

**THE RELATIVE IMPORTANCE OF POPULATION SIZE, COLONIST
QUALITY, AND COLONIST ARRIVAL FREQUENCY FOR
POPULATION SUCCESS**

by

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Abstract

Colonization is a fundamental ecological process structuring populations, communities, and ecosystems. Promoting, or preventing, colonization is also integral to solving applied ecological problems, such as biocontrol, invasive species, community recovery, and conservation. Because of its central role in many of the theory and applications of ecology, it is essential that we determine what drives colonization success. One of the primary, and most commonly managed, factors influencing colonist success is population size. Larger populations are more likely to colonize because they are less vulnerable to the demographic, environmental, and genetic processes that can drive extinction. However, there are other, less studied, factors that could also affect colonist success, such as the quality of colonists or the frequency with which new individuals arrive, but we do not know their relative importance compared to that of population size. This is a crucial piece of missing information necessary for improving our understanding, and management, of the colonization process. I conducted three field experiments that examined variation in the importance of multiple colonist characteristics for success in new populations. The first investigated the relative importance of population size and arrival frequency in introduced populations of *Hemimysis anomala*. The second investigated whether the relative importance of population size, colonist quality, and arrival frequency varied between two zooplankton species (*Daphnia pulicaria* and *Skistodiaptomus oregonensis*). The third experiment determined whether the benefits of increasing colonist quantity and genetic diversity varied based on colonist identity. These experiments showed that the value of colonist quantity, quality, arrival frequency, and/or genetic diversity can depend upon the species and individuals involved. Additionally, I reviewed the mechanisms that drive specific colonists to enter, survive, and exit from human vectors. Human-mediated dispersal is a common driver of both intentional and unintentional colonization, and this review details how human-assisted colonization is caused by the interplay between biological- (e.g. organism characteristics and their abiotic/biotic interactions) and human-based (e.g. human movement ecology) processes.

Co-Authorship

This thesis conforms to the publication format as outlined in the Department of Biology Guide to Graduate Studies, with consolidated Acknowledgements and Literature Cited sections. The contributions made by co-authors are outlined below:

Chapter 2 Dr. Shelley Arnott provided assistance with experimental design, interpretation of data analysis, and manuscript preparation.

Chapter 3 Dr. Shelley Arnott provided assistance with experimental design, interpretation of data analysis, and manuscript preparation.

Chapter 4 Dr. Shelley Arnott provided assistance with experimental design, interpretation of data analysis, and manuscript preparation. Katie Millette performed clone genotyping, genetic analysis, and assisted with manuscript preparation. Dr. Melania Cristescu provided assistance with experimental design and manuscript preparation.

Chapter 5 Dr. Julie Lockwood edited the manuscript and wrote portions of it. Dr. Shelley Arnott provided advice on framework design and edited the manuscript. Sarah Hasnain assisted with background research and edited the manuscript.

Manuscripts directly from this thesis

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Manuscripts from experiments conducted alongside thesis

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List of Abbreviations, Acronyms, and Symbols

ANOVA – Analysis of variance

Av. TP – Average total phosphorus

DOC – Dissolved organic carbon

Ca – Calcium

CAISN – Canadian aquatic invasive species network

Cond. – Conductivity

GLM – Generalized linear model

HQ/LF – high quantity, low frequency

LM – Linear model

LMM – Linear mixed model

LQ/HF – Low quantity, high frequency

LQ/LF – Low quantity, low frequency

LRT – Likelihood ratio test

MAM – Minimum adequate model

Na – Sodium

PCA – Principal components analysis

QUBS – Queen's university biological station

TP – Total phosphorus

ϕ – Dispersion parameter

Glossary

- Colonist arrival frequency** – the frequency with which new colonists arrive in a location
- Colonist genetic diversity** – the number of genotypes/phenotypes in a set of arriving colonists
- Colonist quality** – the physiological condition in which colonists arrive
- Colonist quantity** – the number of arriving colonists
- Colonization** – arrival and establishment of colonists
- Human-mediated dispersal** – direct dispersal of organisms by humans or vectors which humans control
- Human-mediated transport** – see ‘Human-mediated dispersal’
- Invasive** – a non-native species with ecological and/or economic impacts
- Native range** – the natural range of a species (i.e. not established by human-mediated movement)
- Non-native** – species introduced outside their native range by human actions
- Pathway** – mechanism by which non-native species are introduced from one location to another
- Propagule** – a set of non-native individuals
- Propagule number** – the number of introduction events
- Propagule pressure** – the number of non-native individuals introduced to a region
- Propagule size** – the number of non-native individuals in a single introduction event
- Release** – exit of an organism from a human vector or human control
- Route** – the geographic path over which a vector travels
- Source** – the habitat from which an organism originates
- Success** – successful progression of colonists through the colonization process, can also encompass successful population growth and persistence following establishment
- Uptake** – entrance of an organism into or onto a human vector
- Vector** – a method of conveyance in organism dispersal

Chapter 1

General Introduction

Colonization

Organism movement at small (e.g. foraging) and large (e.g. long-distance dispersal) scales is a fundamental ecological process that can determine the fate of individuals, populations, communities, and ecosystems (Nathan et al. 2008). Of particular theoretical and practical ecological importance are the consequences of organism arrival and establishment in new areas ('colonization'). Colonization was first studied in regards to the establishment of new species on oceanic islands (primarily as speculation on mechanisms of arrival, Darwin 1845; Wallace 1880; Darlington 1938). These observations of which species successfully colonized which types of islands then served as the foundation for initial explanations of the processes driving and maintaining biogeographic patterns of species diversity (i.e. island biota is determined by a combination of colonization and extinction processes, Preston 1962; MacArthur and Wilson 1967). This research spurred a sudden diversification in research on colonization beyond that of just observing species occurrences. Experiments were conducted to collect data on the island colonization process (e.g. the fumigation and multi-year re-colonization of islands in Florida, Simberloff and Wilson 1969). There were studies on differences between species in their colonization ability (e.g. Diamond 1974; Platt 1975), order of arrival (e.g. Nilsen and Larimore 1973), the role of neutral processes in colonization (e.g. Simberloff 1978), along with research on the role played by colonization in integral ecological processes, such as community assembly (e.g. Diamond 1975; Connor and Simberloff 1979) and species turnover (e.g. Brown and Kodric-Brown 1977). The ways in which colonization and extinction could jointly determine population and community structure were also being examined for ecological systems other than oceanic islands, such as field crops (Levins 1969), inland lakes (Barbour and Brown 1974), dirt mounds in grassland prairies (Platt 1975), empty urban lots (Crowe 1979), and to models of any habitat with a patch-like distribution (Levins and Culver 1971). This marked

the beginnings of a shift in our understanding of the importance of colonization. It was no longer just something that occurred to populate oceanic islands with new species, it was now a central process determining species co-existence and community structure in any ecological system.

Though not necessarily implicit about researching ‘colonization’, parallel work on the role of colonization in ecological processes was being conducted in several burgeoning ecological fields. Researchers seeking to answer questions about the process of succession (e.g. early succession stages require colonization, Clements 1916), range expansion (e.g. Skellam 1951), species invasion (e.g. establishment of non-native species, Elton 1958), and species co-existence (e.g. regional co-existence via colonization of other habitats, Slatkin 1974; Hanski 1983) were all studying, in some shape or form, the ecological role of colonization. In subsequent decades, the ecological fields implicitly, and explicitly, researching colonization multiplied dramatically. Colonization became an integral component of population ecology (e.g. metapopulations, Slatkin 1977; Hanski 1991), community ecology (e.g. metacommunities, Leibold et al. 2004), landscape ecology (e.g. landscape connectivity, Turner 1989), dispersal ecology (e.g. causes of departure, Hansson 1991), invasion ecology (e.g. drivers of invader success and spread, Simberloff 1989; Kot et al. 1996), restoration ecology (e.g. influence of early colonization stages on population success, Montalvo et al. 1997), conservation (e.g. maintenance of spatial connections, Bennett 1999), and biocontrol (e.g. release strategies, Grevstad 1999). Each of these fields has their own unique research questions, and the body of literature within all of them has increased exponentially in subsequent decades. But the overall point of this brief history into the beginnings of our understanding of colonization is that, regardless of the ecological discipline, colonization is an integral mechanism determining the structure and function of any population, community, and ecosystem.

The colonization process

The importance of colonization to numerous theoretical and applied ecological processes necessitates a thorough understanding of how it operates. This is commonly accomplished by viewing

colonization as temporal process with a series of stages and barriers through which an organism must pass to successfully colonize (Fig. 1.1). The precise structure and names of the temporal stages of the colonization process vary, but they generally begin with an organism navigating the geographic barriers, either via active or passive dispersal, that lay between its source habitat and its eventual recipient environment (e.g. Ebenhard 1991; Richardson et al. 2000; Evans et al. 2010; Blackburn et al. 2011; Hoffmann and Courchamp 2016). Once potential colonists have successfully overcome these geographic barriers, and then entered their new habitat, they are considered to have completed the ‘arrival’ stage of the colonization process. Following arrival, the colonizing organism begins the ‘establishment’ stage, in which it must survive and reproduce to form a self-sustaining population (e.g. Ellis-Evans and Walton 1990; van der Valk 1992; Williamson 1993; Liebhold et al. 1995; Catford et al. 2009; Evans et al. 2010). A ‘self-sustaining population’ is one that can persist without human intervention or the need for further immigration, and therefore has a reproductive rate high enough to sustain or increase its population size (e.g. an intrinsic rate of increase of 0 or greater). Sometimes an additional stage following establishment is included in the colonization process wherein the colonizing organism expands its range and/or spreads again to new habitats (termed the ‘spread’ stage, e.g. Szűcs et al. 2014; Hoffmann and Courchamp 2016). However, the inclusion of a spread stage implies that an organism must spread to successfully colonize, and therefore species that remain rare or localized are failed colonizers (e.g. Hoffmann and Courchamp 2016), which is clearly not the case. The logical endpoint for the colonization process is better positioned as the first instance in which the technical definition of establishment (formation of a self-sustaining population) has been satisfied (Fig. 1.1). There is also a distinction to be made between colonization through space versus colonization through time. Spatial colonization occurs when an organism arrives in a new habitat via movement or vector-mediated transit between different geographical locations. Temporal colonization occurs when colonists originate from an already present, but dormant population. Many organisms can produce dormant offspring that remain viable for years and even decades after the

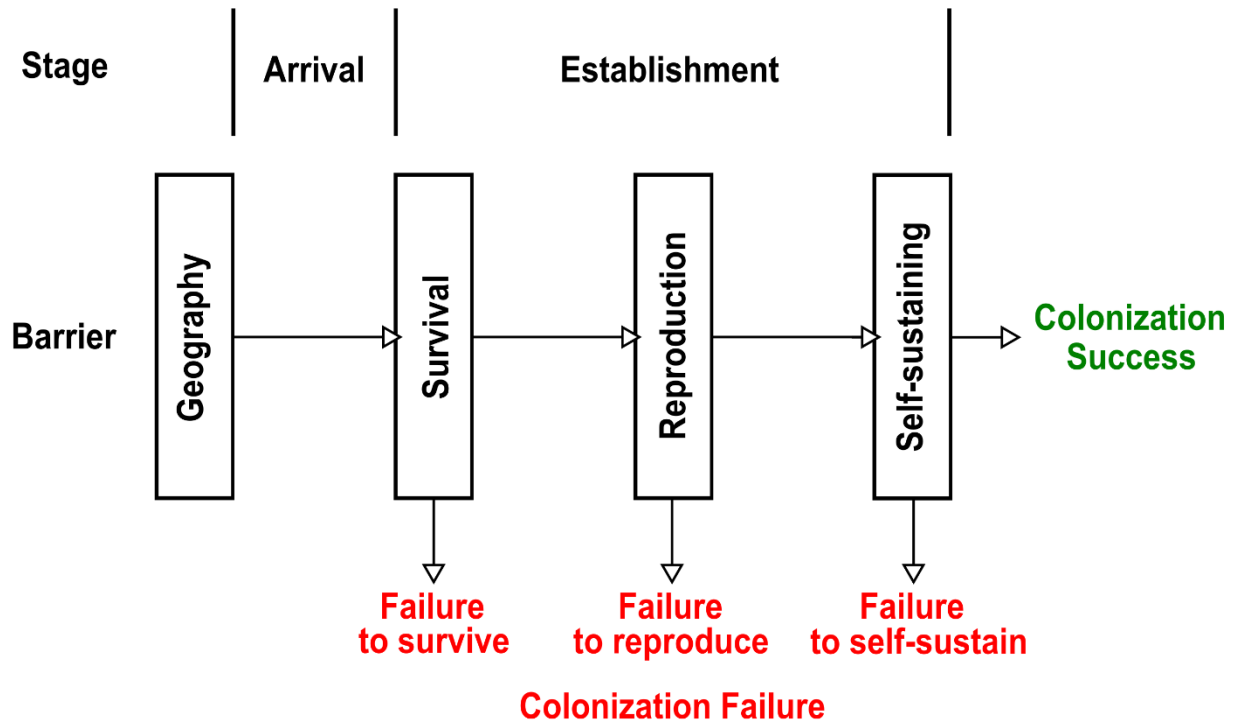


Figure 1.1 The stages and barriers of the colonization process (modified from Hoffmann and Courchamp 2016).

active population has long since died (e.g. seed banks), and these dormant individuals can resume the colonization process once environmental conditions become more suitable. The only difference between the processes of spatial and temporal colonization is the ‘arrival’ stage, which in the case of colonization through time encompasses the emergence of dormant individuals into their ‘new’ environment.

