwoordigheid van bestuiwings-generaliste in sommige ontvangshabitatte, het waarskynlik bygedra tot die positiewe blom- en vrugproduksie van getranslokeerde *F. humilis* bevolkings.

Belowende resultate van hierdie studie moet egter slegs as aanvanklike aanduidings van translokasie sukses geïnterpreteer word. Die literatuurstudie het ´n magdom literatuur uitgewys wat die voortsetting van moniteringprogramme na translokasie vir vyf jaar tot ´n paar dekades, aanbeveel. Hierdie studie bevestig dat suksesvolle vestiging van *F. humilis* na drie jaar bepaal kan word, maar dat volgehoue monitering nodig is om die lang-termyn oorlewing van die bevokings te evalueer.

Sleutelwoorde: Plant translokasie, bedreigde vetplantspesie, bevolkingsdemografie, bestuiwingsekologie

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SOLI DEO GLORIA

Consider the lilies of the field, how they grow: they neither toil nor spin, yet I tell you, even Solomon in all his glory was not arrayed like one of these.

Matthew 6:28, 29

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Chapter 1. Introduction

1.1 *Frithia humilis* – an endangered Highveld succulent

Frithia humilis Burgoyne (Mesembryanthemaceae) is a rare window plant (Figure 1.1A) commonly known as ‘fairy elephant’s feet’ (Burgoyne *et al.,* 2000a, Burgoyne *et al.,* 2000b). This dwarf succulent, rarely protruding more than 30 mm above ground, has contractile leaves enabling the entire plant to retract beneath the soil (Figure 1.1B) during dry winter months (Burgoyne *et al.,* 2000b). This growth habit consequently protects the plant from desiccation and other environmental stressors, while remaining undetectable to herbivores.

Openings in the soil surface left by the contracted cylindrical leaves (Figure 1.1B) are the only way to detect the species in its habitat during dry months (Burgoyne *et al.,* 2000b). These openings allow light to reach the retracted window-tipped leaves, i.e. a concentration of translucent cells on the adaxial leaf surface. Light is effectually concentrated by the translucent tissue, enabling photosynthesis to continue throughout the dry season, without excessive water loss to the plant (Egbert *et al.,* 2008).

Frithia humilis is perennial. After its winter dormancy, and following the first rainfall event of the season (usually during October), the succulent leaves will emerge. Cells in the leaves, arranged in ‘columnar, axial rows’ (Burgoyne *et al.,* 2000b), which were shrunken due to dehydration during dry periods, will rehydrate. Tangential walls of the cells will swell and inflate the leaves until they are pushed above the soil surface.

Its seasonal appearance and small size makes *F. humilis* a cryptic species. It is only conspicuous when it bears small white or pinkish flowers (Figure 1.2A, B) during summer, from November to February. The flowers are 15−20 mm in diameter and it is hypothesised that the flowers turn pink after being pollinated (Burgoyne *et al*., 2000b). Hitherto no studies have been done on the pollination biology of the species. Fruits are formed between the fleshy sepals and are barrel-shaped, hydrochastic capsules that burst open shortly after ripening, releasing a multitude of tiny seed (Burgoyne *et al.,* 2000b). The branching roots of *F. humilis* are able to establish in shallow soil (approximately 50 mm deep) derived from sandstone plates typical of the Vryheid Formation, Ecca

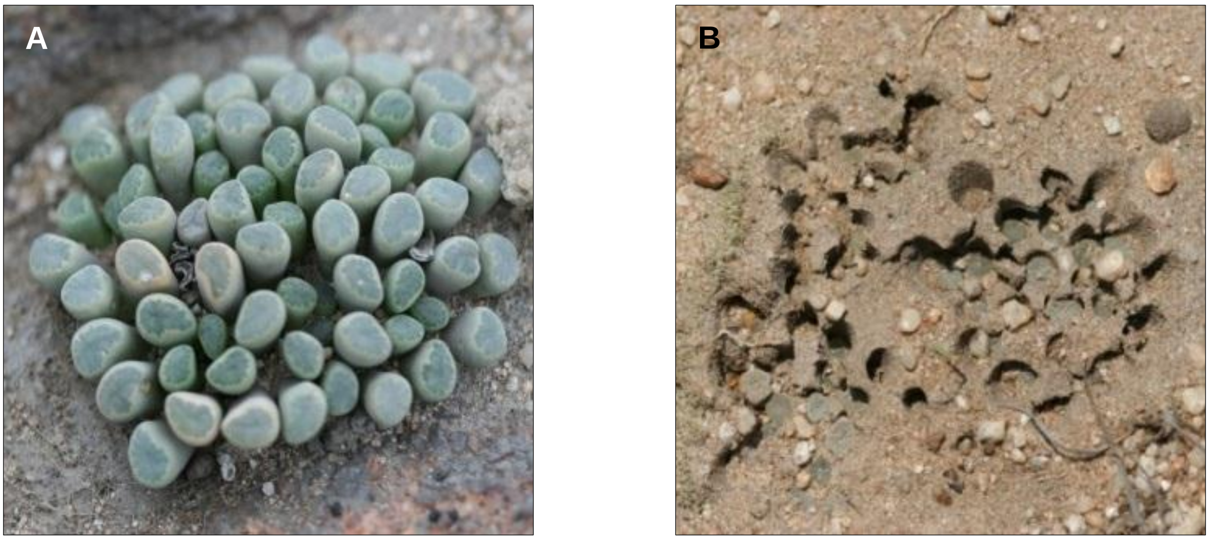

Figure 1.1 The finger-like leaves of *Frithia humilis* (A) is tipped with translucent tissue, enabling light penetration for photosynthesis. Plants have contractile leaves that can retract beneath the soil during dry, winter months (B)– thus protecting the plant from desiccation (Photos: J.H.L. Smit, 2009).

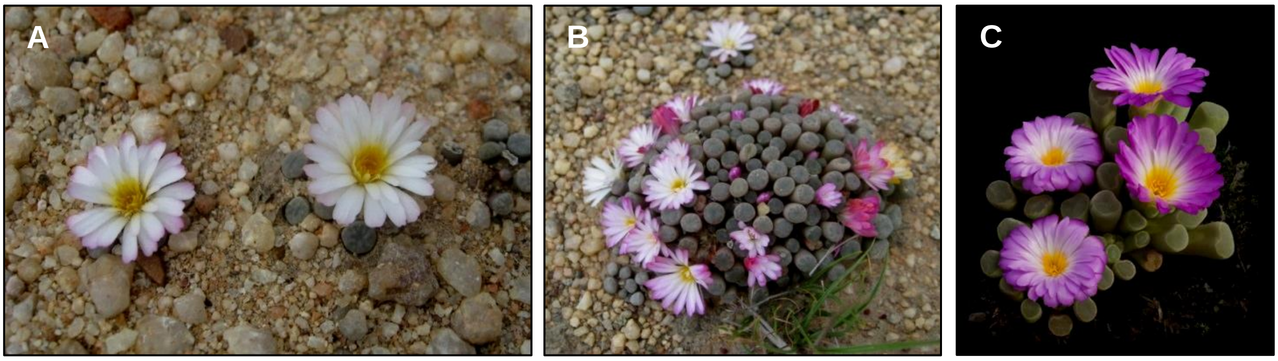

Figure 1.2. *Frithia humilis* bears small, but conspicuous white (A) or pale pink flowers (B) during the summer rainy season, from November to March (Photos: P.M. Harris, 2012). *Frithia pulchra* (C)*,* the only other species in the genus bears bright pink flowers (Photo: Angus, 2006).

Group (Karoo Supergroup) (Figure 1.3).

Frithia humilis, along with the only other member of the genus, *F. pulchra* (Table 1.1 and Figure 1.2C), belongs to the *vygie* family or Mesembryanthemaceae (Aizoaceae). The majority of this extensive family occurs within the Succulent Karoo Biome, a winter rainfall region with an annual rainfall of 10−300 mm (Ihlenfeldt, 1994). *Frithia* species, however, are exceptions. They are of the few mesemb taxa found outside the arid distribution range of the group (Chesselet *et al.,* 2002) and specifically in the moister parts (>600 mm per annum) of the summer rainfall region (Burgoyne *et al.,* 2000b). The two species in the *Frithia* genus have allopatric distribution ranges.

Frithia pulchra occurs on quartzite in the Magaliesberg mountain range in the North-West and Gauteng provinces, whereas *F. humilis* is found on sandstone outcrops in Mpumalanga (Figure 1.4), where it is regarded as endemic (Burgoyne & Krynauw, 2005). In *F. humilis* habitat the weathering product of the porous mother rock is coarse, well-drained gravel. Gravel grains are essential to seedling emergence and establishment of the species, as these substrates are the only

Table 1.1. Comparison between *Frithia humilis* and *F. pulchra,* adapted from Burgoyne *et al.* (2000a).

Characteristic	*Frithia humilis*	*Frithia pulchra*
Morphology		
Roots	Fleshy	Fibrous
Leaves:		
Length	<15 mm	15-25 mm
Colour	Green with brown/purple tinge	Green with blue tinge
Windows	Concave	Convex when turgid, concave when flaccid
Leaf edges	Crenulated markings	Crenulated markings rare
Flowers		
Diameter	15 – 20 mm	25 – 30 mm
Centre colour	Yellow	Yellow / white
Petal colour	White (often pink-tipped) or pink	Bright magenta pink
Petal shape	Acuminate tips	Rounded tips
Petal number	20 – 30	30 – 45
Pollen		
Granules	Arranged in orderly patterns around perforations	Randomly arranged around perforations
Lumen size	Comparatively equal	Differing
Capsules	Thin, fragile tissue enclosing capsule; light brown expanding keels	Thick tissue surrounding capsule; dark brown expanding keels
Seed	Tip attached to funicle rounded	Tip attached to funicle sharper
Ecology		
Distribution	Between Cullinan and Bronkhorstspruit, Gauteng and eMalahleni, Middelburg and Ogies, Mpumalanga	Magaliesburg, Gauteng to Rustenburg, North-West
Lithology	Dwyka & Ecca Groups, Karoo Supergroup	Magaliesberg Formation, Transvaal Supergroup
Habitat	Exposed sandstone plates, very shallow soils derived from coarse sediments	Sandstone plated edges; shallow, coarse quartz soils
Conservation status		
Number of populations	13	1, continuous
Extent of occurrence	2987 km²	± 100 km²
Area of occupancy	± 2.5 ha	± 0.14 km²
Population decline	Continuous	None
Habitat fragmentation	Severe	None
Threats	Expanding informal settlements; over-grazing, alien plant invasion; horticultural collection, coal mining and prospecting	None
Protected populations	1	1
IUCN category	Endangered (EN)	Rare

Figure 1.3. A typical *Frithia humilis* habitat is a xeric patch of shallow, well-drained soil situated in a matrix of grassland. The patch is indicated by a red circle (Photo: J.H.L. Smit, 2009).

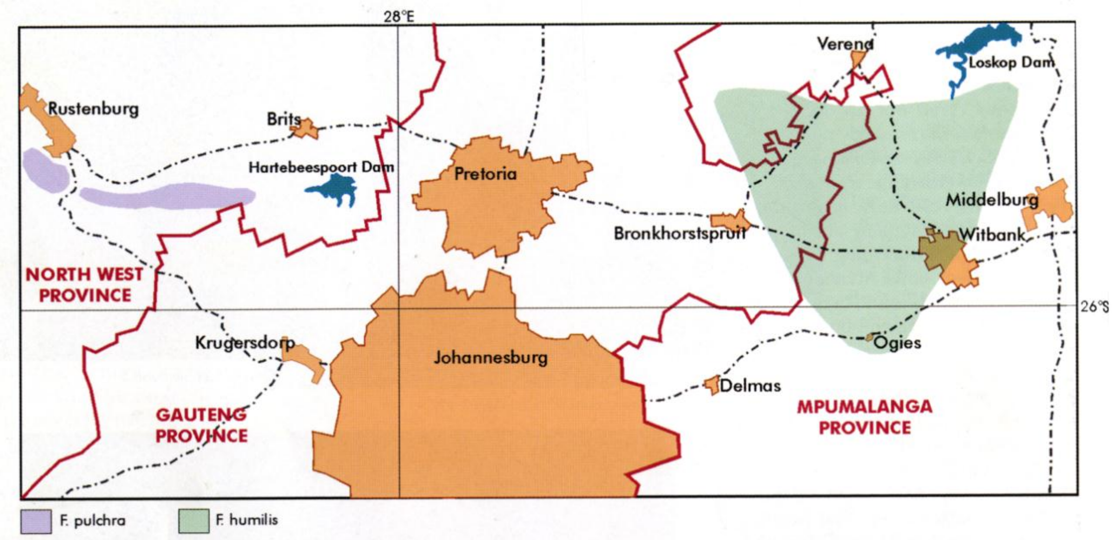

Figure 1.4. Allopatric distribution ranges of *Frithia pulchra* and *F. humilis* (Burgoyne *et al.,* 2000a). Both lie within the summer rainfall region of South Africa.

protection afforded to the minute seedlings on the exposed sandstone plates (Burgoyne *et al.,* 2000b). This preference for shallow soil on elevated rocky outcrops (1369 – 1616 m.a.s.l.), i.e. non-arable land, places it outside the threat of agricultural expansion or urbanisation in Mpumalanga (Burgoyne *et al.,* 2000b).

Frithia humilis is a naturally rare species (Burgoyne *et al.,* 2000a) and its scarceness is often not regarded as requiring urgent conservation action. However, scarcity should encourage conservation, as rarity is deemed an important element in biodiversity conservation (Bevill & Louda, 1999). The lack of its conservation has now been exposed by coal mining, which in turn has influenced its threat status.

1.2 Conservation status of *Frithia humilis*

Frithia humilis was re-assessed and its conservation status upgraded from vulnerable (VU) to endangered (EN) during 2009, the main reason being habitat destruction by coal mining (Table 1.1). The distribution range of the species is limited (the extent of occurrence being less than 3 000 km^2) and its habitat is fragmented (Burgoyne & Krynauw, 2005). Populations of the species are declining, as habitats are increasingly being transformed by expanding coal mining activities, as well as expanding informal settlements, overgrazing, alien vegetation and unscrupulous collecting for the horticulture trade (Burgoyne & Krynauw, 2005).

Mining activities around *F. humilis* habitats are focused on the Witbank coal seam, with opencast mining being the preferred method for coal extraction (Cairncross, 2001). Coal mines threaten *F. humilis* habitats. Ecca Group sandstones, one of the predominant underlying geological structures of *F. humilis* habitats (Burgoyne *et al.,* 2000b), often overlay the coal-bearing seams (Cairncross, 2001).

1.3 Mining in Mpumalanga

Coal is the primary energy source for South Africa (Subramoney *et al.,* 2009). Mpumalanga contains the largest and most easily accessible coal reserves in the country, i.e. the Witbank coal field (Fourie *et al.,* 2008), and collieries dominate the province's mining industry (Mpumalanga Provincial Government, 2009). Energy demands are ever rising to keep pace with the growing economy of the country (Subramoney *et al.,* 2009) and electricity plants within the Witbank Coalfield supplies approximately 75% of South Africa's electricity. However, it is predicted that South African coal production, primarily from the Witbank Coalfield, will peak in 2020, after which mineable coal reserves will diminish or become exhausted (Hartnady, 2010).

1.4 Project history

One of 11 naturally occurring *F. humilis* populations (Burgoyne & Krynauw, 2005), occupying an area north of Witbank, Mpumalanga (quarter degree grid 2529CC) licensed for coal mining by the Exxaro mining group, became threatened by mining activities (McCleland, 2009; De Castro & Brits Ecological Consultants, 2007). *In situ* conservation was deemed unfeasible, due to the future isolated nature of the preserved habitat amidst the rehabilitated mining area and proposed agricultural land-use. Hence, in an attempt to save the population, it was translocated to suitable habitats. The translocation of this taxon was launched in 2009 by representatives of Inyanda coal mine (Exxaro mining group), the South African National Biodiversity Institute (SANBI), and Mpumalanga Tourism and Parks Agency (Burgoyne & Hoffman, 2011). The translocation effort was regarded as viable, since other Mesembryanthemaceae species (*Ruschia*, *Drosanthemum*, *Malephora*, *Delosperma* and *Lampranthus* genera) have been sucessfully translocated in the Succulent Karoo, a winter rainfall region (Blignaut & Milton, 2005).

The donor population was translocated as three separate populations – the largest population was translocated to a geologically similar habitat (Burgoyne & Hoffman, 2011) and two smaller populations were experimentally moved to non-typical habitats. These populations were replanted in the late winter of 2009 (Burgoyne & Hoffman, 2011). SANBI and Exxaro were responsible for decision-making prior to and during the translocation procedure itself. This study only considered post-translocation monitoring which commenced in 2010.

In compliance with IUCN guidelines for species translocations, the following was taken into account during the *F. humilis* translocation (IUCN, 1998; Burgoyne & Hoffman, 2011):

i. Basic ecological data on *F. humilis* was available (Burgoyne *et al.,* 2000a; Burgoyne *et al.,* 2000b; Burgoyne, 2001).
ii. Participants included local farmers and investors, who supported the translocation effort and long-term protection of the populations. No human population was impacted, either socially or economically.
iii. Government permission was obtained via the Mpumalanga Tourism and Parks Agency.
iv. A strategy addressing site preparation for translocation, the translocation process itself and post-translocation monitoring was developed, the latter of which is entailed in this study.

A detailed discussion of the translocation concept and its implementation as a conservation strategy in the instance of *F. humilis*, as well as important actions surrounding the success of a translocation strategy, will be discussed in Chapter 2 (literature review).

1.5 Aims and objectives

The main aim was three-fold and broken down into specific objectives.

1.5.1 Long-term monitoring

The establishment of *F. humilis* populations had to be monitored after the translocation event to determine the successes of the project. A long-term post-translocation monitoring programme (Maschinski *et al.,* 2004) for translocated and control populations were initiated to:

i. gather baseline population numbers six months after translocation for future comparative purposes as no counts were made during the translocation event itself. Baseline data represents an *initial* evaluation of the translocation effort, as at least five to ten years of monitoring would provide enough data to accurately and fairly evaluate translocation success (Jusaitis *et al.,* 2004).
ii. improve knowledge of *F. humilis* population demography and fecundity through the surveillance of the translocated populations and a natural (control) population.
iii. assess population fluctuations to ensure that proper management actions can be taken to combat severe population declines and promote population persistence.

1.5.2 Experimental translocation

The occurrence of coal seams beneath typical *F. humilis* habitat and the rising demand for coal has placed considerable pressure on the remaining intact habitats (for *in situ* conservation or translocation) of this species (Section 1.3).

i. The suitability of atypical geological habitats as translocation receptor sites for this species had to be explored. Successful translocation onto atypical geologies could provide the species with a lifeline.

1.5.3 Plant-insect relationships

Plant-insect relationships at each study site were investigated, as the pollination vectors of *F. humilis* were unknown. Persistence of the species at the translocation sites is not only dependant on habitat suitability, but also reproductive potential. Therefore:

i. pollinators in a control habitat had to be identified.
ii. the presence of potential pollinators in translocation receptor habitats had to be ascertained.
iii. pollinator diversity had to be measured and compared within and between experimental sites, as well as the control population.

1.6 Hypotheses

i. Translocation would cause a mega-disturbance and therefore translocated populations can be expected to show an initial decline in numbers, after which the populations are expected to stabilise.
ii. If translocated populations establish at receptor sites, the number of reproductively mature individuals would increase, which would result in improved reproductive capability (i.e. flowering, fruit-production and seedling germination).
iii. If populations are translocated to geological substrates similar to that of the control and donor sites, these populations would have a higher survival when compared to populations translocated to non-typcial geologies.
iv. If *Frithia humilis* populations are translocated to novel habitats, pollination would take place via a complex of generalist flower-visiting arthopods.

1.7 Format of dissertation

This dissertation complies with guidelines for a standard dissertation at the North-West University. and compasses seven chapters. All references cited in the chapters were recorded in a reference list at the end of the dissertation. The results and discussion chapters include a report submitted to the Exxaro mining company (Chapter 4) and two manuscripts which will be submitted to scientific journals (Chapters 5 and 6). Duplication, especially of literature, methodology and selected results, was unavoidable.

Chapter 2: Literature review

Relevant literature on the topic of translocation and pollination ecology, relevant to the translocation of *F. humilis* is thoroughly discussed in this chapter. The term ‘translocation’ is clarified and actions and/or considerations during and after translocation are succinctly discussed to show the complexity of the procedure. Furthermore, theoretical predictions of possible *F. humilis* pollinators are made.

Chapter 3: Materials and methods

The general methodology applied in this study is described, including a description of the study areas and experimental design. Detailed descriptions of important methods for subsequent chapters are not covered in Chapter 3, to avoid unnecessary duplication. These methods are discussed in detail in the relevant chapters.

Chapter 4: Short-term translocation success of *Frithia humilis* populations in terms of survival and plant growth: a baseline for future studies

Population trends in the translocated populations were studied to determine the initial success of translocation, as well as to establish a baseline for future studies. These trends were quantified in terms of general plant survival and growth, measured over three years of post-translocation monitoring. Subsequently, the results informed an evaluation of translocation procedures and decisions. This chapter is the official report submitted to Inyanda coal mine (Exxaro).

Chapter 5: Feasibility of translocating *Frithia humilis,* an endangered edaphic specialist, to atypical geological habitats

This chapter explores the viability of translocating *F. humilis* populations to different geological substrates. The response of the experimental populations (in terms of demographic and reproductive trends) to different geologies was compared to trends observed in a control population. As one of the first translocation projects of its kind in South Africa, these early responses of populations to different geologies can inform the potential of future efforts to relocate edaphic specialists of the Mesembryanthemaceae. This chapter is being prepared as a manuscript for submission to *Folia Geobotanica*.

Chapter 6: Pollination ecology of *Frithia humilis*

Little is known about the life cycle of the species and this chapter aims to elucidate some aspects thereof, especially pertaining to pollination ecology. Quantitative and qualitative entomological surveys were employed in an attempt to reveal *F. humilis* pollinators in the control habitat and in translocation localities. This chapter is being prepared as a manuscript for a short communication to an entomological journal.

Chapter 7: General conclusions

This chapter summarises the key findings of the study and contributes to our knowledge on *F. humilis* and translocation of Mesembryanthemaceae species.

Chapter 2. Literature Review

2.1 Introduction

Human demands on natural resources are escalating. Consequently, habitat degradation, fragmentation and destruction, as well as invasive species and climate change are realities of the modern ecological context (Godefroid *et al.,* 2011; Weeks *et al.,* 2011). Pressure exerted on natural populations of rare and/or endangered species by the demands and actions (e.g. land use – agricultural, urban or industrial) of ever increasing human populations gives rise to conservation challenges (Wendelberger & Maschinski, 2009). In fact, habitat fragmentation is often the main driver of local species extinction (Heinken & Weber, 2013).

2.2 Translocation

Human threats to endangered and/or endemic plant species necessitate the alleviation of human-wildlife conflict through translocation (Fahselt, 2007; Seddon, 2010; Godefroid *et al.,* 2011). Typical examples were given by Allen (1994), Milton *et al.* (1999), Mueck (2000) and Maschinski *et al.* (2004). In many instances, human assistance in the recovery of endangered species can be vital to enhancing and upholding biodiversity (Weeks *et al.,* 2011).

Therefore, translocation of a population of species can be a valuable ecological mitigation or conservation tool (Griffith *et al.,* 1989), especially in the face of rapid environmental change (Weeks *et al.,* 2011). This holds true for species of which the natural habitat cannot be preserved and when natural recruitment and dispersal cannot be maintained (Seddon, 2010). Translocating species and communities for their recovery from irreparable habitats, for boosting failing populations or for establishing new ones (Fahselt, 2007) have become more frequent during the past two decades (Bullock, 1998; Milton *et al.,* 1999). However, translocations in a conservation context have been reported as early as the 1950's (Murphy *et al.,* 2008).

Concepts from reintroduction biology and restoration biology can be deemed applicable to the context of the *Frithia humilis* translocation, since translocation of any kind is a major disturbance and the newly planted population is in need of restoration (Mueck, 2000).

2.3 Definitions

Considerable confusion and inconsistency exist regarding the terminology of translocation of natural populations (Armstrong & Seddon, 2008). The World Conservation Union (IUCN) has consolidated 'clear, simple and workable' definitions of translocation (IUCN, 1987; Armstrong & Seddon, 2008). 'Translocation' can be used as a 'catch-all term' (Hodder & Bullock, 1997; Armstrong & Seddon, 2008) for any intentional, human-mediated movement of individuals/populations from one area to another. **Translocation** is subsequently categorised as follows (Figure 2.1):

i. **Introduction**: *The human mediated dispersal, either accidental or deliberate, of a living organism outside its historical distribution range, e.g. alien invasive species and/or economically significant crops* (IUCN, 1987). Introductions can be benign, if conservation is envisaged, i.e. conservation introduction (Seddon, 2010), but is recommended only for populations for which there is no viable habitat available within the species' historic range (IUCN, 1998). Seddon (2010) identified two justifiable types of conservation introduction: ecological replacement and assisted colonisation. The former describes the introduction of the most suitable taxon to fill an ecological niche left empty by an extinction event. Such a taxon should preferably be closely related to the extinct species, ideally being sub-specific, or should be functionally similar in order to restore lost ecological function. 'Assisted colonisation' or 'assisted migration' refers specifically to the deliberate movement of a population to a habitat beyond the natural distribution range of the species (Hewitt *et al.*, 2011). The aim of assisted colonisation is to protect the species from human-induced threats, including climate change, urbanisation, industrialisation and agricultural development (Godefroid *et al.,* 2011). It also refers to introductions that take place within the focal species' known distribution range, but to sites where populations have not been known to occur.

ii. **Re-introduction**: *The intentional movement of an organism to a part of its historical distribution range, from which it disappeared due to human activities or natural disaster* (IUCN, 1987). The definition can be refined to indicate the eventual reestablishment of a viable, self-sustaining population in a habitat from which the species has been extirpated (IUCN, 1998, Seddon, 2010), thereby possibly increasing the distribution range of the species (Rout *et al.,* 2007). Prior to re-introduction, the causes of initial species decline should have been investigated and removed from the receptor habitat (IUCN, 1998).

iii. **Re-stocking**: *The movement of organisms with the purpose of reinforcing, supplementing, augmenting or enhancing a population of con-specifics in a natural habitat* (IUCN, 1987; Seddon, 2010). In a botanical context, re-stocking is a method used to overcome natural dispersal barriers (in a fragmented habitat, for example), to accelerate population growth or increase population size (Godefroid *et al.,* 2011), as well as to improve genetic diversity and

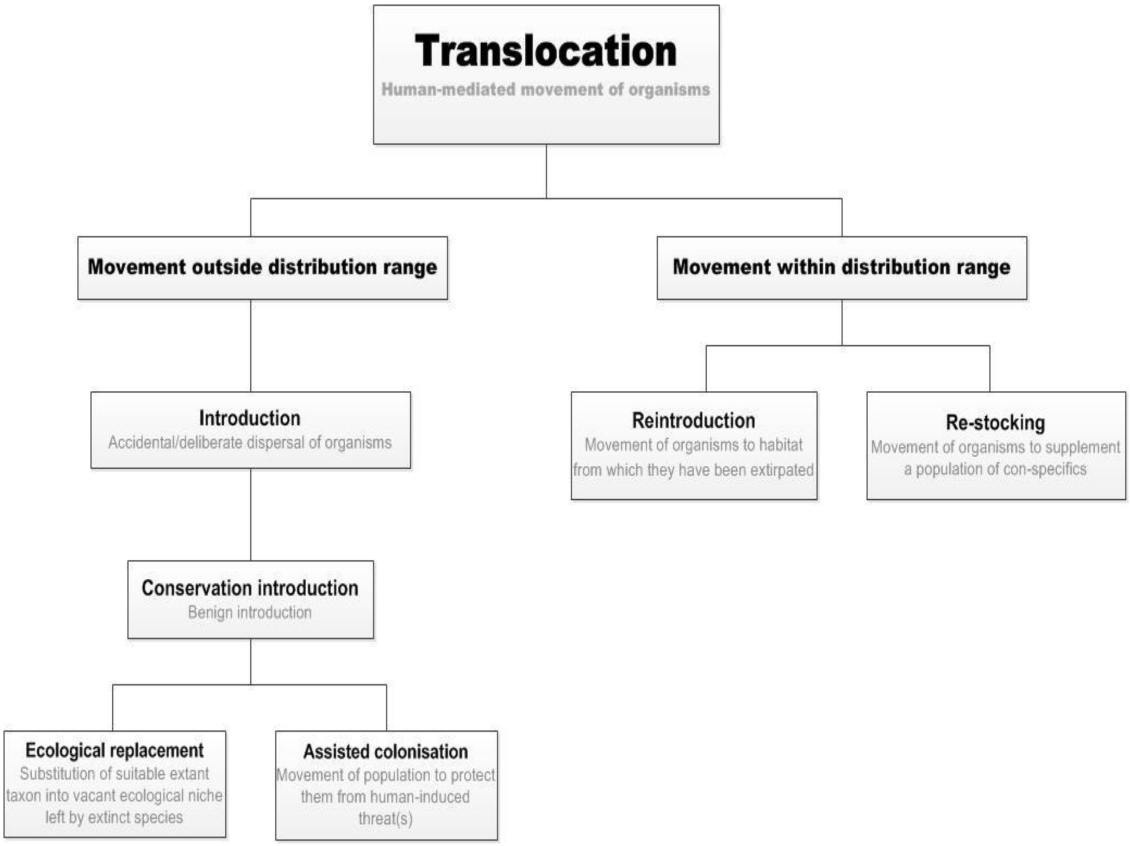

Figure 2.1. A simplified classification of translocation terms (adapted from Seddon, 2010)[1].

> prevent inbreeding depression (Seddon, 2010). However, if the management (i.e. ‘mitigation of limiting factors’) of such augmented populations is poor, the goal of re-stocking – to improve the viability of a population – will not be reached (Seddon, 2010).

The term ‘translocation’ can also be viewed as distinct from *introduction*, *reintroduction* and *re-stocking* (Gordon, 1994; Guerrant & Kaye, 2007), albeit functionally equivalent (Pavlik, 1996). Translocation, in this sense, infers the removal and transplantation of a naturally occurring population from one area to another within its distribution range.

Translocation efforts may differ in focus – a single species (refer to Section 2.3.10 for examples) or a community (e.g. Bullock, 1998; Fahselt, 2007) may be translocated. Considering the focus of this study, this review will focus on single species translocations, which are the most frequently described scale of translocation and are ‘the most relevant management unit’ for species conservation (Albrecht & Maschinski, 2012).

In summary, Gordon (1994) distinguished three general goals of translocation: (1) rescue or mitigation – to move individuals or a population that would alternatively be destroyed by

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[1] Although not explicitly documented in literature, assisted colonisation within the target species’ distribution range is practiced as a conservation measure in South Africa (Siebert, 2014).

encroaching human development or other forms of habitat loss, (2) restoration of populations, communities and/or ecological processes, especially within or to protected areas and (3) research on target species. Translocations can therefore increase the number of populations, enhance metapopulation dynamics and establish viable, self-sustaining populations (Menges, 2008), increasing the likelihood of species survival (Guerrant & Kaye, 2007).

The following literature review will discuss the broad principles of translocation to facilitate an objective view of the *F. humilis* translocation process. The term ‘translocation’ will be applied broadly to include mainly introduction and reintroduction efforts to unoccupied receptor sites for conservation and population restoration purposes (Drayton & Primack, 2012).

2.3.1 Ecological restoration and conservation significance

Translocation is generally viewed as a restorative conservation method (Seddon, 2010). As a conservation strategy, it has been investigated for almost three decades and it is viewed as a ‘powerful tool for the management of the natural...environment’ (IUCN, 1987). Drayton & Primack (2012) described conservation translocation as a ‘well-established standard technique in conservation and restoration ecology’, with numerous governmental bodies and international agencies implementing translocation regimes (Hewitt *et al*., 2011). In this context, translocations can attempt to initiate new populations of endangered species, can introduce populations to more suitable or safe habitats, can increase the total number of conserved populations and can improve genetic diversity amongst isolated and/or small populations. Translocation can also aid the long-term survival of species and eventually ensure the evolution of that species (Milton *et al.,* 1999; Fahselt, 2007), as well as attempt to initiate new populations of species that are endangered (Fahselt, 2007).

The success of a translocation may be positive beyond the establishment of new populations. Translocations can be conducted to improve ecosystem functioning where a loss of biodiversity has occurred by introducing keystone predators, herbivores or mutualists and the presence of keystone species may also trigger beneficial ecological effects (Menges, 2008). Overcrowded populations can be managed via translocation (e.g. through re-stocking) as opposed to culling (Milton *et al.,* 1999). Translocated species may facilitate the establishment of other species (increasing local diversity) or inhibit the establishment of detrimental species (weeds/alien invasives). Furthermore, translocating ecological communities can aid the stabilisation of degraded habitats (Blignaut & Milton, 2005; Fahselt, 2007).

However, translocation is a risky and invasive conservation strategy (Allen, 1994). It is nevertheless an important conservation tool, especially due to rapid climate change and dispersal limitations (Seddon, 2010) resulting from impeding human activities, such as coal mining in the case of *F. humilis* (Burgoyne & Hoffman, 2011).

2.3.2 When is translocation successful?

Two important concepts are applicable to the measurement of translocation success: Firstly, the establishment of a population in a novel environment and secondly, the long-term survival (sustainability) of that population (Armstrong & Seddon, 2008). A population can only be viewed as fully established when it is self-sustaining and persistent (Griffith *et al.,* 1989; Menges, 2008). Furthermore, such populations should have adequate genetic resources to ensure adaptive evolution (Guerrant & Kaye, 2007).

Pavlik (1996) mentioned four milestones by which translocation success can be measured: abundance, extent, resilience and persistence. The former two goals may develop over the short term (1-10 years after translocation), while the latter two can only be tested over long time spans – one to several decades for most species. Newly planted populations should be able to perform basic life cycle processes, such as establishment, reproduction and dispersal ‘in the wild’ (Pavlik *et al.,* 1993). Parsons & Zedler (1997) argued that population stability should be assessed via long-term monitoring (5-15 years) across varying climate conditions.

Initial stages of population establishment and viability can be measured by seedling recruitment (as well as germination and emergence), plant growth and plant reproduction. Accordingly, reproductive success should be quantified by, for instance, the seed output per individual (Menges, 2008) or by the extent of flowering and fruit-bearing (Godefroid *et al.,* 2011).

Tracking the survival of transplants is not the only measurement of initial translocation success. Individual plant growth can also indicate the suitability of the receptor habitat (Menges, 2008) – if the habitat were not suitable, plants would not be able to grow. Seedling recruitment is an especially important parameter to measure, as it indicates the ability of the population to develop successive generations (Godefroid *et al.,* 2011).

Fahselt (2007) mentioned the retention of transplant function within the ecosystem. This includes productivity, nutrient recycling, seed dispersal, pollination, allelopathic interactions and food chain relationships. Such parameters can be compared with that of natural populations to determine the similarities and ‘limitations’ within the translocated population.

A restoration target is an important part of measuring translocation success (Seddon, 2010). Setting such targets can, however, be a challenge in the face of expanding human influence and rapid climate change, as well as the dynamic nature of ecosystems (Seddon, 2010). Restoration targets are usually prescribed by intact natural populations. Fahselt (2007) emphasised the importance of natural (control) populations as a benchmark to quantify changes in translocated populations. Such populations can aid the study of succession, ecosystem functioning, reproduction, interspecific interactions and stress responses in particular species. It can also display vegetation characteristics best suited for particular habitats and serve as genetic reservoirs (Fahselt, 2007).

2.3.3 Challenges and considerations

Although translocation is increasingly being viewed as a suitable conservation method for endangered species (Seddon, 2010), the financial and genetic risks undertaken are great (Milton *et al.,* 1999). Translocations are infamous for their low probability of success (Mueck, 2000) and present a significant disturbance to the translocated population (Fahselt, 2007). Endangered or rare species are most often targets of translocation. Their narrow habitat requirements are, however, more complex than that of common species (Fahselt, 2007) and therefore the outcomes of endangered species translocations are more dubious.

Despite decades of research on the subject of species translocation (Guerrent, 2012), many conservation biologists still view this science as being a debatable solution to biodiversity conservation issues (Hewitt *et al*., 2011), as the efficacy thereof has not yet improved significantly (Fahselt, 2007). In all probability, results of many translocation outcomes remain unpublished, presumably due to failure, which is deemed unsuitable for publication (Fahselt, 2007). Protocols and case studies of failed translocation efforts are also not prevalent in scientific literature (Godefroid *et al.,* 2011). Guerrant & Kaye (2007) argued that if a translocation attempt is approached as a scientific experiment, with clear, publishable methodology, valuable information can be gained, whatever the outcome may be. Hypotheses about demographical processes, population genetics, ecological interactions and other factors influencing population establishment can be (re)formulated and elucidated.

The end-result of any translocation remains uncertain until a population is unquestionably established (Allen, 1994; Menges, 2008) and able to reproduce and adapt to a changing environment (Milton *et al.,* 1999). However, translocations often ‘fail to create a restoration trajectory towards reference sites’ (Menges, 2008).

Although each translocation effort is unique regarding the focal species, purpose of translocation, hypotheses posed and external influences, some influential factors are common to most translocations (Guerrant & Kaye, 2007) (Figure 2.2).

2.3.4 General/technical considerations

Investigation into and planning of the translocation is vitally important. The desirability of the translocation should be critically assessed to improve translocation success rates (IUCN, 1987). Due to factors threatening the immediate survival of the focal species, translocation is usually applied urgently at short notice (Allen, 1994; Burgoyne & Hoffman, 2011). In such cases, translocation is an ‘emergency response’ to a conservation dilemma and applied as a ‘last resort’.

2.2.5.1 Species-specific knowledge

Biological and ecological information on the target species is essential (Gordon, 1994 & 1996), especially with regard to critical life stages, factors determining habitat quality, species interactions

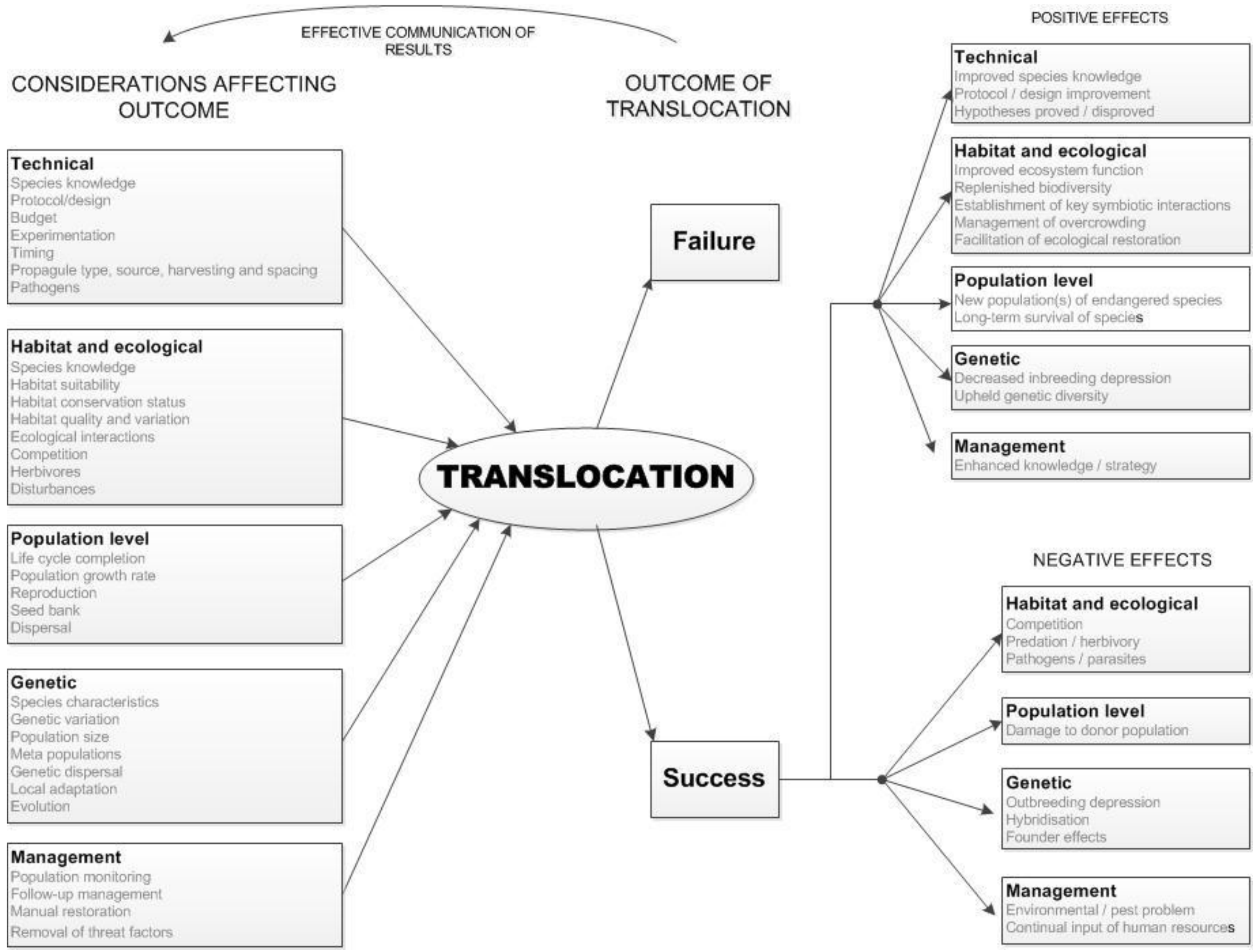

Figure 2.2. Factors relevant in species translocations for conservation purposes (adapted from Hodder & Bullock, 1997). These parameters need to be considered prior to, during and after the translocation event.

and minimum size of the receptor habitat (Griffith *et al.,* 1989). In fact, Parsons & Zedler (1997) reported a lack of such knowledge to be a direct cause of translocation failure. Further understanding of factors restricting establishment and growth of habitat specialists (e.g. edaphic endemics such as *F. humilis*) can aid the identification of suitable translocation habitats (Maschinski *et al*., 2004). Unfortunately, habitat requirements of endangered or rare species are generally poorlyunderstood (Fahselt, 2007).

2.2.5.2 Translocation protocol/design

Translocations of plant species can benefit greatly from attentive protocol design, site selection, appropriate planting techniques and follow-up monitoring (Maschinski *et al.,* 2004), even more so when a translocation attempt is approached as a scientific experiment (Guerrant & Kaye, 2007). Consequent results should supplement deficient biological information on the target species and inform management needs of the species (Gordon, 1996). Gordon (1994 & 1996) emphasised the importance of documenting the rationale and methodology before translocation ensues.

2.2.5.3 Budget allocation

Financial aspects of translocation should not be neglected, as this will determine the extent, perpetuation and efficacy of all other considerations, especially regarding endangered/rare species translocation (Haight *et al.,* 2000). Managerial and post-translocation follow-up is of particular financial concern as future funding is often uncertain (Haight *et al.,* 2000).

2.2.5.4. Technique/strategy: translocations as scientific experiments

A well-devised translocation strategy is vital (Fahselt, 2007) and involves parameters such the number of plants translocated to specific/various suitable habitats and the distribution of those populations (Pavlik, 1996). Experimenting with such parameters may optimise future translocation attempts (Armstrong & Seddon, 2008). Trial translocations to multiple sites should preferably be conducted to increase the probability of future translocation success (Jusaitis *et al.,* 2004). It may also reveal clues regarding species' habitat requirements (Falk *et al*., 1996). Through trial translocations the type and number of founder propagules, micro-habitat requirements, community dynamics, population establishment and expansion rates can be assessed (Jusaitis *et al.,* 2004).

However, experimentating with different translocation strategies, e.g. population size or age of transplants, is mostly not feasible, since it requires 'multiple reintroduction attempts', for which time and financial resources might not be available (Armstrong & Seddon, 2008). Fahselt (2007) pointed out that even if experimentation with translocation strategies were feasible, it probably cannot guarantee translocation success. However, Pavlik *et al.* (1993) argued that if a translocation 'lacks the design and statistical strength of a good experiment', it will fall short of success.

2.2.5.5 Timing of translocation

Guerrant & Kaye (2007) described the timing of translocation as the season or climate in which a translocation was conducted, as well as the number of translocation attempts. Burgoyne & Hoffman (2011) mentioned the dry season as an optimal time for translocating succulents, since fungal infections are less likely to occur.

2.2.5.6 Type of propagule

Direct seeding

Translocation success can depend on the type of propagule used, i.e. adult plants, seedlings or seeds (Jusaitis *et al.,* 2004; Guerrant & Kaye, 2007; Menges, 2008). Seeds can be harvested from wild-growing populations (Ellis & Weis, 2006) or *ex situ* individuals and sown into the novel habitat. Since seeds are easily collected and transported, the use of seeds as propagules reduces risks associated with transplanting seedlings or adult plants (Milton *et al.,* 1999). For this reason they are often the preferred propagules during many translocations (Menges, 2008). Seeding also reduces the potential for artificial selection that takes place in greenhouses and botanical gardens (Menges, 2008). Furthermore, dispersing seeds in a natural manner may possibly increase the eventual genetic diversity of the population (Jusaitis *et al.,* 2004). Seeds should only be used when prolific amounts are available, as seedling emergence and population establishment via seed-sowing are usually low (Milton *et al.,* 1999; Fahselt, 2007). Seedling translocation should therefore be considered if seeds are in short supply (Menges, 2008).

Seedlings

Seedling founders are likely to establish more effectively (Jusaitis *et al.,* 2004). Seedlings also have higher survival and establishment rates than seed propagules (Menges, 2008). These transplants may reach reproductive maturity earlier than seeded propagules, with seedling propagules producing higher, earlier seed yields than seed propagules (Jusaitis *et al.,* 2004). Seedling translocations can be timed to coincide with favourable environmental conditions and the planting position of seedlings can be optimised to reduce propagule wastage (Jusaitis *et al.,* 2004).

However, the above mentioned advantages may be reduced if seedlings were raised in an artificial environment, e.g. a greenhouse or nursery. Such seedlings are likely to have been subjected to different selective pressures than those prevalent in natural conditions (Jusaitis *et al.,* 2004). Disturbances to seedling root systems are sometimes unavoidable and initial root connectivity to the soil may be slow – this might prevent optimal seedling growth (Fahselt, 2007). Seedlings may be contaminated with pathogens, weeds or congeneric genes (Jusaitis *et al.,* 2004). These disadvantages can be minimised by using *in situ* seedlings from other populations or simply reducing the time seedlings spend in *ex situ* conditions (Jusaitis *et al.,* 2004). Also, using seedlings as propagules during translocations can be time-consuming and expensive (Menges, 2008).

2.2.5.7 Source material

Propagules may be sourced from a single or multiple populations. Reasons for using single-source propagules can be practical (e.g. only one population may be available for harvesting) or biological (e.g. preserving genetic integrity) (Guerrant & Kaye, 2007). Harvesting propagules from a single source population is viewed as the general practice in translocation attempts (Guerrant, 2012). To minimise potential genetic effects of multiple-source translocated populations, Guerrant & Kaye (2007) suggested the collection of propagules from areas near the translocation site.

Furthermore, a translocation effort may target wild-growing (*in situ*) or captive-bred (*ex situ*) individuals or populations (Haight *et al.,* 2000). *In situ* wild donor populations are more likely to deliver successful translocations than *ex situ* off-site populations, as *in situ* individuals are more likely to be specifically adapted to prevailing natural conditions (Griffith *et al.,* 1989).

2.2.5.8 Harvesting of adult individuals

Adult plants can be removed from the soil, depending on the size of the species. Larger plants have often completely removed been from soil/substrate (Blignaut & Milton, 2005). In some cases, the substrate with intact vegetation has been removed (‘turving’; Bullock, 1998), translocated and replanted along with individual propagules (Mueck, 2000) or with groups of propagules (Burgoyne &

Hoffman, 2011). Roots and/or the seed bank (Burgoyne & Hoffman, 2011) as well as certain abiotic conditions are thus safeguarded. Soil fauna can also be preserved during turving (Good *et al.,* 1999). Vegetative propagules, such as cuttings (Mueck, 2000) or rhizomes (Volis *et al.,* 2011) have also been used.

2.2.5.9 Spacing of propagules

Appropriate spacing of transplants can determine translocation success, as microsite conditions may be influenced by the proximity of transplants to one another (Milton *et al.,* 1999). Maschinski *et al.* (2004) also considered proximity to other plant species, e.g. potential competitors to influence translocation outcome.

2.2.5.10 Establishing multiple populations

Transplanting multiple populations may increase the chances of overall translocation success, as this decreases the chances of a single population being wiped out by a catastrophe (Jusaitis *et al.,* 2004). Therefore, the dispersal of the translocated populations across various habitats can protect/buffer against events such as fire, disease and overgrazing (Menges, 2008).

Genetic connectivity between populations should be maintained and is facilitated by pollinator flight distance (Schmitt, 1980). Different pollinators have varying flight distances. Robust pollinators, such as large birds, bats and moths may travel up to 25 km, whereas smaller pollinators, such as small birds, bees, wasps and noctuid moths may travel only up to 1 km (Harris & Johnson, 2004).

2.2.5.11 Pathogens

The introduction or spread of soil and/or plant pathogens may occur during the translocation process (Hodder & Bullock, 1997) and should be avoided (Milton *et al.,* 1999), as this can compromise translocation success (Gordon, 1994). Reintroductions from *ex situ* sources are especially susceptible to the transfer of pathogens (Hodder & Bullock, 1997).

2.3.5 Habitat/ecological considerations

An important concept in translocation is the ecological context of the target population. Preserving the population in a healthy habitat enables evolutionary processes to continue (Pegtel, 1998). A population of organisms interacts with biotic and abiotic factors in its environment (Fahselt, 2007). Receptor habitat conditions in a translocation attempt can, therefore, be expected to influence the establishment and persistence of the newly planted population. The limiting factors required for population survival are vital (Griffith *et al.,* 1989).

Maschinski *et al.* (2004) summarised the complexity of rare species translocation as follows: ‘Selecting suitable sites ... for translocation of rare species must consider physical, biological, logistical and historical criteria; however, it is far from self-evident what suitable sites will be’. The latter statement refers to the lack of knowledge on rare species ecology.

2.2.6.1 Species-specific knowledge

Habitat requirements of the focal species have to be known prior to any translocation attempt (Armstrong & Seddon, 2008). Rare species often have unique ecological requirements for optimal seed production, germination, seedling emergence and recruitment. Especially the germination, survival and population expansion of such species may be constrained by being transplanted to previously unoccupied/novel soils (Mustart & Cowling, 1993; Maschinski *et al.,* 2004).

2.2.6.2 Habitat suitability

A translocation attempt may fail due to the receptor habitat's inability to support the translocated population's ecological needs (Armstrong & Seddon, 2008). 'Source and sink sites' (Seddon, 2010) should preferably have similar biotic and abiotic conditions – with emphasis on geomorphic and edaphic characteristics (Gordon, 1994; Jusaitis *et al.,* 2004; Guerrant & Kaye, 2007), as dissimilar donor and receptor sites have been found to reduce transplant fitness (Fahselt, 2007).

A major challenge in translocation biology is the assessment of receptor habitat conditions, since there usually is no data available for the focal species at that site (Armstrong & Seddon, 2008). Microsite conditions of donor and receptor sites are rarely identical, further complicating the selection of an appropriate location (Fahselt, 2007). The receptor habitat may be manually prepared to accommodate transplants prior to translocation, e.g. clearing ground cover vegetation (Guerrant & Kaye, 2007).

The size of a translocated population has to be well beneath the carrying capacity of the receptor habitat (Armstrong & Seddon, 2008). Novel habitats or habitats previously unoccupied by similar species may be poor prospective sites for reintroductions of rare plants/edaphic specialists, due to deficient biotic and abiotic conditions required for optimal seedling survival (Maschinski *et al.,* 2004). The latter authors theorised that, due to genetic connectivity, translocation has a higher probability of success in areas near or in the original range of the donor population.

2.2.6.3 Conservation status of receptor habitat

Habitat suitability should also be measured by the degree of protection it receives (Griffith *et al.,* 1989), e.g. whether the site falls within a conservation area, whether private, municipal or national, since land-management factors also influence translocation success (Guerrant & Kaye, 2007). The site should therefore be biologically and logistically suitable to sustain a translocated population in the long run (Guerrant & Kaye, 2007; Crosti & Fabrini, 2010). Choosing multiple protected receptor sites can play an important role in species preservation, albeit dependent on the degree of connectedness between the sites (Lubow, 1996).

The question of land-ownership should also be addressed prior to translocation: Researchers/agents responsible for the translocation itself and for post-translocation management should be able to attain long-term access to the site (Guerrant & Kaye, 2007).

2.2.6.4 Habitat quality and environmental variation

The quality of the novel habitat is a crucial factor determining the outcome of translocation (Griffith *et al.,* 1989). Local habitat quality should not be compromised in any way, as it may cause increased mortality or reduced fecundity (Seddon, 2010). Habitat quality can be influenced by predation (e.g. herbivory) and encroachment by other species (Jusaitis *et al.,* 2004). Low environmental variation enhances the possibility of translocation success (Griffith *et al.,* 1989).

2.2.6.5 Soil microbes

Mutualisms between transplants and soil biota (e.g. mychorrizal fungi) and/or insects should also be investigated, but are generally neglected during translocation planning (Fahselt, 2007). Soil microbes contribute significantly to soil structure, nitrogen fixing (plant nutrition and nutrient cycling) and to the control of soil pathogens (Fahselt, 2007).

2.2.6.6 Competition

Interspecific competition may affect the outcome of translocations (Griffith *et al.,* 1989). Although competition is inevitable, it should not threaten the survival of the translocated population, especially if that population is within its natural range and at natural abundance levels (Gordon, 1994). Low levels of effective competition or lower numbers of competitors of similar life forms in the receptor habitats can result in higher success (Griffith *et al.,* 1989). Aggressive weeds and pioneers can hamper translocation success (Jusaitis & Polomka, 2008). Manual control (post-translocation management) of such competitors, e.g. by clipping, has been shown to positively influence translocation success (Gordon, 1996).

2.2.6.7 Herbivores

Interaction of translocated plants with herbivores may influence the outcome of a translocation. Herbivory of seeds, seedlings and mature plants may inhibit seedling recruitment and plant growth which are the initial indicators of translocation success (Menges, 2008). Indirect positive influences of herbivory on translocated grassland species, however, have also been observed (Martin & Wilsey, 2006).

2.2.6.8 Disturbances

Stochastic events can affect the outcome of translocation efforts during and after translocation (Menges, 2008). However, disturbance regimes, such as fire or herbivory may support translocation efforts of certain species (Parsons & Zedler, 1997), e.g. grassland and fynbos vegetation (Martin & Wilsey, 2006). Soil disturbances, e.g. digging of planting holes, trampling, fencing and mechanical preparation (Fahselt, 2007) should be avoided or kept to a minimum (Milton *et al.,* 1999). This will minimize the invasion of weeds (Mueck, 2000) and ensure the maintenance of important soil biota, proper aeration and soil structure (Fahselt, 2007).

The effects of the species being translocated on the ecosystem of the receptor site should also be considered (Conant, 1988). The translocated species might interact in undesirable ways with some components of the receptor ecosystem (Fahselt, 2007). For example, hybridisation with sister species (Hodder & Bullock, 1997) may result in the loss of one or more species if the hybrid is an aggressive competitor (Mehrhoff, 1996) or if an outbreeding depression should occur (Gordon, 1994). Another example of detrimental ecological disturbances is the release of allelopathic chemicals by the translocated species, which may have an inhibiting effect on nearby plants (Stinson *et al.,* 2006).

2.3.6 Population level and life cycle considerations

Demographic parameters have to be measured in order to enhance the evaluation of translocation success (Menges, 2008). Menges (2008) viewed the completion of individual life cycles as ‘a critical benchmark’ for ultimate translocation success – individuals should be able to flower and yield fruit. High rates of population increase, low variance in growth rate (Griffith *et al.,* 1989) and positive reproduction rates (Jusaitis *et al.,* 2004) could point towards successful translocation.

2.2.7.1 Reproduction

Should a population of species be unable to reproduce, the translocation can be considered a failure (Menges, 2008). Causes of poor reproductive viability include the lack of appropriate mating types and could necessitate stocking the translocated populations with genotypes from other populations (Menges, 2008). Species with higher reproductive output have better chances of establishing, thereby increasing translocation success (Armstrong & Seddon, 2008).

The ability of a plant species to build a long-term and persistent seed bank can positively influence translocation success, in that population recovery is aided by the soil seed bank (Menges, 2008). However, seed sources should be readily and continuously available for seed dispersal to the seed bank (Menges, 2008).

2.2.7.2 Dispersal

Dispersal of a translocated population across a receptor habitat may also indicate translocation success (Pavlik *et al.,* 1993; Menges, 2008), as the translocated population is able to occupy areas other than those initially selected for translocation. Gordon (1994), however, warned against translocated species that aggressively disperse, consequently threatening other species in the receptor habitat.

2.3.7 Genetic considerations

Genetic traits of species can be viewed as possible limitations to translocation success (Pavlik *et al.,* 1993; Menges, 2008). Despite the important role of genetics in determining the outcome of translocation, genetic analyses are generally not conducted prior to translocation (Fahselt, 2007). Gordon (1994) summarised four key genetic aspects that should be considered prior to translocation: the spatial structure of genetic variation within the species (which is generally unknown), distribution of genetic diversity within the donor population, the potential for the development of an inbreeding depression and the probability of founder effects causing a loss in genetic variability. Genetic issues can manifest in breeding systems (e.g. inbreeding or outbreeding depressions), genetic mixing, genetic representativeness and founder effects, e.g. genetic drift/bottleneck effects (Menges, 2008).

2.2.8.1 Species characteristics

The intrinsic characteristics (embedded in the genotype) of a species determines the population growth rate (Armstrong & Seddon, 2008). The latter eventually influences the survival of the translocated population (Griffith *et al.,* 1989), i.e. the newly planted population could fail to grow due to an inbreeding depression[2] or the inability to adapt to receptor habitat conditions (Armstrong & Seddon, 2008).

Self-compatibility can affect inbreeding, since a plant bearing multiple flowers may be pollinated by pollen from that same individual (Schmitt, 1980). Also, if numerous flowers from an individual plant are visited by a pollinator, this will diminish the chances of cross-pollination and pollen carryover, in effect compromising genetic diversity (Schmitt, 1980).

2.2.8.2 Genetic variation

High genetic diversity in translocated populations paves the way for successful translocation (Griffith *et al.,* 1989). Ideally, translocated populations should reveal similar genetic variation to natural populations (Menges, 2008) and be genetically representative of the donor population (Milton *et al.,* 1999). Moreover, the distribution of rare alleles in translocated populations should be comparable to that of the source population (Menges, 2008). Insufficient genetic diversity in the translocated population might also require the introduction of genotypes from other populations (Menges, 2008).

Precautions should, however, be taken against the possibility of an outbreeding depression – the dilution of adaptive genes, from cross-breeding between individuals from different source populations, resulting in the loss of local ecotypic variation (Menges, 2008). Therefore, to enforce genetic variability in a population, individuals from similar/local populations should be used to supplement the translocated population (Menges, 2008). However, unrestricted gene flow, e.g. genetic swamping, might reduce local population fitness, lead to an outbreeding depression and hamper adaptation to current selection pressures (Storfer, 1999).

Founder effects may manifest in the translocated population under the following conditions if (1) representative propagules were not sampled from the donor population, (2) a limited number of plants supplied most of the propagules or (3) the translocated population is small (Fahselt, 2007;

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[2] *Inbreeding depression* refers to genetic impoverishment due to a decreased number of genotypes in a population. The likelihood of related individuals mating increases – the frequency of homozygotes increases and harmful recessive genes are expressed more regularly. This results in offspring often having poorer reproduction rates and survival (Heinken & Weber, 2013).

Menges, 2008). If a portion of the population were to die off, as often occurs immediately after translocation (Allen, 1994), a genetic impoverishment could occur – increasing the probability of an inbreeding depression (Armstrong & Seddon, 2008). Such a loss of genetic variance could jeopardise the success of the translocation effort (Pavlik *et al.,* 1993).

2.2.8.3 Population size

Population size, i.e. the number of individuals shifted to the receptor habitat, can influence translocation success, since genetic problems accompanying small populations might be prevalent (Griffith *et al.,* 1989; Rout *et al.,* 2007; Menges, 2008). Small populations may fail to establish, due to demographic stochasticity[3] or to low reproduction/survival rates (Armstrong & Seddon, 2008). Harris & Johnson (2004) found reduced pollination success in small populations of several perennial species, which might result in less viable populations.

The size of translocated populations will always be smaller than that of the donor population, especially if translocation techniques regarding the establishment of multiple populations are kept in mind (Menges, 2008). Also, high mortality rates directly after translocation generally decreases the size of the translocated population (Allen, 1994). Inbreeding depressions may occur especially in small populations (Heinken & Weber, 2013) and outcrossing species, where the genetic diversity is limited, thus reducing the survival rate of a translocated population (Menges, 2008).

2.2.8.4 Establishing metapopulations

A ‘metapopulation approach’ can be adopted to uphold genetic diversity during translocation efforts and gene flow should be possible between adjacent populations (Menges, 2008). Gene flow is an important consideration during translocations, since adequate genetic migration between populations prevents inbreeding depression by maintaining genetic variation (Storfer, 1999). The isolation of populations (where no or little gene flow is possible) may cause a loss of genetic variation (Storfer, 1999).

2.2.8.5 Genetic dispersal

Gene flow can be determined by pollinator flight distance (how far pollen is dispersed) and path (to which plants pollen is dispersed to), as well as seed distribution distances (where individual plants end up) (Schmitt, 1980). Pollinators, therefore, play an important role in the genetic upkeep of a population of plants.

Documented pollinators of certain plant groups, such as the Mesembryanthemaceae, have been found not to travel far and therefore pollen flow between populations is restricted (Ihlenfeldt, 1994). In addition, seed dispersal of most Mesembryanthemaceae does not exceed 1 m from the seeding

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[3] Demographic stochasticity will arise in a small population due to individuals having independent vital rates – population demographics and survival will therefore be more variable in small populations (Heinken & Weber, 2013)

plant, impeding genetic export from one population to another (Ihlenfeldt, 1994). Lack of gene flow between populations is no cause for evolutionary concern in the mesemb group. In fact, Ihlenfeldt (1994) stated that self-sterility and mass flowering promote gene exchange within a population. Therefore, in an evolutionary sense, flowering and pollen exchange within a population is vital for the survival of the species.

2.2.8.6 Local adaptation and evolutionary potential

Menges (2008) duly stated: ‘Locally adaptive genetic variation is a key to restoration (translocation) success.’ Fahselt (2007) underscored this statement by suggesting that translocation success depends largely upon the ability of transplants to adapt to a habitat ‘somewhat different’ than the original.

Successful translocation requires the establishment of evolutionary patterns within the transplanted population (Allen, 1994) and the retention of a population’s evolutionary potential is vital (Menges, 2008). Translocating a population of endangered species to a novel habitat may cause it to genetically diverge from remaining natural populations (Conant, 1988), especially if genetic variation between populations was high (Gordon, 1994).

2.3.8 Management considerations

2.2.9.1 Population monitoring

Monitoring is the traditional way of determining the success of a translocation attempt (Armstrong & Seddon, 2008). It can encompass the target population, meta-populations and the ecosystem (Armstrong & Seddon, 2008). Two main focal points when monitoring a translocated population would be the establishment and persistence of that population (Armstrong & Seddon, 2008). Menges (2008) proposed measuring population ‘vital signs’ (survival, growth and fecundity) against a reference population. Although wild populations of some species may reveal higher vital rates, the translocated population(s) should be just as ‘demographically capable’ as wild populations (Menges, 2008).

Frequent monitoring of a translocated population enables the detection of any changes in demography and plant growth over time (Menges, 2008). Pavlik *et al.* (1993) recommended that population monitoring be demographically orientated. Thus, the initial stages of translocation success (seed germination, seedling emergence, seedling recruitment, plant growth and reproduction) can be tracked (Menges, 2008). Factors limiting population establishment and growth can also be identified through focused monitoring.

Long-term monitoring of the translocated population will yield the most reliable and accurate results. It can reveal the long-term viability of translocations, since results of short-term monitoring efforts can be misleading, especially in variable habitats (Menges, 2008). Slow-growing and long-lived translocated species, trees for example, would require long-term monitoring to reveal the outcome of the translocation attempt (Menges, 2008).

Monitoring for up to 25 to 50 years, or even longer, has been suggested by some authors to yield satisfactory answers when gauging the success of translocation attempts (Allen, 1994; Franklin *et al.,* 1994; Sperry, 1994). Unfortunately, monitoring programmes are often abandoned a few years after translocation, due to labour and financial intensity of the undertaking or because transplants seem stable, with 'nothing of merit to report' (Fahselt, 2007), or simply due to time constraints (Crosti & Fabrini, 2010).

Focused monitoring, i.e. the act of monitoring a translocated population to answer research questions or test hypotheses (Armstrong & Seddon, 2008) can be valuable for future translocation attempts, even if the effort fails (Menges, 2008). Unfortunately, post-translocation monitoring has been disregarded in some translocation attempts, resulting in unknown outcomes, the reasons for translocation failure remaining unidentified (Weeks *et al.,* 2011).

2.2.9.2 Post-translocation/follow-up management

Active follow-up management along with population monitoring can determine the fate of translocated populations (Griffith *et al.,* 1989; Armstrong & Seddon, 2008). Jusaitis *et al.* (2004) stressed the importance of understanding restrictions on population growth and stability during critical life stages, e.g. prescribed burning (Gordon, 1994), for effective management of endangered plant populations.

Some researchers advocate on-going post-translocation management for re-established populations. The complex nature of any translocation process may render the goal of establishing self-sustaining populations, doubtful (Parsons & Zedler, 1997). Said authors suggested perpetual post translocation management in cases where population declines occur in two or three successive years.

2.2.9.3 Manual restoration

Although translocation is aimed at the conservation of a population (Seddon, 2010), the translocation process in itself is a major disturbance to the ecological interactions of the target/donor population(s). A translocated population needs to recover from translocation stress and acclimatise to the receptor habitat for successful population establishment (Armstrong & Seddon, 2008).

Armstrong & Seddon (2008) suggested a strategic and adaptive management approach in the post-translocation period, as this allows for the manipulation of habitat conditions to improve chances of population growth. In restoration ecology, manual restoration may be necessary when natural processes cannot fulfil the requirements of a translocated species (Wendelberger & Maschinski, 2009). Therefore, when ecological limiting factors of an endangered species cannot be met, manual, intervening management actions have to be taken, e.g. the caging of emerging seedlings

(Maschinski *et al.,* 2004) or manual control of above-ground weeds/competitors (Menges, 2008) and invasive species (Fahselt, 2007).

To ensure the persistence of translocated population, certain ecological restoration principles have to be applied in the post-translocation management of such populations. These translocated populations ought to be restored to the vigour possessed by the stable, persistent donor population (Menges, 2008).

2.2.9.4 Removal of threat factors

Finally, the removal of threat factors, that rendered translocation necessary in the first place, is vital to ensure the persistence of a translocated population (IUCN, 1998).

Hodder & Bullock (1997) summarised seven points from the IUCN Species Survival Commission's guidelines for reintroductions (IUCN, 1995) that are applicable for translocations in general:

i. Feasibility study and background research: Taxonomic, genetic and ecological research on natural populations is required, assessments of previous translocations of similar taxa should be performed and translocation outcome(s) should be modelled.
ii. Selecting suitable receptor site(s): The site(s) should be within the natural/historic distribution range of the species and should assure a significant conservation benefit to the species, as well as long-term protection of the translocated population(s).
iii. Suitable donor population: The donor stock should be genetically and ecologically resilient, as well as pathogen and parasite free.
iv. Donor population should not be endangered: In case of re-stocking, the donor population should not be imperilled by the removal of individuals.
v. Involvement of anthropogenic factors: Socio-economic assessments should be made to determine the impact of the translocation on local human populations. Negative anthropogenic influences on the translocated population should be minimised.
vi. Careful planning of translocation process: Planning of a translocation endeavour includes attaining the approval of relevant entities, assembling a multidisciplinary team, identifying success indicators and designing a monitoring strategy.
vii. Post-translocation activities: These activities should include post-translocation monitoring, management interventions when necessary (for population(s) and habitat), public relations activities, evaluation of techniques and costing. Lastly, results, irrespective of the outcome, should be published.

The nature of translocations is complicated ('multivariate') (Griffith *et al.,* 1989) and a combination of factors and actions described above is likely to drive the success of a translocation (Guerrant & Kaye, 2007). Ultimately, the outcome of any translocation attempt will be determined by the intrinsic characteristics of the species being translocated (Parsons & Zedler, 1997; Jusaitis *et al.,* 2004).

In situ conservation is still deemed the primary, most feasible way to preserve species (Godefroid *et al.,* 2011), due to the complex nature of ecosystem interactions that have to be replicated and maintained for positive translocation outcomes (Fahselt, 2007). Also, *in situ* populations are exposed to natural selective pressures and the upkeep of evolutionary processes is thus possible (Pegtel, 1998). Gordon (1994) made the following reminder: 'Translocation should never be seen or justified as a substitute for protection of high quality natural areas'.

2.3.9 Population modelling

Population viability analyses provide the most dependable prediction of species survival (Heinken & Weber, 2013), but require 'many years of data and/or projections based on data-hungry demographic models' (Menges, 2008). For example García (2003) monitored an *in situ* population of the dioecious herb *Borderea chouardii* (Dioscoreaceae), a tropical relict occurring only on two adjacent vertical cliffs, for eight years before employing population viability analyses. Long-term persistence of a population, the key to successful translocations, can be projected by means of population viability analyses (Menges, 2008). Predicting future population size, age structure, population growth rates, probability of extinction and time-to-extinction require input data on survival, growth, fecundity, recruitment and dispersal (Menges, 2000).

Further analysis may reveal the sensitivity of population growth rates to individual vital rates, relate past fluctuations in population growth rate to ecological factors/treatments and accordingly evaluate effects of past and potential management on demography and persistence of populations (Menges, 2008). In fact, translocation efforts may even provide opportunities to refine population viability analyses, as starting conditions are known (e.g. number of transplants and date of introduction) (Menges, 2008).

2.3.10 Single species translocation

The *F. humilis* translocation can be viewed as the first of its kind in South Africa (Burgoyne & Hoffman, 2011). The relevance of this study can thus be more fully appreciated when considered in the light of international (Table 2.1) and national (Table 2.2) examples. Only examples of vascular plant translocations are listed in the following tables. Attempts at translocating cryptogams (bryophytes and lichens) have also been made (Brooker *et al.,* 2011). Numerous translocations of invertebrate, bird, reptile and mammal species have been performed throughout the last thirty years (Fischer & Lindenmayer, 2000).

2.4 Pollination ecology

The survival of a population of sexually reproductive plants may depend on mutualistic relationships with biotic components of its ecosystem, especially with pollinators (Kearns & Inouye 1997; Harris & Johnson, 2004). Biological aspects, particularly pollination, play an enormous part in determining

Table 2.1: International examples of single species translocations.

Type of translocation	Region	Target species	Conservation status	Reference
Assisted colonisation	Northern California, United States of America (USA)	*Amsinckia grandiflora* (Boraginaceae)	Nationally endangered	Pavlik *et al.* (1993)
Reintroduction	Florida, USA	*Conradina glabra* (Lamiaceae)	Nationally endangered	Gordon (1996)
Assisted colonisation	Southern California, USA	*Cordylanthus maritimus* ssp. *maritimus* (Scrophulariaceae)	Nationally endangered	Parsons & Zedler (1997)
Reintroduction	Victoria, Australia	*Diuris fragrantissima* (Orchidaceae)	Critically endangered (IUCN)	Smith *et al.* (2007)
Conservation introduction	Southern Florida, USA	*Amorpha herbacea* var. *crenulata* (Fabaceae)	Nationally endangered	Wendelberger *et al.* (2008)
Experimental translocation	Eyre Peninsula, South Australia	*Acacia whibleyana* (Fabaceae)	Nationally endangered	Jusaitis & Polomka (2008)
Assisted colonisation	Southern Italy, Europe	*Cyperus polystachyus* (Cyperaceae)	Critically Endangered (IUCN)	Crosti & Fabrini (2010)
Conservation introduction	Lahav, southern Israel	*Iris atrofusca* (Iridaceae)	Endangered	Volis *et al.* (2011)

Table 2.2. National examples of translocation[4].

Type of translocation	Region	Target species	Conservation status	Reference
Conservation translocation	Witbank, Mpumalanga	*Frithia humilis* (Mesembryanthemaceae)	Endangered (IUCN)	Burgoyne & Hoffman (2011)
Introduction	Succulent Karoo	*Othonna cylindrical* (Asteraceae) *Ruschia versicolor, Lampranthus suavissimus* (Aizoaceae), *Zygophyllum morgsana* (Zygophyllaceae)[4]	-	Blood (2006)

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[4] As translocations are poorly documented in South Africa (Milton *et al.,* 1999), examples of community/multispecies translocations will also be given.

Type of translocation	Region	Target species	Conservation status	Reference
Reintroduction	Succulent Karoo	*Aridaria noctiflora* ssp. *noctiflora;* *Drosanthemum deciduum;* *Psilocaulon dinteri*[5] (Mesembryanthemaceae)	-	Blignaut & Milton (2005)
Reintroduction	Cederberg, Western Cape	*Widdringtonia cedarbergensis* (Cupressaceae)	Critically Endangered (IUCN)	Mustart *et al.* (1995)

the extent to which a translocated plant population thrives in its new environment (Heinken & Weber, 2013).

2.4.1 Importance of pollination

Insect pollination plays a vital role in a multitude of natural ecosystems, since it ensures the fertilisation of sexually reproducing plants (Harris & Johnson, 2004). As pollination is the vector-system for plant reproduction, it can be viewed as a very basic ecosystem service, upholding many food webs (Mayer, 2004).

Furthermore, pollinators are important as agents of gene dispersal in plant populations (Schmitt, 1980; Gordon, 1994) and can be viewed as 'agents of selection', as they play an important role in gene flow via pollen distribution (Kearns *et al.,* 1998). Pollen flow and cross-fertilisation impacts the genetic viability and evolutionary dynamics of a plant population (Cuartas-Hernández *et al.,* 2009; Harder & Aizen, 2010). Pollination success is recognised as an important ecological factor influencing the quantity and quality of seed-set in a population of plants (Harder & Aizen, 2010).

2.4.2 Relevance of plant-pollinator interactions in translocation

Pollination interactions, i.e. between angiosperms and their insect pollinators, may be directly influenced by anthropogenic activities/disturbances (Harris & Johnson, 2004; Memmott *et al.,* 2007) such as habitat fragmentation, land-use changes and alien species introduction (Mayer, 2004), often with devastating effects on the plant population (Heinken & Weber, 2013). It can therefore be expected that translocation, a human-induced disturbance, albeit for conservation purposes, would also disrupt pollination interactions. Even a change in the pollinator composition in a habitat would likely affect plant fitness (Ashworth *et al.,* 2004).

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[5] These species were re-planted on mine spoils as a rehabilitation effort. Conservation of the species was therefore not the primary goal.

Care should be taken to ensure the restoration of plant-pollinator interactions in a population of translocated plants, because pollination is fundamental to successful restoration actions (García-Robledo, 2010). In the context of post-translocation restoration, plant-pollinator interactions are critical to ensure the persistence of the translocated populations. Pollination is vital to bring about fruit-set, seed-set, germination and seedling recruitment (Pufal *et al.,* 2008).

The conservation of pollinators is important to ensure the evolutionary persistence of a translocated population of flowering plants (Kearns *et al.,* 1998). However, the time needed for plant-pollinator interactions to normalise after a translocation is not described in the literature.

The disruption of pollination systems may have the following consequences:

i. An increased probability of heterozygosity loss and inbreeding depression (García-Robledo, 2010), due to an increased likelihood of autogamy/self-fertilisation in some plant species (Kearns & Inouye, 1997). Consequently, less vigorous offspring are produced (Kearns & Inouye, 1997), compromising descendant survival.
ii. Genetic differentiation by means of random processes, such as genetic drift (García-Robledo, 2010), potentially causing the loss of genetic diversity.
iii. The subdivision of populations into semi-isolated demes increases the possibility of extinction (García-Robledo, 2010). Isolated populations may have pollination systems different from continuous populations (Cuartas-Hernández *et al.,* 2009).
iv. Decreased reproductive success, as well as loss of or reduction in fruit production and seed-set, resulting in poorer plant fitness (Heinken & Weber, 2013) and ultimately a reduction in population viability (Harris & Johnson, 2004) or the extinction of a population (Kearns & Inouye, 1997).

The importance of pollination/pollinator visitation as a limiting factor for the persistence of numerous Mesembryanthemaceae species need to be tested, as low seed-set may also result from a nutrient shortage in the environment, as well as from reduced pollen availability (Mayer & Pufal, 2007).

Pollinator-plant interactions may not be established immediately after translocation, due to the disturbance caused by the translocation process. The full importance of pollinators in the aftermath of translocation will ultimately depend on whether the plant-pollinator relationship is facultative or obligate (Kearns & Inouye, 1997). However, most plants are not restricted to one insect pollinator and the interactions are of a generalist nature (Kearns *et al.,* 1998). Pollinator-plant interactions may vary amongst different vegetation communities (Kearns & Inouye, 1997), rendering plant-pollinator relationships flexible in general. Hypothetically, a translocated angiosperm population should, therefore, not be affected by pollinator deficiency in the long term.

Another factor that determines the importance of pollinators in a post-translocation scenario is the reproductive system of the target plant species. Should the species mainly depend on vegetative reproduction, pollination via anthophilous insects would not play a major role in the stabilisation of population demography (Kearns & Inouye, 1997). A long-lived plant species or one producing a large seed bank will not be immediately threatened by the absence of insect pollinators (Kearns & Inouye, 1997) or if pollinators are slow to discover the 'new' (translocated) flowers.

Plant species at the highest risk from pollinator absence are those that are dioecious, self-incompatible and pollinator-specialists, as well as those angiosperms that reproduce by seed propagation (Kearns & Inouye, 1997; Ashworth *et al.,* 2004).

Generally, members of the Mesembryanthemaceae/Aizoaceae family are self-incompatible (Mayer, 2004), even if artificially pollinated and are therefore xenogamous (Peter *et al.,* 2004). There are, however, exceptions, e.g. *Carpobrotus acinaciformis* and *C. edulis*, which are partially and fully self-compatible (Mayer & Pufal, 2007). Self-compatible plants are 'reproductively safe' should pollinators be absent, largely due to the independence of their reproductive success from pollen exchange between individuals (Kunin, 1997).

2.4.3 Asymmetry of plant-pollinator relationships

Habitat disturbances, such as fragmentation (or translocation), may disrupt pollinator systems and therefore plant reproduction (Harris & Johnson, 2004). It is generally accepted that pollinator-specific plants (specialists) would be more affected by disturbances than generalist plants (plant pollinated by several to many pollinator taxa) (Ashworth *et al.,* 2004) (Figure 2.3). However, several studies have shown that generalist plants respond as negatively to habitat disturbance as specialists do (Aizen *et al.,* 2002; Vázquez & Simberloff, 2002; Aguilar *et al.,* 2006) and that plant-pollinator systems are more dynamic than once thought (Waser *et al.,* 1996).