Conceptualizing colonization as a stage-based framework is beneficial because it distills this process down to the most important mechanisms that determine whether colonization succeeds or fails. A successful colonization is one in which an organism proceeds through each of the barriers and stages of the colonization process, while a failed colonization (i.e. extirpation) occurs when an organism arrives, but fails to survive and reproduce, or passes through these initial barriers, but fails to form a self-sustaining population, such as when offspring die prior to their own successful reproduction.

Understanding the colonization process, and the mechanisms that control it, therefore requires a

determination of the factors driving an organism down the path of success, or failure.

Drivers of success or failure in colonizing populations

The factors influencing whether colonization is or is not successful can be divided into three broad categories: i) abiotic factors, ii) biotic factors, and iii) the characteristics of the arriving colonizers (these are based on similar categories used by Barney and Whitlow 2008, and Catford et al. 2009, for predicting invasibility). In terms of abiotic considerations, the most frequently discussed are the suitability of the environment and the amount of available abiotic resources. If an organism is not suited to its abiotic environment (e.g. Marshall et al. 2010; Duncan 2016), or it is unable to attain sufficient resources (Davis et al. 2000), then it cannot survive and reproduce in its new habitat. Similarly, biotic factors, such as the biotic resources required for successful survival and reproduction, must be considered. Colonization success is also likely higher for habitats in which the colonizing species experiences few or no negative biotic interactions (e.g. predators, competitors, pathogens, and parasites, Wellborn et al. 1996; Williamson 1996; Cazelles et al. 2016), and in which positive biotic interactions, such as mutualists or facilitators, are present (Alpert 2006; Cazelles et al. 2016). Finally, the characteristics of the colonizers are also important for determining success or failure. This can include traits associated with successful colonization, such as rapid growth (MacArthur and Wilson 1967), a wider habitat tolerance (Zerebecki and Sorte 2011), and greater phenotypic plasticity (Yeh and Price 2004), but of particular importance is the size of the colonizing population.

The importance of population size

One of the most commonly recognized and studied factors determining success or failure in the colonization process is the size of the colonizing population ('population size', or potentially referred to as 'propagule size'). The benefit of higher population sizes was recognized early in the study of the colonization, with initial observations from intentional introductions of game animals (Philips 1928),

experimental introductions to islands (Crowell 1973), and early attempts at biocontrol (Beirne 1975) all reporting that larger introductions were generally more successful than smaller ones. This observational evidence of the importance of colonist population size was in agreement with early theoretical work on the potential problems encountered by populations founded by a small number of individuals, or that had declined to a smaller size. As population size decreases, so too does genetic diversity (e.g. due to founder effects, Mayr 1942), and smaller populations are also more likely to experience reductions in genetic diversity over time due to inbreeding and genetic drift (Wright 1931). Low genetic diversity can subsequently reduce colonization success by reducing adaptability, by lowering the chance that pre-adapted phenotypes (those already suited to the recipient environment) are present in the colonizing population, by increasing intraspecific competition (since individuals are more likely to share the same genotype and utilize the same resources), and by reducing facilitation between different genotypes (Forsman 2014).

In addition to the effects of genetics, smaller populations are also more vulnerable to extinction due to a lack of numbers. Growth rates tend to fluctuate more dramatically in smaller populations, compared to larger ones, due to stochastic shifts in population demographics (termed ‘demographic stochasticity’, May 1973) and the environment (termed ‘environmental stochasticity’, May 1973). This tendency towards wider fluctuations in growth rate can result in sudden swings into negative population growth, which can further reduce population size to unrecoverable levels, and eventual extinction. Beyond just influencing the severity of growth rate fluctuations, the number of individuals can also determine whether a population has a positive or negative growth rate (‘density-dependence’). As population size declines, so too can its growth rate (termed ‘positive density-dependence’ or the ‘Allee effect’, Allee 1931). This can occur due to previously discussed genetic mechanisms (a ‘genetic Allee effect’, Courchamp et al. 2008), but it can also occur for reasons related solely to the number or density of conspecifics. For example, a species whose fitness is determined by intraspecific cooperation, such as through predator defense or cooperative mating, can experience reduced fitness and growth at lower

population sizes (Courchamp et al. 2008). This dependence of growth on size can be strong enough to create a ‘critical population size’, which is a particular number of individuals that must be present to maintain a self-sustaining population. Falling below the critical size threshold results in inevitable extinction because there are not enough individuals in the population to maintain a neutral or positive growth rate.

Colonist quality and arrival frequency

Over the last several decades, there have been innumerable experiments, surveys, and models across ecological disciplines that have reinforced the relationship between the size of a population and its chance of extinction (e.g. see Lande 1993; Frankham 1995; Grevstad 1999; Stephens et al. 1999; Keller and Waller 2002; Fauvergue et al. 2012; Blackburn et al. 2015 and references therein). However, there are other less-studied characteristics of a colonizing population that could be playing as much of a role in colonist success as that of population size.

Completion of transit to a new environment exacts an energetic cost at all phases of the movement process (Bonte et al. 2012). Source habitat conditions (Stamps 2006), difficulty of the transit process (Baker and Rao 2004), timing of departure/arrival (e.g. Nunes et al. 1998), and difficulty of integration into the new habitat can all affect the physiological condition in which a colonizing individual arrives (termed ‘colonist quality’). This is equally true for human-mediated transport which can levy similar costs at each stage of the transportation process (Carlton and Ruiz 2005; Johnston et al. 2009), with subsequent consequences for colonist quality upon arrival. The quality of an individual can, in turn, determine its likelihood of colonization success. ‘Good’ quality colonizers can exhibit higher survival and reproduction in their new habitat than ‘poor’ quality colonizers (Burgess and Marshall 2011; Van Allen and Rudolf 2013; Estrada et al. 2016; Lange and Marshall 2016). Some species also require a specific amount of energetic reserves to successfully colonize. For example, organisms that must undergo metamorphosis to become a reproductive adult can fail to establish if their energy reserves have been

sufficiently depleted by the transit process to prevent metamorphosis (e.g. Tremblay et al. 2007). Poor-quality individuals are also potentially less successful at coping with predation (Zhang and Buckling 2016), competition (Łomnicki 1988), parasites/disease (Hall et al. 2009; Simpson et al. 2015), and environmental stress (Carere et al. 2010), all of which are important components in successfully surviving and reproducing in a new environment.

Likewise, the frequency with which additional colonists arrive (termed ‘colonist arrival frequency’) could also influence establishment. Colonizing populations can remain connected to other source populations via dispersal (e.g. in a metapopulation, Hanski 1991), or via human-mediated introduction, providing a continued flow of new arrivals. While the effects of increasing arrival frequency are often equated with an increase in population size because additional arrivals serve to further augment the population, it can also have effects separate from the combined demographic, environmental, and genetic benefits of higher population sizes. New colonists from the surrounding region can help to maintain declining populations (Brown and Kodric-Brown 1977; Pulliam 1988), allowing additional time for the formation of a self-sustaining colony. Increasing the frequency of new arrivals also allows for multiple chances at successful establishment, and increases the probability that colonists arrive during favorable environmental periods (Simberloff 2009). Additionally, the genetic diversity of source populations changes through time, and a higher colonist arrival rate provides a better sampling of these temporal changes in source population diversity (Roman and Darling 2007), potentially improving colonization success.

Relative importance of colonizer characteristics

Although we have plenty of evidence for the individual effects of population size, colonist quality, and colonist arrival frequency on colonization success, we have a poor grasp of the relative importance of these characteristics in driving successful colonization (Carlton and Ruiz 2005; Lockwood et al. 2005; Simberloff 2009; Burgess and Marshall 2011; Blackburn et al. 2015). There is some

experimental evidence that quality and arrival frequency can play as important a role in colonization success as that played by population size. Lande and Marshall (2016) found that the establishment success of *Bugula neritina* was determined by a combination of the amount and quality of introduced colonists. Similarly, introductions of *Daphnia magna* by Drake et al. (2005) showed that both the population size and arrival frequency of colonists, not just one factor alone, determined abundance, persistence, and time to extinction in new populations. However, there are only a handful of such studies. We therefore do not know how frequently this equal influence of quality or arrival frequency may occur, nor the circumstances that may cause the influence of these factors to equate to, or even dominate over, the demographic and genetic effects of population size. There are also indications that certain types of organisms, such as those that can increase quickly from a small number of founding individuals (e.g. Burkey 1997; Belovsky et al. 1999) or that do not experience strong Allee effects (e.g. Fauvergue et al. 2007), may not rely as strongly on their initial population size for successful establishment, potentially resulting in a greater importance of quality or arrival frequency in these species. Intraspecific variation in the benefits of higher population sizes for colonist success, such as may occur due to individual differences in the severity of Allee effects (Kadam and Velicer 2006), could also produce circumstances in which particular individuals depend more on quality or arrival frequency for colonization success than they do on population size.

This potential for variation in the relative importance of population size, compared to that of colonist quality and arrival frequency, has serious implications for our understanding and practical management of the colonization process. Practical efforts to establish biocontrol organisms (Memmott et al. 1998; Grevstad 1999; Shea and Possingham 2000; Fauvergue et al. 2012), control invasive species (Taylor and Hastings 2005; Reaser et al. 2008; Blackburn et al. 2015), and restore depauperate habitats (Montalvo et al. 1997; Armstrong and Seddon 2008) often focus on manipulating population size, either by increasing or decreasing the number of colonizers, with less attention paid to managing colonist quality or arrival frequency. While such an approach is consistent with the long history of research into

the importance of population size, it does not account for situations in which quality or arrival frequency could be of equal importance, or even the driving factors in colonization success. Determining the relative importance of these various colonizer characteristics is also valuable for conservation. The factors that determine colonization success are the same that can determine survival in populations that have declined to become small (e.g. population size, Shaffer 1981; Lande 1988; individual quality, Stevenson and Woods 2006; arrival of individuals from other source populations, McCullough et al. 1996; Novaro et al. 2000). Investigating the individual and relative importance of each of these colonizer characteristics is therefore essential for determining which is responsible for driving colonization success, for determining the circumstances under which their relative importance may shift, for informing practical management efforts to control colonizing populations and conserve at-risk species, and for continuing the development of our understanding of the colonization process.

Research and chapter objectives

Understanding which colonizer characteristics – population size, colonist quality, and colonist arrival frequency – are driving successful establishment, and the circumstances under which their individual and relative importance can change, requires experiments in which multiple factors are combined and manipulated in different species. To accomplish this, I conducted three field mesocosm experiments (Figure 1.2), each comprising a single chapter of this thesis, that examined the importance of different combinations of colonizer characteristics across multiple zooplankton species. My first experiment (Chapter 2) investigated the relative importance of population size and colonist arrival frequency in the survival, performance, and extinction of introduced populations of *Hemimysis anomala* (a mysid zooplankton). My second experiment (Chapter 3) examined the relative importance of population size, colonist quality, and colonist arrival frequency in the success of introduced populations of *Daphnia pulicaria* (a cladoceran zooplankton) and *Skistodiaptomus oregonensis* (a copepod zooplankton). My third experiment (Chapter 4) investigated variation within *Daphnia pulex* in the



Figure 1.2 Site and general layout of mesocosm experiments at the Queen’s University Biological Station (QUBS). All experiments utilized the same site and 200L mesocosm tanks pictured above.

benefits of higher population sizes by examining whether the effects of increased colonist quantity and genetic diversity depended upon the identity of the arriving colonists. These experiments were designed to, as a whole, determine the relative benefits provided by increasing population size, colonist quality, and/or colonist arrival frequency among species and individuals. The first two experiments determined whether colonist quality or arrival frequency can play as strong a role in colonist success as that of population size, and whether the relative importance of these factors can shift between species, while the third experiment determined whether there is potential variation within a species in the importance of colonist quantity and genetic diversity.

Zooplankton were used as study organisms for these experiments because they reproduce quickly, allowing for the influence of initial colonizer characteristics to be studied over multiple generations in a single field season. Different zooplankton species also share approximately the same habitat requirements, but differ in a variety of traits that could influence colonization success (e.g. mating systems, trophic group, predator escape mechanisms; Barnett et al. 2007), allowing for an investigation of potential differences between species in the relative importance of various colonizer characteristics. They are also useful for investigating the relative importance of different colonizer characteristics within species because some zooplankton can reproduce asexually (e.g. *Daphnia spp.*), enabling comparisons between asexual clones that differ in both genotype and phenotype.

Finally, I also conducted an additional project (Chapter 5) that examined the conceptualization of, and mechanisms driving, human-mediated dispersal. Colonization is a shared and integral process across ecological disciplines, and our current understanding of the mechanisms involved is founded on research of both 'natural' (i.e. arrival via natural wind, water, and animal vectors) and 'human-mediated' (i.e. arrival via human and human-controlled vectors) colonization. However, the process of colonization is often approached differently depending upon whether natural or human vectors are involved. Natural organism arrival into new habitats is conceptualized as being controlled by ecological and evolutionary mechanisms that drive interactions between organisms and their dispersal vectors (Clobert et al. 2012). Conversely, human-mediated organism movement is conceptualized as being driven by human behaviour and movement patterns that lead to unintentional and intentional organism transport (e.g. Carlton and Ruiz 2005; Auffret et al. 2014). These varying approaches are logical given the differing questions involved in natural versus human-mediated colonization, but using a natural- or human-focused concept can lead to a downplaying, or exclusion, of some of the mechanisms that could be at work within the colonization process. For example, a focus on organism interactions with natural vectors can neglect the potential influence of humans, leaving us with a poor understanding of the degree to which humans are involved in organism dispersal (the lack of which is noted by Bullock 2012; and Auffret et al. 2014).