This could be explained by asymmetric specialisation, where pollinator-specific plants are pollinated by a few generalist pollinator species and generalist plant species are pollinated by a wide array of both generalist and specialist pollinator species (Vázquez & Aizen, 2004).

2.4.4 Theoretical prediction of *Frithia humilis* pollinator(s)

No literature exists to explain the pollination system of *F. humilis*. Examining what little there is to know about Mesembryanthemaceae/Aizoaceae pollination in general may hint at the identity of *F. humilis* pollinator(s) and give certain guidelines as to investigating it, even though most literature refers to Karoo mesembs (Hartmann, 1991; Mayer, 2004; Peter *et al.,* 2005).

The search for *F. humilis* pollinator(s) may start at the most common orders of insects known for their pollination services: Hymenoptera, Lepidoptera, Diptera and Coleoptera (Kearns *et al.,* 1998). Gess & Gess (2004) reported Mesembryanthemaceae/Aizoaceae species in the Succulent Karoo and Nama-Karoo Biomes being visited by bees, wasps and pollen wasps (Table 2.3). Species of pollen wasps were found to visit members of the mesemb family more frequently than other angiosperm families in these biomes. However, the distinction between social and solitary bees is not made in the aforementioned study.

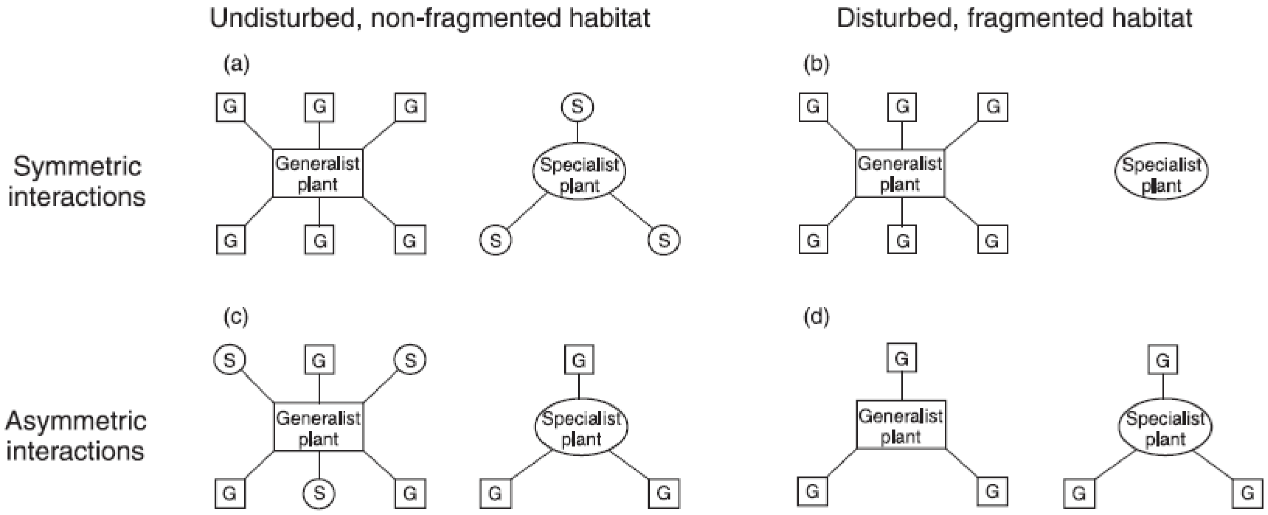

Figure 2.3. Representation of symmetric *vs* asymmetric specialised pollination systems in disturbed habitats. (a) Symmetric interactions imply the generalist plant would be pollinated by generalist pollinators (G) and specialist plants by specialist pollinators (S). (b) Consequently, symmetric specialist-interactions would be more severely disrupted by habitat disturbances or fragmentation, due the sensitivity of S to habitat disturbances. (c) In asymmetric interactions generalist plants may be pollinated by an array of specialist and generalist pollinator taxa. Specialist plants may be pollinated by fewer generalist taxa. (d) Similar responses to habitat disturbances are observed in generalist and specialist plants (Ashworth *et al.,* 2004).

Table 2.3. Bee species found to pollinate mesembs in the Succulent Karoo, Nama Karoo and Fynbos Biomes, as well as populations from the coastal Namib desert[6] (Gess & Gess, 2004).

Order	Family	Species/genera	Common name
Hymenoptera	Andrenidae	*Meliturgula. flavida* (Friese)	Panurgine bees[7]
Hymenoptera	Andrenidae	*Melturgula. haematospila* (Cockerell)	Panurgine bees
Hymenoptera	Andrenidae	*Meliturgula minima* (Friese)	Panurgine bees
Hymenoptera	Apidae	Species of the subfamily Nomadinae	Cuckoo bees
Hymenoptera	Apidae	Species of the subfamily Xylocopinae	Carpenter bees
Hymenoptera	Colletidae	*Colletes* sp.	Plasterer bees
Hymenoptera	Colletidae	*Scrapter bicolor* (Lepeletier & Serville) (on an afternoon flowering mesemb)	Plasterer bees
Hymenoptera	Halictidae	*Lasioglossum* sp.	Sweat bees
Hymenoptera	Megachilidae	Eleven species of the subfamily Fideliinae	Leaf cutter bees
Hymenoptera	Melittidae	*Hesperapis* sp. (one species, unmentioned, appears to be a specialist)	Melittid bees

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[6] These are arid, winter rainfall regions, in contrast to the temperate summer rainfall region of the Grassland biome where *F. humilis* occurs.

[7] Mining, solitary or ground nesting bees

Hartmann (1991) described four pollination syndromes for the Mesembryanthemaceae, i.e. characteristics of mesemb flowers that accommodate pollination by certain insect taxa (Table 2.4). However, more recent literature has questioned the reliability of predicting pollinators via pollination syndromes in that several visitors could be responsible for pollination (Symes *et al.,* 2009). Effective pollen transfer only takes place if a pollinator is morphologically (its body size, feeding apparatus and hairiness) and behaviourally (degree of its contact with anthers and/or stigmas) fitted to a plant species' floral dimension, as this determines the pollen load carried by the insect (Harris & Johnson, 2004; Johnson *et al.,* 2009). The movement patterns of an insect, e.g. flower constancy, also determines its efficacy as pollinator (Harris & Johnson, 2004).

Most Mesembryanthemaceae flowers are adapted to insect pollination on account of their bright petals (Hartmann, 1991; Zietsmann, 2013) and are pollinated by a range of generalists (Peter *et al.,* 2004; Mayer & Pufal, 2007). Nevertheless, specialised plant-pollinator relationships do exist (Ihlenfeldt, 1994; Peter *et al.,* 2004), e.g. some species of Masaridae wasps (Hymenoptera, Vespoidea) prefer flowers of the mesemb genus *Quartinia* (Hartmann, 1991). In some instances, wind pollination may occur in natural habitats, especially in plant species where numerous flowers with reduced petals and profuse amounts of dry, powdery pollen are produced (Hartmann, 1991; Ihlenfeldt, 1994), though this remains to be investigated (Peter *et al.,* 2004).

Peter *et al.* (2004) reported pollination specialisation of female *Allodapula variegata* (Hymenoptera) wasps on a Mesembryanthemaceae species, *Bergeranthus multiceps*, endemic to the Albany Centre of Endemism. Such specialisation seemed restricted to a specific time of year and geographic locality. Therefore, it can be expected that *F. humilis*, in certain populations, might be

Table 2.4. Pollination syndromes for the Mesembryanthemaceae as described by Hartmann (1991).

Pollination syndrome	Pollinator	Pollination method	Flower traits	Opening time
Anemophilous	Wide array of pollinators, including masarid wasps		- Open - Pollen abundant, dry and powdery*	
Melittophilous	- Bees - Butterflies	- Crawling/walking over flowers - Insertion of proboscis/crawling into cone-like flowers	- Open (cone-like in some subtypes) - Pollen abundant, dry and powdery* - Hidden nectaries - Bright, shiny petals (yellow, purple or white)	Diurnal (11:00-17:00)
Phalaenophilous	Moth		- Fragrant - Tapering petal tips - Petals shades of white, even greenish (often yellow towards centre)	Nocturnal
Psychophilous	Butterflies	Insertion of proboscis	- Long, narrow tube - Nectar at base of tube	

* possible wind pollination

pollinated by a specific insect taxon, most probably solitary bees or masarid wasps. However, the same authors also observed other benevolent insect visitors on *B. multiceps*, i.e. butterflies (nectar-foragers), Bombyliidae, Tachinidae and Syrphidae flies (nectar and pollen feeders), the majority of which carried only small pollen loads, in contrast to hymenopteran visitors.

Mesemb species in the Fynbos and Succulent Karoo Biomes have been found to be both fed on and pollinated by monkey beetles (Scarabaeidae). Monkey beetles do not commonly occur in the Grassland Biome (Picker & Midgley, 1996, Picker *et al.,* 2004), but such findings suggest that beetles should not be excluded from the list of possible pollinators of *F. humilis*.

Due to the fragmented nature of the *F. humilis* distribution range, as well as the large, often inhospitable distances between populations, the probability of shared pollinators between populations is low (Cuartas-Hernández *et al.,* 2009). In other words, it can be expected that different *F. humilis* populations have varying pollinators. This could indicate a broad spectrum of possible pollinators.

Peter *et al.* (2004) speculated that the daily timing of flower-opening plays a role in determining which insects act as pollinators. It seemed as though the late afternoon opening of *Bergeranthus multiceps* excluded generalist pollinators, commonly most active over high noon. Flowering time of individuals in most populations of Mesembryanthemaceae is synchronised and short-lived, where flower opening is restricted to a certain time of the day, typically to around midday (Ihlenfeldt, 1994). The time of day when *F. humilis* flowers are open should, therefore, give a good indication of possible insect pollinators. Generally, mesemb flowers are protandrous (anthers mature before stigmata do) and they are maximally fertile on the fourth or fifth day after opening. On average, the flowers of most mesembs may last for up to a week (Hammer, 1995).

Another factor which might influence plant-pollinator relationships in certain mesemb species is ultraviolet (UV) light reflecting properties of some flowers, e.g. *B. multiceps* (Peter *et al.,* 2004). Again, no such information is available for *F. humilis*.

It is possible that, in the absence of the ‘traditional’ pollinator of a species, flowers may be pollinated by an unexpected (‘reserve’) insect (Johnson *et al.,* 2009). It can, therefore, be expected that translocated *F. humilis* plants could be pollinated by new pollinators in novel receptor habitats.

Different pollinators have different energy requirements and the quality and distribution of nectar rewards in a flowering plant population may affect pollinator behaviour (Schmitt, 1980). Many angiosperm species provide a specific quality/quantity of nectar, as well as having specific nectar presentation features, specifically to attract pollinators (Schmitt, 1980). Therefore, if the quantity and quality of nectar/pollen rewards produced by *F. humilis* can be estimated, a more accurate prediction about the identity of the pollinator can be made. Once more, no literature exists on the nectar production of *F. humilis* flowers, or on the pollen quantity produced.

Proximity to other plants has been shown to significantly influence pollination intensity of an insect species (Johnson *et al.,* 2003). Therefore, studying the flowering plant species in the community where *F. humilis* occurs may lead to the identification of the pollinators(s).

Kearns *et al.* (1998) mentioned the importance of knowing and understanding the natural history of the target plant species and its relation to pollinators, especially in a conservation context. Therefore, studying *F. humilis* pollination systems would benefit post-translocation conservation of this endangered plant species.

Chapter 3. Materials and Methods

3.1 Introduction

Jusaitis *et al.* (2004) stressed the importance of empirical assessment of translocation success via surveys, observation, experimentation and data collection. This chapter provides background information on the study areas, as well as general methodology applied during this study. For methods used in specific areas of the study, please refer to subsequent chapters.

3.2 Study areas

South African grasslands are home to several rare plant species (Neke & Du Plessis, 2004), such as *Frithia humilis*. Parts of the South African Grassland Biome, covering 16.5% of land area in the country, is considered critically endangered (Neke & Du Plessis, 2004), with approximately 26% being anthropogenically transformed and degraded (Reyers *et al.,* 2001). Although conservation of this biome is deemed very important, inadequate protection has been afforded (Neke & Du Plessis, 2004).

Translocation localities (receptor sites, Figure 3.1) were chosen within the Rand Highveld Grassland vegetation type (Mucina & Rutherford, 2006). The study was conducted near eMalahleni (formerly Witbank) and Balmoral, Mpumalanga Province, and Bronkhorstspruit, Gauteng Province. *Frithia humilis* populations are endemic to this area (Burgoyne & Krynauw, 2005). Receptor sites were chosen primarily based on characteristics outlined in Table 3.1. Scouting for suitable habitat was done by helicopter and thereafter ground-truthed (Burgoyne & Hoffman, 2011).

A number of potential translocation ‘patches’ was identified at each receptor site. These patches were screened for suitability (the characteristics of which are summarised in Table 3.2) before the rescued population was transplanted.

3.2.1 Witbank Nature Reserve (*W*)

Witbank Nature Reserve is a municipal park of 847 hectares, proclaimed in 1979 (Smit *et al.,* 1997). It is situated along the northern outlet of the Witbank Dam, a reservoir in the Olifants River, directly

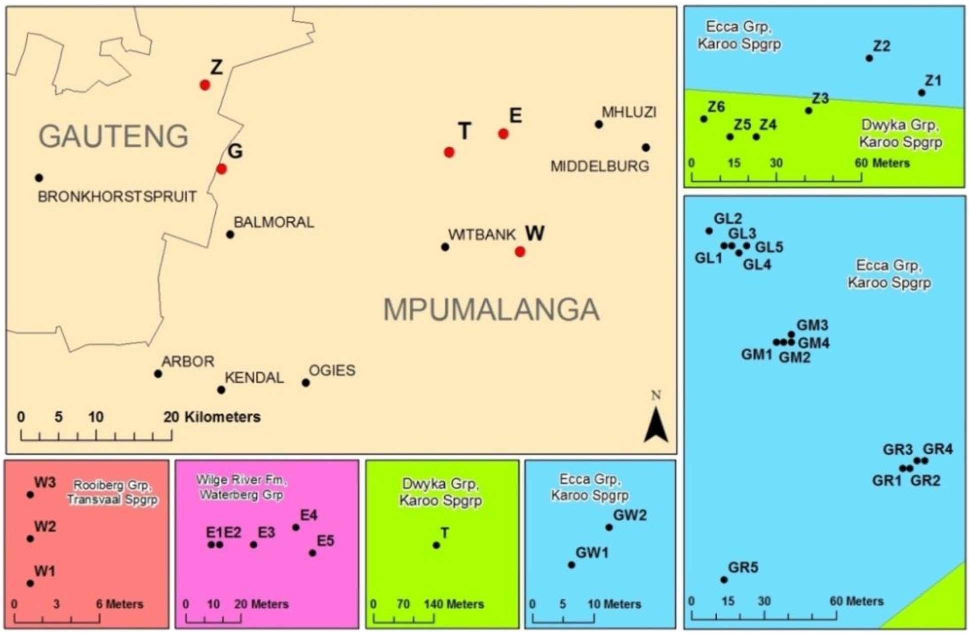

Figure 3.1. A threatened population of *Frithia humilis* (T) was translocated to three suitable receptor sites (E, Eagle Rock Private Estate, G, Goedvertrouwdt farm and W, Witbank Nature Reserve). A control population was identified at Ezemvelo Nature Reserve (Z). All translocation sites occur on sedimentary rocks, except for W, which occurs on felsitic outcrops. Grp, group; Spgrp, supergroup.

Table 3.1. Habitat specifications considered in the translocation of the habitat specialist, *Frithia humilis* (Burgoyne & Hoffman, 2011).

Habitat characteristic	Significance
Geological stratum: i) Porosity ii) Sedimentary rocks (Dwyka and Ecca sandstones, Karoo Supergroup) iii) Plate-like outcrops	i) Prevention of water accumulation and inundation ii) Weathering products, i.e. quartz-rich pebbles, protect vulnerable seedlings from environmental factors iii) Shallow ‘grit pans’ on flat outcrops offer an ideal micro-habitat: shallow, sandy soil, with a top layer of gravel
Slope inclination	A smaller incline prevents plants from being washed down-stream, while still facilitating seed dispersal via water flow
Species composition of the greater habitat	Similar ecological processes would presumably minimise post translocation stress
Intact habitat	Few ecological disruptions prevent further disturbance of populations
Distance from donor site	A shorter distance leads to less disruption of genetic variation
Absence of existing *F. humilis* population	Avoidance of gene pool contamination
Protected/conserved area	Guaranteed conservation of the translocated population(s)

Table 3.2. Translocated populations of *Frithia humilis* and their micro-habitat conditions.

Receptor site	Number of patches	Soil depth	Quartz pebbles	Slope	Species encroachment	Drainage
GL	5	Sufficient	Present, but washes away easily with rainwater runoff, leaving unconsolidated soil and exposed rocks	Steep enough for rainwater to cause plants damage at *GL*1-4	Considerable peripheral presence of *Selaginella dregei*, acting as catchment for washed away seeds	Good, no waterlogging
GM	4	Sufficient	See *GL*	Steep enough for rainwater to cause damage to *GM*1, 2, 4	See *GL*	See *GL*
GR	5	Sufficient	Present at all sites except *GR*4 and *GR*5, where pebbles wash away	Steep enough for rainwater to cause damage to *GR*1, 2 and 4. Population expansion is promoted by slope at *GR*5, as downslope area is suitable for seed germination	*S. dregei* present only at two patches (*GR*1 and 2), leaving the remaining sites dry and prone to evaporative water loss. The miniature *Crassula setulosa* is present at *GR*5	See *GL*
GW	2	Sufficient	Present and stable	Very slight with little rainwater runoff	Peripheral encroachment of *S. dregei* at both sites	See *GL*
E	4	Sufficient	Imported and prone to washing way	Steep enough for rainwater to remove top layer of pebbles at *E*1-3	*S. dregei* encroachment at *E*4. *Microchloa caffra* mainly present at *E*1	*E*4 prone to waterlogging
W	3	Shallower, more compact soil	Absent, instead a more angular pebble is present	Very slight	*Anacampseros subnuda*, *Crassula setulosa* and *M. caffra* present	Waterlogging apparent at all patches
Z	6	Sufficient	Present in a thin layer	Very slight	*M. caffra* present	Good, no waterlogging, except on Dwyka sediments

east of the town of eMalahleni. The *F. humilis* population was planted into a *Myrothamnus flabellifolius-Ursinia nana* herbland, one of the 11 plant communities of the reserve (Smit *et al.,* 1997).

W translocation patches (Figure 3.2) were not underlain by the typical sedimentary outcrops preferred by *F. humilis*. Instead, the population was translocated onto the Selons River Formation (Rooiberg Group) of the Bushveld Igneous Complex, an igneous rock type also referred to as felsite (Smit *et al.,* 1997; Johnson *et al.,* 2006). The microhabitat created by this volcanic rock differs from that of sedimentary rocks. Felsitic rock is more compact and less porous (Grotzinger *et al.,* 2007), inhibiting moisture drainage from patches and therefore increasing the potential for seasonal inundation.

Pebbles were observed to be more angular in shape, are generally larger and less abundant than the quartz pebbles produced by sedimentary rocks. The area had a very slight slope and, in addition to the low drainage potential of the rock, water run-off was minimal. Outcrop peripheries were closely surrounded by grass-dominated vegetation communities (Smit *et al.,* 1997).

As the smallest receptor site, *W* hosted only three translocation patches (*W*1−3) of 0.5 x 0.93 m, 0.62 x 0.64 m and 0.3 x 0.78 m respectively, which were 1 – 2 m apart at most and are 1550-1553 metres above sea level (m.a.s.l.). Witbank Nature Reserve, along with Eagle Rock Private Estate (Section 3.2.2) was chosen as translocation receptor sites to test *F. humilis* growth and survival on non-typical geologies. In addition, *W* had the advantage of being a formally protected area (Smit *et al.,* 1997).

3.2.2 Eagle Rock Private Estate (*E*)

The second experimental translocation was performed to a privately owned farm of the Eagle Rock Home Owners Association, north-east of eMalaheni. Situated on the Wilge River Formation of the Waterberg Group (Transvaal Supergroup), the sedimentary rocks of *E* were denser (less porous)

Figure 3.2. A portion of the donor *Frithia humilis* population was experimentally translocated to Witbank Nature Reserve, where rock plates consist of igneous felsites (Rooiberg Formation) (Photo: T. Orlekowsky, 2011).

than that of typical *F. humilis* habitats (Figure 3.3). Quartz pebbles were imported from the original destroyed *F. humilis* locality to cover and protect the topsoil.

Five translocation patches were established at *E* (1529 m.a.s.l.) the first two patches being 1.1 x 1.7 m and 0.6 x 1.8 m respectively. The third patch consists of four ‘sub-islands’ sized 0.5 x 1.2 m, 0.7 x 1.2, 0.5 x 1 m, 0.3 x 0.9 m respectively. The fourth and fifth patches, due to their small sizes and close vicinity to one another, were eventually treated as one patch consisting of two sub-islands of 0.3 x 0.7 m and 0.7 x 1.7 m. The outcrops were bordered by tall grasslands. Down-slope areas of these patches were prone to water-logging, potentially minimising population expansion. Grazing ungulates roamed the area surrounding the translocated population and trampling seemed to occasionally be a minor threat to the population.

3.2.3 Goedvertrouwd (G)

The Goedvertrouwdt receptor site is located on farm property (portions 9 and 24, Goedvertrouwd farm 499 JR), at the Gauteng-Mpumalanga border, east of the town of Bronkhorstspruit. As the largest receptor site, *G* has 16 patches (Table 3.3) which were spread out across a low hill overlying an abandoned coal mine shaft. Plants translocated to receptor site *G* were planted in patch clusters according to the availability of appropriate sandstone plates, e.g. *GW*, *GL*, *GM* and *GR*. Each cluster was treated as a separate population. Dividing the *G* population in this manner also enabled more accurate statistical analyses, as sizes of the translocated populations (including *E* and *W*) would thus be more equal.

This habitat was characterised by outcrops of the Ecca Group and was therefore the only site that was geologically suitable. Quartz pebbles which facilitate seed germination (Burgoyne *et al*., 2000b) were present at this site. Top soil layers were intact, protecting plants during the dry season. The

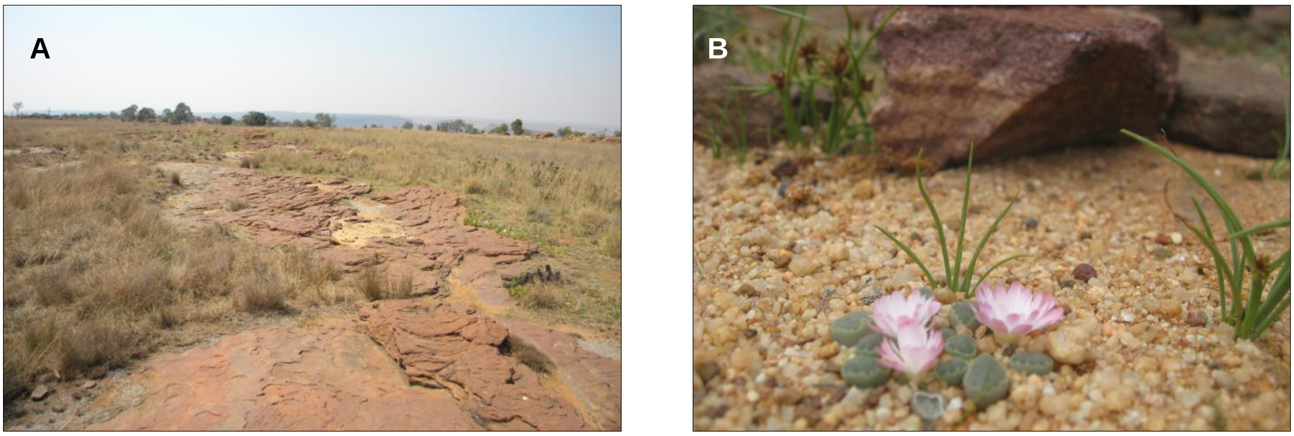

Figure 3.3. The Eagle Rock Private Estate receptor habitat was a geologically atypical habitat, as (A) rock plates are of the sedimentary Wilge River Formation and (B) lack of pebbles necessitated the import of pebbles to protect vulnerable plants (Photos: J.H.L. Smit, 2009 & P.M. Harris, 2012).

Table 3.3. Sizes and altitude of translocation receptor patches at the Goedvertrouwd receptor site.

Patch	Size (m)	Altitude (m.a.s.l.)	Patch	Size (m)	Altitude (m.a.s.l.)
***GW*1**	0.82 x 3.12	1547	***GL*3**	1.5 x 3.1	1555
***GW*2**	1.57 x 1.65	1550	***GL*4**	0.36 x 1.85	1556
***GM*1**	0.56 x 1.74	1556	***GL*5**	1.86 x 2.71	1554
***GM*2**	0.48 x 0.5	1557	***GR*1**	0.75 x 1.39	1552
***GM*3**	0.67 x 1.38	1556	***GR*2**	0.34 x 1.31	1553
***GM*4**	1.7 x 2.45	1556	***GR*3**	0.98 x 1.22	1552
***GL*1**	0.6 x 1.94	1556	***GR*4**	0.95 x 1.690	1554
***GL*2**	1.11 x 1.78	1557	***GR*5**	0.82 x 1.56	1561

slope was <3º. However, this receptor site is not protected by law. It forms part of an abandoned coal mine with acid mine water drainage along the footslopes of the hill, which does not affect the higher lying, upslope translocated populations. Furthermore, past mining activities have resulted in major structural cracks which impede population expansion, due to severe water runoff.

An established population of invasive *Acacia mearnsii* (black wattle trees) is present close to the populations, but no invasion of the receptor patches has been noticed. Furthermore, a small *F. humilis* population of approximately 150 plants were occupying a restricted area less than 1 km away from the translocation receptor patches.

3.2.4 Ezemvelo Nature Reserve (*Z*)

The *F. humilis* population in Ezemvelo Nature Reserve forms part of one of 22 major plant communities in the 11 000 ha nature reserve, namely the *Frithia humilis*-*Microchloa caffra* community on quartz (Figure 3.4) (Swanepoel, 2006). It was the only naturally occurring population of *F. humilis* under protection (Burgoyne & Hoffman, 2011). *Z* was chosen as a control site due to the permanence of the population. The population's area of occupancy was more extensive (in excess of 1 km²) than that of the translocated populations. Six representative 1 m^2 patches, equal distances apart, were chosen (*Z* 1−6) for monitoring.

The habitat is situated on a low-lying hill and is underlain by Dwyka and Ecca Group sediments which outcrop as exposed patches in a grassland matrix. The habitat is covered by a thin layer of sandy soil and quartz pebbles, with no visibly harmful encroachment by other grassland species. In fact, tufts of the grass *Microchloa caffra* seemed to protect neighbouring *F. humilis* individuals from environmental factors, such as desiccation and radiation (Figure 3.4). Plants growing in close proximity to tufts were larger and more turgid than those growing in open spaces. Ideal environmental and habitat conditions seemed to enable *F. humilis* to conquer a very large area at Ezemvelo Nature Reserve, with very high population numbers. Such high numbers are unusual for populations of Mesembryanthemaceae, with taxa in the Succulent Karoo Biome usually occurring in

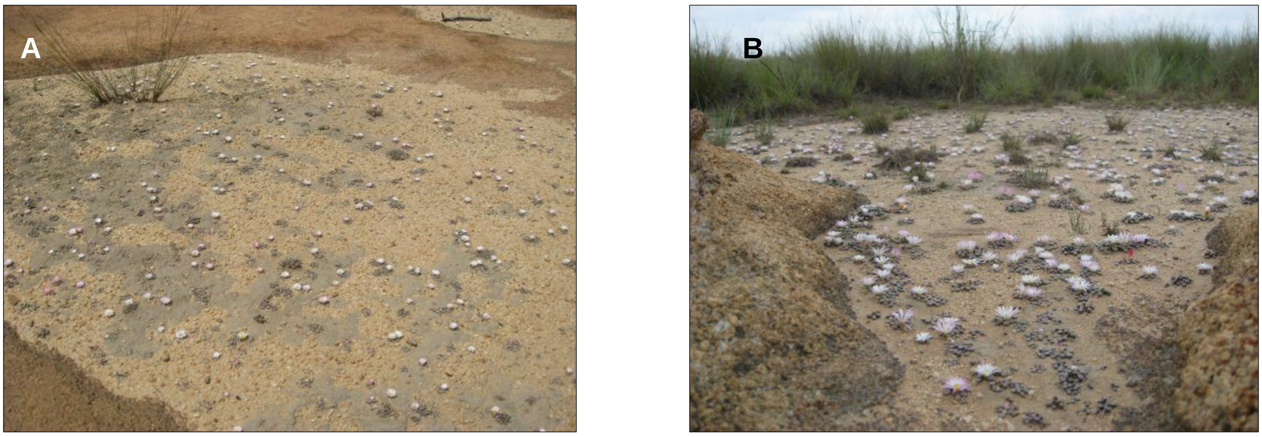

Figure 3.4. Ezemvelo Nature Reserve hosts the only protected natural population of *Frithia humilis*; this population occupies outcrops of the Ecca and Dwyka Groups (e.g. A) and forms part of the *F. humilis-Microchloa caffra* community (B) (Photos: P.M. Harris, 2012).

small populations, due to the ecological restrictions (Ihlenfeldt, 1994).

3.3 Monitoring

Monitoring forms a critical part of any translocation process, as it enables researchers to determine the extent of population establishment (Maschinski *et al.,* 2004). Hence, a labour-intensive monitoring programme was implemented in February of 2010 to collect accurate population data. Recording plant numbers and changes in population size or population trends are effective measures of initial translocation success (Joseph *et al.,* 2006; Menges, 2008). Measuring the abundance of individuals growing in each patch *per se* was not the only objective. Population relative age structure and demography were also determined, along with trends in flowering, fruit production and seedling recruitment, which are prerequisites for estimating population persistence (Mueck, 2000; Godefroid *et al.,* 2011).

3.3.1 Monitoring season

Monthly surveys of the translocated populations (Figure 3.5) were required to keep track of seedling emergence and survival, as well as adult growth, survival and reproduction (Menges, 2008). However, due to the seasonal appearance of *F.humilis* (populations being cryptic during the dry, winter months) (Burgoyne *et al.,* 2000b), populations were monitored only during the peak rainy season, from November to February. Counting plants during dry months would have caused habitat disruption, as the quartz layer had to be disturbed to find the plants. Monitoring was further reduced to the months of November and February specifically on account of time and budget constraints and began in February 2010.

3.3.2 Age classification system

The seed bank, juvenile and adult individuals were translocated in soil sods (Burgoyne & Hoffman, 2011). Relative age (RA) classes were required to measure changes in population trends, demographics and seedling recruitment. Age states in plant demographic studies are more impor-

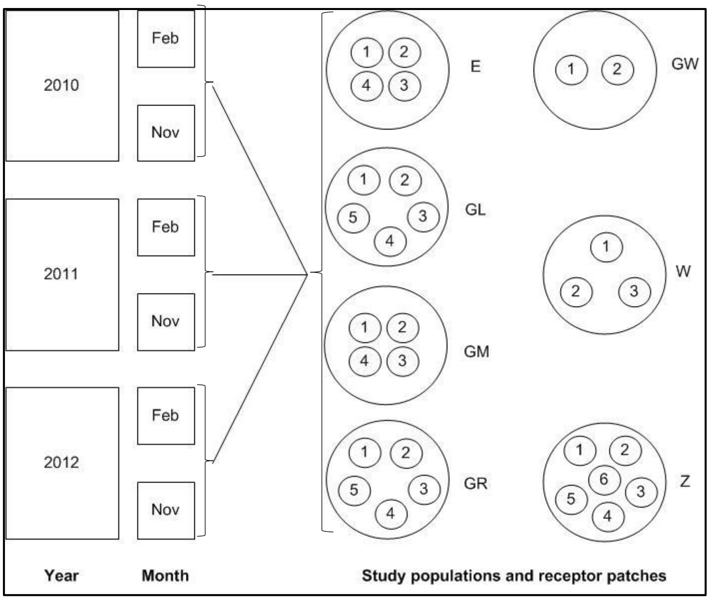

Figure 3.5. Data sampling structure: All receptor patches were sampled twice a year (February and November) for three years.

tant in analysing population structure than the use of calendar ages of individual plants. Age states are indicative of ontogenological stages of a plant, but more importantly of its ecological role (Gatsuk *et al.,* 1980).

A RA classification system was therefore designed, based on the number of leaves that each individual bears (Table 3.4). Age groups of succulents are generally determined by number of branches in the case of shrubs (e.g. *Euphorbia*; Knowles & Witkowski, 2000), rosette diameter when studying large forbs (e.g. *Aloe*; Pfab & Scholes, 2004) or number of leaves where small forbs are studied (e.g. *Haworthia*; Biko'o *et al.,* 2011).

Furthermore, results from *ex situ* studies on the species revealed that no individual with less than

Table 3.4. A relative age (RA) classification of *Frithia humilis* plants based on the number of leaves per plant, as well as reproductive capability.

RA class (leaves)	Reproductive group
<3	Seedling
3−5	Sub-adult (reproductive)
6−10	Adult
11−15	Adult
16−20	Adult
21−30	Adult
>30	Adult

three leaves produced flowers (Orlekowsky *et al.,* 2010; Glatz *et al.,* 2012). Such individuals were regarded as juveniles (non-reproductive individuals). The remaining groups were viewed as flower-bearing, reproductive adults with the exception of RA group 3−5. The latter was classified as sub-adults, as they represent a transition between juveniles and reproductive adults. Individual plants were classified according to Table 3.4.

Other characteristics were recorded, including the number of flowers, seed capsules and damaged/unhealthy leaves per individual. Therefore, data on population size, age structure, flower frequency, fruit production and general health were collected. In order to satisfy the broader aims of this study the counts were done in two ways, which will be discussed below.

3.3.3 Total counts

The first comprehensive counts were made six months thereafter to serve as a baseline for future comparative purposes and are discussed in Chapter 4. Total counts required that all individuals at each translocation site were counted using the RA system. Baseline data required that total counts be done exclusively for the translocated populations and did not include the control population, *Z*. Total counts provided data to measure the response of populations to the translocation disturbance, to create a record of total individual abundance per size class, as well as to keep track of fluctuations in total translocated population age structure and reproductive capabilities.

3.3.4 Grid counts

The number of plants per 1 m^2 was counted to gather statistically workable and comparable data on population dynamics and the results are discussed in Chapter 5. The experimental feasibility of the study was supplemented by the addition of a control – a naturally occurring population at Ezemvelo Nature Reserve which served as a ‘benchmark’ for population trends. Monitoring of this population commenced in February of 2011.

Grid count data was recorded within a 1 x 1 m metal frame (‘grid’) which was placed at fixed points within a patch, so as to be representative of the population at a particular locality and within the respective patches. Sampling points were fixed with markers drilled into the sandstone at each translocation patch to provide permanent reference points for consistent data gathering over time. Each individual plant was classified according to its RA class and counted along with flowering and fruiting data.

3.3.5 Analyses of population data

The processing of data acquired through this study is emperical in nature, as it investigates changes in populations over time (Devore & Farnum, 2005). Analyses of variance (ANOVA) are typically applied to data when differences (variance) between multiple groups (in this case translocated population and RA class means), as well as variance within those groups, have to be detected (Devore & Farnum, 2005). ANOVA is a commonly used statistical tool in ecological studies

(Bennington & Thayne, 1994). Descriptive statistics, i.e. population and RA group means and standard deviations, were used in the presentation of results.

However, in this case ANOVAs did not yield satisfactory, reliable results: The data did not exhibit normality/normal distribution due to variation in and smallness of population sizes (N), and did not display constant variance. Shapiro-Wilk and Levene's tests, as well as Q-Q and Box-plots confirmed that data did not satisfy the respective ANOVA assumptions. Furthermore, random and independent sampling methods could not be employed during monitoring, due to the small size of the populations.

A linear mixed model (LMM) was applied to the data, allowing for a variety of correlation patterns to be modelled. Population numbers of one year were dependent on the population numbers of previous years, as individuals can switch between RA classes from season to season due to leaf growth or senescence. Data were also collected from fixed points over time. This created a dependence of data (covariance), which a linear mixed model can account for. Since multiple measurements per translocation patch were performed biannually over three years, i.e. sampling was replicated over time and across populations, correlated data arose in the statistical analyses.

LMMs are often employed by ecologists when data are not distributed normally, including data generated by counts (Bolker *et al*., 2009), as well as to account for dependence or relatedness between data (Noël *et al*., 2011). Although ANOVA tests are robust enough to withstand non-normality, count data cannot typically be transformed, due to the occurrence of zero numbers (Bolker *et al.,* 2009).

IBM SPSS version 21 was used for all statistical analyses (IBM corp., Released 2012).

3.4 Entomological studies

It is essential for translocated individuals to complete their life cycle (Menges, 2008). For plant populations, this would imply the presence of active pollinator(s) in the habitat. The pollinator(s) of *F. humilis* have not yet been identified and their presence at the receptor sites was therefore not verified prior to translocation. Quantitative and qualitative methods were employed in an attempt to identify pollinators in each of the study sites, i.e. *E*, *GL*, *GM*, *GR*, *GW*, *W* and *Z*. These methods will be described in Chapter 6.

Chapter 4. Short-term translocation success of *Frithia humilis* populations in terms survival and plant growth: a baseline for future studies.

4.1 Introduction

A 2008 ecological survey by De Castro & Brits Ecological Consultants confirmed the presence of *Frithia humilis* Burgoyne, an endangered succulent plant species of the Mesembryanthemaceae family, at Inyanda Coal Mine (Exxaro) in Mpumalanga, where mining activities had already begun (McCleland, 2009). This species is declared as Endangered (EN), mainly due to its limited distribution range (the extent of occurrence being less than 3 000 km^2) and its fragmented habitat. Populations of the species are declining further, as habitats are increasingly being threatened by expanding mining activities, informal settlements, overgrazing, alien vegetation and unscrupulous harvesting for the horticultural trade (Burgoyne & Krynauw, 2005; Burgoyne & Hoffman, 2011).

Current mining activities pose a major threat, as much sought after coal deposits of the Mpumalanga Highveld often underlie the sandstone outcrop habitats of *F. humilis* populations. These sandstone outcrops are typically of the Dwyka and Ecca Groups, Karoo Supergroup (Cairncross, 2001). Hence, remaining natural populations of this plant species are at imminent risk from complete habitat transformation or loss.

One of the 11 known naturally occurring populations of *F. humilis* (Burgoyne & Krynauw, 2005) became threatened by mining activities after a mining license had been granted to Inyanda Coal Mine (Figure 4.1). In an attempt to save the population from extirpation, Inyanda Coal Mine in collaboration with the South African National Biodiversity Institute (SANBI) and Mpumalanga Tourism and Parks Agency translocated the population to one suitable receptor habitat (Burgoyne & Hoffman, 2011) and two experimental receptor sites during 2009.

This was the first conservation translocation attempt involving this species. However, reintroductions of other members of the Mesembryanthemaceae have been performed for restoration (Blignaut &

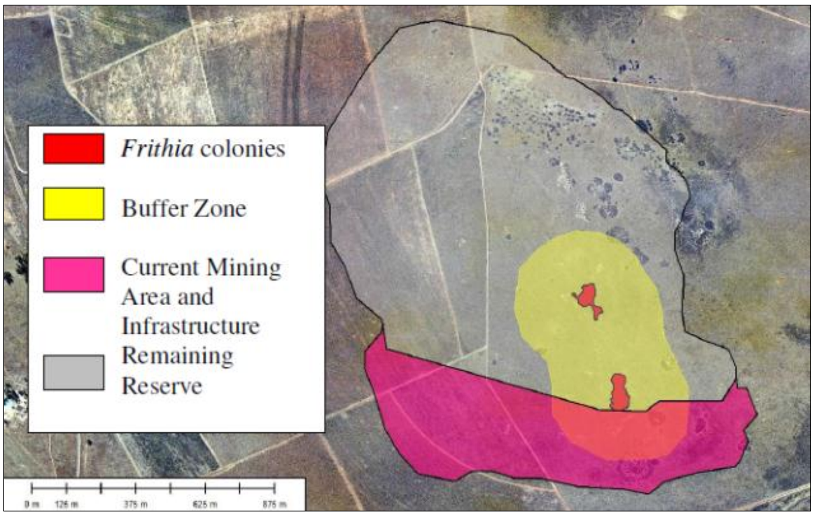

Figure 4.1. Location of the original donor *Frithia humilis* population *in situ*, prior to translocation in 2009 (Courtesy of De Castro & Brits Ecological Consultants).

Milton, 2005) and experimental purposes (Ellis & Weis, 2006). Generally, conservation translocations in South Africa have been applied to plant species in the Fynbos Biome and charismatic animals, such as big game species (Milton *et al.,* 1999). Consequently, the translocation of *F. humilis* and the resultant study can be viewed as a benchmark in the South African conservation context.

The translocation itself was not the endpoint, but rather the species' long-term survival at receptor sites. The success of a translocation can be determined once population establishment and long term persistence (including genetic/evolutionary viability) of the population has been ascertained (Griffith *et al.,* 1989; Armstrong & Seddon; 2008, Menges, 2008). Certainty can only be gained via long-term post-translocation monitoring of populations (Griffith *et al.,* 1989), focusing on plant survival, growth, and reproduction, as well as seedling recruitment (Pavlik *et al.,* 1993; Menges, 2008).

Corresponding with the scientific norm in translocation ecology, a *F. humilis* monitoring programme was initiated in February 2010. Firstly, the aim was to establish repeatable methodology for continued long-term monitoring, which allows, for a change in participants without disrupting data acquisition. Secondly, short-term indicators of translocation success (Menges, 2008) were investigated, including survival, plant growth, reproduction and seedling recruitment.

The possibility of translocation success could thus be predicted by initial indicators of population response to translocation, which would, in turn, determine human, financial and time resource allocation to the monitoring programme. A baseline of population data, particularly to inform future studies on the species and translocation of similar taxa, was also established.

4.2 Methods

Establishing an effective monitoring programme meant designing it in such a way so as to answer questions regarding effects of translocation on the populations (Armstrong & Seddon, 2008; Menges, 2008). Such questions referred to the immediate response of the translocated populations to the disturbance of translocation in terms of plant survival, growth and reproductive potential (Menges, 2008). Plant survival and growth will be discussed in Chapter 4, whereas the reproductive response of translocated populations will be discussed in Chapter 5.

Population monitoring was initiated in February of 2010, approximately six months after translocation took place. Data presented in this study represent comprehensive baseline figures and does not include individuals lost during the first six months after the translocation event. Data were gathered by implementing a relative age (RA) classification system, as outlined in Section 3.3.2 of Chapter 3.

A complete picture of translocated cohort (i.e. all individuals in the translocated populations, across all RA groups) response could be gained by means of total counts, i.e. population censuses, whereby each translocated individual, as well as plants that emerged after translocation, were counted. The populations at Eagle Rock Private Estate (*E*), Goedvertrouwd farm (*G*) and Witbank Nature Reserve (*W*) were surveyed. Censuses usually are impractical with regards to time, finances and other resources (Devore & Farnum, 2005). The small size of populations and the close proximity of receptor sites, however, rendered population censuses feasible in this case.

Population growth could be determined by monitoring changes in population size from year to year. Plant growth, on the other hand, was problematic to determine directly (e.g. Pfab & Witkowski, 1999; Biko'o *et al.,* 2011). Due to the small size of the species and its seasonal leaf retraction, as well as the exposed habitats, the marking of individual plants was not feasible. Collective growth had to be inferred from fluctuations in RA group size. For instance, a surge in the sub-adult group from one season to the next could have indicated seedling growth (individuals shifting from RA group <3 to the 3-5 group). However, such inferences were not ideal, since the source of RA group fluctuation could also stem from leaf senescence (individuals shifting to 'smaller' RA groups). Therefore, an increase in the number of individuals in one RA group, not accompanied by an equal decrease in subsequent RA groups was viewed as indicative of plant growth.

A linear mixed model analysis was applied to the data. Significant differences between population means were acquired by applying pairwise comparisons on estimated marginal means (EMM)[8]. By employing Sidak adjustments, multiple population and RA group means could be accurately compared and differences over time (2010 – 2012) and habitats (receptor sites) determined.

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[8] 'Estimated marginal means' (EMM) represent the transformed means of populations/samples of unequal size, enabling the comparison of sample means across different treatments, revealing significant differences between means. EMM represent a statistical prediction of population means and are values derived from linear mixed models applied to data with a non-normal distribution.

Plant productivity is often correlated with rainfall (Jürgens *et al.,* 1999). Therefore, the relationship between mean seasonal rainfall and population size was investigated in the receptor habitats. Rainfall figures were obtained via the South African Weather Service (SAWS). Rainfall data from the greater Witbank area were used for *E* and *W* and rainfall data from the greater Bronkhorstspruit area for *G*. Graphs representing correlations were applied to three different groups within each translocated population: seedlings (RA group <3), sub-adults (RA group 3-5) and adults (RA group 6-10 and up) (Table 3.9, Chapter 3).

Six superficial soil samples (15 cm x 5 cm x 5 cm) were taken from within *F. humilis* receptor habitats, i.e. *E*, *G* and *W*. Two were taken from the control population at Ezemvelo Nature Reserve (*Z*). Four soil samples from outside *E* and *G* sites were additionally taken. Soil of the donor population was also sampled prior to translocation in 2009. All samples were analysed for nutrient status, exchangeable cation amounts, particle size distribution and soil metal content to determine differences in edaphic characteristics of the donor and control populations, as well as the *F. humilis* receptor habitats. Soil analyses were conducted by Eco-Analytica Laboratory, Potchefstroom (http://www.nwu.ac.za/content/eco-analytica-laboratory-index).

Nutrient status analyses included tests for calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and phosphorus (P) concentrations ($mg.kg^{-1}$), pH measured in water (H_2O) and potassium chloride (KCl), as well as electrical conductivity (EC; $mS.m^{-1}$). The pH represents the acidity of soil on a scale from 0 to 14, where 7 is neutral; values below 7 are considered acid and values above 7, alkaline. pH H_20/KCl was measured by 1:2.5 extraction. EC indicates soluble salt content of soil and was measured through saturated paste soil extract; higher EC values are indicative of higher amounts of soluble salts (Bui, 2013).

Exchangeable cation analyses included tests for Ca^{2+}, Mg^{2+}, K^+ and Na^+ concentrations, cation exchange capacity (CEC) S-value and base saturation (BS%). Exchangeable cations were extracted by 1M ammonium acetate (pH = 7). The CEC of soil represents its capacity to retain nutrients. Higher CEC in soil is indicative of nutrient richness; CEC for sandy soils tends to be lower, since cations are easily leached and that of clayey soils is higher (Horneck *et al*., 2011; Hardy, *et al*., 2013). CEC for this study was determined by saturating the soil with 1M ammonium acetate at a pH of 7. S-values are the sum of interchangeable basic cations and are indicative of soil fertility. BS% represents the percentage of soil CEC taken up by basic cations (Ca, Mg, Na and K) at the existing soil pH and is considered an indicator of pH (Horneck *et al*., 2011), since higher BS% is correlated with increased pH (Hardy, *et al*., 2013).

Particle size distribution analyses tested for soil texture in the following four categories: >2mm, sand, silt and clay (the latter three being <2mm). Soil texture can be indicative of nutrient leaching and water retention (Ihlenfeldt, 1994; Bui, 2013). Soil metal content for each sample was also analysed. The following metals were tested as the most prominent: beryllium (Be), boron (B), Na, Mg, aluminium (Al), P, K, Ca, titanium (Ti), manganese (Mn), iron (Fe), zinc (Zn) and barium (Ba).

4.3 Results

4.3.1 Population size

No accurate records of pre-translocation figures have been made public. Burgoyne & Hoffman (2011) reported the presence of 3092 individual plants (422 adults, 1144 sub-adults and 1515 seedlings) at the 16 receptor patches at *G*. However, since population numbers are not elaborated on by said authors, the data presented in this study can be regarded as detailed baseline figures. This study commenced in February of 2010 and, therefore, a 'data gap' of six months exists between the translocation procedure and the first monitoring session. The total number of individuals at the onset of monitoring was 3433 (Table 4.1). Initial total population size (i.e. the total number of translocated individuals, across all receptor habitats) could be estimated, assuming that a loss of individuals did take place during and after translocation (Allen, 1994). The translocated cohort showed a 22% decline in numbers from 2010 to 2011, i.e. over 12 months. Also assuming that the initial reduction in total population numbers was no less than 22%, the original total number of individuals could have been 4400.

The translocated cohort (i.e. the translocated individuals in totality) did not show any statistically significant shifts in mean numbers over the monitoring period, since p-values of pairwise comparisons of yearly means ranged between 0.398 and 0.968 (Table A.0.1, Appendix A). Type III Tests of Fixed Effects[9] (Table 4.2) also indicated that no significant differences existed between the

Table 4.1. Mean total population size in the translocated populations per annum. % g represents the growth in a population between years. 2010 figures were used as a baseline. Figures based on actual census data, where the mean is derived from February and November counts in one year.

Receptor site	2010	2011	%g	2012	%g	3 year	%g
E	673	382	-43	364	-5	364	-46
GL	1226	1153	-6	1366	19	1366	11
GM	375	260	-31	280	8	280	-25
GR	947	636	-33	1301	105	1301	37
GW	157	138	-12	316	129	316	101
W	55	126	129	86	-32	86	56
Overall	3433	2695	-22	3713	38	3713	8

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[9] Type III Tests of Fixed Effects tests the significance of fixed effects/factors, e.g. the effect of study site.

Table 4.2. Type III Tests of Fixed Effects, depicting the effect that relative age (RA) group, receptor site and year, as well as their interactions, had on mean population size. * indicates a significant effect.

Effect	F-value	Significance
Year	1.079	0.342
RA group	20.287	0.000*
Receptor site	5.502	0.000*
Year/RA group	1.015	0.434
Year/Receptor site	0.634	0.783
RA group/Receptor site	3.465	0.000*
Year/RA group/Study site	0.445	1.000

mean size of the translocated cohort over time, as year had no significant effect on the mean size of populations. RA group size (number of individuals per RA group) had a significant effect in some populations, since the interaction between RA group and study site was significant. Receptor sites also significantly affected population means (i.e. all individuals across RA groups in a certain population) (Table 4.2). Therefore, populations at each receptor site had to be considered separately.

Since the onset of this study, in February 2010 comprehensive population censuses revealed an overall population growth of 8% (2010 – 2012). However, this was not true for all populations in the respective receptor habitats. The population at *E* showed a reduction in numbers of 46% over time and *GM* declined by 25%, while other receptor sites at *G* and *W* increased in size (Table 4.1).

Most populations declined from 2010 to 2011, but population numbers escalated during 2012 (Table 4.1; Figure 4.2; Table A.0.2, Appendix A), resulting in *GL*, *GR* and *GW* exhibiting higher numbers at the end of study period than in 2010; *W* showed an opposite pattern by having increased during 2011, followed by a decline during 2012. This population nevertheless showed a net increase in size from 2010 to 2012 (Table 4.1). By 2012 the *E* and *GM* populations showed declines of 46% and 25% respectively. *GL*, *GR*, *GW* and *W* increased in size by 11 %, 37%, 101% and 56% respectively (Table 4.1), over the monitoring period.

Pairwise comparisons indicated that *E* was the only receptor site with a significant decline in population size from 2010 to 2012 (Table 4.3). No other receptor site showed significant fluctuations (differences in mean total size), as p-values from pairwise comparisons ranged between 0.264 and 1.000 (Table A.0.3, Appendix A). *GM* and *W* differed significantly from *GL* (p=0.004 and 0.001

respectively), in terms of population means over the entire monitoring period; *W* also differed significantly from *GR* (p=0.027) (Figure 4.2; Table A.0.4, Appendix A).

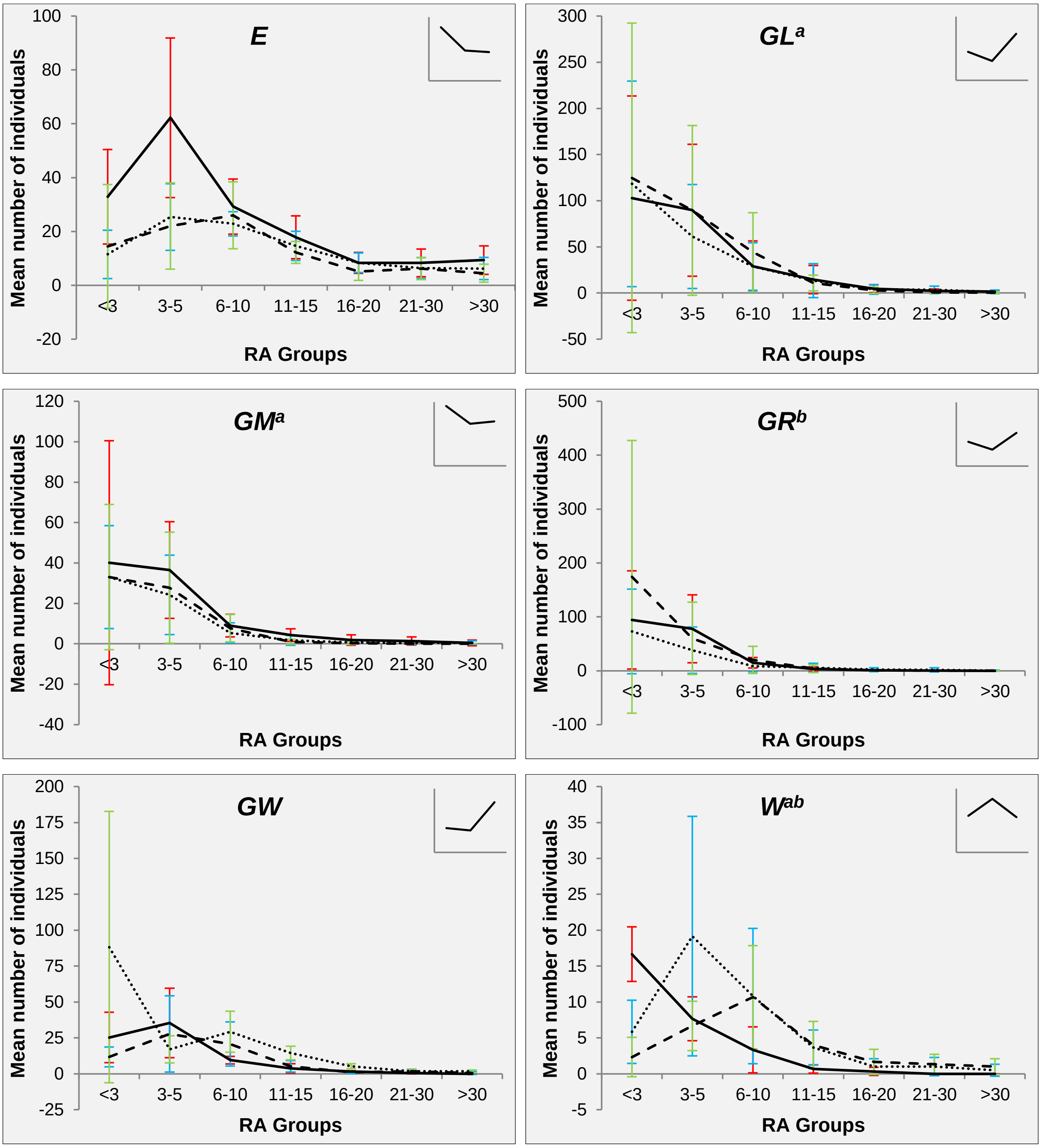

Figure 4.2. Mean number of individuals per relative age (RA) group in translocated populations during 2010-2012. Charts marked with corresponding letters indicate significant differences in the estimated marginal means of total individuals. Coloured bars indicate standard deviation (SD) of each mean; significant differences in yearly RA group occur where SD does not overlap. ***Curves inserted in top right corner of each chart represent the general trend in mean population size over three years; y-axis, mean number of individuals; x-axis, monitoring period*****. — 2010 (red) ···· 2011 (blue) – – – 2012 (green).**

Table 4.3. Pairwise comparisons of the estimated marginal means (EMM) of the total Eagle Rock population over the study period. * indicates significant difference in EMM (p<0.05) using Sidak adjustments for multiple comparisons.

Year		Mean difference	Standard error	p-value
2011 compared to	**2010**	-10.55	2.940	0.003*
2012 compared to	**2010**	-11.649	2.995	0.002*
	2011	-1.094	2.940	0.976

The translocated cohort as a whole did not reveal statistically significant fluctuations in yearly mean sizes of separate RA groups (Table A.0.5, Appendix A). However, standard deviation bars of *W* did indicated a probable significant decline in the seedling group (<3), since SD bars of 2010 means did not overlap with those of 2012 (Figure 4.2).

Population fluctuations over time were visibly more pronounced (though only significant for *W*) in the seedling/juvenile (<3), sub-adult (3-5) and 6-10 RA groups (Figure 4.2, Table A.0.2, Appendix A). The EMM of these groups were generally significantly higher than that of other RA groups over the monitoring period (Figure 4.3). Population increases in *GL*, *GR* and *GW* stemmed primarily from seedlings and sub-adults, although 'older' RA groups in *GW* and *W* also contributed to increased population size (Figure 4.2). Observed population fluctuations (population growth and decline) were therefore seated in the younger RA groups (<3, 3-5 and 6-10), while sizes of 'older' groups (11-15, 16-20, 21-30 and >30) remained more stable.

Plant growth could be inferred from shifting peaks in yearly RA group size within a population. *E* showed a decline in all RA groups over the monitoring period, apart from RA group 6-10 in 2012, with the addition of an average of 12 plants (Table A.0.2, Appendix A). However, the decrease of 'older' plants during the same year (an average of 21 plants from RA group 11-15 and up) exceeded the increase of individuals in RA group 6-10. Therefore, plant growth (i.e. a shift of seedlings and sub-adults to subsequent RA groups) could not be unambiguously deduced.

RA groups up to 6-10 at *GL* showed increased numbers by 2012, despite a decline in RA group 3-5 during 2011. There was an overall decline in the number of 'older' plants (RA groups 11-15 and above) and an overall increase in the number of sub-adults and individuals from RA group 6-10. The increasing amount of sub-adults and individuals from RA group 6-10 (with an average of 98 and 73 individuals, respectively) at *GL* were considered as indicative of plant growth, since the aforementioned increase exceeds the loss of larger individuals (an average of 21 plants) (Table A.0.2, Appendix A). The increased number of sub-adults, especially, was viewed as evidence for seedling recruitment – individuals passing from the seedling stage into the reproductive stage.

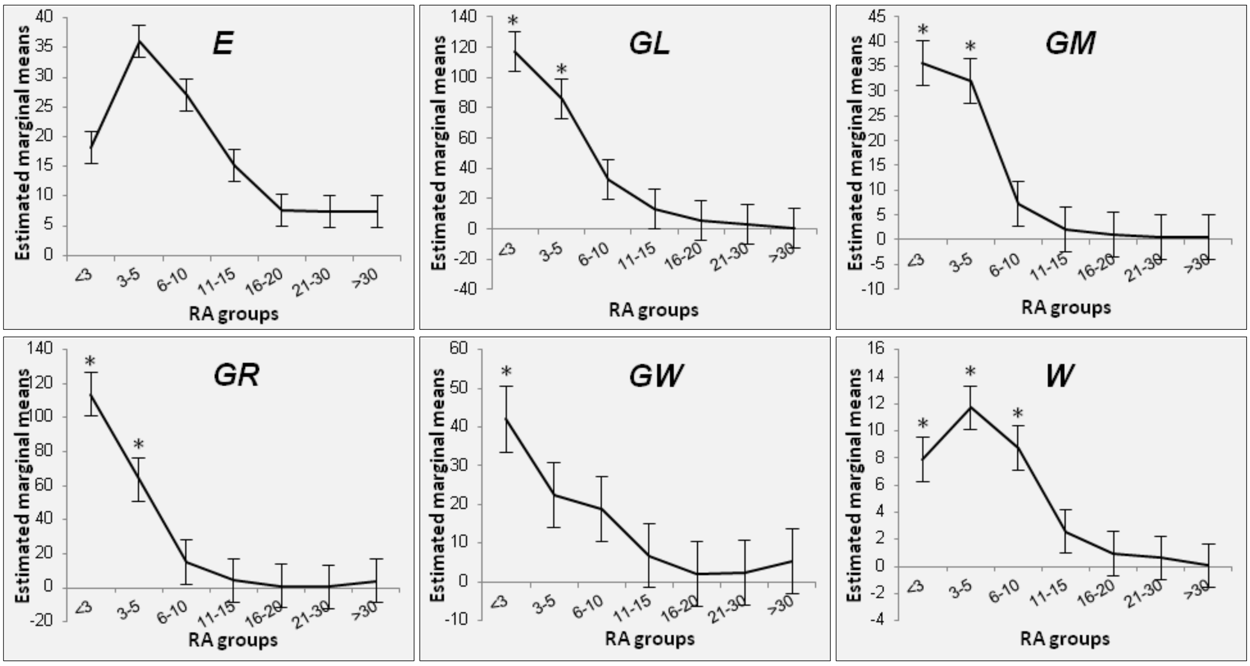

Figure 4.3. Relative age (RA) group estimated marginal means of the translocated populations, spanning all monitoring seasons. * indicates RA groups that differ significantly from other RA groups per translocated population. Bars indicate the standard error of each mean.

The translocated population at *GM* decreased across all RA groups during 2011. The decline continued in 2012 for RA groups 11-15 and ‘older’, with a loss of 12 individuals, whereas RA groups <3, 3-5 and 6-10 increased in size. RA groups 3-5 and 6-10 increased with an average of 15 individuals (Table A.0.2, Appendix A), thus exceeding the loss of individuals in subsequent RA groups. The addition of individuals to RA groups 3-5 and 6-10 was viewed as indicative of plant growth at the GM receptor site.

Plant growth was also apparent at receptor site *GR*. Despite an initial reduction in the sizes of RA groups <3, 3-5 and 6-10 during 2011, these groups showed increased numbers during 2012. A contrasting pattern was observed for RA groups 6-10 and ‘older’: during 2011 there was an overall increase in these groups, but numbers declined during 2012. Since RA groups 3-5 and 6-10 increased with an average of 168 and only 21 individuals were lost in ‘older’ RA groups during 2012, plant growth could be inferred at *GR*.

The population at *GW* showed unmistakable plant growth since the population increased in size from 2010 to 2012, with the exceptions of RA groups <3, 3-5 and 11-20 during 2011 and RA group 3-5 during 2012 (Table A.0.2, Appendix A). However, by 2012 the loss of individuals from the above mentioned RA groups were exceeded by the addition of individuals to other RA groups.

The *W* population exhibited growth throughout the monitoring period, apart from a loss of seedlings during 2011 and 2012 and sub-adults during 2012. An increased number of individuals in ‘older’ RA groups in the same year can be viewed as indicative of plant growth.

4.3.2 Rainfall effects

Upon inspection of the relationship between average seasonal rainfall figures and mean size of seedling, sub-adult and adult groups respectively (Figure 4.4), it was apparent that 'correlation patterns' (the trend line slope of each curve) of the above mentioned reproductive groups differed between receptor habitats (For an overview of mean seasonal rainfall figures, refer to Table A.0.6, Appendix A). *E* showed positive correlation patterns for seedlings, sub-adults and adults, i.e. high rainfall figures are generally correlated to high population numbers of all reproductive groups.

Most Goedvertrouwd populations (*G*) exhibited correlation patterns similar to one another in that seedlings and adults correlated negatively with mean seasonal rainfall. Sub-adult groups at receptor sites *GL*, *GR* and *GW* showed positive correlation patterns between mean plant numbers and mean seasonal rainfall. The *GM* population, however, exhibited a correlation pattern similar to that of *E*.

RA groups at *W* showed correlation patterns different to that of the *G* and *E* receptor sites, since seedling and sub-adult groups showed positive correlation patterns and the mean adult group size was negatively correlated with mean rainfall figures.

4.3.3 Soil effects

Differences in soil properties between study sites were to be expected, since geological substrates varied. Nutrient content typical of *F. humilis* edaphic environments were considered to be demarcated by values of the donor and control populations, i.e. 10.5 – 38.0 mg.kg^{-1} for Ca, 9.5 – 15.5 mg.kg^{-1} for Mg, 29.0 – 30.5 for K, 4.5 – 11-5 mg.kg^{-1} for Na and 12.7 – 15.6 mg.kg^{-1} for P (Table 4.4). Samples deviating most frequently from the above mentioned limits were collected from outside *E* and *G* habitats, as well as from *W* ('Outside' refers to soil samples taken 50 m away from the *F. humilis* population at a particular study site). These deviations were generally higher than limits of typical *F. humilis* habitats for Ca, Mg and K. P concentrations from all receptor site and 'outside' soil samples, except for *W*, were lower than the limits set by the donor and control populations.

Nutrient contents of *G* habitats agreed more frequently with the limits for *F. humilis* habitats, although lower than normal concentrations of Mg, Na and P were found (Table 4.4). Deviations from pH limits (H_2O: 4.19 – 4.50, KCl: 3.75 – 3.86) were found in samples from outside *E*, outside *G* and *W* of which the pH was slightly higher. However, all soil samples can be considered acidic, since the pH at all study sites were smaller than seven. EC at receptor sites *E, G* and outside *G* were lower than the typical range for *F. humilis* habitats (40 – 52 mS.m^{-1}), that of *W* and the outside sample of *E* were within the range.

Exchangeable cationic ranges for typical *F. humilis* habitats were regarded as being delimited by values of the donor and control populations (Ca: 0.05 – 0.19 cmol(+).kg^{-1}, Mg: 0.08 – 0.13 cmol(+).kg^{-1}, K: 0.07 – 0.08 cmol(+).kg^{-1}, Na: 0.02 – 0.05 cmol(+).kg^{-1}) (Table 4.5). No receptor site

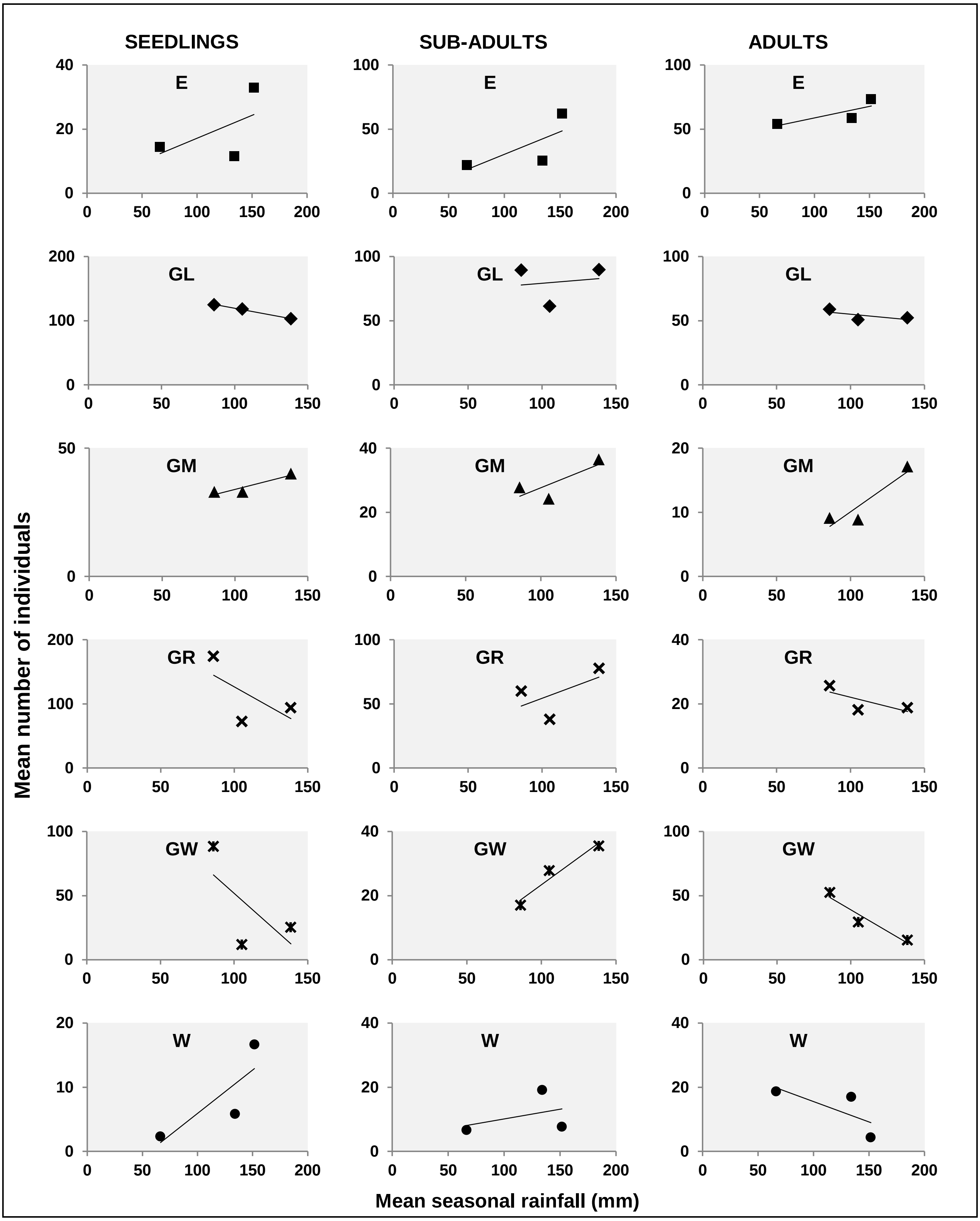

Figure 4.4. Relationships between the mean sizes of reproductive groups (seedlings, sub-adults and adults) in each receptor habitat over the monitoring period and the average rainfall per season (November-February). Trend lines with positive gradients are indicative of positive correlations between rainfall and reproductive group size and *vice versa*.

Table 4.4. Analysis of soil nutrient status (mg.kg^{-1}) and electrical conductivity (EC) in study areas, including Ezemvelo Nature Reserve, a natural *Frithia humilis* habitat serving as a control site. 'Outside' refers to soil samples taken 50 m away from the *F. humilis* population at a particular study site.

Study site	Ca	Mg	K	Na	P	pH		EC (mS.m^{-1})
						H_2O	KCl	
Donor population	10.50	9.50	29.00	11.50	15.60	4.19	3.75	40.00
E	17.00	7.00	40.00	7.00	12.30	4.42	3.70	36.00
E (outside)	94.50	19.00	44.50	7.50	6.17	4.94	4.11	41.00
G	18.50	3.50	29.00	2.00	9.60	4.40	3.74	17.00
G (outside)	52.00	14.50	38.00	1.00	10.97	4.61	3.83	19.00
W	101.00	30.00	113.50	9.50	21.30	4.44	3.87	42.00
Z	38.00	15.50	30.50	4.50	12.70	4.50	3.86	51.00

Table 4.5. Analysis of soil exchangeable cations (cmol(+).kg^{-1}), S-value and base saturation in study areas, including Ezemvelo Nature Reserve (*Z*), a natural *Frithia humilis* habitat serving as a control site. 'Outside' refers to soil samples taken 50 m away from the *F. humilis* population at a particular study site.

Study site	Ca	Mg	K	Na	CEC	S-value	Base saturation (%)
Donor population	0.050	0.080	0.070	0.050	7.660	0.250	3.330
E	0.080	0.060	0.100	0.030	8.150	0.280	3.380
***E* (outside)**	0.472	0.156	0.114	0.032	5.970	0.770	12.970
G	0.090	0.030	0.070	0.010	7.110	0.200	2.870
***G* (outside)**	0.259	0.119	0.097	0.004	7.030	0.480	6.840
W	0.500	0.250	0.290	0.040	10.290	1.080	10.530
Z	0.190	0.130	0.080	0.020	6.660	0.410	6.230

coincided frequently with the exchangeable cationic ranges of *F. hum*ilis habitats. Higher amounts of exchangeable cations were measured in receptor sites *E* (K = 0.1 cmol(+).kg^{-1}) and *W* (Ca: 0.5 cmol(+).kg^{-1}; Mg: 0.25cmol(+).kg^{-1}; K: 0.29 cmol(+).kg^{-1}). Samples from outside receptor sites *E* and *G* also yielded higher amounts of exchangeable cations (*E*, Ca: 0.472 cmol(+).kg^{-1}; Mg: 0.156 cmol(+).kg^{-1}; K = 0.114 cmol(+).kg^{-1}; *G*, Ca: 0.259 cmol(+).kg^{-1}; K: 0.097 cmol(+).kg^{-1}).

CEC values of soil samples taken within and outside *G* were typical for *F. humilis* habitats, while CEC values of *E* and *W* were higher (8.15 and 10.29 respectively) than the typical CEC range and that of the outside sample at *E* was lower (Table 4.5). All receptor sites, apart from *E* measured S-values outside the typical range for *F. humilis*. The two external samples of *G* and *E* measured higher S-values (0.48 and 0.77), *W* measured the highest S-value (1.08), while *G* measured a lower S-value (0.2) (Table 4.5). Soil samples of *E* were within base saturation limits for *F. humilis* habitats and that of *E* and *G* outer samples (12.97 and 6.84), as well as *W* (10.53) were higher than typical, while the base saturation value for the *G* receptor site was lower than typical (2.87).

As with nutrient content and exchangeable cation ranges of soil from *F. humilis* habitats, the range for soil particle distribution (Table 4.6) was represented by percentages measured at the donor and control sites respectively. Soil particle distribution of both the *G* receptor site and the outer soil sample at *G* were within limits for most soil particle sizes (>2 mm, sand, silt and clay). The only exception was the percentage of silt in receptor site *G* being lower than the typical range (5.85%) (Table 4.6). Soil samples of most study sites, apart from *W* (24.44 %), had clay percentages within the range for *F. humilis* habitats.

Receptor site *E* soil had fewer coarse (>2 mm) particles (4.01 %) and silt (5.34 %) than typical *F. humilis* habitats, but was more sandy (88.2 %). The outer soil sample at *E* yielded results similar to that of receptor site *E*, since the percentages of coarse particles (0.03 %) and silt (3.06 %) were lower and that of sand particles was higher (90.67 %) than typical habitats. *W* exhibited a higher percentage of coarse particles (53.6 %) and silt (31.75 %), but was less sandy (43.81 %) than typical *F. humilis* habitats, making *W* soil the least typical, in terms of soil particle distribution.

Only selected elements were visually presented to showcase the most dramatic differences in soil metal content (Figure 4.5). A more complete account of soil metal analyses is provided in Table A.0.7, Appendix A. Soil metal content of *E* and *G* outer samples were always higher than that of receptor sites (*E*, *G* and *W*) and of the donor and control (*Z*) populations (Figure 4.5). Receptor

Table 4.6. Analysis of soil particle size distribution in study areas, including Ezemvelo Nature Reserve (*Z*), a natural *Frithia humilis* habitat serving as a control site. 'Outside' refers to soil samples taken 50 m away from the *F. humilis* population at a particular study site.

Study site	**> 2 mm (%)**	**Sand (%)**	**Silt (%)**	**Clay (%)**
Donor population	15.313	70.00	11.69	18.31
E	4.01	88.20	5.34	6.47
***E* (outside)**	0.30	90.67	3.06	6.27
G	9.00	87.07	5.85	7.08
***G* (outside)**	12.10	75.82	8.85	15.33
W	53.60	43.81	31.75	24.44
Z	7.78	87.72	7.82	4.45

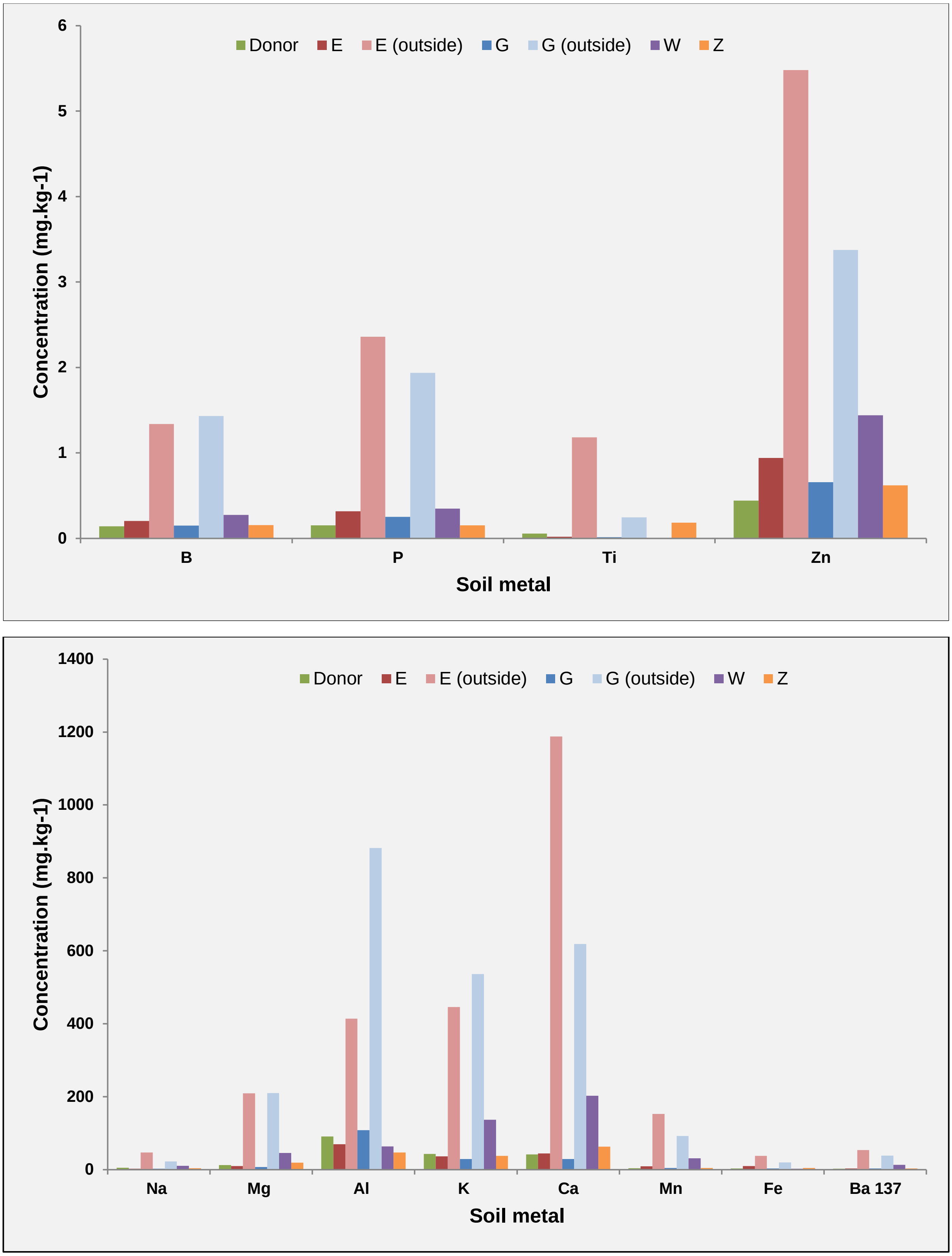

Figure 4.5. Soil metal concentrations in the translocated populations (*E*, *G* and *W*), the donor population and the control population (*Z*). A, maximum values < 6mg.kg^{-1} and B, minimum values > 6 mg.kg^{-1}. 🟩 Donor population 🟥 *E* 🟥 *E* (outside) 🟦 *G* 🟦 *G* (outside) 🟪 *W* 🟧 *Z*.

site *W*, the soil of which often deviated from typical values in terms of nutrient status (Table 4.4), exchangeable cation values (Table 4.5) and soil particle distribution (Table 4.6), also frequently exhibited higher values of soil metal content than other study sites (excluding soil outside *E* and *G*, but including the donor and control populations). This was true for the following elements: B, Na, Mg, P, K, Ca, Mn and Zn. *W* soils scored the lowest values of Ti and Fe compared to all other study sites.