Similarly, a human-focused approach to organism movement can miss the ecological and evolutionary mechanisms influencing natural organism movement, which could be as relevant for human vectors as they are for natural ones (e.g. individual behaviour, Chapple et al. 2012). The purpose of my final project was therefore to integrate the biological- and human-based processes involved in human-mediated dispersal to develop a general framework outlining the mechanisms that determine which individuals enter, survive, and exit from human vectors. Such a conceptualization highlights both the organism- and human-based mechanisms involved in human-mediated dispersal, and unifies our understanding of the mechanisms driving the colonization process between fields focused on either natural or human-mediated colonization.

Chapter 2

Strength in size not numbers – propagule size more important than number in sexually reproducing populations

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Abstract

Propagule pressure has consistently been identified as a primary factor in invader success, and reducing it can be one of the most effective methods for preventing the establishment of non-native species. However, when policy is implemented to reduce propagule pressure it almost exclusively focuses on the size of individual introduction events ('propagule size'), with little confirmation that controlling this single aspect of propagule pressure is the most effective strategy. The number of introduction events ('propagule number') can play as much, or more, of a role in invader success, yet only a small portion of propagule pressure research has studied the relative importance of size and number. We investigated the relative roles of propagule size and number in the establishment of a sexually reproducing species using a field mesocosm experiment that introduced *Hemimysis anomala* (a non-native mysid) across a range of propagule sizes and numbers. We found that single, large introductions had higher abundances and probabilities of survival than smaller, more frequent additions. This experiment illustrated that, for sexual reproducers, focusing on lowering propagule size can be the most effective method for reducing non-native establishment.

Introduction

Propagule pressure, the product of the size of each introduction ('propagule size') and the number of introduction events ('propagule number'), is a principal factor driving the establishment and spread of non-native species (Lockwood et al. 2005; Simberloff 2009; Blackburn et al. 2013). Enacting policies to reduce propagule pressure can be one of the most effective methods for preventing invasions (Mack et al. 2000; Verling et al. 2005), and can be more effective than mitigation or eradication strategies that target already established invaders (Pyšek and Richardson 2010). However, regulation of propagule pressure is generally achieved by reducing propagule size and not number, but there are numerous reasons why controlling either size or number might reduce non-native establishment: 1) smaller populations are more prone to random extinctions from demographic/environmental fluctuations (Shaffer 1981; Fauvergue et

al. 2012); 2) reducing the size of introductions can increase their vulnerability to extinction via Allee effects (reviewed by Courchamp et al. 1999); 3) less frequent introductions can prevent the rescue of already established but declining populations (Brown and Kodric-Brown 1977); 4) invaders that arrive less often cannot easily recover from periods of adverse conditions, and are less likely to arrive in favorable periods or sites (Simberloff 2009); and 5) reducing introduction size and frequency lowers genetic diversity, resulting in less adaptable populations (Hanski 1999). If current and future propagule policy is going to focus primarily on only a single aspect of propagule pressure, then it is important to confirm that this is the most broadly effective strategy.

Although we have a large body of research on the overall effect of propagule pressure in driving the success of introduced populations, very few studies have attempted to disentangle the respective roles of size and number. Early modeling work by Grevstad (1999) and Shea and Possingham (2000) on the optimal release strategies for biocontrol insects indicated that the importance of propagule size could depend upon the relative severity of demographic versus environmental stochasticity. In their models, increasing propagule size increased establishment primarily in new populations that experience Allee effects, but smaller and more numerous propagules had greater success if establishment was not hindered by low population sizes. The results of these early models were further reinforced by Cassey et al. (2014) who simulated the establishment success of sexually reproducing birds with different life histories. Larger bird populations introduced in a single event always performed the best, even in more variable environmental conditions, since introducing everything at once maximized future reproductive potential. All three models indicate that controlling propagule size could be effective for limiting establishment, but the results from Grevstad (1999) and Shea and Possingham (2000) suggest that this might only apply when Allee effects are involved.

Even if we assume that targeting propagule size is a broadly effective strategy because many non-native species probably experience some type of Allee effect (as suggested by Liebhold and Bascombe (2003) and Tobin et al. (2011)), we have little experimental support for this conclusion. Studies by Drake

et al. (2005), Hedge et al. (2012), and Britton and Gozlan (2013) are three of the best examples of experimental investigations of the relative importance of propagule size and number. However, none of these studies provides a thorough assessment of a population likely to exhibit Allee effects. Drake et al. (2005) used the asexual reproductive phase of *Daphnia magna*. Hedge et al. (2012) studied settlement in Pacific oyster larvae (*Crassostrea gigas*), which exhibited greater success when introductions were smaller and more frequent due to effects similar to negative density dependence. Additionally, the relative effect of propagule number on the sexually reproducing fish (*Pseudorasbora parva*) from Britton and Gozlan (2013) was difficult to determine as propagule number was only manipulated at a single level of propagule pressure, and all introductions that varied in propagule number successfully established. Further experimentation is sorely required to test modeling predictions and assess whether propagule size, not number, is the principal driver of establishment in organisms with Allee effects.

To determine the relative importance of propagule size compared to number in a species with the potential for Allee effects, we conducted a field mesocosm experiment with a sexually reproducing zooplankton as our study organism. We compared the survival of small, introduced populations of *Hemimysis anomala* (a sexual mysid non-native to North America, hereafter referred to as *Hemimysis*) across a gradient of propagule pressures each divided over one, two, or four additions. Sexually reproducing zooplankton experience mate-finding Allee effects (Kramer et al. 2008), and mysids also exhibit swarming behavior. Mysid swarms provide a variety of benefits related to density that could factor into the establishment of new populations, such as facilitation of reproduction, increased foraging efficiency, and reduced energy costs of swimming (Ritz 1997; Ritz et al. 2001). We predicted that, based on the previously discussed models and the potential for Allee effects in the mysids, a larger propagule size would be more important than a larger propagule number for successful establishment.

Considering the overwhelming consensus on the importance of propagule pressure in the success of species introductions, and policy focusing specifically on propagule size, developing a better understanding of the mechanisms by which propagule pressure operates is crucial to the future

management and prevention of invasions. Additionally, investigating the factors that influence non-native establishment is not just relevant to invasion ecology and management policy, but has applications to any ecological field concerned with the survival of small populations.

Methods

The field mesocosm experiment was conducted at the Queen's University Biological Station (QUBS, 44°34'00.2"N, 76°19'26.5"W) in Ontario, Canada between June 12 and August 21, 2014 using *Hemimysis* as our study organism. *Hemimysis* is a littoral mysid that was first detected in Lake Ontario and Lake Michigan in 2006 (Pothoven et al. 2007). It has a wider tolerance range for temperature and conductivity compared to other mysids, and the adults are omnivorous but primarily consume zooplankton (Ricciardi et al. 2012). It can produce up to four broods between April and October, with between 2 to 70 offspring in each brood, and the juveniles can mature in less than a month (Pothoven et al. 2007).

We employed a regression design for our experiment in which *Hemimysis* was introduced across a gradient of propagule sizes (2, 4, 8, 12, 16, 20, and 24 individuals per mesocosm). Introductions were also divided over 1, 2, or 4 weeks (the propagule number treatment), with all additions occurring over the first four weeks of the experiment. Since we endeavored to keep an even ratio of males:females in each treatment, propagule sizes of 2 could only be added once, and propagule sizes of 4 were divided over just 1 or 2 additions. For propagule sizes of 12 and 20 divided over 4 additions, females were favored in additions 1 and 3 (a male:female ratio of 1:2 or 2:3 respectively), with males similarly favored in additions 2 and 4. All possible combinations of propagule size and number were replicated 6 times.

We established the mesocosm experiment between June 12-13 using one-hundred and eight 200L cylindrical tanks (84cm diameter x 53cm high). Each tank was filled with 180L of lake water from nearby Lake Opinicon (see Appendix A: Table A1 for information on all lakes involved in the experiment) filtered through an 80µm mesh to remove large zooplankton while allowing most phytoplankton and

some small zooplankton to pass through. Shards of a 14cm diameter and an 8cm diameter unglazed ceramic pot were added to each mesocosm to provide daytime cover as *Hemimysis* is sensitive to daytime light levels and prefers rocky rather than soft substrates (Ricciardi et al. 2012). Tanks were also covered with 1mm mesh to provide additional shade and to protect from insects and debris. We provided a natural resident community and food source by collecting zooplankton from the pelagic and littoral regions of four nearby lakes uninvaded by *Hemimysis*. We added zooplankton to each mesocosm at an ambient density on June 20. All mesocosms were then allowed to acclimate for one week prior to commencement of the *Hemimysis* additions.

Hemimysis were collected after sunset on June 25, July 2, July 9, and July 16 from the St. Lawrence River at Montreal, QC, CA (45°29'58.9"N, 73°33'01.7"W) using vertical plankton tows with a 250µm net. All individuals were then transported that same night in dark containers to a laboratory near the field site. Over the following two days, all *Hemimysis* remained in dark containers and were fed Cyclop-eeze® daily. The second night after capture, all individuals were identified and added to the mesocosms. Only adult males and non-gravid females (based on Pothoven et al. 2007) were used in the experiment, with approximately equal numbers of large (about 9-11mm) and small (about 6-8mm) individuals used for each treatment. Tanks were haphazardly assigned to treatments, and *Hemimysis* were transported from the lab to the field site in 1L of 80µm filtered lake water and added to the mesocosms. Initial introductions for all propagule number treatments were made on June 27, with additional introductions for propagules divided over two additions made on July 4, and for those divided over four additions on July 4, 11, and 18.

To account for accruing waste and nutrient/phytoplankton loss over time, 45L from each mesocosm was removed and replaced every two weeks following initial *Hemimysis* additions. Water was first gently mixed, then removed via siphons with 80µm filters, and fresh 80µm filtered lake water was slowly added. This process was performed during the day on July 9, July 23, and August 6.

Sampling protocol

Hemimysis abundance was monitored weekly beginning on July 10 to allow for two weeks between initial introduction and initial sampling to minimize stress. For each tank, ceramic cover was carefully removed, *Hemimysis* were then captured and counted using aquarium nets (10cmx15cm), stored in mesocosm water, then returned following replacement of their cover. All sampling began one hour after sunset and concluded prior to sunrise (~9pm to 5:30am) and was performed using red-light headlamps. Sampling generally required two nights, with any *Hemimysis* additions occurring on the second night. If we were unable to detect *Hemimysis* for three consecutive weeks after the last addition, we considered the population extirpated and sampling was not continued.

Statistical analysis

All statistical analyses were performed in R 3.1.2 (R Core Team, 2015) using the 'MASS' (v.7.3-37, Venables and Ripley 2002) and 'glmmADMB' (v.0.8.1, Fournier *et al.*, 2012; Skaug *et al.*, 2013) packages with $\alpha=0.05$.

Experimental data was analysed using metrics of *Hemimysis* abundance and presence/absence (1/0, calculated as whether or not a single individual was detected). To standardize the amount of growth time across the experiment (since introductions concluded in different weeks based on the specific propagule number treatment), only data for four consecutive weeks following 2-2.5 weeks of average growth time were used. Therefore, data for *Hemimysis* introduced in a single event (propagule number = 1) encompassed four sampling weeks beginning on July 10 (2 growth weeks since initial introduction) and ending on July 31. Data for the two addition treatments (propagule number =2) encompassed four sampling weeks beginning on July 17 (three growth weeks for the initial additions and two for the second = 2.5 average growth weeks) and ending on August 7. Data for the four addition treatments (propagule number = 4) encompassed four sampling weeks beginning on July 24 (four, three, two, and one growth weeks for the first, second, third, and fourth additions respectively = 2.5 average growth weeks) and

ending on August 14.

Differences in *Hemimysis* abundance were compared between treatments using data over all sampled weeks, and differences in presence/absence were compared with data from just the final week (the 4th week following 2-2.5 average growth weeks). All metrics were analysed using generalized linear models (GLMs). Presence/absence data was converted to proportion surviving out of 6 replicates for each treatment to conform to the requirements of logistic regression (using a Binomial distribution with a logit link function). Abundance was modeled using a Negative Binomial distribution with a log link function.

Model selection was performed for all metrics following Crawley (2005). Selection began with a full model comprised of the interaction between the discrete continuous term for propagule size and the categorical term for propagule number, with an additional discrete continuous term for time (weeks 1-4) in the abundance model. Minimum adequate models were determined by comparing the deviance of successively reduced models using likelihood ratio tests. Terms were discarded if the deviance of a simpler model vs. a more complex model was not significantly different ($P > 0.05$). Model selection continued until a higher order term was retained, or the null model was determined to be the minimum adequate model (see Appendix A: Table A2 for the minimum adequate models and descriptive statistics for all metrics). Significance of interactions or single fixed parameters was assessed by dropping them from the minimum adequate model, then comparing the deviance of models with and without the dropped term using likelihood ratio tests ($P < 0.05$).

Assumptions of models and distributions were checked using Pearson residual vs. fitted plots, scale-location plots, and dispersion (with ϕ in the results indicating the dispersion of each model). Additionally, visual comparisons of predicted relationships against the actual data were useful for evaluating model fits.

Results

For *Hemimysis* abundance, there was a significant interaction between the effects of propagule

size, propagule number, and time (Negative binomial multiple regression, $n=432$, $\phi=1.23$, $\text{Deviance}_{2,14}=6.42$, $P=0.040$). In populations introduced in a single event, higher propagule sizes generally resulted in higher abundances (Fig. 2.1a). However, the effect of propagule size weakened as propagules were divided over two additions (Fig. 2.1b), with little effect of increasing propagule size in populations divided over four additions (Fig. 2.1c). Additionally, over time the abundances in populations introduced over one or two additions tended to decline (Fig. 2.1a and 2.1b), but these declines were more rapid in populations divided over four additions (Fig. 2.1c).