Metal content of the *E* receptor site soils were higher than that of donor and control site soils for elements B, P, Mn, Fe and Zn, the former three of which are heavy metals. *E* soils scored a metal content lower than or similar to the control and donor sites, as well as receptor site *G*, for the following elements: Na, Mg, Al and K. *G* receptor site soils were similar to that of the donor and control population for elements B, P, Mn, Fe and Zn, but scored lower metal content for elements Na, Mg, K, Ca and Ti. Receptor site *G* soils exhibited the highest Al content of study sites excluding the exterior samples.

4.4 Discussion

The loss of translocated individuals is inevitable (Allen, 1994). Jusaitis (2005) reported that experimentally translocated populations of *Prostanthera eurybioides* (Lamiaceae) experienced primary loss of individuals during the first year after transplantation. No further losses were recorded and one population continued to establish successfully, with 80% of the translocated population surviving. Blignaut & Milton (2005) viewed the survival of translocated mesemb species in the succulent Karoo (with very dry and hot summer conditions) as good when 68% of the total translocation cohort survived after 17 months. Mueck (2000) considered the survival of >60% of translocated *Pimelea spinescens* ssp. *spinescens* (Thymelaeaceae), an endangered species endemic to Victoria, Australia, as successful two years after translocation.

E was the only population to show a statistically significant decline in population size from 2010 to 2012. Less than 60% of the population survived three years after translocation. However, during 2012 the population at receptor site *E* seemed to stabilise, as there was no further significant decline in population size. Stabilisation of this population in the future may thus be possible, provided that reproductive outset, i.e. seedling production, can be upheld (Knowles & Witkowski, 2000). Short term translocation success at receptor site *E* was, therefore, generally poor, since the majority of plants did not survive. Moreover, plant growth could not be explicitly proven and seedling emergence was dubious. The meagre ‘performance’ of *E* as a receptor site was despite attempts to improve germination conditions and recruitment with imported gravel from the donor site. The latter was continually washed away during rainfall events, leaving plants and their root systems exposed. Furthermore, population numbers of *E* correlated with rainfall figures, since the overall number of seedlings, sub-adults and adult plants at *E* decreased over the study period, associated with the decline in mean seasonal rainfall (Jürgens *et al*., 1999; Maschinski *et al*., 2004). This suggested that plants experienced desiccation. *E* could therefore benefit from manual watering during below average rainfall seasons as a mitigation measure (e.g. Ellis & Weis, 2006; Murphy *et al.,* 2008).

Receptor site *GM* showed a 25% reduction in population size from 2010 to 2012, with all reproductive groups correlating with decreasing seasonal rainfall in the area. This receptor site was the only site on Goedvertrouwd farm to show a negative response to translocation. Although *GM* did not host the smallest population (*GW* did), it was home to the population with the least amount of adult plants. Reductions in RA groups 'older' than 6-10 measured in *GM* (as well as *GL*) were most likely caused by rodent herbivory (Figure 4.6). Holes at these receptor patches were indicative of herbivory by omnivorous striped field mice (*Rhabdomys pumilio*) (Dewsbury & Dawson, 1979), identified by droppings collected around the holes (Venter, 2012). Herbivory was especially prevalent during the dry season, presumably due to lack of other food sources. However, the perpetual seedling emergence over the monitoring period at *GL*, which was lacking at *GM*, counteracted the loss of larger individuals. *GM* was not further than 60 m from either *GL* or *GR* and the complete absence of pollinators could thus not have been the cause of reduced seedling numbers and this issue will be further explored in Chapter 5.

Translocated populations at *GL*, *GR*, *GW* and *W* responded more positively to translocation, since these populations showed net increase in numbers over the monitoring period. Plant growth, an increase of individuals in higher RA groups, was also apparent at these receptor sites, indicating habitat suitability, as well as early signs of post-translocation establishment (Menges, 2008). *GR*5 was the only population patch to show expansion, since many seedlings were counted outside the original translocation receptor area. Seed dispersal into surrounding habitats is deemed an indicator of population persistence and eventual translocation success (Drayton & Primack, 2012).

Figure 4.6. *Frithia humilis* plants uprooted and removed from populations at some Goedvertrouwd receptor site (*GL* and *GM*). Holes were dug by striped field mice, *Rhabdomys pumilio* (Photo: D. Zaayman, 2011).

Differential survival of translocated populations in similar receptor habitats has been reported by other authors, e.g. McClain & Erbinger (2008) for *Tetraneuris herbacea* (Asteraceae) and Severns (2003) for *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Jusaitis (2005) also mentioned that receptor habitats situated within a few meters of one another may produce different outcomes for translocated plant populations, due to slightly differing habitat conditions. These habitat conditions may not always be perceptible or explicable (Albrecht & Maschinski, 2012).

F. humilis populations in the Goedvertrouwd receptor habitat showed the largest increase in population sizes and the property therefore warrants protection, to ensure the survival of the most successful transplanted populations.

W showed an increase in adult (RA groups 6-10 and 'older') numbers despite declining rainfall, i.e. there was no correlation between rainfall and adult plant numbers. This could be linked to the high clay content of the soil and therefore water retention capacities. It is proposed that soil remains moister in drier seasons and enables established individuals to grow. Moreover, large rocks in the patches seemed to protect adult *F. humilis* individuals from environmental elements, as plants growing next to rocks were notably healthier and more turgid than other plants. Herbivory was also noted at *W*. Larval lepidopterans were found feeding on adult plants during 2012 (Figure 4.7). Adult plants at this receptor habitat increased in number, however, and herbivores were not viewed as a significant threat to population survival at that time. Continued monitoring will reveal whether herbivory of this kind will imperil the population in the future, since herbivory has been linked to impaired translocation success (Jusaitis, 2005; Godefroid *et al*., 2011).

The population at *W* did not exhibit an increase in seedling numbers. Indeed, there was a significant loss in the amount of plants in the RA group <3. The reduction of seedlings at *W* can be associated with drier climatic conditions. Pebbles at *W* were observed to be more angular in shape, were

Figure 4.7. A larval lepidopteran found feeding on adult *Frithia humilis* plants at Witbank Nature Reserve (Photo: D.M. Komape, 2012).

generally larger and less abundant than the quartz pebbles produced by sedimentary rocks. The decline in seedling numbers could be linked to the larger above ground particles, which do not provide sufficient protection for seedlings (Burgoyne *et al.,* 2000b). Low pollination frequency may also account for reduced seedling numbers. Low juvenile numbers could be an early indication of future population extinction (Knowles & Witkowski, 2000) and the *W* population is therefore at risk.

RA groups <3, 3-5 and/or 6-10 contributed significantly to *F. humilis* population size at all receptor sites. The presence of seedlings and young individuals has been reported as critical in the perpetuation of certain succulent species (*Euphorbia barnardii*, Knowles & Witkowski, 2001; *Haworthia koelmaniorum* var. *mcmurtryi*, Biko'o, *et al*., 2011). Younger RA groups can therefore be viewed as important in determining post-translocation success of *F. humilis* populations, since the growth of translocated populations at *GL*, *GR* and *GW* can be attributed to these groups. Furthermore, larger individuals were generally targeted by herbivory, narrowing the age structure to age classes <3, 3-5 and 6-10. Consequently, these age classes can become the 'engine' of population perpetuation in translocated *F. humilis*.

This observed contribution will be referred to as the 'Frodo[10]-effect' for the purpose of this study. It is suggested that the survival and establishment of a translocated *F. humilis* population is not dependent on the fittest or largest individuals, but more on the 'weaker', smaller individuals, which seemingly have the ability to adapt to environmental conditions posed by novel environments. Larger individuals were exposed to herbivory, erosion and drought stress in the new habitats. Contrary to these findings, younger individuals during translocation attempts have been found to be more vulnerable to mortality than older plants (e.g. Helenurm, 1998). However, the response of different species to translocation is bound to vary (Noël *et al*., 2012).

Seedlings at *GL*, *GR* and *GW* responded differently to seedlings at *E* to declining rainfall in the broader area. Seedling numbers at *GL*, *GR* and *GW* were negatively correlated to rainfall: seedlings increased despite drier environmental conditions. Seedlings seemed more resilient against drought stress due to the presence of possible 'nurse plant' species, *Selaginella dregei* (Selaginellaceae), especially abundant among *G* receptor sites. Nurse plants have been associated with protective environments for emerging succulent seedlings (Valiente-Banuet & Ezcurra, 1991). Maschinski *et al*. (2004) also found that shrubs protected translocated seedlings of *Purschia subintegra* (Rosaceae) for three years following translocation. Similarly, Lanno & Sammul (2014) reported that the survival of transplanted populations of *Ligularia sibirica* (Asteraceae) responded positively to the

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[10] 'Frodo' is a reference to the unlikely protagonist in J.R.R. Tolkien's classic fantasy novel, *The Lord of the Rings*, where a small, seemingly insignificant 'hobbit', and not a strong (typical) hero, was burdened with a task to save Middle-Earth, the setting of the novel, from impending doom.

presence of neighbouring vegetation in receptor sites. *Frithia humilis* seedlings were especially prevalent amongst the foliage of this procumbent fern at *GL*, *GR* and *GW*.

Some *GR* patches (*GR*1 – 4), however, exhibited sparser vegetation cover, as well as extensive disturbance by ants. The increase in seedlings at *GR* was primarily measured at *GR*5, even though this receptor patch had a steeper slope and therefore increased risk of damaging water flow. Seedlings emerged successfully presumably due to the presence of *S. dregei* colonies, as well as additional cover from the low-growing suffrutex *Searsia magaliesmontanum*, resulting in less exposed down-slope habitats. Vegetation cover at this patch diminished evaporative water loss, which could account for the increase in plant numbers despite lower rainfall figures in 2012, since seedling numbers did not correlate with lower seasonal rainfall. *Selaginella dregei* grew less abundantly at receptor sites *E* and *W*, while the fern was not present in downhill area of *GM*, possibly contributing to the decline in seedling numbers throughout post-translocation monitoring.

Selaginella dregei colonies presumably created suitable conditions for *F. humilis* seeds to germinate in, since quartz pebble sediments were also layered more thickly among these colonies. It was deduced that rain water washed a portion of the top gravel layer, along with *F. humilis* seeds into the fern colonies. *Frithia humilis* seeds, being dispersed in typical Mesembryanthemaceae fashion from capsules to the top gravel layer next to mother plants, are subject to being washed by rainwater (Burgoyne *et al.,* 2000b). *Selaginella dregei* colonies therefore acted as 'traps' for *F. humilis* seeds and possibly for additional nutrients (e.g. 'fertile islands', Stock, *et al*., 1999).

Contrarily, Anderson *et al*. (2004) found that small-seeded Karoo mesemb species tended to germinate in open spaces as opposed to vegetated areas (Esler, 1999). De Villiers *et al*. (2001) likewise did not find conclusive evidence that shrub species in the Succulent Karoo was significantly advantageous to seedling recruitment and survival. These authors did, however, mention that ecological restoration efforts may benefit from protective shrubs, in that transplanted vegetation on rehabilitated mine spoils may be protected from environmental elements, such as wind. Translocated *F. humilis* seedlings may, therefore, benefit from a protected micro-environment created by *S. dregei*.

Much fewer sub-adult plants were observed among *S. dregei* plants. Competition for space and nutrients among the two species could pose a problem for growing seedlings and sub-adults (Anderson *et al*., 2004), but this effect had not been observed by the end of the study period. The presence of *S. dregei* was not viewed as a limiting factor in post-translocation seedling emergence, since seedlings were present in areas lacking the fern. Nonetheless, *S. dregei* colonies may be beneficial to the establishment of translocated *F. humilis* populations.

A number of studies have highlighted the benefits and disadvantages of different propagule types in the successful establishment of translocated plant populations, i.e. seeds, seedlings and/or whole plants (Milton *et al*., 1999; Jusaitis *et al*., 2004; Fahselt, 2007; Menges, 2008; Godefroid *et al*., 2011). Godefroid *et al*. (2011) reported that transplants sourced from a stable donor population are more likely to survive than when sourced from declining populations. The donor *F. humilis* population, being stable, consisted of a natural array of potential propagules, i.e. seeds in the 'seed bank', seedlings and mature, reproductive plants.

Albrecht & Maschinski (2012) concluded from their comprehensive review of translocation efforts that populations founded with adult plants had a higher probability of survival, growth and reproduction than populations initiated with seeds or seedlings. The distribution of RA groups in the founding *F. humilis* populations (recorded during the first monitoring session during February 2010) revealed that all translocated populations hosted more seedlings and sub-adults than adult plants. The heterogeneity of propagule type, as well as the RA group distribution, in the donor *F. humilis* populations may have influenced the ability of the translocated populations at *GL*, *GR*, *GW* and *W* to survive and grow.

The initial size of a translocated population is an important factor for population persistence (Bottin *et al*., 2007). The translocation process in effect fragmented the donor *F. humilis* population into three smaller populations. Although this was necessary due to spatial constraints in receptor habitats and genetic considerations, e.g. genetic contamination of natural populations (Burgoyne & Hoffman, 2011), the small size of the 'new' populations gave rise to some concerns, both genetically and ecologically. Small populations may fail to establish, owing to demographic stochasticity or to low reproduction/survival rates (Armstrong & Seddon 2008) and are also at increased risk of extinction via amplified genetic drift and inbreeding depression (Kearns *et al.,* 1998; Godefroid *et al*., 2011). Godefroid *et al*. (2011) summarised suggestions from a number of authors regarding the minimum number of requisite plants to establish a translocated population, ranging from 500 – 2500 individuals. The number of individuals recorded at translocation receptor sites in February 2010 (672 at *E*, 1226 at *GL*, 375 at *GM*, 947 at *GR*, 157 at *GW* and 55 at *W*) was often less than suggested. Albrecht & Maschinski (2012), however, emphasised that reintroduced plant populations of 50 individuals or less are at a greater risk of extinction through demographic stochasticity. Populations at *GW* and *W* (the two smallest populations) increased in size over the monitoring period.

Where population survival and plant growth are concerned, results from the *F. humilis* translocation study suggest that this species can be translocated in small numbers (approximately 50 – 1300 individuals), with various degrees of success, over the short-term, These results were supported by post-translocation findings over a long period (30 years) of Cieślak *et al*. (2007) for a single translocated population of the endangered *Cochlearia polonica* (Brassicaceae), as well as by shorter term post-translocation results (5-10 years) of Maschinski & Wright (2006) for *Jacquemontia reclinata* (Convolvulaceae) and Jusaitis (2005) for *Prostanthera eurybioides*. Small populations are capable of persisting in habitats of sustained suitable quality (Matthies *et al*., 2004). The on-going management and protection of all *F. humilis* receptor sites are therefore imperative. Genetic implications for small populations of *F. humilis* should nonetheless be investigated and Albrecht & Maschinski (2012) suggest monitoring translocated populations with small founder populations over a timescale of at least five years.

Measurements of soil characteristics indicated that receptor site *W* deviated frequently from the proposed range of soil characteristics, in having higher nutrient content, different soil particle distribution and higher content of certain metals. *W* contained more silt and clay (is less sandy) than other translocation sites and contains more coarse particles, which is typical of *F. humilis* habitats (Burgoyne *et al*., 2000b). The donor population was less sandy than all other study sites (apart from *W*) and had the second highest clay content, possibly indicating that *F. humilis* plants are able to survive in less sandy conditions. Schmiedel & Jürgens (1999) reported that some contractile mesemb species in the Succulent Karoo can occupy shale outcrops, granite bedrock fissures and other ‘desert pavements’.

Coarse particles (>2 mm) in the soil of most study sites were less than that measured for other quartz-field mesemb species, such as *Argyroderma fissum* and *A. pearsonnii*, in the Succulent Karoo (Ellis & Weis, 2006). Stone content in receptor site *W* was similar to that found by Ellis & Weis (2006) for the above mentioned species. However, *W* was covered not by quartz gravel, but by felsitic pebbles.

Metal content from soil outside of *F. humilis* receptor sites (at *E* and *G*) were notably higher than soil from the donor and control sites (Figure 4.5). Survival of *F. humilis* individuals dispersed to areas outside receptor sites *E* and *G* would thus be unlikely, even though other soil characteristic did not differ as dramatically from the suggested limits of typical *F. humilis* habitats. Some mesemb species, however, are able to grow in soil with high metal content (Ellis & Weis, 2006) and even metal toxic soils such as serpentine (Siebert *et al*., 2001).

Soil at *G* was less saline (had a lower EC value) than other study sites. Salinity in all study sites was higher than found for quartz habitat specialist mesembs in the Succulent Karoo (Ellis & Weis, 2006). The pH-values were relatively similar between sites, in that all sites had acidic soils (pH < 5), which is lower than that of other quartz-occupying mesemb species in the Succulent Karoo (pH 6.4 - 8.4) (Ellis & Weis, 2006). Schmiedel & Jürgens (1999) reported higher stone content than at the *F. humilis* study sites (apart from *W*), lower EC and comparable pH in quartz fields associated with high proportions of endemic mesemb species.

CEC values at receptor site *G* were within the proposed limits set by the donor and control population soil parameters, which indicate that *F. humilis* prefers to grow on nutrient poor soil, since survival and growth at most *G* receptor patches were notable. Ihlenfeldt (1994) also reported that some mesemb species occupy nutrient poor edaphic habitats in the Succulent Karoo. Soil particle distribution of *G* also mostly coincided with said limits, except for lower silt content.

Generally, soil characteristics varied between the donor, control and receptor, sites. *Frithia humilis* populations were able to occupy these soils with some degree of survivorship and growth, even though many Mesembryanthemaceae species, especially in the succulent Karoo, are limited to specific soil types (Ihlenfeldt, 1994).

4.5 Recommendations

A minimal interference policy was upheld after the *F. humilis* translocation. In so doing, plants sensitive to the translocation disturbance or unable to adapt to the new environments would phase out of the population gene pools. This was not a typical policy, but followed an approach similar to that of Colas *et al.* (2008) for the experimentally translocated *Centaurea corymbosa* (Asteraceae), namely that no post-translocation management was implemented. These authors emphasised the importance of an intensive monitoring programme (in lieu of a management programme as such) in order to identify possible causes of translocation failure, as well as to assess the extent of translocation success. Guerrant (2012) mentioned the completion of a translocated population's life cycle *without* human intervention as a parameter for long-term translocation success. Therefore, the 'hands-off' approach to post-translocation management of *F. humilis* populations has grounds.

Beneficial manipulation of conditions under which translocated populations can establish, such as watering of plants, caging against herbivores or redistribution of quartz and quartz-like pebbles cannot entirely compensate for the disturbance caused by coal mining activities and the translocation procedure alike. However, intensive post-translocation management may optimise population establishment and translocation success (Godefroid, *et al*., 2011). Translocation is a human-induced disturbance (albeit potentially advantageous to species conservation), and since the conservation of an endangered species was the ultimate goal of the translocation described in this study, post-translocation management may have benefited the short-term outcome of the *F. humilis* translocation.

Seedlings and juveniles contributed significantly to the size of translocated populations. Anderson *et al.* (2004) recommended that seedlings and cuttings planted during ecological restoration be watered. Maschinski *et al*. (2004) also proposed that transplanted propagules could benefit from water supplementation. 'Post-germination rainfall' is viewed as an important factor in vegetation restoration attempts (De Villiers *et al*., 2001). Murphy *et al.* (2008) also reported the watering of orchid transplants during below average rainfall periods. Since plant numbers, across various age categories in translocated populations of *F. humilis* were correlated with rainfall, the watering of transplants may prove beneficial for population establishment. However, Burgoyne & Hoffman (2011) reported that the translocated population were not watered for a month after translocation lest fungal infections occur. Thereafter, only small amounts of water have been administered for an unspecified time-period.

Since herbivory has been connected with population extinction (Pfab & Witkowski, 1999), rodent activity may have been curbed by erecting exclosures around the at-risk Goedvertrouwd populations, e.g. Milton (1994) and Crosti & Fabrini (2010). Jusaitis (2005) and Lanno & Sammul (2014) emphasised that translocated populations are especially vulnerable during the first year after translocation. However, three years have passed since translocation. Caging *F. humilis* populations at this time might prove to be unnecessarily laborious and costly.

Given the poor seedling yield in *E*, *GM* and *W*, hand pollination could have been an implemented to improve reproductive output (Parsons & Zedler, 1997).

To prevent 'excessive optimism' (Godefroid *et al*., 2011) about the outcome of the *F. humilis* translocation effort, a long-term monitoring programme should be upheld, since initial positive results, e.g. high survival rates, may be followed by reversals (Fahselt, 2007). Nevertheless, the possible establishment of three new populations of *F. humilis* should be viewed as an essential step towards conserving this species (Colas *et al*., 2008). Lanno & Sammul (2014) also viewed three years of post-translocation monitoring as too short a time scale to adequately evaluate translocation success. Initial results may, however, prove useful in terms of improved knowledge of species ecology, as well as translocation and monitoring methodology.

Furthermore, Colas *et al*. (2008) mentioned the importance of additionally monitoring a natural population for comparative purposes. The monitoring results from a control population at Ezemvelo Nature Reserve (Gauteng) will therefore be presented in the succeeding chapter.

4.6 Conclusion

A repeatable methodology for post-translocation monitoring, as well as scientifically sound baseline data for future comparative purposes were successfully established, as proven by the statistically robust results. Initial results showed that the translocated *F. humilis* populations responded positively to translocation thus far, despite lower than ideal survivorship and poor plant growth at *E* and *GM* and decreasing juvenile numbers at *W*. Populations at *GL, GR* and *GW* showed the best response to translocation, in that populations increased in numbers and plant growth was evident. The ability of *F. humilis* populations, translocated with a multiple array of propagules, to survive in habitats with a wide range of habitat and soil parameters (but especially in acidic soil) is evident over the short term.

Considering the amount of literature recommending the continuance of monitoring programmes for five years to several decades, the results in this study, however promising, should be viewed as initial indications of translocation success. A long-term monitoring programme is strongly advised to document future population survival/growth. Results from further monitoring efforts will elucidate the more permanent effects of translocation disturbance on *F. humilis* populations. The possible establishment of three new populations of *F. humilis* should be viewed as an essential step towards conserving this species.

Chapter 5. *Frithia humilis*: an endangered habitat specialist translocated to atypical geological substrates

5.1 Introduction

Frithia humilis is an endangered succulent species, endemic to a region in the western part of Mpumalanga and a small part of Gauteng (Burgoyne & Krynauw, 2005). This species occupies shallow quartz 'grit pans' on sedimentary outcrops of the Ecca and Dwyka groups (Karoo Supergroup) (Burgoyne *et al.,* 2000b), similar in appearance to 'quartz fields' described by Schmiedel & Jürgens (1999).

During a 2009 ecological survey, a population of *F. humilis* was found in an area designated for coal mining. As no other option was available to preserve the population it was translocated to three geologically distinct habitats (Burgoyne & Hoffman, 2011). Only one geologically suitable habitat, Goedvertrouwd farm near Balmoral, Mpumalanga, was found within the species' distribution range (Figure 3.1, Chapter 3). The majority of the donor (original) population was translocated to this receptor site. Two additional habitats were identified within protected areas near eMalahleni, Mpumalanga, namely Witbank Nature Reserve and Eagle Rock Private Estate. The available habitats in these protected areas, however, did not contain the geological outcrops typical to *F. humilis* habitats. Translocation to these receptor sites had to be regarded as experimental, to test a hypothesis that *F. humilis* is an edaphic specialist and unable to establish on non-typical geology (Burgoyne & Hoffman, 2011).

This study, which commenced six months after translocation, was aimed at testing the above hypothesis by investigating the trends in population demography at each of the three geological substrates. Each substrate was viewed as a separate 'treatment' and the response of each population to these treatments was measured by means of population censuses. The response of translocated populations was assessed in terms of population numbers, reproductive potential (flower production), reproductive output (fruit production) and seedling emergence (development of subsequent generations) over the monitoring period and related to that at the control population (Colas *et al*., 2008), so as to glean insight into the drivers of observable population establishment (Menges, 2008; Drayton & Primack, 2012). In this way, the viability of translocating *F. humilis* populations to non-typical geology could be investigated as a conservation measure, especially since extant native habitats are threatened by coal mining activities. While survival and plant growth as indicators of translocation success were discussed in Chapter 4, the focus of this chapter will be on reproductive function of the translocated populations, as well as that of the control population.

At the rate of human-induced environmental change, the urgency of conserving endangered endemic species cannot be questioned. Slow-growing plant species, with limited seed distribution mechanisms and occupying a fragmented habitat are priorities for obvious reasons (Seddon, 2010). Therefore, despite the controversy surrounding translocations of any sort, such conservation strategies may become the only salvation for at-risk species. Studies such as the one discussed in this dissertation are important to elucidate translocation challenges.

The translocation of *F. humilis* was a last resort rescue attempt of a population of an endangered species. It can, nonetheless, be viewed as a trial or experimental translocation (Albrecht & Maschinski, 2012), which can contribute to knowledge of plant species ecology, translocation and monitoring procedure, as well as the effects thereof (Noël *et al*., 2011; Drayton & Primack, 2012; Guerrant, 2012). Such information might provide invaluable assistance to future recovery and/or conservation attempts (Godefroid *et al*., 2011), since detailed population surveys following translocation attempts are rarely published (Colas *et al*., 2008). Enhanced knowledge on translocation of rare and/or threatened plant species translocation may prove critical in the mitigation of species extinction, especially on a local scale (Noël *et al*., 2011).

To effectively test whether *F. humilis* populations could be successfully translocated to non-typical geological substrates, populations at each of the respective receptor sites had to be evaluated on the basis of plant survival and reproductive capabilities (Menges, 2008; Godefroid *et al*., 2011; Albrecht & Maschinski, 2012).

5.2 Methods

5.2.1 Receptor habitats

The translocated populations were replanted to three receptor habitats, each with a different geological substrate: Goedvertrouwd Farm (*G*), Eagle Rock Private Estate (*E*), and Witbank Nature Reserve (*W*). A naturally occurring population at Ezemvelo Nature Reserve (*Z*) was surveyed as a control.

G habitats consisted of sedimentary outcrops of the Ecca Group (Karoo Supergroup) typical of *F. humilis* habitats (Burgoyne *et al.*, 2000b). These rocks provided a well-drained, level habitat. A sufficient top-layer of quartz gravel, a weathering product of the rock, was also present. *Frithia* *humilis* plants were subsequently not subjected to inundation or aggressive water runoff during rainy periods. Plants, especially vulnerable seedlings, were also protected from environmental damage. Four distinct receptor areas, *GL*, *GM*, *GR* and *GW*, all located on Ecca sandstone, contributed to the larger *G* habitat. For analytical purposes each area was considered a distinct receptor habitat, hosting separate populations.

Receptor habitat *E* hosted a single translocated population of *F. humilis* on sedimentary outcrops of the Waterberg Group (Transvaal Supergroup). The habitat provided by these rocks was less well-drained than Ecca sediments, with a steeper slope in some areas (>3°). The *E* habitat was at a higher risk of water-logging in some areas and water runoff during wet seasons. The availability of quartz-gravel was also limited and had to be imported from the donor site. *E* was therefore regarded as an atypical habitat.

The receptor habitat at *W* was also viewed as non-typical, since the Rooiberg Formation (Transvaal Supergroup) outcrops were not sedimentary in origin but igneous. Felsitic rocks of the Rooiberg Formation have poor drainage and yielded weathering products (pebbles) that were larger and more angular in shape than found in typical for *F. humilis* habitats.

The control population at *Z* was situated on Ecca and Dwyka sediments (Karoo Supergroup) and was therefore geologically similar to that of the donor population at Inyanda Coal mine and receptor site *G*. The inclusion of a control population was essential in the establishment of an effective post-translocation monitoring programme. Colas *et al.* (2008) emphasised that the comparison between demographic trends in translocated populations is essential in the assessment of translocation success. By including a natural control population, this study also addresses a gap in literature regarding rare plant translocations (Colas *et al.,* 2008).

5.2.2 Population censuses

Population trends were monitored via population censuses. Grid counts (Section 3.3.4, Chapter 3) were employed as a representative sampling method, where a fraction of each population was surveyed to draw conclusions of the entire population (Devore & Farnum, 2005), using the relative age (RA) classification system (Section 3.3.2, Chapter 3). A linear mixed model was applied to data sets to consider the discrepancies in population sizes when comparisons between relevant data were made. Statistical effects based on estimated marginal means (EMM) were used to determine significant differences between population numbers, flower and fruit production, as well as seedling numbers per 1 m^2.

5.3 Results

Yearly fluctuation in mean population size per 1 m^2 corresponded to that presented in Chapter 4. These results will not be elaborated on, but only briefly summarised below. Most translocated populations showed some loss of individuals throughout the monitoring period, but populations at most *G* receptor sites, except *GM*, recovered and even increased in size by 2012 (Figure 5.1). The size of the *GM* population remained stable from 2011 to 2012, after a decrease from 2010. *E* was the only receptor site at which the population continually declined in overall individuals per 1 m^2 over time. The population was significantly reduced in size from 2010 to 2011 ($p = 0.003$) (Figure 5.1) and diminished further from 2011 to 2012, albeit not statistically significant. 2012 population figures at *E* were significantly lower than those of 2010 ($p = 0.000$). The decline in population size was seated in all RA groups (Figure 5.1). The population at *W,* despite an increase in numbers from 2010 to 2011, was reduced in size by 2012. The per 1 m^2 response in population numbers measured at *W* was less positive than measured by total counts.

The mean number of individuals over the extent of the monitoring period did not differ significantly between translocated populations, excluding *GR* and *W* ($p =0.033$) (Table 5.1; Figure 5.1). Population *Z* did, however, exhibit a significantly higher mean per 1 m^2 population size than any translocated population ($p = 0.000$) (Table 5.1; Figure 5.1). The control population experienced an increase in size across all RA groups from 2011 to 2012, but the rise in numbers was not statistically significant.

A more detailed inspection of population EMM revealed that the above mentioned differences between translocated populations and the control were seated in all RA groups besides the seedling (<3) group (Table 5.2). A comprehensive table of all pairwise comparisons is presented in Table B.0.1, Appendix B. The significant difference between mean size of populations at *GR* and *W* was not seated in any particular RA group, but in overall individuals per 1 m^2.

Generally, seedlings (RA group <3) and sub-adults (RA group 3-5) were significantly more abundant per 1 m^2 than adult plant groups (Table 5.3). This was true for all populations (Figure 5.2, Table B.0.2, Appendix B), except for *GW*, where no significant differences between numbers of RA groups per 1 m^2 were found (p-values varied between 0.102 and 1.000). RA group 6-10 was also significantly larger than 'older' groups at *E*, *W* and *Z*.

The 'younger' RA groups (<3, 3-5 and 6-10) contributed significantly to the size of the study populations over the monitoring period (Figure 5.1). These results also largely corresponded with the findings in Chapter 4.

Fluctuations in the 'older' RA groups (11-15, 16-20, 21-30 and >30) were less pronounced than in the younger groups. Collectively, 'older' groups continually decreased in size from 2010 to 2012 at receptor site *E* and *GM* (Figure 5.1). At *GL*, older plants showed an overall decline from 2010 to 2012, despite a slight increase in numbers during 2011. 'Older' RA groups at *GR* showed a net increase in collective numbers from 2010 to 2012, in spite of a decrease in numbers from 2011 to 2012. *GW* showed a continual increase in the joint numbers of 'older' RA groups, while at *W* the combined numbers of larger groups remained stable from 2011 to 2012, after an upsurge from 2010

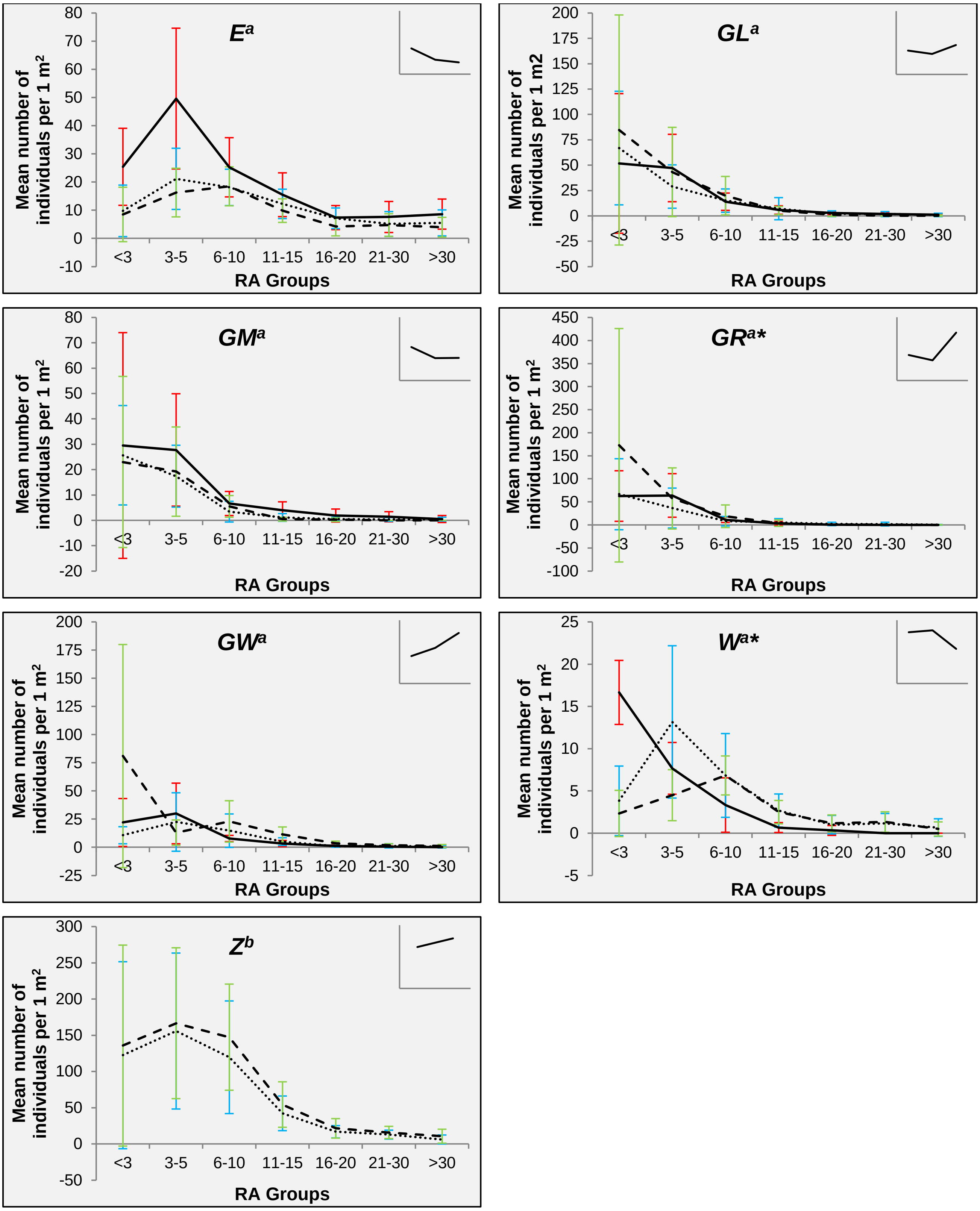

Figure 5.1. Mean number of *Frithia humilis* individuals per 1 m^2 in the respective study populations over the monitoring period. Charts of populations marked with dissimilar letters or * differ significantly ($p<0.05$) in overall estimated marginal means. Coloured bars indicate the standard deviation (SD); significant differences in yearly RA group size occur where SDs do not overlap. *Curves inserted in top right corner of each chart represent the general trend in mean population size over three years; y-axis, mean number of individuals; x-axis, monitoring period*. — 2010 (red) ···· 2011 (blue) – – – 2012 (green).

Table 5.1. Pairwise comparisons of the estimated marginal means (EMM) of the size of each population per 1 m² over three years of monitoring. The EMM and the standard error (SE) are also presented to indicate per 1 m² density per relative age group. * indicates significant differences in EMM (p<0.05) using Sidak adjustments for multiple comparisons.

Receptor habitats	EMM ± SE	p-values					
		GL	*GM*	*GR*	*GW*	*W*	*Z*
E	13.619 ± 4.337	1.000	1.000	0.718	1.000	0.966	0.000*
GL	19.357 ± 3.879	-	0.702	1.000	1.000	0.317	0.000*
GM	8.171± 4.337	-	-	0.100	1.000	1.000	0.000*
GR	24.701 ± 3.884	-	-	-	0.845	0.033*	0.000*
GW	12.128 ± 6.133	-	-	-	-	0.999	0.000*
W	3.692 ± 5.309	-	-	-	-	-	0.000*
Z	72.324 ± 4.322	-	-	-	-	-	-

Table 5.2. Pairwise comparisons of the per 1 m² relative age (RA) group estimated marginal means (EMM) in each study population over the monitoring period. * indicates significant difference in EMM (p<0.05) using Sidak adjustments for multiple comparisons. (Only populations between which significant differences occurred, were tabled).

RA group	Populations	*E*	*GL*	*GM*	*GR*	*GW*	*W*
<3	***Z***	0.066	0.888	0.153	1.000	0.711	0.086
3-5	***Z***	0.002*	0.003*	0.001*	0.028*	0.009*	0.001*
6-10	***Z***	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
11-15	***Z***	0.000*	0.000*	0.000*	0.000*	0.001*	0.000*
16-20	***Z***	0.001*	0.000*	0.000*	0.000*	0.000*	0.000*
21-30	***Z***	0.002*	0.000*	0.000*	0.000*	0.000*	0.000*
>30	***Z***	0.998	0.016*	0.018*	0.007*	0.114	0.037*

Table 5.3. Pairwise comparisons of the estimated marginal means (EMM) of individuals per relative age (RA) group per 1 m² over the study period and in all populations. * indicates significant differences in inter-group EMM (p<0.05) using Sidak adjustments for multiple comparisons.