For the probability of survival in the final week, increasing propagule number reduced the probability of survival (Fig. 2.2a-c, Logistic regression, $n=18$, $\phi=0.89$, $\text{Deviance}_{2,4}=10.84$, $P=0.004$), but there was no effect of propagule size. Propagules introduced only once had ~36% probability of survival after four weeks (Fig. 2.2a), with this probability declining to ~31% in propagules introduced twice (Fig. 2.2b), and ~0% in populations divided over four additions (Fig. 2.2c).

Discussion

Our field experiment demonstrated that propagule size was the most important aspect of propagule pressure in determining the establishment of our non-natives. In our experiment, larger introductions that arrived in a single event fared better than smaller introductions arriving more frequently. This likely occurred because reducing the size and increasing the number of propagules brought each introduction further below the critical size required to persist, increasing their vulnerability to Allee effects and stochasticity. This prevented the steady accumulation of individuals over multiple introduction events since previous populations had either declined or gone extinct prior to the arrival of the next propagule.

The results of our short-term establishment experiment were consistent with our predictions: single, large introductions of *Hemimysis* had higher abundances and probabilities of survival, with both declining as propagules were divided over two and four additions. Although the same overall amount of

individuals were introduced at all levels of propagule pressure, smaller repeated introductions failed to achieve the same abundances as populations introduced in single events. This suggests that, over multiple additions, *Hemimysis* populations tended to decline or die before the next set of arrivals, failing to accumulate individuals across multiple introduction events. Whether the reduction in survival at lower propagule sizes and higher propagule numbers was due to demographic stochasticity, mate finding difficulties, or the inability to swarm is difficult to determine. It is unlikely that individuals had trouble finding mates in our mesocosms since mysids are fast swimmers (Borcherding et al. 2006), and readily locate other zooplankton with mechanoreception (Viherluoto and Viitasalo 2001) and potentially mates via some form of chemoreception (Clutter 1969). We also believe there was plenty of time to reproduce during the experiment, and we began capturing juveniles in the larger introductions after the first few weeks following initial additions. However, restricting swarming can also induce mate limitation since mysid swarms can facilitate reproduction by keeping individuals in close contact, increasing the probability that males encounter a receptive female (Clutter 1969).

While we infer that the negative effects of both lower propagule size and higher propagule number in our experiment were likely due to positive or inverse density dependence, we do not know what portion of this relationship was driven by lowered genetic diversity. Unless specifically controlled, reducing propagule size also lowers diversity, which can similarly reduce survival (Forsman 2014). Additionally, while increasing propagule number would normally increase the diversity of non-native populations (Roman and Darling 2007; Handley et al. 2011), in our experiment higher propagule numbers had to come at the expense of lower propagule size to disentangle their relative effects. Further experimentation is required to determine what role genetic diversity played in our results since we did not account for shifts in propagule diversity with size.

Survival was generally low in our experiment, but this is not uncommon for establishment experiments using non-native species (e.g. Hopper and Roush 1993; Memmott et al. 2005), and was mostly driven by the negative effect of higher propagule number. The gradual decline in abundance over

time in all treatments was also not surprising. *Hemimysis* swarms can consist of tens of thousands of individuals (Ricciardi et al. 2012) and it is probable that the critical size required for maintaining the population is considerably larger than the introduction sizes used in our experiment. While some of our experimental mortality could have been caused by unfavorable mesocosm conditions, it is unclear whether our mesocosms were more or less favorable compared to natural lakes or rivers. Aquatic field mesocosms closely track daily temperature fluctuations (see Appendix A: Fig. A1 and A2 for temperature variability at our field site), potentially creating a less stable environment for establishment, but as mentioned our mesocosms were shaded to reduce variability and *Hemimysis* has a relatively wide temperature tolerance (Ricciardi et al. 2012). Additionally, the absence of predators and potentially more stable water chemistry in our experiment may have instead improved *Hemimysis* survival. In either case, based on model predictions from Cassey et al. (2014), the severity of the environment may not have affected our broader experimental conclusions of the relative importance of propagule size and number as the outcome is predicted to be the same regardless of environmental favourability.

There are three important limitations to our study, the first of which is the duration of the experiment. Short-term survival provides an indication of success in the initial establishment phase, but longer-term experiments are required to assess how the effects of propagule size and number might influence the later stages of population growth. However, our conclusion of the greater importance of propagule size in species that experience Allee effects may also apply across longer timescales because the initial effects of propagule pressure can persist for multiple generations (e.g. Fauvergue and Hopper 2009), potentially influencing the spread (Carlton 1996) and impact (Ricciardi et al. 2011) stages of an invasion. Secondly, it is unlikely that our results would apply across all organisms that exhibit Allee effects as susceptibility to reductions in propagule size could change depending upon differences in growth rate, reproductive strategy, or ability to disperse to nearby populations (Barrett 2011). Finally, the broader applicability of propagule size policy is important to assess not just in one species, but across a range of organisms that exhibit a variety of types of density dependence. The influence of propagule

number could be much stronger in populations that do not suffer as extensively from Allee effects (e.g. asexual reproducers), particularly in systems that experience greater environmental variability.

Implications

Our experimental design is relevant to policy that aims to manage propagule pressure as large/infrequent or small/frequent propagules. Regulation focused on propagule size would reduce the amount of individuals in each propagule, but leave the frequency at which they arrive unchecked (smaller/frequent). Targeting propagule number would result in less frequent introductions, but the amount of individuals per event would remain unchanged (larger/infrequent). We have illustrated that focusing on reducing propagule size can be effective at limiting the establishment of organisms with Allee effects, even when introduction frequency remains high, as long as individual introductions are as small as possible. Propagule size will also be the aspect of propagule pressure most often, and more easily, regulated as it is unlikely propagule number could be controlled for a majority of the highest volume and most lucrative introduction vectors (e.g. ballast water, wood materials, or the pet/horticultural trade). Practical applications of propagule size policy have been effective in reducing non-native establishment in high traffic vectors transporting a variety of species. For example, no new aquatic non-natives from ship ballast water have been detected in the Laurentian Great Lakes since the enactment of regulations reducing the number of transported organisms (Bailey et al. 2011). Controlling propagule pressure, and particularly propagule size, is a broadly applicable and simple policy approach that can reduce the establishment of non-native species (see Reaser et al. (2008) for a review of how such policy might be implemented). These methods do not have to be based around the identification of already known invaders, which can prove more expensive and often less efficient (Smith et al. 1999), but instead on reducing the overall introduction rate of all propagules. A focus on decreasing the size of introduction vectors is a necessary step in slowing the rate of new invasions and preventing the future impacts of a broad array of non-natives that have yet to establish and spread.

However, there are circumstances under which implementing regulations only for propagule size may not be sufficient. If the critical size threshold is not known or cannot reliably be achieved then focusing on reducing propagule pressure may not be an effective use of resources. Additionally, if propagule number is high enough then eventually even small propagules could encounter the right conditions for establishment or accrue enough individuals over a short period of time (e.g. the successful establishment of non-native baitfish transported by anglers at low propagule sizes but in high numbers, Drake and Mandrak 2014). In these cases, reductions in propagule number may be required to provide enough time between introduction events for populations to succumb to stochastic dynamics and Allee effects. Finally, vectors transporting asexual species (e.g. parthenogenetic zooplankton, Drake et al. 2005) or those that exhibit negative density dependence (e.g. gregarious settling mussels, Hedge et al. 2012) could experience little effect, or even an increase in establishment, at lower propagule sizes. While reducing propagule size is a good first step in enacting propagule pressure-based policy, it is unlikely to be the most important aspect of propagule pressure in all cases. As management becomes more specific to certain vectors or species, the potential for shifts in the importance of the other aspects of propagule pressure, such as number or diversity, must be considered and investigated.

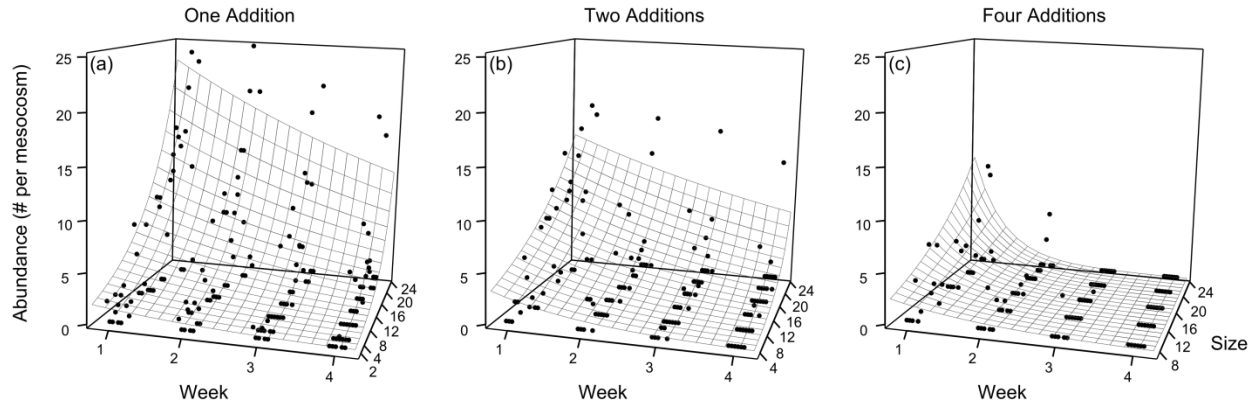


Figure 2.1 Change in *Hemimysis* abundance (number of individuals per mesocosm) with increasing propagule size across all sampling weeks for populations divided over (a) one, (b) two, or (c) four introduction events. Abundance data is shown as individual points (black circles) and jittered slightly along the ‘Week’ axis to better show all 6 replicates at each level of size. A best fit plane (grey mesh) is plotted for each propagule number treatment based on coefficients from the minimum adequate model for *Hemimysis* abundance (Appendix A: Table A3).

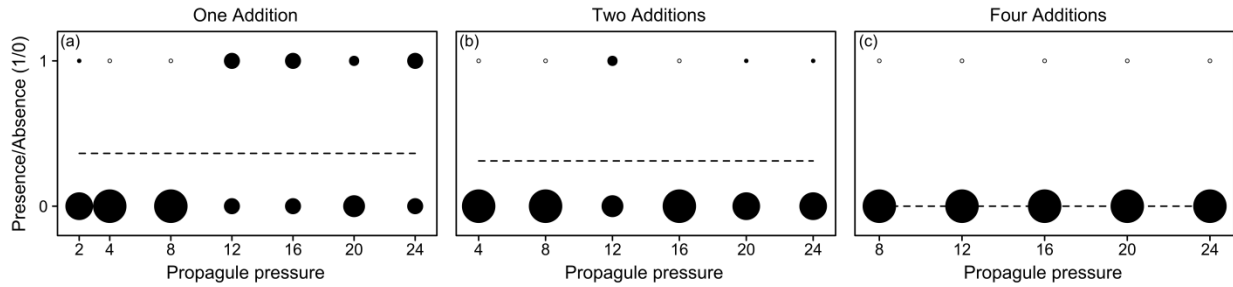


Figure 2.2 Presence/absence (1/0) of *Hemimysis* in the final sampling week (the 4th week following 2-2.5 average growing weeks). Changes in presence/absence are represented by the size of filled circles, with the circle size representing the proportion (between 0 and 1) of replicates in each category (empty circles represent no replicates in that category). A best fit line (dashed line) is plotted for each propagule number treatment based on the minimum adequate model for *Hemimysis* presence/absence (Appendix A: Table A2).

Chapter 3

Relative importance of colonist quantity, quality, and arrival frequency to the extinction of two zooplankton species

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Abstract

Colonist quantity, quality, and arrival frequency can all individually drive the dynamics and extinction of new populations. However, we do not understand which has the strongest influence, nor the circumstances under which their relative importance may change. We conducted a field mesocosm experiment that manipulated colonist quantity, quality, and arrival frequency in two zooplankton species (*Daphnia pulicaria* and *Skistodiaptomus oregonensis*). Individuals of each species were cultured under either high or low food concentrations to produce, respectively, ‘good’ and ‘poor’ quality colonists. Each species was then introduced at either small (2 individuals) or large introduction quantities (8 individuals) divided over single or multiple introduction events. We found that the extinction of *Daphnia pulicaria* was not particularly affected by any of our treatments. Introductions of just two individuals performed as well as larger or more frequent introductions, regardless of quality. Conversely, *Skistodiaptomus oregonensis* extinction was strongly driven by arrival frequency. Populations that arrived in a single event exhibited high rates of extinction (75-83%), with this probability declining dramatically when colonists were introduced over multiple events (33% extinction). Our results show that other less studied aspects of the colonist pool, such as arrival frequency, could be as important to population persistence as the initial quantity of arriving colonists. Additionally, there are potentially numerous species that are well suited to succeeding with a small number of founders, and whose success is therefore not necessarily dependent upon colonist quantity, quality, or arrival frequency.

Introduction

Successful establishment, subsequent population dynamics, and extinction have all repeatedly been shown to be affected by the quantity of initial colonists (Szűcs et al. 2014; Blackburn et al. 2015). Smaller populations experience wider fluctuations in growth rates due to demographic and environmental stochasticity (Fauvergue et al. 2012), they are more vulnerable to Allee effects (Courchamp et al. 2008), and tend to have lower genetic diversity (Wright 1969; Charlesworth and Willis 2009), all of which can

increase the probability of failure.