RA groups	3-5	6-10	11-15	16-20	21-30	>30
<3	0.516	0.000*	0.000*	0.000*	0.000*	0.000*
3-5	-	0.001*	0.000*	0.000*	0.000*	0.000*
6-10	-	-	0.668	0.241	0.185	0.092
11-15	-	-	-	1.000	1.000	0.998
16-20	-	-	-	-	1.000	1.000
21-30	-	-	-	-	-	1.000

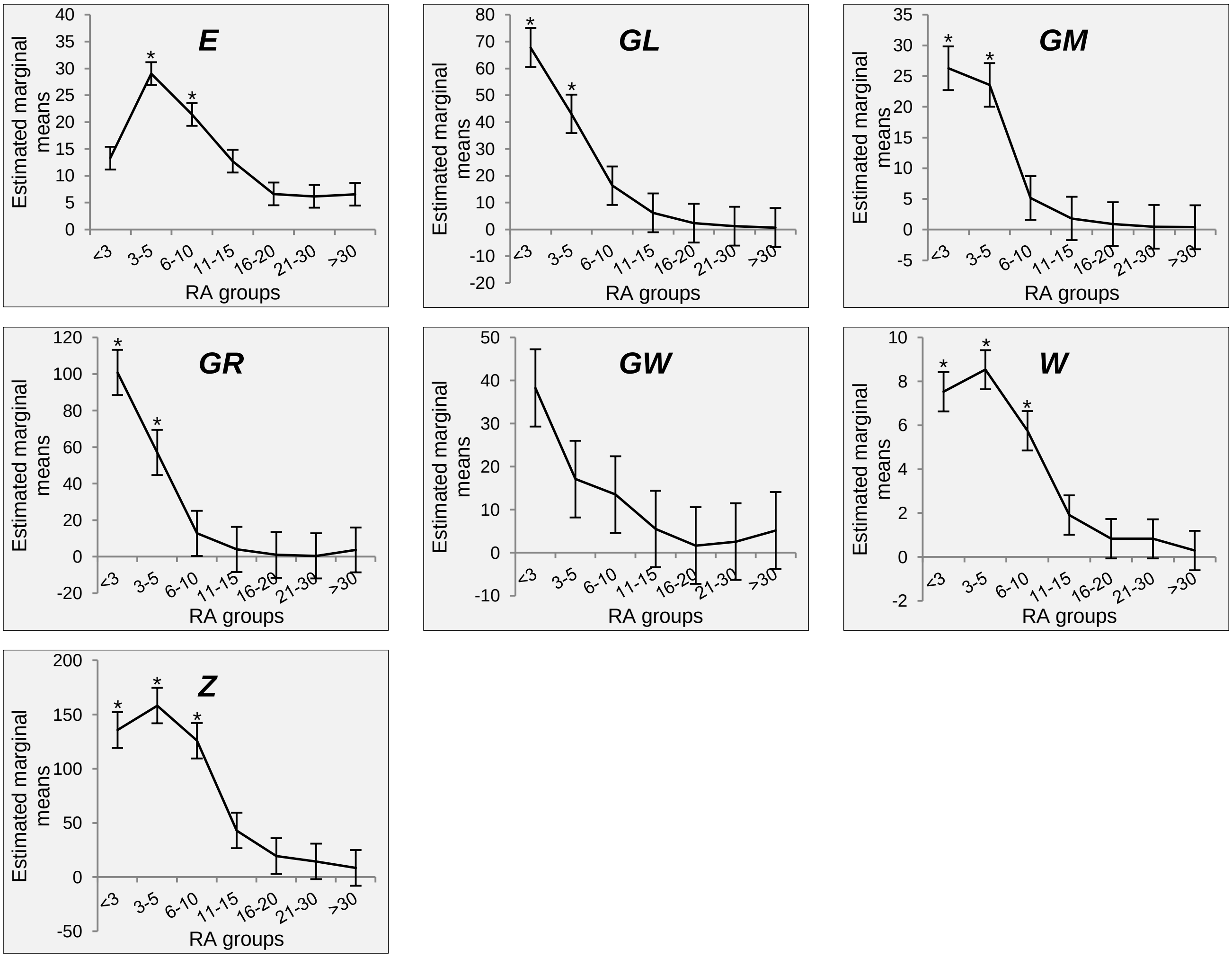

Figure 5.2. Relative age (RA) group per 1 m² estimated marginal means of the study populations, spanning all monitoring sessions. * indicates RA groups that differed significantly from the rest. Bars indicate the standard error of each mean.

to 2011 (See Table B.0.3, Appendix B for detailed numbers).

‘Younger’ RA groups 3-5 and 6-10 hosted significantly larger numbers of flowering individuals than ‘older’ RA groups (16-20, 21-30 and >30) and seedlings (non-flowering individuals) (Figure 5.3). Flowering individuals were significantly more abundant in RA group 3-5 at *GM* ($p = 0.010 – 0.023$), and in RA group 6-10 at *E* ($p = 0.000$), *GL* ($p = 0.000 – 0.002$), *GR* ($p = 0.003 – 0.006$), *GW* ($p = 0.007$), *W* ($p = 0.000 – 0.029$) and *Z* (0.000 – 0.001). RA groups 3-5 and 6-10 carried more flowers per individual than other adult RA groups, although never significantly more (Figure 5.4), at translocation sites *GM*, *GR*, *GW* and *W*. RA group 11-15 carried the largest number of flowers per individual at receptor sites *GL* and control *Z*, whereas individuals from RA group >30 bore the largest amount of flowers per plant at receptor site *E*.

Control site *Z* hosted a significantly larger number of flowering individuals per 1 m² than receptor habitats ($p = 0.000$). Plants in this population produced significantly more flowers per individual than the translocated populations (Table 5.4). The mean number of flowers did not differ significantly between the various receptor sites, but flowering individuals were the most abundant at *E* and *GL*, while *GM* and *W* had the least amount of flower-bearing individuals (Table 5.5).

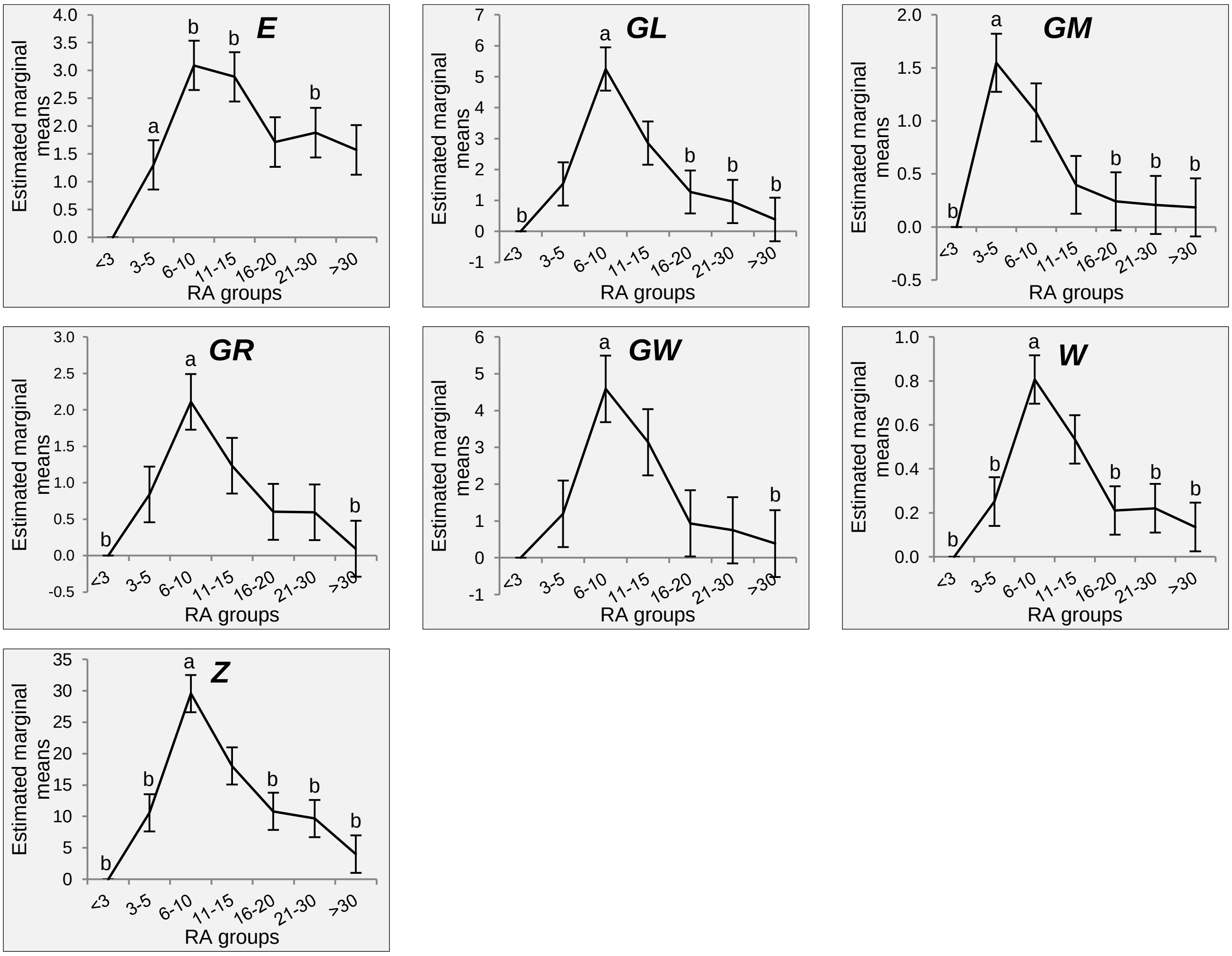

Figure 5.3. Relative age (RA) group estimated marginal means of flowering individuals per 1 m² the study populations, spanning all monitoring sessions. Differing letters indicate RA groups that differed significantly from one another. Bars indicate the standard error of each mean.

Table 5.4. Pairwise comparisons of the per 1 m² mean number of flowers per individual plant at each receptor site, over three years of monitoring. The estimated marginal mean (EMM) and the standard error (SE) are also presented to indicate per 1 m² density per RA group. * indicates significant differences in EMM ($p<0.05$) using Sidak adjustments for multiple comparisons.

Receptor habitats	**EMM ± SE**	**p-values**					
		GL	***GM***	***GR***	***GW***	***W***	***Z***
E	0.605 ± 0.044	0.007*	0.000*	0.000*	0.488	0.002*	0.001*
GL	0.394 ± 0.039	-	0.893	0.304	1.000	1.000	0.000*
GM	0.298 ± 0.043	-	-	1.000	0. 702	1.000	0.000*
GR	0.262 ± 0.039	-	-	-	0.252	0.995	0.000*
GW	0.442 ± 0.061	-	-	-	-	0.989	0.000*
W	0.339 ± 0.050	-	-	-	-	-	0.000*
Z	0.861 ± 0.044	-	-	-	-	-	-

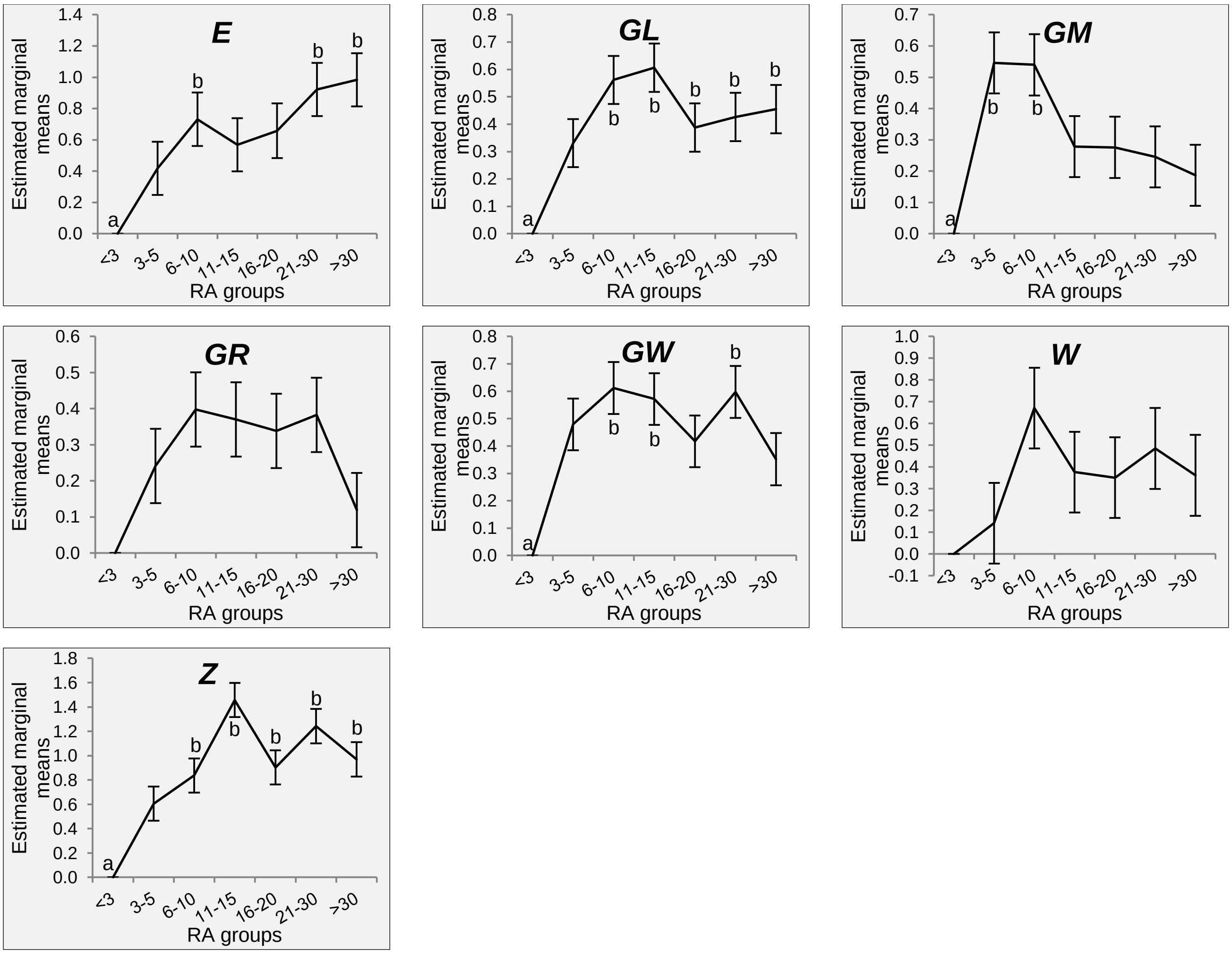

Figure 5.4. Relative age (RA) group estimated marginal means of the number of flowers per individual per 1 m² at the study populations, spanning all monitoring sessions. Differing letters indicate RA groups that differed significantly from one another. Bars indicate the standard error of each mean.

Table 5.5. Pairwise comparisons of the number of flowering plants per 1 m² at each receptor site over three years of monitoring. The estimated marginal mean (EMM) and the standard error (SE) are also presented to indicate per 1 m² density per RA group. * indicates significant differences in EMM (p<0.05) using Sidak adjustments for multiple comparisons.

Receptor habitats	**EMM ± SE**	**p-values**					
		GL	***GM***	***GR***	***GW***	***W***	***Z***
E	1.759 ± 0.426	1.000	0.603	0.855	1.000	0.430	0.000*
GL	1.758 ± 0.381	--	0.508	0.786	1.000	0.352	0.000*
GM	0.536 ± 0.426	-	-	0.100	0.987	1.000	0.000*
GR	0.781 ± 0.381	-	-	-	1.000	1.000	0.000*
GW	1.511 ± 0.603	-	-	-	-	0.937	0.000*
W	0.309 ± 0.492	-	-	-	-	-	0.000*
Z	11.839 ± 0.428	-	-	-	-	-	-

All translocated populations showed an increased number of flower-bearing plants in 2011, with a decline occurring during 2012 (Figure 5.5). This increase from 2010 to 2011 was statistically significant (p = 0.000 – 0.011) at all receptor sites apart from *GW* (p= 0.393) (Table 5.6). The number of flowering plants decreased at most translocated populations from 2011 to 2012 and the decline was significant at receptor sites *E*, *GL* and *W* (p = 0.000 – 0.008). *GW* was the only receptor site at which a continual increase in the number of flowering plants per 1 m^2 took place. The number of flowering individuals also increased from 2011 to 2012 at control site *Z*. Despite the decline in flowering individuals from 2011 to 2012 at translocation receptor sites, the number of flowering plants was still slightly more by 2012 than at the onset of monitoring in 2010 (Figure 5.5).

Fruit-bearing individuals suffered a continual decrease in per 1 m^2 abundance over time at *E*, with 2012 numbers being significantly lower than in 2010 (p = 0.028). *GW*, *W* and *Z* showed a consistent increase in fruit-producing plants over the monitoring period, albeit not statistically significant (p = 0.372 – 0.986 for *GW*, p = 0.282 – 0.805 for *W* and p = 0.767 for *Z*) (Figure 5.6). Receptor sites *GL*, *GM* and *GR* showed an increase in the number of fruit-producing plants from 2010 – 2011, although the rise in numbers was only significant at *GL* and *GR* (p = 0.008 and p = 0.006, respectively). Per 1 m^2 number of fruit-bearing plants declined for the three aforementioned populations, but was only significantly decreased at *GM* (p = 0.040) and *GL* (p = 0.026).

Receptor site *GL* hosted more fruit-producing individuals than other study sites and numbers were significantly higher than other translocation receptor sites (p = 0.001 – 0.040), but not the control site, *Z* (p = 0.235) (Table 5.7). These differences were seated in RA group 6-10, since *GL* hosted significantly more fruit-bearing individuals in this RA group than receptor sites *E*, *GM*, *GR* and *W* (p = 0.013 – 0.032), but not significantly more than receptor site *GW* (p = 0.108) and control site *Z* (0.709). *GM* and *W* hosted the smallest number of fruit-bearing plants (Table 5.7).

The translocated populations showed similar, but not identical, RA group distribution to the control population. ‘Younger’ plants (RA groups <3, 3-5 and/or 6-10) were significantly more abundant in most populations (*GW* exempted, where no significant difference in RA group numbers were found).

Younger individuals (RA groups 3-5, 6-10, 11-15) produced more fruit than individuals in older RA groups (Figure 5.7) in all translocation receptor sites, supporting the Frodo hypothesis (Chapter 4); differences in mean numbers of RA groups at these sites were not significant. RA group 6-10 at *Z*, however, had the largest number of fruit-producing individuals of all RA groups (Table 5.7)

Plants from RA group 6-10 flowered the most abundantly in and throughout all study populations, apart from *GM*. However, the population at *GW* was the only population to show an increase in the number of flowering and fruit-producing individuals from 2011-2012, similar to the natural population, whereas most other translocated populations showed reductions in these areas. *W* was the only other translocated population to show a similar trend in fruit production to the population at

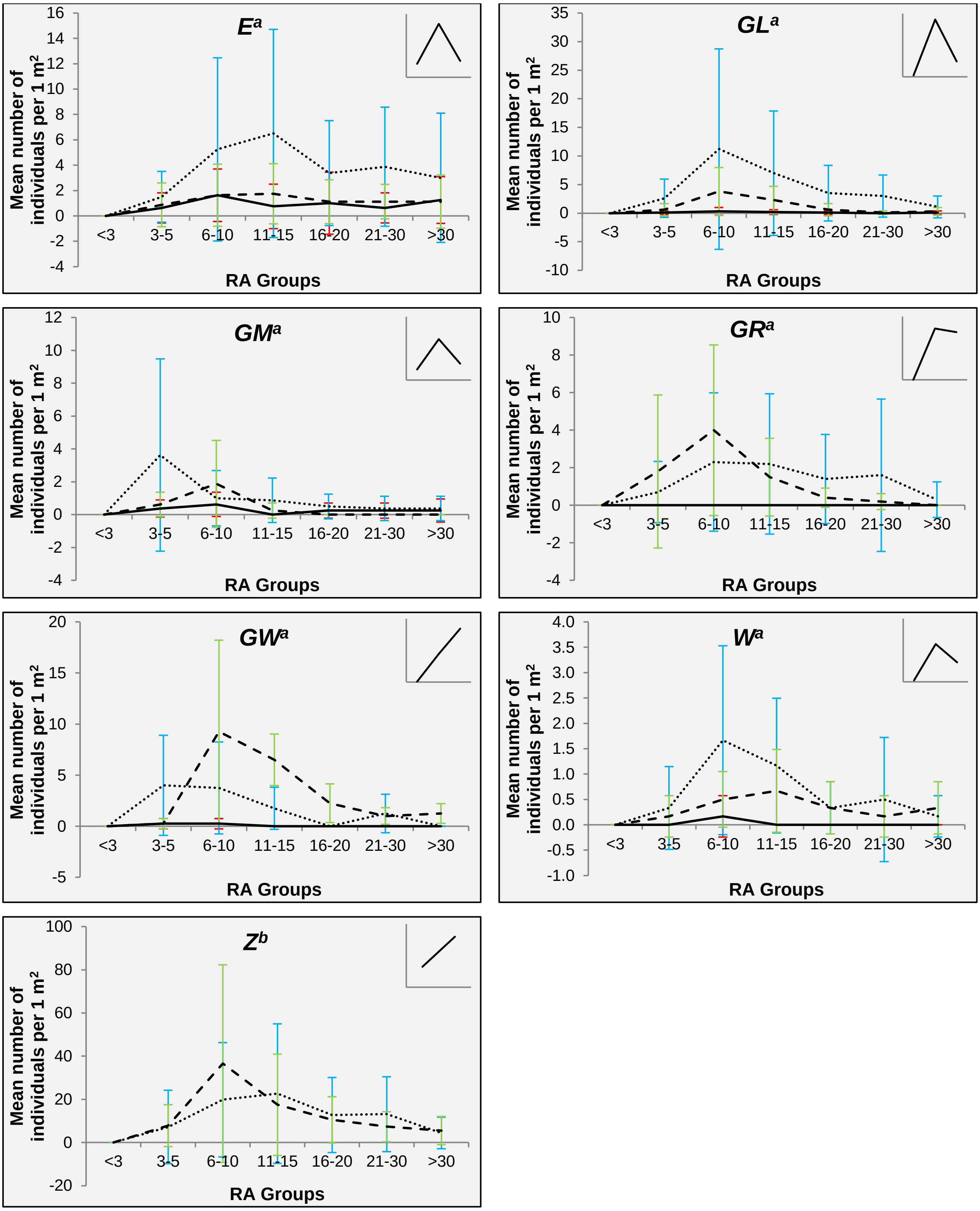

Figure 5.5. Mean number of flower-bearing individuals per 1 m^2 in the respective *Frithia humilis* populations, over the monitoring period. Charts of populations marked with dissimilar letters differ significantly ($p<0.05$) in overall estimated marginal means. Coloured bars signify the standard deviation (SD); significant differences in yearly relative age (RA) group size occur where SDs do not overlap. *Curves inserted in top right corner of each chart represent the general trend in mean population size over three years; y-axis, mean number of individuals; x-axis, monitoring period*. — 2010 (red) ···· 2011 (blue) – – – 2012 (green).

Z. RA group 6-10 produced significantly more fruit in the control population. This group produced more fruit than other RA groups only in one translocated population (*GL*).

Table 5.6. Pairwise comparisons between yearly mean number of flowering individuals per 1 m^2 over the monitoring period. * indicates significant differences in estimated marginal means ($p<0.05$) using Sidak adjustments for multiple comparisons.

E		**Mean Difference**	**Standard Error**	**p-value**
2010 compared to	**2011**	-2.524	0.367	0.000*
	2012	-0.174	0.364	0.951
2011 compared to	**2012**	2.351	0.367	0.000*
GL				
2010 compared to	**2011**	-3.955	0.590	0.000*
	2012	-0.977	0.586	0.268
2011 compared to	**2012**	2.978	0.590	0.000*
GM				
2010 compared to	**2011**	-0.719	0.237	0.011*
	2012	-0.139	0.236	0.913
2011 compared to	**2012**	0.580	0.237	0.051
GR				
2010 compared to	**2011**	-1.215	0.351	0.003*
	2012	-1.128	0.350	0.006*
2011 compared to	**2012**	0.087	0.351	0.993
GW			SE	p-value
2010 compared to	**2011**	-1.534	0.992	0.393
	2012	-2.926	1.011	0.053
2011 compared to	**2012**	-1.392	0.992	0.471
W				
2010 compared to	**2011**	-0.574	0.092	0.000*
	2012	-0.284	0.091	0.009*
2011 compared to	**2012**	0.289	0.092	0.008*
Z				
2011 compared to	**2012**	-0.709	2.029	0.728

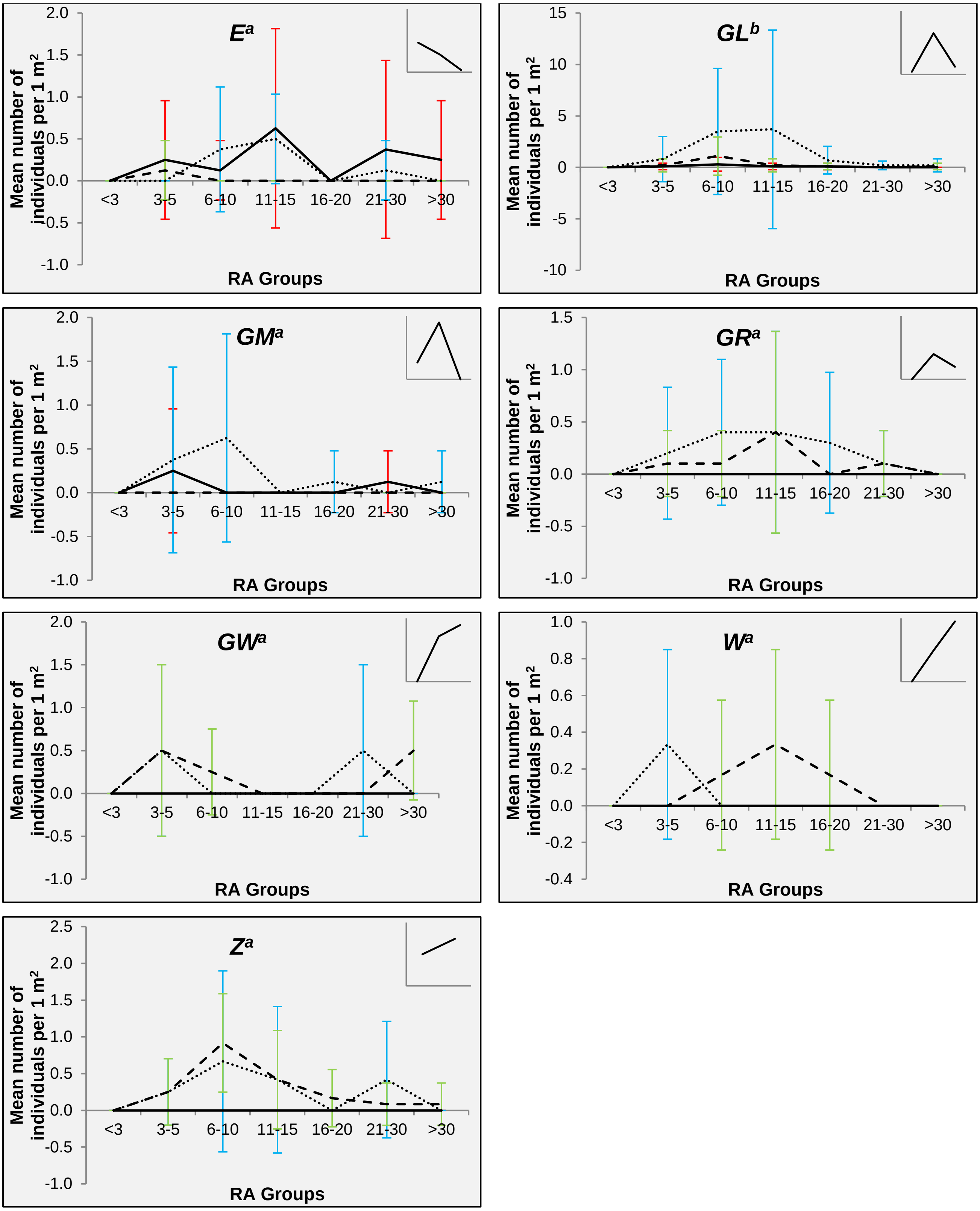

Figure 5.6. Mean number of fruit-bearing individuals per 1 m^2 at the respective sites, over the monitoring period. Charts of populations marked with dissimilar letters differ significantly ($p<0.05$) in overall estimated marginal means. Coloured bars signify the standard deviation (SD); significant differences in yearly relative age (RA) group size occur where SDs do not overlap. *Curves inserted in top right corner of each chart represent the general trend in mean population size over three years; y-axis, mean number of individuals; x-axis, monitoring period*. — 2010 (red) ···· 2011 (blue) – – – 2012 (green).

Table 5.7. Pairwise comparisons of the number of fruit-bearing plants per 1 m² at each receptor site over three years of monitoring. The estimated marginal means (EMM) and the standard error (SE) are also presented to indicate per 1 m² density per RA group. * indicates significant differences in EMM (p<0.05) using Sidak adjustments for multiple comparisons.

Receptor habitats	EMM ± SE	p-values					
		GL	*GM*	*GR*	*GW*	*W*	*Z*
E	0.131 ± 0.084	0.006*	1.000	1.000	1.000	1.000	0.999
GL	0.543 ± 0.075	-	0.001*	0.001*	0.040*	0.001*	0.235
GM	0.077 ± 0.084	-	-	1.000	1.000	1.000	0.930
GR	0.100 ± 0.075	-	-	-	1.000	1.000	0.966
GW	0.107 ± 0.118	-	-	-	-	1.000	0.999
W	0.048 ± 0.096	-	-	-	-	-	0.873
Z	0.262 ± 0.084	-	-	-	-	-	-

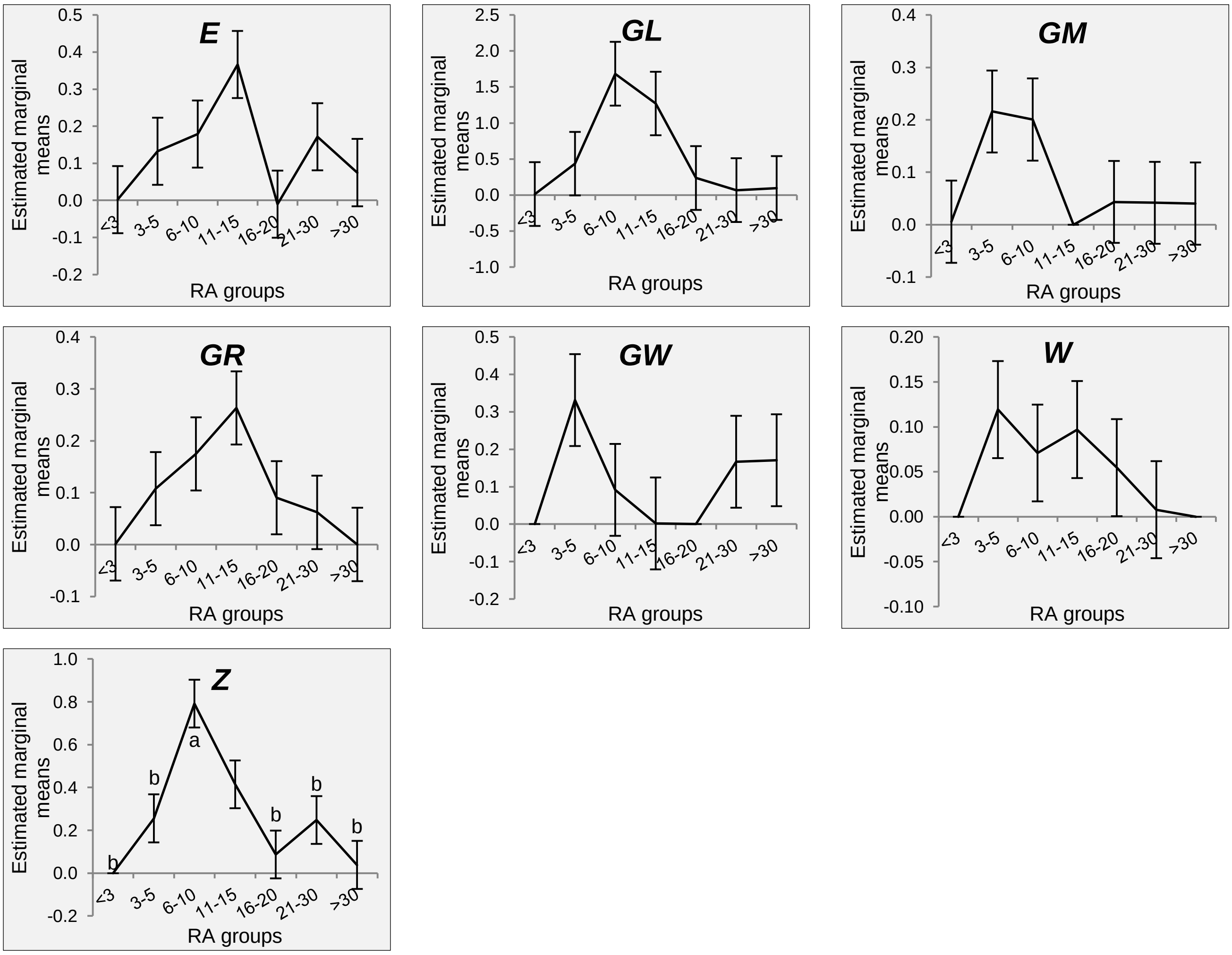

Figure 5.7. Relative age (RA) group estimated marginal means of fruit-bearing individuals per 1 m² at the study populations, spanning all monitoring sessions. Differing letters indicate RA groups that differed significantly from one another. Bars indicate the standard error of each mean.

5.4 Discussion

Results by Helenurm (1998) and Bottin *et al*. (2007) highlighted the importance of receptor site-specific effects on the survival of translocated populations. Jusaitis (2005) reported that microsite conditions were critical in determining the outcome of experimental translocations of an endangered Australian plant species, *Prostanthera eurybioides* (Lamiaceae), a species preferring granite or schist outcrops. Plant populations transplanted to different micro-habitats might vary in their response to translocation. Moreover, Maschinski *et al.* (2004) suggested that novel habitats or habitats previously unoccupied by similar species are poor prospective sites for rare plant reintroductions. This was clearly illustrated by the varying reproductive responses of translocated *F. humilis* populations to different geological substrates. The *F. humilis* populations transplanted to the Goedvertrouwd receptor sites responded most positively to this new habitat, despite varying responses of the subpopulations (*GL*, *GM*, *GR* and *GW*). Populations translocated to *E* and *W*, receptor sites with non-typical geological substrate responded less positively to translocation.

In their study of rare and endangered wetland species, Noël *et al*. (2011) reported that the most important factor contributing to the establishment of translocated populations was ecological similarity of receptor site and donor habitat. The Goedvertrouwd sites were the only receptor sites geologically similar to the donor habitat. *GL*, *GR* and *GW* showed overall population growth, in contrast with the findings of Godefroid *et al*. (2011), who reported poor survival rates for reintroduced plant populations. Survival and population growth in these three Goedvertrouwd populations can therefore be interpreted as a positive response to translocation to native geological substrates.

Population numbers at *GM* remained stable in 2012 after an initial decline in 2011. The population at *GM* experienced significant declines in fruit-production from 2011 to 2012. Reduced reproductive output could explain the decline in seedlings during that time period. The poor reproductive response of the population at *GM* may be due to slightly different ecological conditions (see Chapter 4).

Successful translocations can manifest in the following ways: reproductive potential (flower production), reproductive output (fruit production) and seedling emergence. Plant survival (across all demographic or RA groups) indicates the ability of a population to occupy a habitat successfully. Reproductive output is indicative of a population’s ability to persist (Colas *et al*., 2008). Drayton & Primack (2012) regarded the continued presence of reproductive individuals in translocated populations as indicative of population establishment two years after translocation. McClain & Erbinger (2008) similarly viewed the persistence of translocated *Tetraneuris herbacea* (Asteraceae) populations as unlikely, since flowering was extremely low after three to six years of monitoring. By 2012, flowering and fruiting plants of *F. humilis* were present at most translocated populations, albeit with reduced abundance at *E* and *GM*. Lanno & Sammul (2014) considered sparse flowering in translocated populations of *Ligularia sibirica* (Asteraceae) as an incentive to prolong monitoring until the reproductive viability of such populations could be adequately evaluated. Long-term monitoring is therefore essential in evaluating the long-term viability of translocating *F. humilis* populations to different geological habitats.

Translocated *F. humilis* populations flowered significantly less per plant per 1 m² than the control, possibly indicating lower reproductive potential in translocated populations. The control population at *Z* had higher population numbers, as can be expected from a population with a naturally higher per 1 m² density of plants. This was similar to the findings of Colas *et al*. (2008) that showed translocated populations of *Centaurea corymbosa* (Asteraceae) had fewer flowering plants than natural populations.

The population at *GL* hosted more fruit-bearing plants than the control (albeit not significantly more). Helenurm (1998) viewed fruit-production in experimentally translocated populations of the annual species, *Lupinus guadalupensis* (Fabaceae) as an indication of population fitness, i.e. the ability to adapt to receptor site conditions. Despite the reduction in fruit-production from 2011 – 2012, the population at *GL* produced significantly more fruit per 1 m² than any other translocated population. The reproductive output of the population at *GL* was therefore not impaired by the translocation disturbance. There was an increase in the number of seedlings at *GL* and *GR*, regardless of reduced flowering and fruit-production from 2011 to 2012, demonstrating seedling emergence in favourable germination conditions, despite perceived reduced fecundity during that time period. It is also unlikely that the population at *GW* suffered impaired reproductive response as a result of translocation, since flowering and fruit-production continually improved over the monitoring period. Even though seedlings at the *GW* population were reduced during 2011, the number of seedlings improved drastically during 2012, indicating proper germination conditions.

Translocation attempts may fail due to inadequate seed production or inappropriate germination conditions, which lead to insufficient seedling emergence (Albrecht & Maschinski, 2012). The reproductive response and seedling production of the translocated populations at *GL*, *GR* and *GW* could therefore be viewed as a positive step toward translocation success.

Colas *et al*. (2008) found that translocated populations showed lower mortality in pre-reproductive individuals than natural populations. In the context of this study, some *F. humilis* populations translocated to typical geological habitats (i.e. *GL*, *GR* and *GW*) showed similar trends to the control population (*Z*) regarding seedling emergence (pre-reproductive individuals), i.e. an increase in the number of seedlings. This was most likely due to similarities in germination conditions between the control habitat and geologically typical receptor sites (Albrecht & Maschinski, 2012).

The population at *W* decreased in 2012 after a slight improvement in numbers during 2011. *W* was the smallest translocated population and consequently also hosted the smallest number of flowering and fruit-bearing plants. Survival of transplants as well as continual fruit-production (despite decreased flowering in 2012) throughout the monitoring period showed that translocation to this atypical habitat is possible, but with limited success. Poor seedling emergence despite increased fruit-production at *W* indicated sub-optimal germination conditions, making long-term, multi-generational survival of the population at this receptor site improbable (Knowles & Witkowski, 2001; Biko'o *et al*., 2011). The lowest numbers of flowering and fruit-producing individuals were found at *GM* and *W*. Flowering and seedling emergence was low at these sites and long-term monitoring will be essential for the evaluation of translocation success in the future.

Seedling numbers did not differ significantly between translocated populations and the control population. The only populations to show trends similar to that of the control were those translocated to typical geology (*GL*, *GR* and *GW*), suggesting vigour in short term seedling emergence at these receptor sites. This holds especially true for translocated populations, where seedling numbers have been sustained or increased over the monitoring period, i.e. *GL*, *GR* and *GW*. Seedlings emerging in translocated populations during 2010 probably stemmed from sods containing a translocated seed bank. Since mesemb seedlings germinate under favourable conditions and do not remain dormant for long (Ihlenfeldt, 1994), it is likely that the seedlings observed during 2011 and 2012 were the result of effective pollination of flowers, successful fruit production and seed set in suitable environments, especially in typical *F. humilis* receptor habitats at *GL*, *GR* and *GW*.

'Younger' RA groups, 3-5 and 6-10, as well as seedlings, were present in significantly higher numbers than other RA groups in the study populations. Since seedling production and the presence of younger plants play an important part in population persistence (Maschinki *et al*., 2004; Knowles & Witkowski, 2001; Biko'o *et al*., 2011), the younger RA groups in translocated *F. humilis* populations can be viewed as essential to translocation success. Maschinski *et al*. (2004) found seedling survival to be a limiting factor in the translocation of *Purschia subintegra* (Rosaceae) to novel soils. Seedling numbers in both non-typical habitats, i.e. *E* and *W*, were steadily reduced over the monitoring period, matching the findings of Maschinski *et al*. (2004).

Furthermore, results showed that younger RA groups (i.e. smaller plants) 3-5, 6-10 and 11-15 played an important part in the survival, reproductive potential and output (i.e. population persistence) of translocated populations, since plants were more abundant in these groups than any other RA group throughout the monitoring period. Not only were flowering individuals more abundant in the younger RA groups, but these individuals flowered more abundantly than other RA groups as well. This confirms the Frodo-effect mentioned in Chapter 4, where 'weaker' individuals contributed significantly toward the survival and possibly toward the population perpetuation (and ultimately translocation success).