In addition to the effects of quantity, colonist quality must also be taken into account to understand and predict population dynamics and extinction. Individual colonist quality is influenced by a variety of factors, such as environmental conditions in the source habitat (Stamps 2006), maternal effects (Mousseau and Fox 1998), and the severity of transit (either natural or human-assisted, Carlton and Ruiz 2005; Bonte et al. 2012). Populations that contain a greater proportion of ‘good’ quality colonists can exhibit higher survival and reproduction than those composed primarily of ‘poor’ quality individuals (Burgess and Marshall 2011; Van Allen and Rudolf 2013; Estrada et al. 2016; Lange and Marshall 2016), improving their likelihood of persisting.

Less well studied is the role played by the frequency of colonist arrival (but see Drake et al. 2005, Hedge et al. 2012, Britton and Gozlan 2013, and Sinclair and Arnott 2016 for examples). Following initial establishment, new populations can remain connected to other nearby populations via dispersal (e.g. in a metapopulation, Hanski 1999), providing a continued flow of new arrivals. Even in the case of introduced populations (i.e. biocontrol or non-native species), additional colonists can be transported and introduced multiple times (Shea and Possingham 2000; Roman and Darling 2007). While the effects of increased arrival frequency are often equated with those of increased quantity because additional arrivals further augments population size, it can also have effects separate from the demographic, environmental, and genetic effects of colonist quantity. Higher colonist arrival frequency can help to maintain declining populations by providing a continued flow of individuals from source populations (Brown and Kodric-Brown 1977; Pulliam 1988), it can allow for re-establishment following local extinction (Hanski 1999), and can increase the probability that colonists arrive during favorable environmental periods (Simberloff 2009). Colonist arrival frequency could therefore have as strong an influence on population dynamics and extinction as colonist quantity or quality.

Identifying which of these population factors – colonist quantity, quality, and arrival frequency (hereafter referred to as ‘quantity’, ‘quality’, and ‘frequency’, respectively) – is driving persistence, and

the circumstances under which this may change, is crucial for focusing management on strategies that best promote, or prevent, the extinction of new or declining populations. While we know that each can individually influence population dynamics and extinction, we do not understand how they interact, nor the strength of their effects compared to one another (Carlton and Ruiz 2005; Lockwood et al. 2005; Burgess and Marshall 2011; Blackburn et al. 2015). Additionally, the studies that have investigated their relative importance generally only involve two factors (either quantity and frequency or quantity and quality) in single-species study systems, with their results showing a clear, persistent effect of quantity (Table 3.1). However, we know that the success of particular types of organisms may be unrelated to initial colonist quantity, such as those that can increase quickly from a small number of founding individuals (e.g. Burkey 1997; Belovsky et al. 1999), and could instead be more strongly related to colonist quality or frequency. More work is needed that examines the interaction between all of these factors simultaneously, and across multiple species under similar experimental conditions.

We used two field mesocosm experiments to investigate the relative importance of, and interactions between, colonist quantity, quality, and frequency to the extinction of two zooplankton species: *Daphnia pulicaria* and *Skistodiaptomus oregonensis*. These two species were chosen for our experiments to assess how quantity, quality, and frequency can vary in importance between different species that exhibit similar habitat requirements (*D. pulicaria* and *S. oregonensis* are both zooplankton that are often found in the same lakes in our study region of Southern Ontario, Canada). These species also differ widely in various traits that could influence the relative importance of colonist quantity, quality, and frequency. For example, *D. pulicaria* differs from *S. oregonensis* in terms of mating system (cyclic parthenogenesis versus obligate sexual reproduction, respectively), trophic group (herbivorous versus omnivorous), feeding mode (filtration versus stationary suspension), and predator vulnerability (they have different escape mechanisms and the average body size of *D. pulicaria* is larger than *S. oregonensis*) (Barnett et al. 2007).

To manipulate colonist quality, both *D. pulicaria* and *S. oregonensis* were cultured under high

and low food concentrations to manipulate their lipid stores (quality in this study therefore refers to the energetic condition of an individual colonist), creating ‘good’ and ‘poor’ quality colonists. Each species was then introduced to separate field experiments at two founding population sizes (small or large), and over two introduction schedules (all introduced in a single event or divided over multiple events) to disentangle the relative influences of quantity, quality, and frequency on extinction. Cladoceran and copepod zooplankton also exhibit dormancy, either in the form of diapause or quiescence (Gyllström and Hansson 2004). Dormant individuals or life stages may re-colonize environments when conditions become more favorable (sometimes years later), and arguably these populations are not truly extinct until all dormant stages have become non-viable. ‘Extinction’ in our study therefore refers only to measurements of the currently active population.

Our predictions for this study system were based on three experimental studies that involved similar, or the same, zooplankton species, and all investigated the effect of colonist quantity on population success: Drake et al. 2005 (using *Daphnia magna*), Kramer and Drake 2010 (using *D. magna*), and Gray and Arnott 2012 (using *Daphnia retrocurva* and *S. oregonensis*). Drake et al. (2005) found no effect of quantity alone on *D. magna* abundance or extinction, though there was an effect when combined with increased frequency. Kramer and Drake (2010) observed a strong effect of quantity on a single clone of *D. magna* in no-predator treatments (~50% reduction in extinction when population size was increased from 2 to 8 individuals). Conversely, Gray and Arnott (2012) found no effect of quantity on *D. retrocurva* growth rates, but *S. oregonensis* growth rates benefited from larger introduction sizes (r increased from ~0.03 to ~0.045 in introductions of 4 versus 8 individuals). While there are likely species-specific differences between our *D. pulicaria* and the *D. magna* and *D. retrocurva* from these studies, these experiments are the closest to our own system in terms of experimental design and species involved. Combined, these three studies suggest a mixed importance of colonist quantity in *Daphnia* species, with potentially greater importance of quantity for *S. oregonensis*. Additionally, there are clear differences in the demographic limitations of *D. pulicaria* and *S. oregonensis* that could influence the effect of colonist

quantity. The asexual phase in *D. pulicaria*, a cyclic parthenogen, dominates during the summer when our experiments are conducted, while *S. oregonensis* must reproduce sexually. Therefore, both a male and female must survive for *S. oregonensis* to persist, while *D. pulicaria* can potentially persist with even just a single surviving individual. Based on the studies detailed above, and the inherent demographic differences between these species, we predicted for our study that colonist quantity would be more important in *S. oregonensis* compared to *D. pulicaria*, and that the influence of colonist quality or frequency would be stronger (or just more apparent) in *D. pulicaria*, compared to *S. oregonensis*.

Methods

Our study began as a pilot experiment that commenced on 11-Apr-2015, the purpose of which was to assess what food treatments would result in individuals with either high or low lipid stores in both *D. pulicaria* and *S. oregonensis*. Zooplankton store energy primarily as lipids, and the amount of stored lipids affects their survival and reproductive capabilities (Lee et al. 2006; Arts et al. 2012), therefore potentially influencing colonist success in a new environment. Lipids are stored by cladoceran and copepod zooplankton as visible droplets which can be assessed and scored using a lipid index (Tessier and Goulden 1982; Arts and Evans 1991; Vanderploeg et al. 1992). The lipid index typically ranges from 0 (no visible lipids) to 3 (high quantity of visible lipids). The goal of the pilot experiment was therefore to assess what food treatments produced a mean lipid score around 0 and 3 in each zooplankton species. Such extremes in zooplankton lipid stores are commonly observed over the course of the growing season in natural populations (e.g. Tessier and Goulden 1982; Vanderploeg et al. 1992), and are therefore relevant to natural fluctuations in reproduction and food availability. Both species were fed using a laboratory culture of *Chlamydomonas reinhardtii*.

On 11-Apr-2015, we collected *D. pulicaria* and *S. oregonensis* from the pelagic region of Elbow Lake (see Appendix B: Table B1 for information on all lakes involved in the experiments) using vertical tows with an 80 μ m, 15cm diameter net. On the same day, they were then transported in cooled containers

to temperature- and light-controlled environmental chambers at Queen's University in Ontario, Canada. For *D. pulicaria*, we initially isolated 150 adult females into separate 100mL containers of Elbow Lake water that had been filtered through a G4 glass fibre filter (1.2 μ m pore size; Fisher Scientific, Waltham, Massachusetts, USA). This ensured enough offspring were produced on any given day for use in our experiments. Only third generation female neonates (juveniles newly released from the brood pouch) were used in our experiments to minimize maternal effects. For *S. oregonensis*, since juvenile sex determination is unreliable, only adult male and non-gravid female individuals were used from a collective pool maintained in filtered Elbow Lake water. All lab zooplankton were fed an amount of *C. reinhardtii* equivalent to 20 μ g C individual⁻¹ day⁻¹, and slowly acclimated over the course of two weeks in environmental chambers to a 16:8h day-night cycle, with 20°C daytime and 15°C nighttime temperatures, similar to conditions they would eventually encounter when introduced to the field experiment (Arnott, unpublished data).

The pilot experiment occurred between 21-May-2015 and 28-May-2015. Twenty individuals from each species (10 males and 10 females for *S. oregonensis*) were maintained in isolation under food treatments of 2.5, 5, 10, and 20 μ g C individual⁻¹ day⁻¹. For *S. oregonensis*, an additional treatment of 1 μ g C individual⁻¹ day⁻¹ was also used since lower food concentrations can be required to starve copepods compared to cladocerans. Due to the extensive lipid stores observed in some individual *D. pulicaria* in our high food treatments, a *Daphnia* lipid index ranging from 0 to 4 was used instead (following Larsson and Wathne 2006). Only the *D. pulicaria* fed 2.5 μ g C individual⁻¹ day⁻¹ (mean lipid index of 0.5) and *S. oregonensis* fed 1 μ g C individual⁻¹ day⁻¹ (mean lipid index of 0.25)(Appendix B: Fig. B1) achieved lipid scores close to 0, which required at least seven days in isolation to achieve. High lipid stores were also observed after this period in both *D. pulicaria* and *S. oregonensis* fed 20 μ g C individual⁻¹ day⁻¹ (mean lipid index of 3.2 and 2 respectively, Appendix B: Fig. B1). While it is unlikely that the natural dispersal period for non-resting stage zooplankton would last a week or more, it is not unusual for live freshwater zooplankton colonists to survive for several days or longer within human vectors (e.g. Verling et al.

2005).

The field experiments were conducted from 1-June-2015 to 3-Sept-2015 at a field site at Queen's University Biological Station (44.566850°N, 76.324796°W), Ontario, Canada. All experimental mesocosms were set up on 1-June-2015. Seventy-seven cylindrical tanks (84 cm diameter × 53 cm high) were filled with 180L of water from nearby Lake Opinicon (Appendix B: Table B1) filtered through a 50µm mesh (to remove zooplankton but allowing edible phytoplankton to pass through), then covered with 1mm mesh to protect from insects, debris, and litter. Mesocosms were haphazardly assigned to experimental treatments, and to receive either *D. pulicaria* (36 mesocosms) or *S. oregonensis* (36 mesocosms), with 5 mesocosms assigned to a 'control' treatment that received no experimental introductions to determine what, if any, natural colonization occurred during the experiments.

The experiments employed two quality treatments crossed against three quantity/frequency treatments. Zooplankton were first cultured under either high (20µg C individual⁻¹ day⁻¹) or low (*D. pulicaria*: 2.5µg C individual⁻¹ day⁻¹; *S. oregonensis*: 1µg C individual⁻¹ day⁻¹) food concentrations (referred to as 'good' and 'poor' quality individuals respectively), then introduced to field mesocosms as either: 1) 2 individuals introduced in a single event (referred to as low quantity, low frequency; LQ/LF); 2) 8 individuals introduced in a single event (referred to as high quantity, low frequency; HQ/LF); and 3) 2 individuals introduced in each of 4 separate events (low quantity, high frequency; LQ/HF). This design allowed us to distinguish the relative effects of increasing frequency from increasing quantity by introducing the same total number of individuals in the larger introductions, but divided over either single or multiple introduction events. Two individuals were used for the smallest introductions since populations of this size would be vulnerable to the effects of demographic stochasticity (Fauvergue et al. 2012), and this is also the minimum possible population size for *S. oregonensis* to ensure that one male and one female could be included in each introduction. Copepods can potentially establish a new population without males, since females can store sperm for long periods or carry it within a spermatophore, but we wanted to allow for the potential for sexual reproduction in each initial *S.*

oregonensis introduction. Eight individuals were chosen for the larger introductions since this is at or above the quantities at which introduced populations of other *Daphnia* species (e.g. *Daphnia magna*, Kramer and Drake 2010) and *S. oregonensis* (e.g. Gray and Arnott 2012) have exhibited a relatively low probability of extinction (0-25%). Each combination of the quality and immigration treatments was replicated six times for a total of 36 replicates for each species (72 total mesocosms).

In order to properly disentangle the relative effects of quantity and frequency, the amount of time within the experiment had to be standardized between the single and multiple addition treatments prior to sampling. For example, if both the single and multiple addition treatments began at the same time and were sampled two weeks later, every individual within the single addition treatment would have experienced a full two weeks to survive, reproduce, and/or die, while only part of the population of the multiple addition treatments would have experienced the same amount of time. To standardize timing, the four introductions for the high frequency treatments were conducted over four consecutive weeks, while the low frequency introductions occurred in a single event halfway between the 2nd and 3rd introduction events for the high frequency treatment. Therefore, once all introductions had concluded, zooplankton in the single and multiple addition treatments had all experienced an average of ~11 days per individual within the experimental mesocosms (Table 3.2).