The number of older plants remained more stable, indicating resilience and the ability to adapt to the novel environments. Adult plants at *E* and *GM* seemed incapable of utilising the habitat, since their numbers declined over the monitoring period (presumably due to the washing away of top soil and gravel by rain and consequent increased exposure to environmental elements, as well as herbivory at *GM*), possibly indicating poor habitat suitability, even though *GM* is located on typical *F. humilis* geological substrate. However, adult plants at *W* seemed to thrive, despite the different geological microhabitat.

Kunin (1997) and Colas *et al*. (2008) mentioned a relationship between plant density and reproductive success. The natural density of the *F. humilis* donor population was upheld during the translocation procedure: propagule-containing sods were transplanted randomly to receptor sites. Density-effects were not the focus of the study and will therefore not be examined in detail. The control population at *Z*, had the greatest number of plants per 1 m² and was also the population with the highest number of seedlings and significantly more flowering individuals, but it did not produce the largest amount of fruit-bearing plants (*GL* did). *W* hosted the smallest population as well as least number of seedlings, flowering and fruit-bearing individuals. *GR* was home to the largest translocated population and accommodated the largest number of seedlings of any translocated population, but did not produce the largest number of flowers or fruit. Therefore, density effects in the translocated populations may be more complex and beyond the scope of this study.

The donor population of *F. humilis* provided an advantage in its having been well-established prior to translocation (Godefroid *et al*., 2011) and having adequate population density. Both of these qualities improved the probability of persistence after translocation (Griffith *et al.,* 1989). Population density (the distance between individuals in one patch (Kunin, 1997)) may influence pollinator visitation rate, with consequences for the plant population’s reproductive success. Kunin (1997) mentions a decrease in pollination and plant reproductive success in sparse plant populations.

Most of the translocated ‘patches’ of *F. humilis* populations were geographically restricted, i.e. the chances of patch expansion were low due to habitat and ecological constraints or resource limitations. According to Armstrong & Seddon (2008) an increase in density of certain patches can be viewed as a form of population expansion and possibly persistence. If the size of a population in a receptor patch remains constant, that population would probably be able to persist (Armstrong & Seddon 2008). Therefore, even if the translocated populations do not expand geographically, but can at least stabilise – i.e. mature individuals producing enough progeny to replace senescent or dead individuals, population persistence might be ensured. However, only prolonged monitoring will confirm the above mentioned possibility.

Drayton & Primack (2012) reported that long-term post-translocation monitoring can reveal the true extent of population establishment. Translocated populations of rare flowering plants were resurveyed by the authors 15 years after translocation. These populations failed to persist, despite the initial appearance of establishment, inferred from the presence of reproductive individuals, after two years following translocation. The authors concluded that translocation as a conservation mitigation tool should be applied very cautiously. The results from this study on translocated *F. humilis* populations should therefore be affirmed or corrected by studies after at least a decade, as recommended by Drayton & Primack (2012).

Evaluating the success of the translocation would require reliable documentation of the monitoring results (Milton *et al.,* 1999). It is therefore imperative that the current *F. humilis* monitoring programme should continue for at least another seven to ten years. The results of an experimental translocation, whether successful or not, can aid the management of extant populations or even of successfully translocated populations: '...failures, if well documented, can be instructive' (Menges, 2008).

The value of this study was to contribute to the growing knowledge on rare/endangered species translocations. The translocation methods utilised in this procedure, including transplanting populations to atypical habitats, can inform future translocation efforts (Drayton & Primack, 2012). Although a protocol for the translocation of fynbos species has been drafted (Milton *et al.,* 1999), no such guidelines exist for succulent species in other parts of southern Africa. Therefore, recording (through monitoring) the results of the *F. humilis* translocation attempt/experiment may in future inform guidelines for the translocation of succulent species in grassland ecosystems of southern Africa. This is essential, as more information on translocation attempts on a larger variety of species may improve the predictability of future translocation outcomes (Griffith *et al.,* 1989; Menges, 2008).

Furthermore, Jusaitis (2005) mentioned that plant species translocations to multiple receptor sites might give insight into a species' ability to adapt to various environmental conditions. The conservation implication of such insights, according to said author, would be the possibility of translocating a population of species beyond its natural or known distribution range. Multiple populations may also be established in the case of a single remaining natural population. In the case of *F. humilis*, such knowledge may prove invaluable to the conservation of the species, since its natural habitats are likely to be continually threatened by coal mining. However, the initial results reported in this chapter indicated that the viability of introducing *F. humilis* population to habitats containing non-typical geological substrates (*E* and *W*) is doubtful. Indeed, translocation to typical habitats (e.g. *GM*) may even yield varied results.

Allen (1994) introduced an interesting argument concerning the consequences of translocation: If translocation is proved a viable conservation method, developers may have incentive to use any land, despite the presence of an endangered/rare species. A disturbing allegation against translocation as a conservation method made by Fahselt (2007), supported the concerns raised by

Allen (1994): ‘Another of the problems identified was that habitat destruction becomes more acceptable once translocation is proposed.’

Despite the possibility for the success of any well-researched translocation attempt, failure remains highly probable. Ultimately, a species’ intrinsic characteristics make it a good or doubtful candidate for translocation (Godefroid *et al*., 2011). Translocation as a conservation strategy should therefore ideally be practised as a last resort or damage control measure. It should be practised with great responsibility, as it has the potential of becoming an ‘easy way out’ for developers/land users interested in developing sensitive areas (Allen, 1994).

5.5 Conclusions

Short-term results indicated the viability of translocated *F. humilis* populations and supported the decision to translocate populations to typical geologies for conservation purposes. Populations translocated to Goedvertrouwd receptor sites *GL*, *GR* and *GW* performed well in terms of survival, reproductive potential, reproductive output and seedling emergence. Encouraging responses to translocation indicated the potential of these populations to establish and persist over time, pointing to translocation success, albeit in the short-term. The possibility of translocation success will impact conservation of the species positively, since only a limited number of known natural populations exist and coal mining, which targets coal seams underlying typical F*. humilis* habitat, is likely to be a continual threat.

However, the viability of *F. humilis* populations being translocated to non-typical geological substrates has shown limited potential, though long-term monitoring (5 – 10 years) will be bound to reveal the true outcome. Poor survival together with inferior reproductive response confirmed that *E* (Wilge River Formation outcrops), made poor receptor sites for translocated *F. humilis* populations. Rooiberg felsite outcrops (*W*) also proved to be a dubious receptor site, since there was a downward trend in seedling emergence over time, suggesting inferior germination conditions. However, translocation to non-native geological substrates did not have disastrous short-term consequences, since such populations continued to flower, produce fruit and seedlings, albeit at reduced rates (or continually declining, in some instances).

Chapter 6. Pollination ecology of *Frithia humilis*.

6.1 Introduction

A population of the endangered succulent species, *Frithia humilis* (Mesembryan-themaceae) was translocated from its original habitat, designated for coal mining, to three novel habitats (Burgoyne & Hoffman, 2011). Translocation is generally regarded as a ‘last resort’ conservation measure (Gordon, 1994; Godefroid *et al.,* 2011) . In this case it was the only way to safeguard the population from certain extinction. The suitability of receptor habitats, in terms of substrate, ecological integrity, genetic impacts and long-term protection was investigated prior to translocation (Burgoyne & Hoffman, 2011). However, the presence of potential pollinator insects was not explored, partly due to pollinator species being unknown for this species of the Mesembryanthemaceae.

The mutualistic relation between a plant species and its pollinator(s) is fundamental in the survival of a plant population (Kearns & Inouye, 1997; Mayer, 2004), all the more so in restoration procedures (García-Robledo, 2010), such as conservation translocations. However, no literature describing the pollination system of *F. humilis* exists. Studies on insect relationships and pollination of Mesembryanthemaceae species in the Karoo have been done (e.g. Hartmann, 1991; Ihlenfeldt, 1994; Gess & Gess, 2004; Mayer, 2004; Peter *et al.,* 2005) and guided the formulation of hypotheses regarding *F. humilis* mutualisms. Flowering time of individuals in most populations of Mesembryanthemaceae is synchronised and short- lived (Ihlenfeldt, 1994). The presence of specific pollinator taxa at the translocation receptor sites may thus be crucial to the viability of translocated *F. humilis* populations.

This study quantitatively explored insect diversity at all study sites, expecting to glean insights into potential pollinator taxa. Qualitative sampling was also employed to determine the presence and identity of possible pollinators. In such a way, insect diversity and the presence/absence of pollinators in different receptor habitats, as well as a control habitat, could be explored. The nature of plant-pollinator relationships could thus be elucidated.

Future translocation endeavours involving *F. humilis* can benefit from knowledge of pollinator species, since the latter may influence the reproductive viability of translocated populations (Heinken & Weber, 2013).

6.2 Methods

The immediate habitat of each translocated *F. humilis* population was qualitatively and quantitatively sampled for insects, i.e. Eagle Rock Private Estate (*E*), Goedvertrouwd farm (*GL*, *GM*, *GR* and *GW*) and Witbank Nature Reserve (*W*). The control population at Ezemvelo Nature Reserve (*Z*) was also surveyed.

6.2.1 Qualitative surveys for pollinators

Observational, qualitative surveys were conducted to identify species that targeted *F. humilis* flowers at anthesis. Flower opening in most mesemb populations is restricted to a certain time of the day, typically to around noon (Ihlenfeldt, 1994). Therefore, observational surveys on *F. humilis* populations were restricted to three hours over midday (11:00-14:00).

Insects visiting *F. humilis* flowers during anthesis were sampled by means of sweep nets and pooters/aspirators (Figure 6.1), pinned and preserved for identification. *W* could not be observationally sampled, on account of rainy/overcast weather during field trips. Representative specimens of insect visitors were identified to species level where possible. This was done at the Biosystematics Division of the Plant Protection Research Institute of the Agricultural Research Council (ARC).

6.2.1.1 Pollinator verification: scanning electron microscopy

An insect may alight on a flower, but successful pollination is not guaranteed. The insect could be a mere visitor, since pollination is only successful if fertilisation in the flower takes place (Johnson *et al.,* 2009). Photographs, using the Nikon Multizoom AZ100M stereomicroscope, were taken of representative specimens to ascertain the presence of pollen on the specimens.

To confirm the pollinator status of an insect of a particular plant species it should be viewed through a scanning electron microscope (SEM) (Peter *et al.,* 2005). Micrographs were subsequently taken employing a FEI Quanta FEG 250 SEM to determine whether *F. humilis* pollen was present on the collected insects, as this would confirm its probable role as pollinator as opposed to a visitor. Insects and pollen-bearing appendages were sputter-coated with a gold/palladium alloy prior to microscopy. *Frithia humilis* pollen grains, manually collected from flowers, were prepared along with the insects for examination under the SEM to verify the pollen structure as depicted by Burgoyne *et al.* (2000b) (Figure C.0.1, Appendix C). Pollen grains on insects were not acetolysed so as to keep them lodged on/in insect appendages.

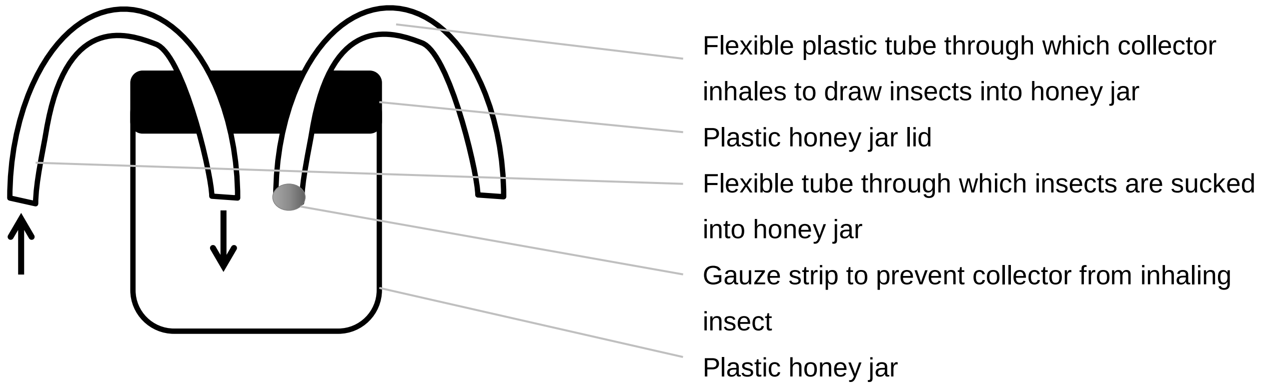

Figure 6.1. Diagram of a pooter/aspirator, used for collecting small insects on or near to *Frithia humilis* plants.

6.2.2 Quantitative surveys: Insect diversity

Seasonal insect diversity was assessed by randomly sampling from insect communities at each study location prior to and after the rainy season. Entomological surveys were conducted in conjunction with plant population monitoring. This arrangement proved time-efficient, since all relevant data collection could be completed during one field trip. Two types of insect traps were constructed: pitfalls and 'sticky traps' (Figure 6.2). All insect traps were set up and left for 24 hours at each study site, resulting in two insect data sets for each study site per year.

6.2.2.1 Pitfall traps

Pitfalls were constructed to sample crawling, flightless insects that might interact with *F. humilis* populations. Deep pitfalls were not suitable for sampling translocation patches. The rock bed was often very shallow, with a thin layer of soil cover. Shallow plastic cups were buried as traps, with the upper rims level with the soil surface. In most cases, the optimal position for placing a pitfall trap – amidst *F. humilis* plants – did not allow even these shallow pitfalls to be buried completely. Hence, soil 'ramps' (Figure 6.3) had to be constructed to facilitate insect movement to the trap opening and into the pitfall, which was partly filled with a solution consisting of 70% ethanol, diluted soapy water and Polyethylene glycol. The cup was fitted and filled three quarters with the alcohol solution, after which the traps were left out for 24 hours. This time span allowed both diurnal and nocturnal insects to be sampled. The contents of each pitfall were subsequently decanted into a marked plastic honey jar filled with 70% ethanol.

The pitfall traps were set out in sets of three or four within each group of translocation patches. Thus, replications for statistical analyses were created, with two sets of three traps each at *GW*, five at *GR*, one at *W* and six at *Z*. Sets of four traps each were set out in *GL* (five) and GM (four). Four traps were set out across *E*1 and 2 and three traps across *E*3 and 4, on account of space constraints at these sites.

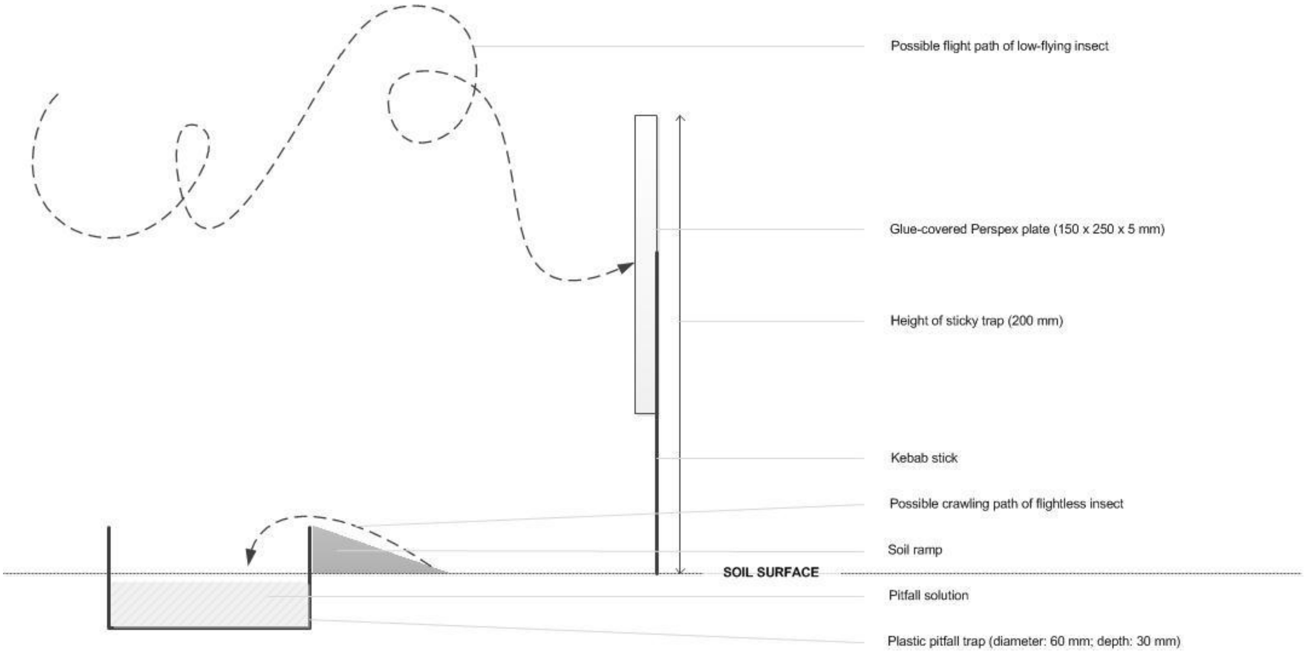

Figure 6.2. Diagram of techniques used for the collection of insects active in and around *Frithia humilis* populations. Left: shallow pitfall. Right: 'sticky trap'.

Figure 6.3. Shallow pitfalls, with soil ramps leading to trap openings, were used to sample crawling insects in and around *Fritiha humilis* populations (Photos: P.M. Harris, 2012).

6.2.2.2 Sticky traps

Sticky traps were constructed to trap low-flying insects active above *F. humilis* patches. Traps were constructed from clear Perspex plates, 250 x 150 mm, mounted on kebab sticks (Figure 6.2). The latter were attached to either end of a plate by a strip of grey duct tape. Plates were covered in *Stick 'Em* perpetually sticky glue on either side. The glue was heated on site with a portable gas stove and immediately applied to the Perspex plates by means of a foam paint roller (A, Figure 6.4). Each sticky trap consisted of two Perspex plates stabilised on the ground by rocks found naturally in the area (B, Figure 6.4). This was seen as the easiest and most natural way to secure the traps.

The two parts of a trap were placed perpendicular to each other to enhance the probability of low-flying insects from all wind directions colliding with the trap-set. Each set of pitfalls was accompanied by a pair of sticky traps. Sticky traps were put out along with the pitfalls for a 24 hour period. Once the traps were collected, each sticky plate was wrapped in clear cling wrap, so as not to contaminate other surfaces with glue, while preserving the specimens for examination.

6.2.3 Analyses of quantitative data

Following field sampling, insects were counted and taxonomically classified to order and family level (where possible) and morphospecies. A stereo microscope was used to sort pitfall samples and a hand lens was used for sticky trap samples. Morphospecies from pitfalls were organised into small bottles containing 70% ethanol and data were captured on Microsoft Excel (2010) spread sheets. Morphospecies from sticky traps could only be roughly identified, due to a warping effect of the glue. These samples were left on the traps.

Pitfall and sticky trap data sets were kept separate, since identification of morphospecies trapped on sticky traps was less accurate, resulting in possible ‘pooling’ of species. Data of pitfalls and sticky traps, respectively, were pooled per study patch and the data from each year were also pooled. Consequently, a composite representation of general insect diversity at each study site during the *F. humilis* flowering season was acquired.

Diversity indices were used to estimate biodiversity (usually in the form of ‘taxonomic units’) in a habitat, enabling comparison between sites. Two concepts are applicable in the measurement of biodiversity by means of indices: firstly, species richness (*S*), the total number of species present in

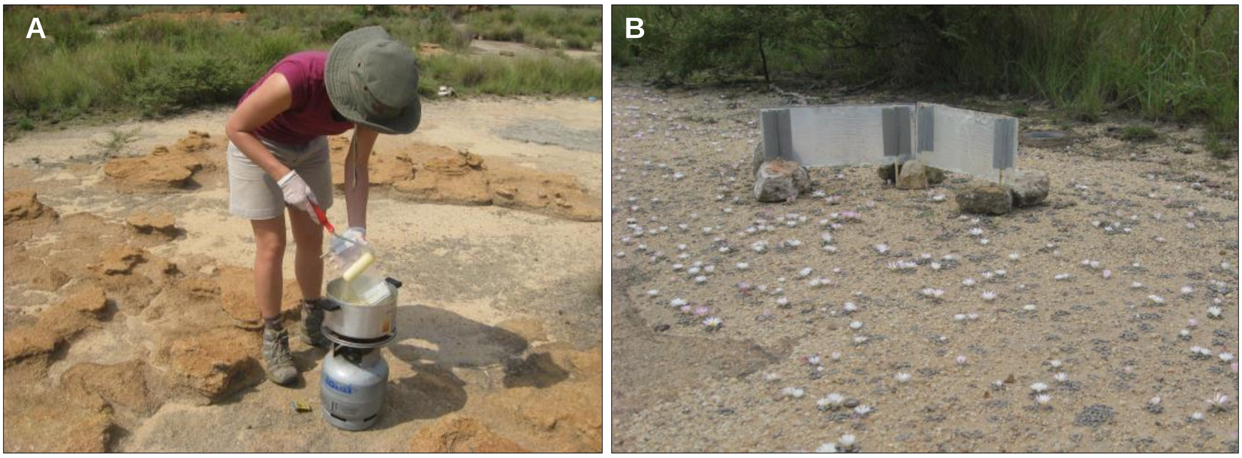

Figure 6.4. Sticky traps were prepared for trapping low-flying insects: (A) *Stick ‘Em* glue was heated on site and applied to Perspex plates before a pair of plates was (B) mounted perpendicular to each other in a study patch. (Photos: P.M. Harris, 2012).

a habitat and secondly, evenness, how evenly the abundance of species is distributed across the community in a given habitat (Heip *et al.,* 1998). Insect diversity data were analysed using PRIMER 6 software (Clarke & Gorley, 2006).

The following diversity measurements were calculated:

6.2.3.1 Margalef's species richness

Margalef's species richness (*d*) is an estimation of *S*:

$$\boldsymbol{d = \frac{(S-1)}{log_e N}}$$

where *N* is the total number of individuals.

6.2.3.2 Simpson's diversity index

Simpson's diversity index (*λ*) considers both *S* and evenness. Values range between zero and one for this index. A higher value indicates a more diverse community.

$$\boldsymbol{\lambda = \sum P_i^2}$$

where P_i is the proportion of *S*, consisting of the i^{th} species.

6.2.3.3 Shannon-Wiener diversity index

The Shannon diversity index (*H'*) also takes into account *S* and evenness. High values for this index are indicative of a diverse community.

$$\boldsymbol{H' = -\sum P_i log P_i}$$

6.2.3.4 Pielou's evenness

Evenness (*J'*) values range from zero to one. A value closer to one represents more evenly distributed species abundance.

$$\boldsymbol{J' = \frac{H'}{log_e S}}$$

6.3 Results

6.3.1 Qualitative surveys

Six insect species were found to alight on open *F. humilis* flowers (Table 6.1). However, not all were confirmed as pollinators. The two *Platylesches* species (Lepidoptera, Hesperiidae) (Figure C.0.2, Appendix C) were void of pollen and were consequently not photographed under the SEM. Of the four remaining species, *Megachile niveofasciata* (Hymenoptera, Megachilidae) (Figure 6.5) carried copious amounts of *F. humilis* pollen grains on its hairy abdomen (Figure 6.6). Pollen grains on *Amegilla fallax* (Hymenoptera, Apidae) (Figure 6.7) could not be clearly identified as belonging to *F. humilis*, even though the shape of most was reminiscent of *F. humilis* pollen (Figure 6.8). *Lipotriches* sp. (Hymenoptera, Halictidae) (Figure 6.9) was not found to carry *F. humilis* pollen (Figure 6.10).

Table 6.1. Insects observed to alight on *Frithia humilis* flowers at various study sites over the study period.

Order	Family	Species	Common name	Population
Lepidoptera	Hesperiidae	*Platylesches* prob. *ayresii* (Trimen) *P*. prob. *moritili* (Wallengren)	Peppered hopper Honey hopper	*Z*
Hymenoptera	Apidae	*Amegilla fallax* (Smith)	Banded bee	*GW, Z*
	Megachilidae	*Megachile niveofasciata* (Friese)	Dauber bee	
	Halictidae	*Lipotriches* sp.	Sweat bee	
Diptera	Bombyliidae	*Exoprosopa eluta* (Loew)	Bee fly	*E, GW, GL* & *Z*

Exoprosopa eluta (Diptera, Bombyliidae) (Figure 6.11) carried very few pollen grains lodged in a cavity in the head of the fly (Figure 6.12).

Pollinator species were clearly more active in *F. humilis* patches at *GW*, *GL* and *Z.* Bee species were only observed at the *Z* and Goedvertouwd sites, while bombylids were present at all study sites (*W* could not be sampled for pollinator species due to overcast and rainy weather conditions).

6.3.2 Quantitative surveys

Pitfall surveys showed that *GM* (S = 56, d = 9.84) and *GR* (S = 57, d = 8.95), were the most species rich sites (Table 6.2). Sticky trap surveys indicated *GL* as the most species rich site (S = 34, d = 6.06). Insects were most abundant in *Z,* however, as indicated by both pitfall (N = 1262) and sticky trap figures (N = 585). With regards to evenness, *W* and *Z* revealed the most even distribution of species in pitfalls and sticky trap (J' = 0.48 at both sites). Study patches with the highest diversity, were *GM* (pitfall $H'(loge)$ = 3.07 and $1\text{-}\lambda$ = 0.90) and *GL* (sticky trap H' = 2.70 and $1\text{-}\lambda$ = 0.88). *W* and *Z* showcased the least diverse insect communities, since Shannon's and Simpson's diversity indices were much lower than those of other sites. This was the case for pitfalls (*W*: $H'(loge)$ = 1.92, $1\text{-}\lambda$ = 0.73; *Z*: $H'(loge)$ = 1.59, $1\text{-}\lambda$ = 0.58) and sticky traps (*W*: $H'(loge)$ = 1.44, $1\text{-}\lambda$ = 0.54; *Z*: $H'(loge)$ = 1.37, $1\text{-}\lambda$ = 0.62).

Figure 6.5 Stereo microscope photo of *Megachile niveofasciata* (Hymenoptera, Megachilidae), a generalist pollinator of *Frithia humilis.*

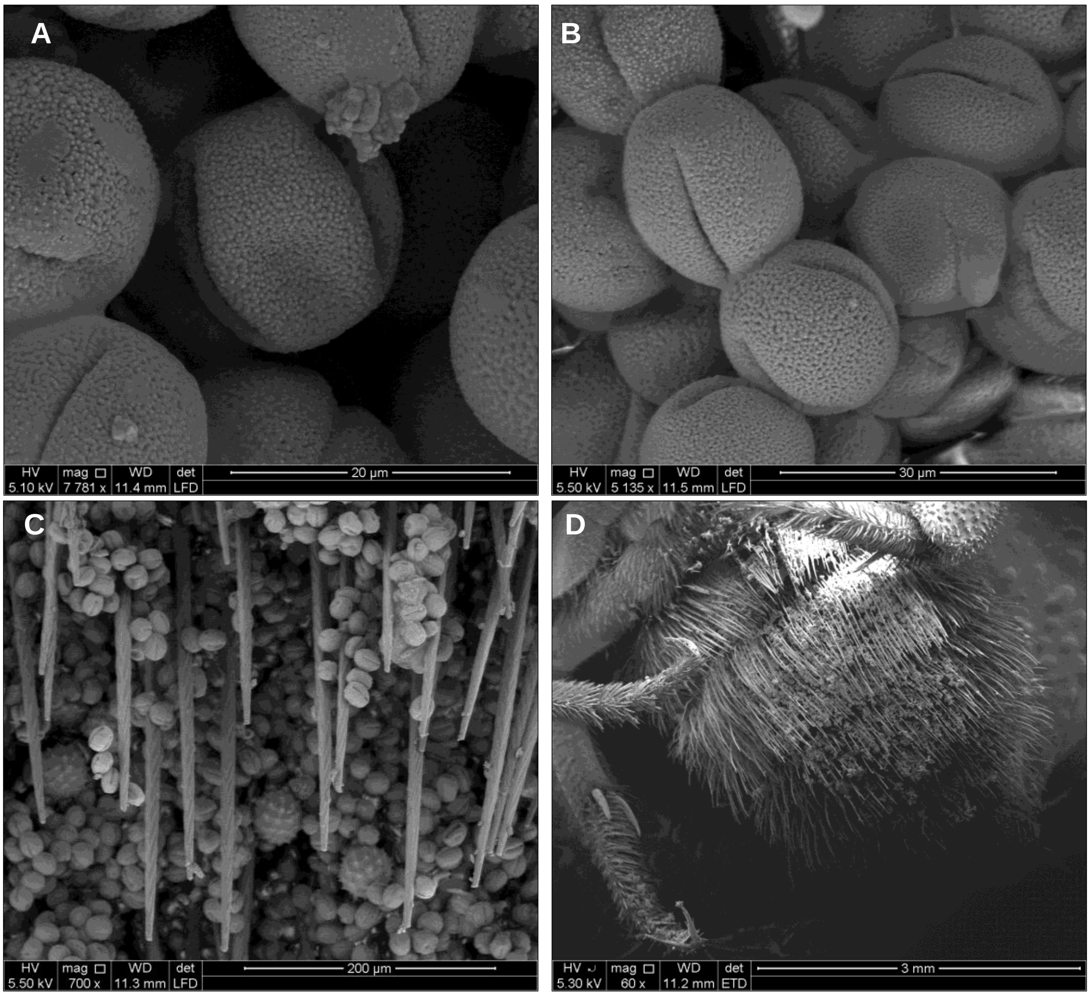

Figure 6.6. Scanning electron microscope micrograph of *Frithia humilis* pollen grains (A & B), among those of other species, on the abdomen of *Megachile niveofasciata* (Hymenoptera, Megachilidae) (C & D).

Figure 6.7. Stereo microscope photo of *Amegilla fallax* (Hymenoptera, Apidae), a generalist possible pollinator of *Frithia humilis.*

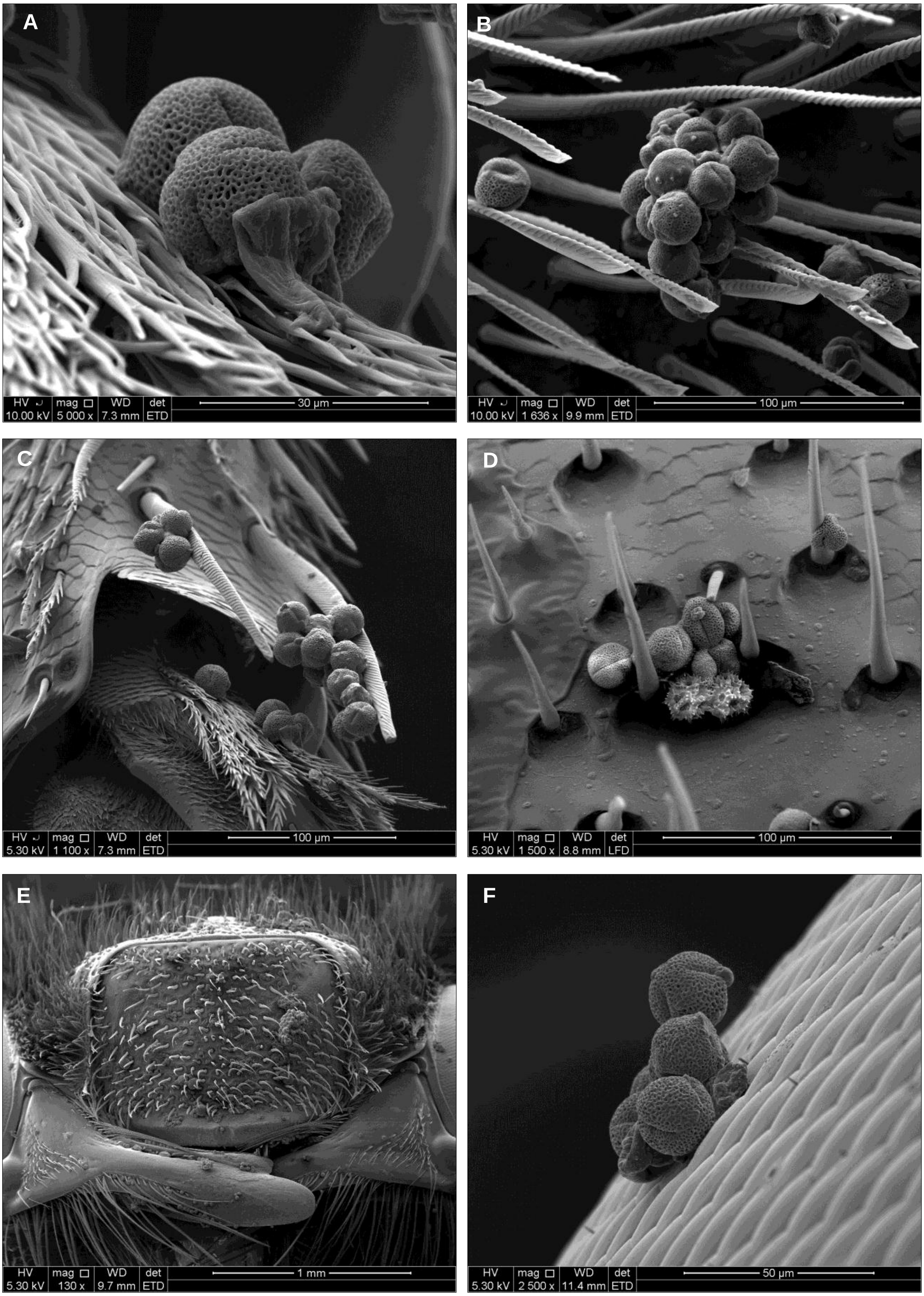

Figure 6.8. Scanning electron microscope micrograph of *Frithia humilis* pollen grains on legs (A, B & C), head (D & E) and eyes (F) of *Amegilla fallax* (Hymenoptera, Apidae).

Figure 6.9. Stereo microscope photo of *Lipotriches* sp. (Hymenoptera, Halictidae) not confirmed as a *Frithia humilis* pollinator.

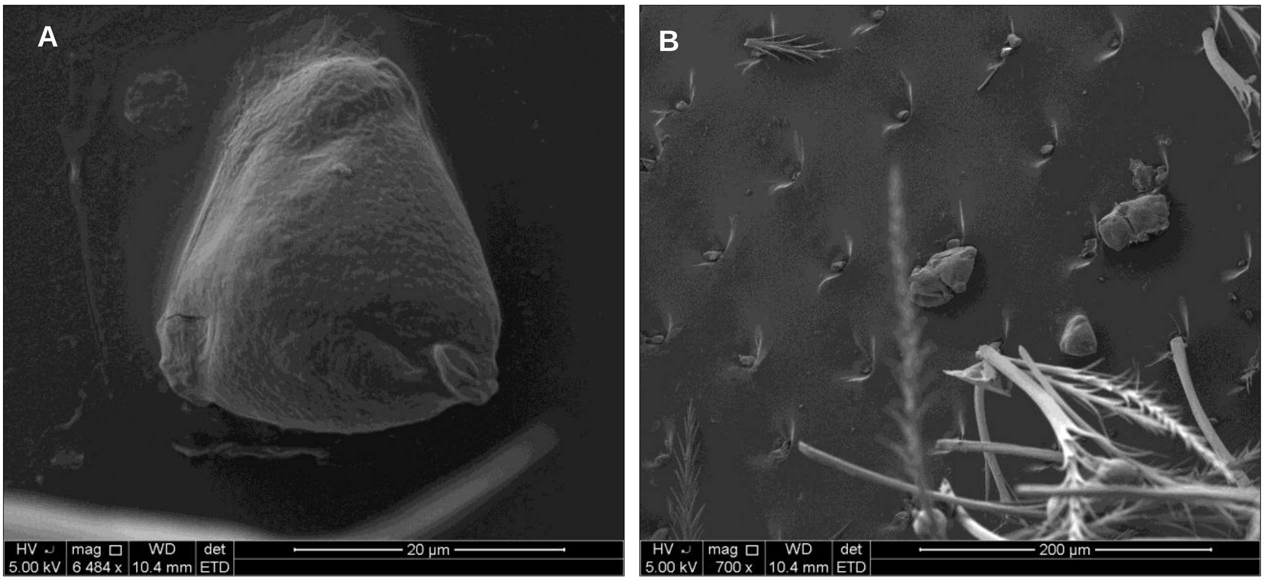

Figure 6.10. Scanning electron microscope micrograph of pollen grains not belonging to *Frithia humilis* (A) were found on *Lipotriches* sp. (Hymenoptera, Halictidae) legs and pollen baskets (B).

Figure 6.11. Stereo microscope photo of *Exoprosopa eluta* (Diptera, Bombyliidae), a possible pollinator of *Frithia humilis*, observed at all study habitats, except at Witbank Nature Reserve.

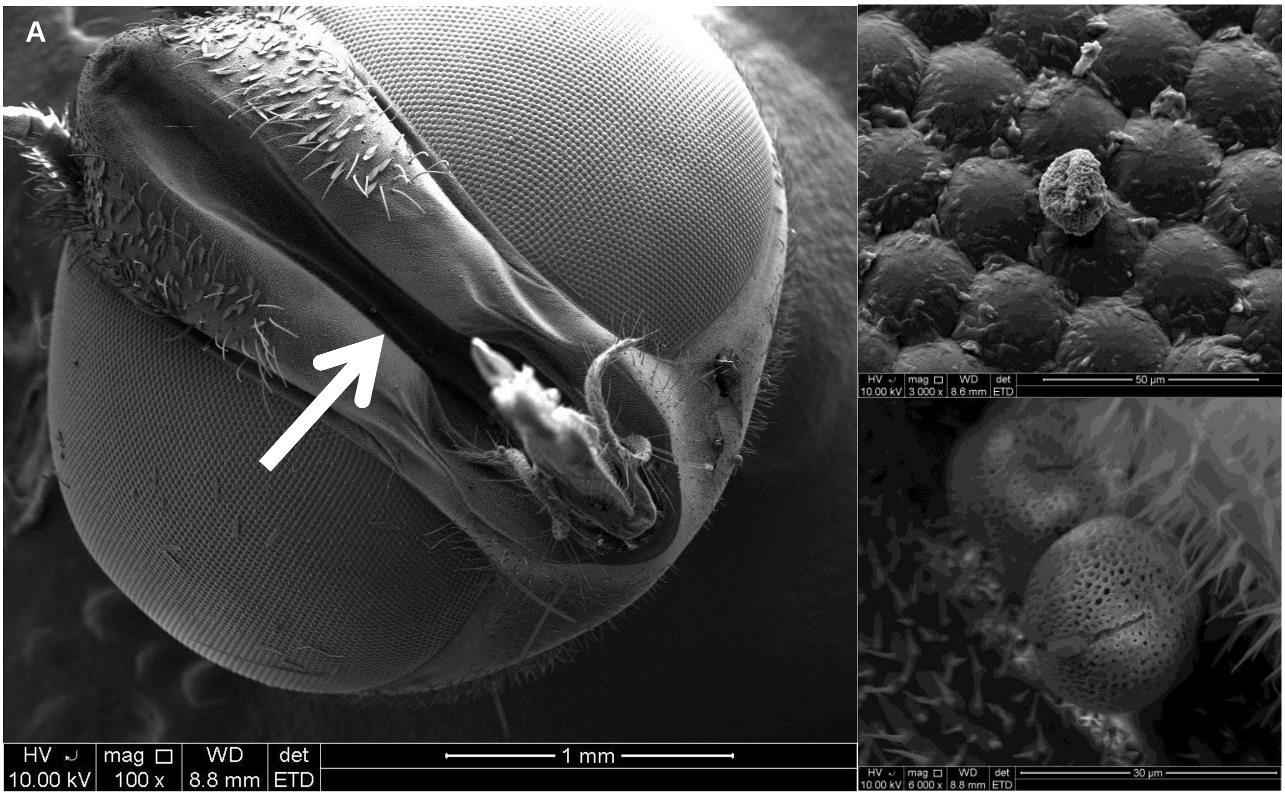

Figure 6.12. Scanning electron microscope micrograph of the head of *Exoprosopa eluta* (Diptera, Bombyliidae) (A), found to carry few pollen grains on its eye (B) and a cavity in its head, indicated by the white arrow (C), resembling those of *Frithia humilis*.