Experiment 1 – Daphnia pulicaria

Multiple additions

The high frequency treatment introductions occurred over a period of several weeks, beginning on 4-June-2015 and concluding on 2-July-2015. The individuals involved in each introduction event were prepared weekly following the same procedure. First, 100 female neonates <48 hours old were isolated in separate 100mL containers of filtered Elbow Lake water. While we only required 24 total individuals for each introduction, isolating as large a quantity of neonates as possible created a pool from which individuals could be randomly chosen. Fifty neonates were randomly assigned to receive 20µg C

individual⁻¹ day⁻¹ for one week, and 50 to receive 2.5µg C individual⁻¹ day⁻¹. Following one week in the quality treatments, we randomly selected 12 *Daphnia* from the surviving individuals in each of the good- and poor-quality pools (mortality for both was ~10%) and transported them in individual, cooled containers from the lab to the field site. Upon arrival at the field site, individuals were randomly paired and introduced to their respective experimental replicates (2 individuals to each of 6 replicates for both the good- and poor-quality treatments). Isolation into quality treatments for the high frequency introductions occurred on June 4, 10, 17, and 24, and mesocosm introductions occurred on June 11, 18, 25 and July 2 respectively.

Single additions

For the low frequency introductions, 200 neonates were isolated on 14-June-2015, with half assigned to the good quality and half assigned to the poor-quality treatments. The size of the neonate pool from which we could select was made as large as was feasible for this treatment since 120 total individuals were required for introduction. On 21-June-2015, individuals were selected, transported to the field site, and introduced to their respective mesocosm treatments (2 or 8 individuals to each of 6 replicates for both the good- and poor-quality treatments) following the same procedures detailed above for the multiple additions.

Experiment 2 – Skistodiaptomus oregonensis

To replenish the exhausted laboratory pool of *S. oregonensis* (which had also declined in abundance in Elbow Lake in the intervening period), additional individuals were collected from nearby Warner Lake (Appendix B: Table B1). Collection procedures followed those used for Elbow Lake, except that *S. oregonensis* from Warner Lake were maintained in filtered Warner Lake water. An additional modification was also made to their quality treatments following the pilot study. Since *S. oregonensis* is omnivorous (Barnett et al. 2007), good quality treatments (20µg C individual⁻¹ day⁻¹) were also supplied

with 10 μ g of crushed Cyclop-eeze (a source of high quality animal protein and fat, Argent Chemical Laboratories, Redmond, Washington, USA) per individual, while poor quality treatments (1 μ g C individual⁻¹ day⁻¹) were fed only phytoplankton.

Multiple additions

The high frequency treatment introductions of *S. oregonensis* began on 11-June-2015 and concluded on 9-July-2015, and the individuals involved in each introduction event were prepared using the same procedure each week. First, 30 adult male and 50 adult female *S. oregonensis* (with no visible egg masses or spermatophores) were captured and isolated in separate 100mL containers of filtered Warner Lake water. We isolated more female than male stock to ensure that any females that developed visible egg masses within the body cavity during their week of isolation could be discarded to reduce the influence of reproduction prior to capture. 15 males and 25 females were randomly assigned to receive 20 μ g C individual⁻¹ day⁻¹ for one week, with the other half receiving 1 μ g C individual⁻¹ day⁻¹. Following one week in the quality treatments, 6 males and 6 females were randomly selected from each quality pool and transported to the field. At the field site, we randomly paired male and female individuals, then introduced them to their respective mesocosms (one male and one female to each of six replicates for both the high- and low-quality treatments). Isolation into quality treatments for each of the four introductions occurred on June 11, 17, 24, and July 1, and mesocosm introductions occurred on June 18, June 25, July 2 and July 9 respectively.

Single additions

For the low frequency introductions, 80 males and 120 females were isolated following the same procedure as the multiple *S. oregonensis* additions on 21-June-2015, with half assigned to good quality and half assigned to poor quality treatments. On 28-June-2015, individuals were selected, transported to the field site, and introduced to their respective mesocosm treatments (2 or 8 individuals to each of 6

replicates for both the good- and poor-quality treatments).

Experiments 1 and 2 ran for 8 weeks following conclusion of their respective final introductions. Experiment 1 finished on 27-Aug-2015, while Experiment 2 finished on 3-Sept-2015. This time period was chosen to ensure at least two generations had passed by the conclusion of the experiments. *D. pulicaria* require approximately 10 days to produce their first brood, thus about 6 generations passed over the course of Experiment 1. Life history information on *S. oregonensis* is scarce. They can produce multiple generations over the summer (Lai and Carter 1970), with a clutch size ranging between 4-20 eggs (Chow-Fraser and Maly 1991), and can quickly progress from clutch formation to the onset of the next gravid period within a few days (Chow-Fraser et al. 1988). Judging by their densities at the conclusion of Experiment 2 (reported below), about 2-3 generations had passed between initial introductions and final sampling.

Sampling protocol

Mesocosms were destructively sampled at the conclusion of each experiment to avoid altering population dynamics by the repeated removal of individuals for sampling. Sampling for final abundances occurred on 27-Aug-2015 and 3-Sept-2015 for Experiments 1 and 2 respectively. Zooplankton samples were collected using an 8cm diameter tube sampler that captured most of the depth of the water column. Mesocosm water was gently stirred prior to sampling, and 2L samples were taken from five different points in each mesocosm (creating a total sample of 10L), passed through a 50 μ m filter, then preserved in at least 70% ethanol. All sampling equipment was carefully rinsed between mesocosms to prevent organism transfer. Additionally, following sample collection at the end of the experiment, all water within each mesocosm was then siphoned through a 50 μ m filter and preserved in at least 70% ethanol. Enumeration of all samples was performed using a Leica MZ16 dissecting scope and a Leica DM E compound microscope. To save undue time and effort, full mesocosm samples were only processed in instances where no individuals were detected in a 10L sample. All abundance counts were converted to

number per litre.

Non-destructive sampling was also conducted for *D. pulicaria* beginning on 1-July-2015 (four weeks following final introductions to allow time for undisturbed acclimation), and occurred once per week until the conclusion of the experiment on 27-Aug-2015. The relatively large body size of *D. pulicaria* makes them clearly visible in macro-photographs (Appendix B: Fig. B2), allowing the assessment of abundance without removing individuals from the population. This is not possible for *S. oregonensis* as we could not distinguish them from various debris and other organisms in the photographs. Photograph samples of *D. pulicaria* were collected by first obtaining a 10L mesocosm sample (following the same procedure detailed above) on a 50 μ m filter. The filter and contents were then placed into a backlit container, photographed, then returned the mesocosm. The sampling process for a single mesocosm required less than 5 minutes to complete, and the filters did not dry out during the procedure. Control treatments were also sampled following these same methods, and all sampling equipment was carefully rinsed between mesocosms. These photograph samples allowed us to not only assess extinction by the conclusion of Experiment 1, but also the effects of our treatments on *D. pulicaria* population dynamics.

Statistical analysis

All statistical analyses were performed using $\alpha = 0.05$ in R 3.2.3 (R Core Team 2015), along with the “lme4” (version 1.1–10, Bates et al. 2015) and “glmmADMB” (version 0.8.3.2, Fournier et al. 2012; Skaug et al. 2015) packages. Response variables for *D. pulicaria* included abundance over all sampled weeks, and presence/absence in the final week (1/0, calculated as whether or not a single individual was detected). Response variables for *S. oregonensis* were abundance and presence/absence from the final sampling week. Populations were considered extinct if no individuals were detected in a mesocosm by the conclusion of the experiment.

Abundance of *D. pulicaria* was analyzed using linear mixed models (LMMs) to account for the

non-independence of repeated measurements through time. Full models included the three quantity/frequency treatments (LQ/LF; HQ/LF; LQ/HF) and the two quality treatments (Good and Poor) as fixed categorical predictors, 'Week' as a fixed continuous predictor (the quadratic term was also included to test for possible non-linear relationships), and a categorical variable for each replicate as a random slopes and intercepts predictor. *D. pulicaria* presence/absence was analyzed with generalized linear models (GLMs) using a Binomial distribution with a logit link function. Full models included only the three quantity/frequency treatments and the two quality treatments as fixed categorical predictors. Metrics for *S. oregonensis* were analyzed using generalized linear models (GLMs) with the three quantity/frequency treatments and the two quality treatments as fixed categorical predictors. Abundance was modeled using a Negative Binomial distribution with a log link function, and presence/absence using a Binomial distribution with a logit link function. Assumptions of LMMs were checked using plots of residual versus fitted values, histograms of model residuals, and plots of residuals against all fixed effects. Assumptions of GLMs were checked using plots of Pearson residuals versus fitted values, scale-location plots, and dispersion (with ϕ in the results indicating the dispersion of the model). Additionally, visual comparisons of predicted relationships against the actual data were useful for evaluating model fits in both experiments.

Model selection was performed for all response variables following Crawley (2005). Selection began with a full model containing the interactions between all fixed predictor variables, along with the random predictor in the case of LMMs. For mixed models, the structure of the random effect was first determined by comparing the full model to models with only a random intercepts term, or with no random effect, using likelihood ratio tests under restricted maximum likelihood. Fixed predictors and interactions required for all models were determined by comparing the deviance of successively reduced models using likelihood ratio tests. Fixed terms were discarded if the deviance of a simpler model versus a more complex model was not significantly different ($P < 0.05$). Removal of unnecessary terms continued until a higher order term was retained, or the null model was determined to be the 'minimum adequate model'

(see Table 3.3 and Appendix B: Table B2 for all minimum adequate models and descriptive statistics for each model). Significance of interactions or single fixed parameters was assessed by dropping them from the minimum adequate model, then comparing the deviance of models with and without the dropped term using likelihood ratio tests ($P < 0.05$).

Results

Control mesocosms were colonized primarily by four zooplankton groups: 1) *Chaoborus punctipennis* (zooplanktivorous larvae of the phantom midge); 2) chironomid larvae; 3) cyclopoid copepods (primarily *Mesocyclops edax*); and 4) rotifers (such as *Keratella cochlearis* and *Polyarthra remata*), and each group was ubiquitous in all experimental mesocosms. Colonization by *D. pulicaria* or *S. oregonensis* was not detected in any of the control mesocosms.

Experiment 1 – Daphnia pulicaria

There was a significant interaction between the effects of the quality treatments and time on *D. pulicaria* abundance ($n=180$, Deviance_{1, 10}=4.92, $P=0.026$) (Fig. 3.1), with no effect of the quantity/frequency treatments (Table 3.3; Appendix B: Table B2 and Fig. B3). Good quality populations achieved a median density of 7.85 individuals L⁻¹ by the initial sampling week (four weeks following final introductions), which increased slowly over the following weeks, reaching 11.55 individuals L⁻¹ by the final sampling date (Fig. 3.1). In comparison, the initial median density for poor quality populations was 31% lower at 5.7 individuals L⁻¹, but increased to around 15 individuals L⁻¹ in subsequent weeks, before declining to 13.35 individuals L⁻¹ by the final week (Fig. 3.1). There was no effect of the quantity/frequency or quality treatments on *D. pulicaria* presence/absence (Table 3.3) as 97% of the experimental populations were still present after an average growth time of 9.5 weeks, and only a single replicate from the ‘low quantity, low frequency’ treatment went extinct.

Experiment 2 – Skistodiaptomus oregonensis

There was a significant effect of the quantity/frequency treatments on the presence/absence of *S. oregonensis* ($n=36$, $\phi=1.09$, $\text{Deviance}_{2,35}=7.5$, $P=0.023$)(Fig. 3.2), but no effect of the quality treatment (Table 3.3 and Appendix B: Table B2) and no effect of any treatment on *S. oregonensis* abundance (Fig. 3.2a, Table 3.3). However, treatment effects on abundance were difficult to conclusively determine since most replicates in the ‘low quantity, low frequency’ and ‘high quantity, low frequency’ populations were extinct by the conclusion of the experiment. The probability of extinction was generally high for all populations in which colonists were introduced in a single event (LQ/LF: 83.3% extinct; HQ/HF: 75% extinct), and comparatively lower for colonists introduced across multiple events (LQ/HF: 33.3% extinct)(Fig. 3.2b).

Discussion

The relative importance of colonist quantity, quality, and frequency to population extinction differed between species. For *D. pulicaria*, none of these factors affected extinction, although colonist quality did have some influence on population dynamics. Conversely, dividing colonists over multiple introduction events reduced *S. oregonensis* extinction. While initial colonist quantity, quality and frequency have been shown in other studies to individually and interactively affect population success, our results show that their relative importance can change between organisms, and in certain species none of these factors may be particularly relevant to population success.

One of the most consistent results in the handful of studies that have experimentally investigated the relative contributions of colonist quantity and quality, or quantity and frequency, is that increasing the amount introduced (via increasing quantity or frequency) has had measurable effects on population success (Table 3.1). Our observed lack of effect of increased quantity or frequency on the population dynamics and extinction of *D. pulicaria* illustrates how these factors may not always be influential in the survival of new populations. We had predicted a potentially lower effect of quantity in *D. pulicaria*,

compared to *S. oregonensis*, but the lack of any effect of either quantity or frequency was unexpected (based on laboratory experiments with *D. magna* by Drake et al. 2005 and Kramer and Drake 2010). However, our result is consistent with studies of *Daphnia* colonization in natural environments that have inferred populations successfully established by just one to two initial colonists (e.g. *D. exilis*, Hairston et al. 1999; *D. magna* and *D. longispina*, Haag et al. 2005). Therefore, while we cannot determine why immigration had no detectable effect on extinction or abundance in *D. pulicaria* compared to *D. magna* from similar experiments, our results, along with studies of natural *Daphnia* colonization, indicate that it isn't unusual for new *Daphnia* populations to succeed with a small number of founders. This suggests that for species like *D. pulicaria* in which new populations can succeed with small quantities of initial colonists, other less commonly studied aspects of colonist introduction, such as quality, could be the primary factors in determining success.

The effect we detected of colonist quality on the abundance trajectories of *D. pulicaria* provides some indication that this could be the case. While quality did not influence extinction, poor quality *D. pulicaria* populations were initially lower in abundance and grew quickly over the sampling period, compared to the good quality populations whose abundances were initially higher (by a median of ~400 total individuals) and remained relatively consistent. *Daphnia* that are starved or receive poor quality food focus on rearing a smaller number of offspring, reducing overall clutch size (Tessier et al. 1983; Guisande and Gliwicz 1992). This initially lower offspring production in our poor-quality populations could have kept them at lower abundances for longer, delaying the onset of rapid growth, while the good quality populations had already expanded and reached a higher density by the time of initial sampling, and were also more stable through time. Additionally, higher mortality or longer acclimation periods in poor quality individuals in the initial introduction could have also prolonged the period of lower abundance in these populations. The poor-quality individuals we initially introduced were potentially less successful at coping with predation (Zhang and Buckling 2016), competition (Lømnicki 1988), parasites/disease (Hall et al. 2009; Simpson et al. 2015), and environmental stress (Carere et al. 2010). Quality-driven changes in

reproductive output, survival, acclimation, etc, are all potentially common allocation tradeoffs in any individual with low energetic resources, with subsequent consequences for population dynamics (Kooijman 2010).

Our experiment using *S. oregonensis* illustrated how dividing colonists over multiple introduction events can reduce their probability of extinction, leading to a stronger effect of arrival frequency compared to colonist quantity or quality. A similar greater importance of frequency compared to quantity has been observed in species in which larger, single event introductions have led to increased competition or reduced temporal facilitation (e.g. gregarious settling larvae, Hedge et al. 2012), resulting in a negative effect of increased quantity and a positive effect of increased frequency. However, we do not believe that this is the cause of our positive arrival frequency effect as positive effects of increased quantity have been observed in *S. oregonensis* introduced in a single event (Gray and Arnott 2012). Instead it is more likely that temporal changes in habitat suitability led to the extinction of colonists introduced at specific time periods. As environmental variability increases, dividing colonists over more introduction events can reduce their probability of extinction (Grevstad 1999; Drolet and Locke 2016). It is also clear that, regardless of quantity, if colonists arrive in unsuitable habitat the likelihood of extinction is high (Duncan 2016). The two processes likely responsible for creating temporal shifts in habitat suitability in our experiment were: 1) natural temporal variability inherent in a field experiment creating fluctuations in environmental conditions and resource availability (Davis et al. 2000; Stachowicz and Byrnes 2006); and 2) temporal shifts in predation pressure as *C. punctipennis* larvae (an ambush predator that can consume diaptomid copepods, Winner and Greber 1980), which naturally colonized our experiments, developed over time. However, we are unable to conclusively determine whether environmental suitability was the cause of the frequency effect in this experiment. A future experiment in which large, single event arrivals occurred at different points in time, with their performance compared to that of smaller introductions divided over multiple events, could be used to determine whether timing of introduction is the mechanism driving population failure. An additional possibility is that there were temporal differences between the

individual colonists selected for introduction. Although all *S. oregonensis* were maintained in the same lab environment, there were possible individual-level differences in genetic background or traits between the single and multiple event introductions (e.g. if certain types of individuals died earlier in the lab and thus weren't involved in the later introductions). Increasing arrival frequency would subsequently reduce extinction if the individuals involved in the single event introductions were, by chance, less capable of persisting than those selected for the multiple introductions that occurred earlier or later. Allowing for temporal differences in colonist composition is an inherent potential benefit of multiple introductions (Roman and Darling 2007; Simberloff 2009). Source populations change through time, and increasing colonist arrival frequency better captures this temporal variance compared to single event introductions.

It should be noted that while quantity and quality had no effect on *S. oregonensis* extinction in this experiment, we do not suggest that they are not involved in population success (for examples see references in Table 3.1). It is possible that weaker influences of quantity or quality were present, but due to the high level of extinction exhibited by our low frequency introductions we could not detect these effects. Instead, our results illustrate some of the circumstances under which the relative importance of quantity, quality, and frequency might change (i.e. between species or in potentially more variable environments), and the need for further studies to investigate the mechanisms that might be driving these shifts.

Although our results show that the relative importance of colonist quantity, quality, and frequency can change between species, our experiment does not address what could be driving these shifts. Trait differences between species are a possible answer. For example, the *Chaoborus* that naturally colonized our experiment can be a more effective copepod predator (Rudstam 2009), potentially increasing the benefit of earlier colonist arrival in the *S. oregonensis* introductions, compared to *D. pulicaria*, leading to the stronger importance of arrival frequency in this species. However, assessing whether the effect of quantity, quality, or frequency is trait-mediated requires a more extensive exploration of their relative importance across numerous species, along with the manipulation of key

traits that can influence vulnerability to the demographic and environmental processes that can drive extinction (e.g. species with asexual versus sexual mating systems, Stephan and Wissel 1994; Legendre et al. 1999; Møller 2003). Additionally, the inclusion of a diverse, native community in the recipient habitat, which was absent in our experiment, would add additional selective pressures that could better reveal trait-mediated differences in the relative importance of colonist quantity, quality, and frequency. This would improve our understanding not only of how these factors can vary in importance between species, but also allow for predictions about what factor(s) are most relevant for a specific organism and environment in question.

Implications

Our experimental design and results are relevant to applied ecological problems that seek to understand the properties controlling extinction. Efforts to restore anthropogenically disturbed habitats, control invasive species, conserve declining populations, and introduce biocontrol organisms all benefit from knowing which properties are responsible for determining whether small populations succeed or fail. Often, management practices focus on altering the size of these populations as this has been proven effective at controlling vulnerability to demographic, environmental, and genetic limitations (Shaffer 1981; Shea and Possingham 2000; Blackburn et al. 2015). However, our results support growing evidence that, depending upon the specific species and environment involved, of equal concern could be the conditions individuals experience in their source habitat, or during transit, as these can affect performance and survival in the new environment (i.e. the influence of quality). Similarly, determining the suitability of the environment into which a species arrives, how dramatically the environment fluctuates, and how often new arrivals occur (i.e. the influence of frequency), could also prove as relevant as accounting for the quantity of individuals. Finally, there are potentially a large number of species that are well-suited to succeeding in new environments with a low number of founding individuals. For such species, the influence of colonist quantity, quality, or arrival frequency could be low, and other aspects of colonist

introduction, such as genetic background, may play a more critical role.

Table 3.1 Experimental studies that have investigated the relative effects of colonist quantity, quality, or frequency on the establishment and growth of new populations. Column headings ‘Positive’ and ‘Negative’ indicate what effects increased immigration (either through increased quantity alone or quantity and frequency combined) had on the response metrics within each study.

Studied Interaction	Dominant Effect	Positive	Negative	Species	Reference
Quantity and Frequency	Quantity	Establishment and abundance	–	<i>Pseudorasbora parva</i>	Britton and Gozlan 2013
	Both	Abundance, persistence, and time to extinction	–	<i>Daphnia magna</i>	Drake et al. 2005
	Both	Settlement and abundance	Proportion colonizing and surviving	<i>Crassostrea gigas</i>	Hedge et al. 2012
	Quantity	Abundance and survival	–	<i>Hemimysis anomala</i>	Sinclair and Arnott 2016
Quantity and Quality	Quantity	Recruitment, biomass, abundance and reproduction	–	<i>Bugula neritina</i>	Burgess and Marshall 2011
	Quantity	Establishment and biomass	–	<i>Imperata cylindrica</i>	Estrada et al. 2016
	Both	–	Survival and individual growth	<i>Stegastes partitus</i>	Johnson 2008
	Both	Establishment, biomass, and reproduction	–	<i>Bugula neritina</i>	Lange and Marshall 2016
	Both	–	Survival	<i>Ciona intestinalis</i>	Marshall and Keough 2003

Table 3.2 Schedule of experimental introductions for each treatment, with both species following the same introduction schedule. ‘Average Time in Experiment’ is calculated across all introduced individuals within each treatment for Day 21, at which time all introductions had concluded. For example, by Day 21 the eight introduced individuals in the HQ/LF treatment had experienced 11 potential days within the experiment. For the LQ/HF treatment, two individuals had experienced 21 days, two 14 days, two 7 days, and two 0 days, which averages to 10.5 days per individual.

Treatment	Introduction Schedule					Average Time in Experiment (days per individual)
	Day 0	Day 7	Day 10	Day 14	Day 21	
LQ/LF	-	-	2 introduced	-	-	11 days
HQ/LF	-	-	8 introduced	-	-	11 days
LQ/HF	2 introduced	2 introduced	-	2 introduced	2 introduced	10.5 days

Table 3.3 Minimum adequate model (MAM) structure, statistical methodology, and descriptive statistics for abundance and presence/absence models. ‘Immigration’ (a categorical variable for the three quantity/frequency treatments: LQ/LF, HQ/LF, LQ/HF), ‘Quality’, and ‘Week’ entries under the ‘MAM’ heading represent the fixed predictors that were retained in the MAMs, while ‘ID’ refers to the unique replicate identifier for each mesocosm when used as a random effect. ‘Null’ indicates that the null model was the MAM. ‘LMM’ and ‘GLM’ under the ‘Model’ heading refers to ‘Linear Mixed Model’ and ‘Generalized Linear Model’ respectively.

Metric	Log	Model	MAM	Descriptive Statistics	Distribution
<i>D. pulicaria</i> abundance	Yes	LMM	Quality + Week + Week ² + Quality:Week + Quality:Week ² + (Week ID)	Mean: 11.6 Variance: 55.06	Normal
<i>D. pulicaria</i> Presence/absence	NA	GLM	Null	Minimum success: 5 Minimum failure: 0	Binomial
<i>S. oregonensis</i> abundance	NA	GLM	Null	Mean: 0.55 Variance: 1.58	Negative Binomial
<i>S. oregonensis</i> presence/absence	NA	GLM	Immigration	Minimum success: 2 Minimum failure: 4	Binomial

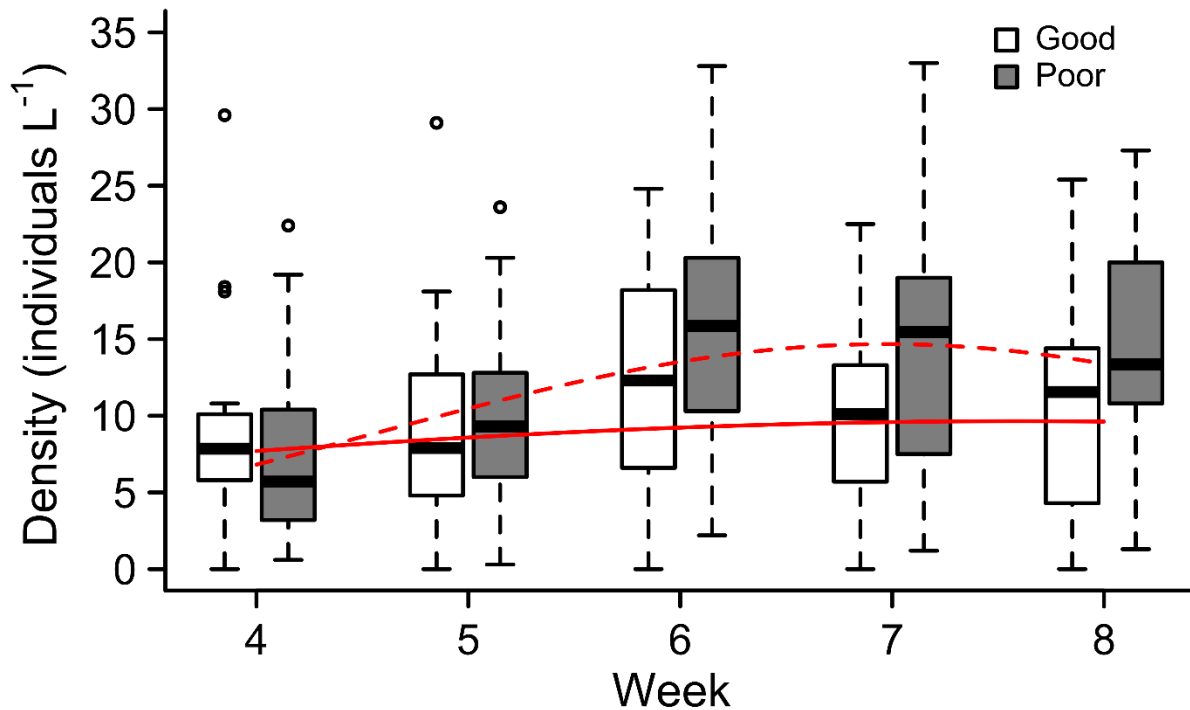


Figure 3.1 Effects of the quality treatments and time on *D. pulicaria* density (individuals L⁻¹, n=18) across all sampling weeks for good (white boxes) and poor (grey boxes) quality populations. Bottom and top edges of boxes represent the first and third quartiles respectively, with a dark, horizontal line as the median. Whiskers encompass data points that lie within 1.5 times the inner quartile range, data outside this range are shown as separate points. Best-fit lines represent the predicted relationships between time and density for good (solid lines) and poor (dashed lines) condition individuals based on the minimum adequate model (Table 3.3).