Table 6.2. Measures for pitfall (P) and sticky trap (ST) insect diversity in study sites. Data collected over the course of two years (2011 – 2012) were pooled per population.

Study site	*S*		*N*		*d*		*J'*		*H'(loge)*		*1-λ*	
	P	ST	P	ST	P	ST	P	ST	P	ST	P	ST
E	50	28	715	308	7.46	4.71	0.56	0.62	2.20	2.06	0.79	0.74
GL	34	34	224	232	6.10	6.06	0.74	0.77	2.61	2.70	0.87	0.88
GM	56	22	267	255	9.84	3.79	0.76	0.77	3.07	2.37	0.90	0.85
GR	57	31	521	194	8.95	5.69	0.63	0.73	2.56	2.49	0.83	0.84
GW	54	30	1179	195	7.49	5.50	0.58	0.71	2.30	2.42	0.83	0.84
W	40	20	834	312	5.80	3.31	0.52	0.48	1.92	1.44	0.73	0.54
Z	37	18	1262	585	5.15	2.65	0.44	0.48	1.59	1.37	0.58	0.62

S: Species richness; *N*: total number of individuals; *d*: Margalef's species richeness; *J'*: Pielou's evenness ; *H'(loge)*: Shannon's diversity index; *1-λ*: Simpson's diversity index

6.4 Discussion

Hymenopteran pollinators listed in Table 6.1 were from families reported by Gess & Gess (2004) to pollinate Mesembryanthemaceae species in winter rainfall areas. These families were Megachilidae, Apidae, and Halictidae bees. Bees generally visit plant species with high reward, in the form of nectar (carbohydrate source) or pollen (protein source) (Peter & Johnson, 2013). *Megachile* (Megachilidae) is an abundant and speciose genus. Bees from this taxon are important pollinators, both in biodiversity conservation (e.g. Goldblatt *et al.,* 2005; Peter & Johnson, 2013) and agricultural contexts (Munyuli *et al.,* 2011b; Eardley, 2012). *Amegilla* species (Apidae) are known 'buzz' (vibrational) pollinators of wild plants (e.g. Goldblatt *et al.,* 1998, 2000, and 2005) and crop species (Munyuli *et al.,* 2011a). *Amegilla fallax* has been found to pollinate indigenous winter-flowering *Aloe inconspicua* (Xanthorrhoeaceae) (Hargreaves *et al*., 2008; Symes *et al*., 2009), as well as

Plectranthus porphyranthus (Lamiaceae) (Edwards *et al.,* 2000). *Lipotriches* species (Halictidae) are known to pollinate naturally occurring plant species (Goldblatt *et al.,* 1998), as well as crops (Munyuli *et al.,* 2011b). Adult *Exoprosopa* (Bombyliidae) individuals are common dipteran pollinators and exhibit bee-like behaviour. These flies tend to hover above flowers in order to feed on nectar and pollen (Scholtz & Holm, 1989).

Sticky traps and pitfalls (quantitative surveys) proved inefficient at collecting *F. humilis* pollinators, since none of the species collected during observational surveys were found in pitfalls oron sticky traps. The species identified from qualitative surveys are considered strong, agile flyers (Scholtz & Holm, 1989) and this probably resulted in them not being captured through the use of sticky traps. One Apidae bee was caught in a pitfall trap at *Z*, but could not be identified to species level. Fourteen lepidopteran individuals were found in pitfalls and six on sticky traps, but none of the specimens resembled Hesperiidae butterflies found to visit *F. humilis* flowers. One specimen could be identified as a Noctuidae moth. *Frithia humilis* flowers open only during sunny days, from 10:00 to approximately 18:00, which excluded the moth as a potential pollinator. The number of non-pollinating insect species collected in pitfalls and sticky traps outweighed the number of potential pollinators (i.e. hymenopteran bees). Hence, qualtitative methods employed in the study could be considered more efficient at collecting pollinator species for *F. humilis*.

Goedvertrouwd sites showed higher diversity than other translocation receptor sites (*E* and *W*) and even the control site, *Z*. There is no pre-translcoation record of insect diversity at the receptor sites. Diversity indices were higher for receptor sites, except for *W*, than for the control site. The vitality and diversity of insect communities in translocation receptor sites is an indication of good habitat quality (Kleijn & Van Langevelde, 2006), with little disturbance (Kruess & Tscharntke, 2002).

The larger Goedvertrouwd receptor site hosted the most diverse insect communities. *GL* and *GM* were the largest of the Goedvertouwd receptor sites, with a total area of 13.497 m² and 6.303 m² respectively (compared to *GW* and *GR* with total respective areas of 5.149 m² and 5.569 m²), and these sites scored higher diversity indices than other study sites. Species richness and abundance of some insect species groups have been associated with larger, non-isolated habitats (Steffan-Dewenter & Tscharntke, 2002). *Frithia humilis* populations occupying larger receptor areas are therefore more likely to inhabit entomologically diverse habitats.

Pollinators played an important role in the initial positive response of the transplanted *F. humilis* populations. Sexual reproduction in plants is facilitated by pollination, and especially by insects in the case of the Mesembryanthemaceae (Hartmann, 1991). The presence of fruit in most translocated populations (excluding *GM* by 2012) was therefore indicative of pollinator activity in receptor habitats. However, not all translocated populations reproduced equally successfully over the short-term, e.g. fruit production in *E* decreased significantly over the monitoring period and was significantly reduced in *GM* by 2012. It is therefore probable that pollinators were less active or inefficient at these habitats.

Translocation success (i.e. the long-term persistence of translocated populations) is likely not to be limited by pollinator specificity and distribution, but rather by flower production (e.g. Kleijn & Van Langevelde, 2006) and germination conditions (Chapters 4 and 5). Generalist pollinators of *Centaurea corymbosa* (Asteraceae) were less attracted to translocated populations that flowered at low density (Colas *et al*., 2008). Two populations with the least amount of individuals, i.e. at *GM* and *W*, also flowered less and produced less fruit than populations at other receptor habitats. These populations may experience reduced fecundity due to pollinators over-looking such populations.

6.5 Conclusion

Pollinator species were not revealed through quantitative surveys of insect diversity. Qualitative surveys proved more efficient and accurate at uncovering pollinator species. This study provided the first evidence of Apidae, Megachilidae (Hymenoptera) and Bombyliidae (Diptera) insect species most likely being part of a pollinator complex that pollinates *F. humilis.* Furthermore, it confirmed the hypothesis that *F. humilis* is pollinated by an array of generalist pollinator species. The generalist nature of the plant-pollinator relationship, as well as the presence of generalist pollinator species at receptor habitats, probably contributed to the initial positive response of *F. humilis* populations to translocation.

Chapter 7. Conclusions

7.1 Introduction

Translocation, as implemented in the case of the *Frithia humilis* population at Inyanda coal mine, is becoming an ever more important conservation strategy (Griffith *et al.,* 1989; Fahselt, 2007; Seddon, 2010; Godefroid *et al.,* 2011; Weeks *et al.,* 2011). However, translocation is still viewed as being in an experimental phase. Research on this topic is rarely published if outcomes are negative (translocation failures) (Fahselt, 2007). The gap in knowledge regarding translocation is especially pronounced in a South African conservation context (Milton *et al*., 1999; Burgoyne & Hoffman, 2011). Scientific attempts to improve knowledge on translocation as a conservation method in South Africa, including this study, are therefore imperative.

The hypotheses regarding translocation as a conservation measure presented in Chapter 1 and the findings in Chapters 4 to 6 will be summarised in this chapter. An overview of the chapters and corresponding hypotheses will be provided for the sake of clarity.

<u>Chapter 4:</u> ***Short-term translocation success of*** **Frithia humilis** ***populations in terms of survival and plant growth: a baseline for future studies***

<u>Hypothesis 1:</u> Translocated populations will show an initial decline in numbers, after which population size will stabilise.

<u>Chapter 5:</u> The feasibility of translocating **Frithia humilis*****, an endangered edaphic specialist to atypical geological habitats***

<u>Hypothesis 2</u>: Establishment of the population would be accompanied by an increase in reproductive capability (i.e. flowering and fruit-producitn).

<u>Hypothesis 3:</u> Translocated plants in receptor habitats with typical *F. humilis* geological substrates should have a higher survival rate than plants translocated to atypical habitats.

<u>Chapter 6:</u> Elements in the life cycle of **Frithia humilis*****: introduction to the pollination ecology of the species***

Hypothesis 4: Pollinators are likely to include a complex of generalist flower visiting arthropods.

7.2 Short-term translocation success

Population figures presented in Chapter 4 serve as a baseline for post-translocation monitoring and future studies on the translocated *F. humilis* populations. These figures showed that the translocated cohort declined in terms of numbers during the first year following translocation.

Population growth was observed during the 2012 growing season, indicating a recuperation of population numbers and stabilisation of population size at the Goedvertrouwd (*G*) receptor habitats. The size increase in the Witbank Nature Reserve (*W*) population was not reciprocated in the seedling component of that population, contrary to trends in *G* populations (excepting *GM*), where seedling numbers increased considerably over the monitoring period. Deficient reproductive output, i.e. seedling production and recruitment, can be an early indication of population demise. Translocations of *F. humilis* populations to *W* and Eagle Rock Private Estate (*E*), the latter of which showed an overall decline in population size over the study period, can therefore be considered as less successful over the short-term, with the possibility of extinction. Conversely, populations at *G* can be considered as successfully translocated over the short-term.

7.3 Translocation to atypical geologies

The experimental translocation of *F. humilis* populations to atypical habitats was of considerable significance. This habitat specialist naturally occurs on geological substrates derived from Ecca and Dwyka sediments, which is often underlain by coal seams. Coal mining will not cease in the near future and effective translocation to safe habitats is likely to become an increasingly important conservation measure for the species. Testing whether the species would be able survive on non-typical, non-coal bearing substrates, with little managerial intervention, was therefore important. Flowering and fruit-production showed a net increase for most populations over the monitoring period. However, translocated *F. humilis* populations responded better to geological habitats typical for the species (*G*), in terms of population survival, flowering and fruit production (reproductive responses), as well as seedling emergence.

7.4 Pollination ecology

Frithia humilis pollinators were previously unknown and this study revealed Apidae and Megachiledae bees and Bombyliidae flies as possible pollinators of the species. The study confirmed that pollination of *F. humilis* flowers occured via a suite of generalist insect species.

7.5 General conclusion

Short-term assessment of this *Frithia humilis* translocation endeavour revealed that the overall response of translocated populations to typical habitats was positive. This positive response, however, was not unanimous across receptor habitats. Biological, geological and edaphic environments determined the short-term outcome of translocation. Continued, long-term monitoring is of vital importance to confirm population persistence and whether experimental populations are prone to extinction.

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Appendix A

This appendix accompanies Chapter 4.

Table A.0.1. Pairwise comparisons, with Sidak adjustments for multiple comparisons, of the estimated marginal means (EMM) of the total translocated *Frithia humilis* cohort. Differences in EMM are significant when p<0.05.

Year		Mean difference	Standard error	p-value
2010	2011	5.072	5.142	0.693
	2012	-2.144	5.232	0.968
2011	2010	-5.072	5.142	0.693
	2012	-7.216	5.060	0.398
2012	2010	2.144	5.232	0.968
	2011	7.216	5.060	0.398

Table A.0.2. Average[11] number of *Frithia humilis* individuals in the various relative age groups per annum in each receptor habitat, with global positioning system (GPS) coordinates.

	GPS co-ordinates	**<3**			**3-5**			**6-10**			**11-15**			**16-20**			**21-30**			**>30**		
		2010	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**
E	25°45'09.5"S 29°17'32.7"E	132	46	60	249	102	88	117	92	104	72	59	49	34	34	21	34	26	25	38	25	18
GL	25°47'42.0"S 28°57'21.5"E	423	514	624	460	350	448	140	148	221	60	58	54	25	17	15	13	15	4	9	6	2
GM	25°47'43.3"S 28°57'22.2"E	161	127	132	146	97	111	36	22	31	18	7	4	8	3	2	6	2	0	2	3	0
GR[12]	25°47'45.0"S 28°57'23.9"E	472	363	872	396	191	301	72	44	102	16	27	20	4	10	4	1	9	2	0	2	1
GW	25°47'40.3"S 28°57'13.6"E	51	24	177	76	56	34	19	42	59	8	11	29	3	2	11	1	4	4	0	1	4
W	25°53'36.7"S 29°18'43.2"E	29	18	7	17	58	21	7	31	32	2	10	12	1	3	6	0	4	6	0	2	2

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[11] The yearly averages of individuals per relative age group were calculated using the total number of individuals per RA group counted during each monitoring session, i.e. in February and November of each year.

[12] *GR*5 was located at 25°47'46.5"S 28°57'21.4"E, approximately 100 m southwest of *GR*1-4.

Table A.0.3. Pairwise comparisons of the estimated marginal means (EMM) of translocated *Frithia humilis* populations over the study period. Only populations that did not show a significant difference EMM (p<0.05) are tabled.

Year		Mean difference	Standard error	p-value
GL				
2010	2011	11.748	16.656	0.864
	2012	-2.982	17.420	0.998
2011	2010	-11.748	16.656	0.864
	2012	-14.730	16.656	0.765
2012	2010	2.982	17.420	0.998
	2011	14.730	16.656	0.765
GM				
2010	2011	4.170	4.908	0.790
	2012	3.582	4.996	0.862
2011	2010	-4.170	4.908	0.790
	2012	-0.588	4.908	0.999
2012	2010	-3.582	4.996	0.862
	2011	0.588	4.908	0.999
GR				
2010	2011	10.139	12.785	0.816
	2012	-11.480	12.873	0.758
2011	2010	-10.139	12.785	0.816
	2012	-21.618	12.785	0.264
2012	2010	11.480	12.873	0.758
	2011	21.618	12.785	0.264
GW				
2010	2011	-2.610	10.004	0.992
	2012	-9.091	10.337	0.790
2011	2010	2.610	10.004	0.992
	2012	-6.480	10.004	0.898
2012	2010	9.091	10.337	0.790
	2011	6.480	10.004	0.898
W				
2010	2011	-2.345	1.876	0.537
	2012	-0.178	1.909	1.000
2011	2010	2.345	1.876	0.537
	2012	2.167	1.559	0.453

2012	2010	0.178	1.909	1.000
	2011	-2.167	1.559	0.453

Table A.0.4. Pairwise comparisons of overall estimated marginal means (EMM) (2010-2012) between translocated *Frithia humilis* populations at different receptor sites. * indicates significant differences in EMM (p<0.05) using Sidak adjustments for multiple comparisons.

Populations		**Mean difference**	**Standard error**	**p-value**
E	*GL*	-19.120	6.596	0.064
	GM	5.765	6.953	0.999
	GR	-11.047	6.596	0.781
	GW	2.649	8.515	1.000
	W	12.253	7.677	0.833
GL	*GM*	24.885	6.596	0.004*
	GR	8.073	6.219	0.962
	GW	21.769	8.227	0.128
	W	31.373	7.355	0.001*
GM	*GR*	-16.812	6.596	0.165
	GW	-3.116	8.515	1.000
	W	6.488	7.677	0.999
GR	*GW*	13.696	8.227	0.788
	W	23.3	7.355	0.027*
GW	*W*	9.604	9.116	0.995

Table A.0.5. Pairwise comparisons of the estimated marginal means (EMM) of each relative age group across all populations over the study period. * indicates significant differences in EMM (p<0.05) using Sidak adjustments for multiple comparisons

Year		**Mean difference**	**Standard error**	**p-value**
<3				
2010	2011	8.865	24.056	0.976
	2012	-24.472	26.438	0.734
2011	2010	-8.865	24.056	0.976
	2012	-33.337	22.960	0.384
2012	2010	24.472	26.438	0.734
	2011	33.337	22.960	0.384
3-5				
2010	2011	9.852	8.755	0.600
	2012	6.027	11.055	0.929

2011	2010	-9.852	8.755	0.600
	2012	-3.825	8.193	0.954
2012	2010	-6.027	11.055	0.929
	2011	3.825	8.193	0.954

6-10

2010	2011	-2.292	3.271	0.863
	2012	-8.284	4.168	0.140
2011	2010	2.292	3.271	0.863
	2012	-5.992	3.054	0.149
2012	2010	8.284	4.168	0.140
	2011	5.992	3.054	0.149

11-15

2010	2011	-1.093	1.957	0.925
	2012	-2.212	2.186	0.678
2011	2010	1.093	1.957	0.925
	2012	-1.119	1.865	0.909
2012	2010	2.212	2.186	0.678
	2011	1.119	1.865	0.909

16-20

2010	2011	-0.376	0.689	0.929
	2012	-0.423	0.766	0.927
2011	2010	0.376	0.689	0.929
	2012	-0.047	0.657	1.000
2012	2010	0.423	0.766	0.927
	2011	0.047	0.657	1.000

21-30

2010	2011	-0.520	0.627	0.793
	2012	0.089	0.652	0.999
2011	2010	0.520	0.627	0.793
	2012	0.609	0.599	0.674
2012	2010	-0.089	0.652	0.999
	2011	-0.609	0.599	0.674

>30

2010	2011	0.140	0.438	0.984
	2012	0.304	0.522	0.916

2011	2010	-0.140	0.438	0.984
	2012	0.164	0.414	0.971
2012	2010	-0.304	0.522	0.916
	2011	-0.164	0.414	0.971

Table A.0.6. Mean season rainfall figures for the period from October to February 2009 – 2012 (Data supplied by the South African Weather Service).

	2009/'10	**2010/'11**	**2011/'12**
E & W	151,75 mm	134,15 mm	66,35 mm
G & Z	138,60 mm	105,30 mm	86,00 mm

Table A.0.7. Analysis of soil metal concentrations in study areas, including Ezemvelo Nature Reserve (*Z*), a natural *Frithia humilis* habitat serving as a control site. Elements are tabled according to their mass numbers.

Metal concentration *(mg/kg)*	***Donor***	***E***	***E* (outside)**	***G***	***G* (outside)**	***W***	***Z***
Be	0,008	0,002	0,035	0,003	0,067	0,018	0,003
B	0,140	0,203	1,339	0,148	1,431	0,273	0,155
Na	5,003	2,825	47,350	2,668	22,340	10,633	4,075
Mg	12,795	9,768	209,575	7,030	209,700	45,650	18,925
Al	90,975	69,800	414,000	108,450	882,000	64,000	47,150
P	0,153	0,315	2,360	0,252	1,936	0,347	0,152
K	43,050	36,600	446,000	28,800	536,500	137,100	37,725
Ca	41,900	44,625	1187,500	28,800	619,000	202,300	62,750
Ti	0,057	0,0178	1,183	0,013	0,247	0,003	0,184
V	0,000	0,000	0,001	0,000	0,001	0,000	0,000
Cr	0,024	0,030	0,214	0,013	0,142	0,022	0,015
Mn	3,905	9,268	152,725	4,590	92,300	31,100	4,390
Fe	3,060	9,732	38,100	3,188	19,828	2,653	4,615
Co	0,056	0,089	1,093	0,067	0,473	0,395	0,132
Ni	0,104	0,196	1,525	0,137	0,799	0,174	0,181
Cu	0,060	0,053	0,397	0,046	0,578	0,086	0,078
Zn	0,441	0,940	5,480	0,657	3,375	1,439	0,620
As	0,016	0,000	0,001	0,003	0,023	0,005	0,000
Se	0,001	0,001	0,007	0,001	0,007	0,001	0,001

Rb	0,541	0,492	4,470	0,423	7,445	1,687	0,299
Sr	0,365	0,328	7,718	0,298	6,318	1,827	0,455
Mo	0,006	0,018	0,092	0,004	0,032	0,051	0,005
Pd	0,010	0,001	0,009	0,002	0,060	0,008	0,003
Ag	0,000	0,000	0,001	0,000	0,001	0,000	0,000
Cd	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Sb	0,012	0,014	0,121	0,011	0,113	0,018	0,012
Ba	2,738	3,448	53,850	3,353	38,250	13,118	2,938
Pt	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Au	0,010	0,015	0,116	0,010	0,094	0,040	0,010
Hg	0,000	0,002	0,005	0,000	0,000	0,003	0,000
Tl	0,000	0,000	0,000	0,000	0,000	0,010	0,000
Pb	0,120	0,118	0,150	0,006	0,001	0,254	0,048
Bi	0,035	0,035	0,348	0,035	0,347	0,038	0,035
Th	0,027	0,027	0,275	0,028	0,277	0,028	0,026
U	0,003	0,002	0,020	0,002	0,032	0,003	0,002

Appendix B

This appendix accompanies Chapter 5.

Table B.0.1. Pairwise comparisons of the per 1 m² relative age group estimated marginal means (EMM) of each study population over the monitoring period. * indicates significant differences in EMM ($p<0.05$) using Sidak adjustments for multiple comparisons.

<3	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
E						
Mean difference	52.392	11.303	79.400	24.550	7.592	107.462
Standard error	33.172	34.966	33.172	42.825	38.918	34.495
Significance	0.935	1.000	0.366	1.000	1.000	0.066
GL						
Mean difference	—	41.089	27.008	27.842	59.984	55.070
Standard error	—	33.172	31.275	41.373	37.314	32.675
Significance	—	0.995	1.000	1.000	0.922	0.888
GM						
Mean difference	—	—	68.097	13.247	18.895	96.159
Standard error	—	—	33.172	42.825	38.918	34.495
Significance	—	—	0.633	1.000	1.000	0.153
GR						
Mean difference	—	—	—	54.850	86.992	28.062
Standard error	—	—	—	41.373	37.314	32.675
Significance	—	—	—	0.989	0.397	1.000
GW						

<3	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
Mean difference	—	—	—	—	32.142	82.912
Standard error	—	—	—	—	46.107	42.441
Significance	—	—	—	—	1.000	0.711
W						
Mean difference	—	—	—	—	—	115.054
Standard error	—	—	—	—	—	38.495
Significance	—	—	—	—	—	0.086

3-5	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
E						
Mean difference	9.721	6.199	30.410	10.352	21.869	114.964
Standard error	24.789	26.130	24.789	32.003	28.276	25.102
Significance	1.000	1.000	.996	1.000	1.000	0.002*
GL						
Mean difference	—	15.919	20.690	20.073	31.590	105.244
Standard error	—	24.789	23.372	30.918	27.042	23.702
Significance	—	1.000	1.000	1.000	.998	.003*
GM						
Mean difference	—	—	36.609	4.154	15.670	121.163
Standard error	—	—	24.789	32.003	28.276	25.102
Significance	—	—	.969	1.000	1.000	.001*
GR						
Mean difference	—	—	—	40.763	52.279	84.554
Standard error	—	—	—	30.918	27.042	23.702
Significance	—	—	—	.991	.753	.028*
GW						
Mean difference	—	—	—	—	11.517	125.317
Standard error	—	—	—	—	33.778	31.169
Significance	—	—	—	—	1.000	.009*
W						

3-5	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
Mean difference	—	—	—	—	—	136.833
Standard error	—	—	—	—	—	27.328
Significance	—	—	—	—	—	.001*

6-10	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
E						
Mean difference	4.693	14.771	6.715	5.877	14.899	101.522
Standard error	15.870	16.729	15.870	20.489	18.086	16.004
Significance	1.000	1.000	1.000	1.000	1.000	0.000*
GL						
Mean difference	—	10.077	2.021	1.184	10.206	106.215
Standard error	—	15.870	14.963	19.794	17.295	15.105
Significance	—	1.000	1.000	1.000	1.000	0.000*
GM						
Mean difference	—	—	-8.056	8.894	.128	116.293
Standard error	—	—	15.870	20.489	18.086	16.004
Significance	—	—	1.000	1.000	1.000	0.000*
GR						
Mean difference	—	—	—	-.838	8.184	108.236
Standard error	—	—	—	19.794	17.295	15.105
Significance	—	—	—	1.000	1.000	0.000*
GW						
Mean difference	—	—	—	—	9.022	107.399
Standard error	—	—	—	—	21.610	19.901
Significance	—	—	—	—	1.000	0.000*
W						
Mean difference	—	—	—	—	—	116.421
Standard error	—	—	—	—	—	17.418
Significance	—	—	—	—	—	0.000*

11-15	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
E						
Mean difference	6.515	10.016	8.087	6.609	10.265	35.195
Standard error	6.454	6.803	6.454	8.332	7.351	6.478
Significance	1.000	0.971	0.995	1.000	0.983	0.000*
GL						
Mean difference	—	3.501	1.571	.094	3.750	41.71
Standard error	—	6.454	6.085	8.050	7.029	6.110
Significance	—	1.000	1.000	1.000	1.000	0.000*
GM						
Mean difference	—	—	1.930	3.408	0.249	45.211
Standard error	—	—	6.454	8.332	7.351	6.478
Significance	—	—	1.000	1.000	1.000	0.000*
GR						
Mean difference	—	—	—	1.478	2.178	43.282
Standard error	—	—	—	8.050	7.029	6.110
Significance	—	—	—	1.000	1.000	0.000*
GW						
Mean difference	—	—	—	—	3.656	41.804
Standard error	—	—	—	—	8.785	8.069
Significance	—	—	—	—	1.000	0.001*
W						
Mean difference	—	—	—	—	—	45.46
Standard error	—	—	—	—	—	7.051
Significance	—	—	—	—	—	0.000*

16-20	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
E						
Mean difference	4.563	5.579	5.643	4.503	5.654	13.287
Standard error	2.621	2.763	2.632	3.384	2.985	2.631
Significance	0.878	0.699	0.603	0.990	0.788	0.001*
GL						

16-20	*GL*	*GM*	*GR*	*GW*	*W*	*Z*
Mean difference	—	1.016	1.080	0.060	1.091	17.85
Standard error	—	2.621	2.483	3.269	2.854	2.482
Significance	—	1.000	1.000	1.000	1.000	0.000*
GM						
Mean difference	—	—	*.065*	*1.076*	*0.075*	*18.865*
Standard error	—	—	2.632	3.384	2.985	2.631
Significance	—	—	1.000	1.000	1.000	0.000*
GR						
Mean difference	—	—	—	1.140	0.011	18.93
Standard error	—	—	—	3.278	2.864	2.494
Significance	—	—	—	1.000	1.000	0.000*
GW						
Mean difference	—	—	—	—	1.151	17.79
Standard error	—	—	—	—	3.568	3.277
Significance	—	—	—	—	1.000	0.000*
W						
Mean difference	—	—	—	—	—	18.941
Standard error	—	—	—	—	—	2.864
Significance	—	—	—	—	—	0.000*

21-30	*GL*	*GM*	*GR*	*GW*	*W*	*Z*
E						
Mean difference	4.342	5.075	5.059	4.637	4.951	8.916
Standard error	1.903	2.006	1.903	2.457	2.169	1.921
Significance	0.499	0.331	0.260	0.792	0.496	0.002*
GL						
Mean difference	—	0.732	0.717	0.295	0.609	13.258
Standard error	—	1.903	1.794	2.373	2.074	1.813
Significance	—	1.000	1.000	1.000	1.000	0.000*
GM						
Mean difference	—	—	0.016	0.437	0.124	13.991

21-30	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
Standard error	—	—	1.903	2.457	2.169	1.921
Significance	—	—	1.000	1.000	1.000	0.000*
GR						
Mean difference	—	—	—	0.422	0.108	13.975
Standard error	—	—	—	2.373	2.074	1.813
Significance	—	—	—	1.000	1.000	0.000*
GW						
Mean difference	—	—	—	—	0.314	13.553
Standard error	—	—	—	—	2.592	2.388
Significance	—	—	—	—	1.000	0.000*
W						
Mean difference	—	—	—	—	—	*13.867*
Standard error	—	—	—	—	—	2.090
Significance	—	—	—	—	—	0.000*

>30	***GL***	***GM***	***GR***	***GW***	***W***	***Z***
E						
Mean difference	5.253	5.598	5.916	5.568	5.641	2.366
Standard error	2.073	2.185	2.073	2.676	2.360	2.075
Significance	0.340	0.323	0.185	0.661	0.432	0.998
GL						
Mean difference	—	0.345	0.663	0.315	0.388	7.619
Standard error	—	2.073	1.954	2.585	2.257	1.956
Significance	—	1.000	1.000	1.000	1.000	0.016*
GM						
Mean difference	—	—	0.317	0.030	0.043	7.965
Standard error	—	—	2.073	2.676	2.360	2.075
Significance	—	—	1.000	1.000	1.000	0.018*
GR						
Mean difference	—	—	—	0.347	0.275	8.282
Standard error	—	—	—	2.585	2.257	1.956

>30	*GL*	*GM*	*GR*	*GW*	*W*	*Z*
Significance	—	—	—	1.000	1.000	0.007*
GW						
Mean difference	—	—	—	—	0.073	7.934
Standard error	—	—	—	—	2.821	2.587
Significance	—	—	—	—	1.000	0.114
W						
Mean difference	—	—	—	—	—	8.007
Standard error	—	—	—	—	—	2.259
Significance	—	—	—	—	—	0.037*

Table B.0.2 Pairwise comparisons of relative age group estimated marginal means (EMM) of each translocated population over the monitoring period. * indicates significant differences in EMM (p<0.05) using Sidak adjustments for multiple comparisons.

E	3-5	6-10	11-15	16-20	21-30	>30
<3	0.000*	0.124	1.000	0.446	0.305	0.282
3-5	–	0.075	0.000*	0.000*	0.000*	0.000*
6-10	–	–	0.021*	0.000*	0.000*	0.000*
11-15	–	–	–	0.340	0.422	0.589
16-20	–	–	–	–	1.000	1.000
21-30	–	–	–	–	–	1.000
GL						
<3	0.140	0.000*	0.000*	0.000*	0.000*	0.000*
3-5	–	.074	0.007*	0.002*	0.002*	0.001*
6-10	–	–	0.999	0.976	0.957	0.885
11-15	–	–	–	1.000	1.000	1.000
16-20	–	–	–	–	1.000	1.000
21-30	–	–	–	–	–	1.000
GM						
<3	1.000	0.001*	0.000*	0.000*	0.000*	0.000*
3-5	–	0.001*	0.000*	0.000*	0.000*	0.000*
6-10	–	–	1.000	1.000	1.000	0.999

11-15	–	–	–	1.000	1.000	1.000
16-20	–	–	–	–	1.000	1.000
21-30	–	–	–	–	–	1.000
GR						
<3	0.179	0.000*	0.000*	0.000*	0.000*	0.000*
3-5	–	0.169	0.058	0.039*	0.032*	0.014*
6-10	–	–	1.000	1.000	1.000	1.000
11-15	–	–	–	1.000	1.000	1.000
16-20	–	–	–	–	1.000	1.000
21-30	–	–	–	–	–	1.000
GW						
<3	0.528	0.554	0.199	0.102	0.098	0.083
3-5	–	1.000	1.000	0.993	0.997	1.000
6-10	–	–	1.000	1.000	1.000	1.000
11-15	–	–	–	1.000	1.000	1.000
16-20	–	–	–	–	1.000	1.000
21-30	–	–	–	–	–	1.000
W						
<3	1.000	0.976	0.001*	0.000*	0.000*	0.000*
3-5	–	0.413	0.000*	0.000*	0.000*	0.000*
6-10	–	–	0.047	0.005*	0.005*	0.001*
11-15	–	–	–	1.000	1.000	0.992
16-20	–	–	–	–	1.000	1.000
21-30	–	–	–	–	–	1.000
Z						
<3	0.999	1.000	0.003*	0.000*	0.000*	0.000*
3-5	–	0.944	0.000*	0.000*	0.000*	0.000*
6-10	–	–	0.003*	0.000*	0.000*	0.000*
11-15	–	–	–	0.998	0.994	0.957
16-20	–	–	–	–	1.000	1.000
21-30	–	–	–	–	–	1.000

Table B.0.3. Per 1 m² means and standard deviation (SD) of relative age (RA) groups in the translocated population throughout the monitoring period, 2010 – 2012.

E	2010		2011		2012	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
<3	25	13.6689	10	9.1456	9	9.6214
3-5	50	24.9682	21	10.8554	16	8.6148
6-10	25	10.5119	18	6.4240	19	6.9076
Younger RA groups total	**100**		**49**		**44**	
11-15	16	7.7828	12	5.2304	10	4.1897
16-20	7	4.2741	7	3.6815	4	3.3700
21-30	8	5.5275	5	4.4219	5	3.9911
>30	9	5.3702	6	4.6291	4	3.5051
Older RA groups total	**40**		**30**		**23**	

GL	2010		2011		2012	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
<3	52	68.8025	67	56.0703	85	113.3608
3-5	47	33.1957	29	21.3802	43	44.0247
6-10	14	8.5823	15	11.5031	20	18.9901
Younger RA groups total	**113**		**111**		**148**	
11-15	6	4.0770	7	10.8878	5	3.9944
16-20	3	1.3375	2	2.8597	1	1.6193
21-30	2	1.6633	2	2.5298	1	0.6749
>30	1	0.9944	1	1.7288	1	0.6749
Older RA groups total	**12**		**12**		**8**	

GM	2010		2011		2012	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
3	30	44.5036	26	19.5955	23	33.7639
3-5	28	22.1472	17	12.2000	19	17.6129
6-10	7	4.7189	3	4.0686	6	4.2426
Younger RA groups total	**64**		**46**		**48**	
11-15	4	3.2950	1	1.5526	1	0.7559
16-20	2	2.5877	1	0.7559	1	0.7071

21-30	1	1.9955	1	0.7440	0	0.0000
>30	1	1.4142	1	0.7559	0	0.000
Older RA groups total	**8**		**4**		**2**	

GR	2010		2011		2012	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
<3	63	54.8497	67	77.0457	173	253.2476
3-5	64	47.3297	36	43.2491	58	66.3780
6-10	11	6.0818	8	10.4185	19	24.6387
Younger RA groups total	**138**		**111**		**250**	
11-15	3	4.0675	5	8.4090	4	6.8872
16-20	1	0.8433	2	3.6818	1	0.8660
21-30	1	0.3162	2	4.0291	1	0.9661
>30	0	0.0000	1	0.9487	1	0.3162
Older RA groups total	**5**		**11**		**7**	

GW	2010		2011		2012	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
<3	22	21.2446	11	7.5884	81	98.9646
3-5	30	26.9568	23	25.9808	13	11.1654
6-10	8	2.7538	15	14.9081	23	18.3485
Younger RA groups total	**60**		**49**		**117**	
11-15	3	2.7538	5	3.4641	12	6.6081
16-20	1	0.9574	1	0.8165	4	1.9149
21-30	1	0.5774	1	1.8930	2	1.2583
>30	0	0.0000	1	1.0000	1	1.2583
Older RA groups total	**5**		**8**		**19**	

W	2010		2011		2012	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
<3	17	3.7859	4	4.1191	2.333	2.7325
3-5	8	3.0551	13	9.0203	4.500	3.0166
6-10	3	3.2146	7	4.9565	6.833	2.3166
Younger RA groups total	**28**		**24**		**13.667**	
11-15	1	0.5774	3	1.9664	3	1.3784

16-20	1	0.5774	1	1.0954	1	0.9832
21-30	0.000	0.0000	1	1.1690	1	1.2111
>30	0.000	0.0000	1	1.0328	1	0.8367
Older RA groups total	**2**		**6**		**6**	

Appendix C

This appendix accompanies Chapter 6.

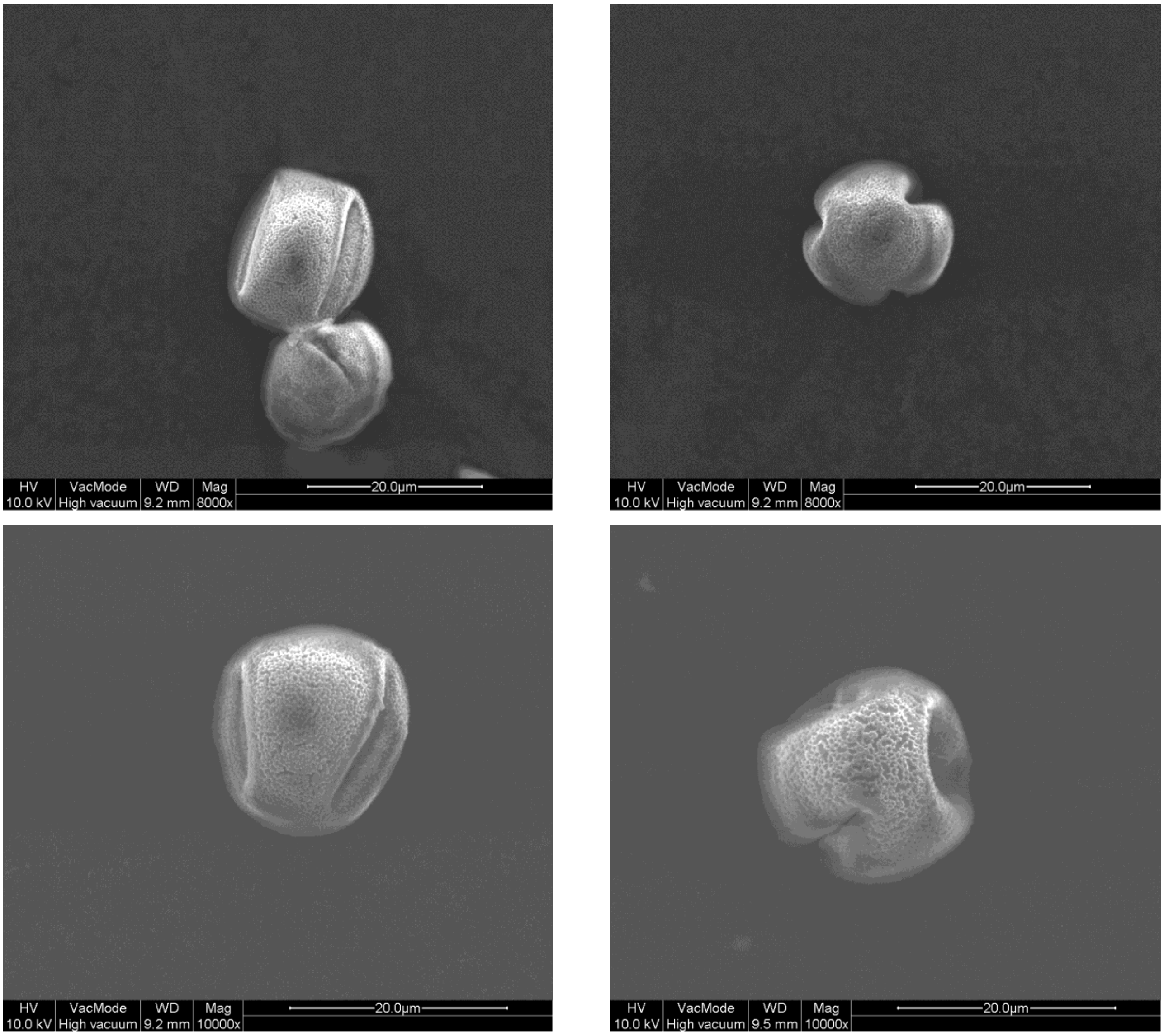

Figure C.0.1. *Frithia humilis* pollen collected from flowers growing in the North-West University Botanical Garden.

Figure C.0.2. ***Platylesches ayresii*** **(left) and** ***P. mortili*** **(right) were void of** ***Frithia humilis*** **pollen.**