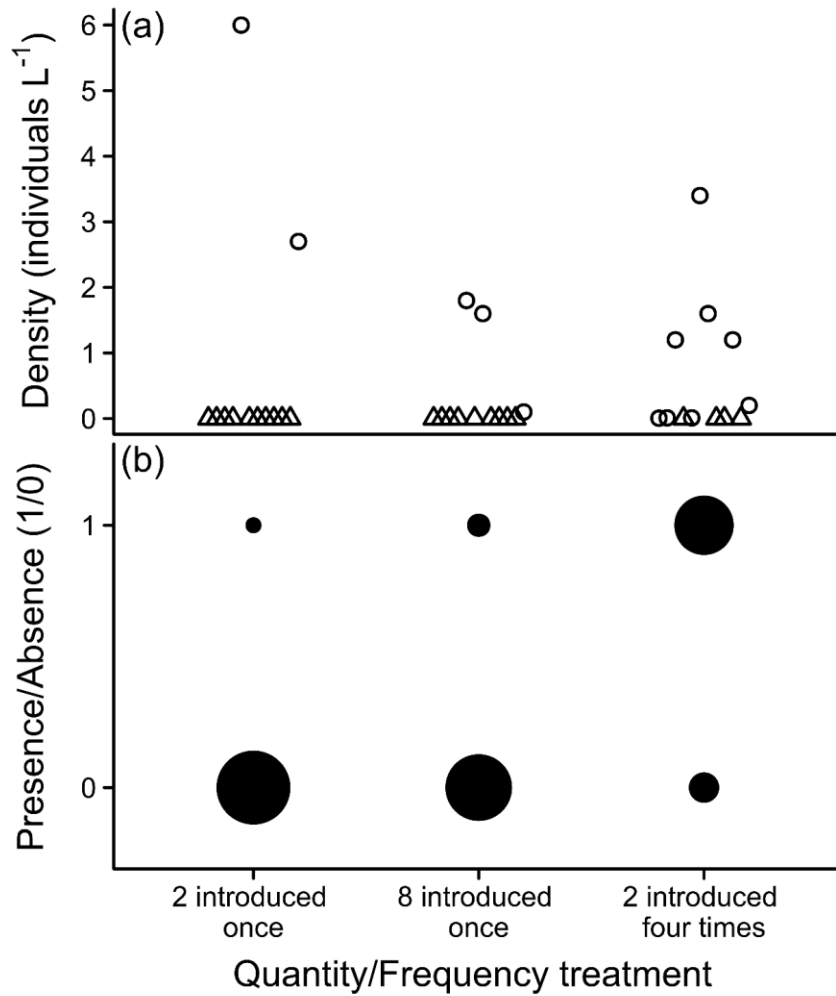


Figure 3.2 Effect of the quantity/frequency treatments (2 individuals once=LQ/LF; 8 individuals once=HQ/LF; 2 individuals four times=LQ/HF) on *S. oregonensis* (a) density (individuals L⁻¹, $n=12$) and (b) presence/absence (1/0, $n=12$) from the final sampling week. In (a), each point represents a single replicate population, with densities greater than zero represented by empty circles, and densities equal to zero by empty triangles. In (b), changes in presence/absence are represented by the size of filled circles (maximum circle size is 12), with circle size representing the total number of replicates, out of 12, in which *S. oregonensis* is either present or absent (2 individuals once: 2/12 present or 16.7%; 8 individuals once: 3/12 present or 25%; 2 individuals for times: 8/12 present or 66.7%).

Chapter 4

Colonist identity determines the benefits of increased quantity and genetic diversity in the success of new *Daphnia* populations

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Abstract

It is generally accepted that larger populations are more likely to establish and spread due to the benefits provided by a higher number of individuals (quantity) and genotypes/phenotypes (genetic diversity). However, the identity and traits associated with the individuals in a population or colonist pool could also determine the importance of quantity or genetic diversity for population success. Poor-performing individuals may benefit from increased quantity or genetic diversity, while high-performing individuals might readily succeed even with a small number of founders. We conducted a field mesocosm experiment that manipulated colonist quantity, genetic diversity, and identity to determine if the effects of quantity or genetic diversity depend upon which particular individuals are involved. Nine distinct genotypes of *Daphnia pulex*, characterized also by phenotype, were introduced at low (one colonist) and high (nine colonists) introduction quantities and at low (monoclonal populations) and high (mixed genotypes) genetic diversities. We found that for some genotypes an increase in the quantity or genetic diversity of colonists was beneficial for overall success, while for others increasing quantity or genetic diversity had little effect. We found that, in *D. pulex*, the benefits provided by increased quantity were related to body size, and the benefits of increased genetic diversity were related to individual performance. Larger-bodied genotypes tended to exhibit reduced effects of increased quantity, while increasing genetic diversity tended to have little effect on high-performing genotypes. Our results provide support that the identity of the individuals present in a colonist pool could, at least partly, determine the importance of colonist quantity or genetic diversity to population success, and also how responsive a population might be to efforts to promote, or prevent, its success through population control.

Introduction

The size of a population has been repeatedly identified as a key component in its successful establishment and growth, with success often positively correlated with the number of individuals in a population (e.g., Shaffer 1981, Montalvo et al. 1997) or the number of arriving colonists (e.g., Blackburn

et al. 2015). This is due to the combined effects of population size on two factors that can determine success: quantity (number of individuals in the population) and genetic diversity (number of genotypes/phenotypes). Fewer individuals renders a population more vulnerable to Allee effects (Stephens et al. 1999), as well as demographic and environmental stochasticity (Fauvergue et al. 2012). Similarly, lower genetic diversity reduces adaptability and the potential for facilitation between genotypes, and increases the likelihood of genetic Allee effects, such as inbreeding depression (Forsman 2014, Luque et al. 2016).

However, establishment success is not always correlated with colonist quantity and genetic diversity. For some species, increasing both can improve success (e.g., Ahlroth et al. 2003, Hufbauer et al. 2013, Szűcs et al. 2014), but for others a higher quantity of colonists provides no benefit (e.g., Fauvergue et al. 2007, Crawford and Whitney 2010), and the same can be true for genetic diversity (e.g., Hovick et al. 2012, Erfmeier et al. 2013). Even within the same group of organisms, such as *Daphnia* which are a common model organism in quantity and genetic diversity experiments, the importance of these factors varies. Some studies show positive effects of increased quantity or genetic diversity on population success (Kramer and Drake 2010, Holmes et al. 2016), while others show no effect of increased quantity (Drake et al. 2005, Gray and Arnott 2012, Sinclair and Arnott 2017), or no effect of increased genetic diversity (Robinson et al. 2013). Investigating the reason for this considerable variation is key to determining whether quantity or genetic diversity will play an integral role in colonization, and for practical efforts to promote (e.g. community recovery or biocontrol) or prevent (e.g. invasive species) population establishment and growth. Population control is commonly accomplished by manipulating population size (Shaffer et al. 1981, Lande 1988, Montalvo et al. 1997, Fauvergue et al. 2012, Blackburn et al. 2015), and knowing when quantity or genetic diversity are important for success is crucial for creating effective management strategies.

Variability in the benefits of increased colonist quantity or genetic diversity has been attributed to a variety of factors. Some species rely less on conspecific density than others (e.g., those that exhibit little

or no Allee effects, Fauvergue et al. 2007), and so increasing colonist quantity does little to improve establishment. The environments into which colonists arrive also differ in their abiotic suitability and severity of interactions with resident species, which can in turn drive differences in the importance of both factors (Pantel et al. 2011, Hovick et al. 2012, Duncan 2016). Experimental design can also determine whether increasing quantity or genetic diversity has an influence on success. For example, manipulating genetic diversity by mixing individuals from multiple source populations could result in either positive or negative effects on population fitness (Forsman 2014). However, there is a crucial underlying factor that is frequently not controlled, but that could determine whether quantity, or genetic diversity, or both, or neither, are important for success. Research on these factors often focuses on amount: how many individuals or genotypes are present and how does this affect population success? Yet at the same time, the identity (genotype/phenotype) of the individuals can also influence population success (e.g., Vanni et al. 1987, Vellend et al. 2010, Pantel et al. 2011). This is due to intraspecific differences in traits, such as vital rates (e.g. Cressler et al. 2017), competitive ability (e.g., Cheplick 1997), resource acquisition (e.g., Manschadi et al. 2008), predator defense (e.g., Duquette et al. 2005), parasite/disease resistance (e.g., Alexander et al. 1996, Carius et al. 2001), and phenotypic plasticity (Pigliucci 2001), that can determine the likelihood of establishment (Shea and Chesson 2002, Prenter et al. 2004, Yeh and Price 2004, Theoharides and Dukes 2007, Forsman 2014). Additionally, certain individuals could be pre-adapted to their colonizing habitat (those in possession of traits already suited to survival and reproduction in the new environment), and are thus more likely to succeed.

The chance inclusion or exclusion of individuals with favorable, invasive traits and/or those pre-adapted to a recipient environment (hereafter referred to as ‘high-performing’ individuals) could determine whether a higher quantity or genetic diversity of colonists is beneficial for population success. A population with high-performing individuals could readily succeed even if colonist quantity or genetic diversity remains low (e.g., genotypes with lower critical population densities or those capable of self-fertilization; Kadam and Velicer 2006, de Groot et al. 2012), and may thus depend less on higher

quantities or genetic diversities for success. Conversely, populations that do not happen to include high-performing individuals, or experiments that by chance only include high-performing colonists in higher quantity or diversity treatments, may experience greater success when either one is increased. This is akin to a type of sampling effect (the probability of selecting species with a specific property, Huston 1997), in which the particular individuals involved can determine the benefits provided by increased colonist quantity or genetic diversity.

Improving the sampling of high-performing individuals is a commonly discussed mechanism by which increasing quantity or genetic diversity can benefit population success (e.g., Crawford and Whitney 2010, Erfmeier et al. 2013). But, there are only two studies of which we are aware that provide evidence that changing colonist identity can alter the effect of increased colonist quantity or genetic diversity. The first is by Maron (2006), which shows potential interpopulation differences in the effect of higher colonist quantity. For one group of colonists (*Hypericum perforatum* individuals sourced from Hamburg, Germany), increasing introduction quantity from 100 to 400 seeds dramatically improved colonization success from 0% to above 70%, but the success of colonists from a different source (Adliswil, Switzerland) was unaffected by increased seed quantity, remaining constant at ~35%. Thus, within the same species, increasing quantity had a beneficial effect on the success of one group of colonists, but not another. It should be noted, however, that this conclusion is not discussed within this paper, we only infer it from their results. The second study is by Vellend et al. (2010), and it indicates that the benefits of increased genetic diversity could depend upon genotype identity. They found that some single genotype monocultures of their dandelion colonist (*Taraxacum officinale*) performed equally to, or better than, a higher diversity mixture of five genotypes, while other monocultures performed more poorly. From this, they inferred that the consequences of reducing colonist diversity may differ depending upon identity. Lower diversity may only reduce population success if it subsequently results in the loss of high-performing individuals.

While these studies provide indications that identity could determine the importance of colonist

quantity or genetic diversity, they only serve as a starting point for addressing this question. Maron (2006) only manipulates colonist quantity, not genetic diversity, and does not control identity as the study was only designed to test differences among multiple source populations. Conversely, Vellend et al. (2010) only manipulates genetic diversity, not quantity, though identity is explicitly controlled. Additionally, interspecific interactions between colonists and residents, a crucial part of the colonization process, are low or absent in both studies. Maron (2006) establishes colonists in a cleared habitat, while Vellend et al. (2010) employs only a single resident competitor. A more comprehensive experimental investigation is required that manipulates the identity (specifically the phenotype), along with the quantity and genetic background of colonists establishing in a resident community of multiple competitors and predators, and that assesses whether the effects of increasing quantity and genetic diversity can differ based on identity. To accomplish this, we conducted a field mesocosm experiment that founded populations with different phenotypes/genotypes and quantities of *Daphnia pulex* sensu lato (Cristescu et al. 2012). *D. pulex* is an excellent study organism for determining how identity may influence the value of quantity and genetic diversity. It is a rapidly reproducing cyclic parthenogen (asexual reproduction during optimal ecological conditions with sexual reproduction occurring during periods of stress). This flexible reproductive strategy allows for the manipulation of the genetic background and diversity of replicated treatments through the use of different clones, and for comparisons of the effects of increased colonist quantity or genetic diversity among clones. Each clone can also be genotyped, and various life-history traits measured, to ensure that clone 'identity' corresponds to a unique genotype and a unique set of phenotypic traits. Additionally, past experiments involving *Daphnia* species have indicated a potential for differential colonizer success among genotypes (e.g., *Daphnia pulex*, Vanni 1987) and possible intraspecific differences in the benefits of increased colonist quantity (e.g., for *Daphnia magna*, Drake et al. 2005 show little individual effect of increased quantity, while Kramer and Drake 2010 show a strong effect).

Our experiment was designed to answer three questions: 1) does the observed effect of increasing

D. pulex colonist quantity depend upon colonist identity? 2) does the observed effect of increasing *D. pulex* colonist genetic diversity depend upon colonist identity? and 3) do the effects of quantity or genetic diversity relate to phenotypic traits or source habitat similarity? We answered our first question by separately introducing 9 different genotypes of *D. pulex* at low (1 colonist) and high (9 colonists) introduction quantities to field mesocosms containing a diverse native community, then assessing whether the effect of increased colonist quantity differed among genotypes. For our second question, we also introduced a mixed colonist pool comprised of 1 individual from each of the 9 genotypes (9 colonists total, a high diversity treatment). Comparing population success between the high diversity introductions to that of the 9 colonist introductions for each of the single genotypes (low diversity monoclonal populations) allowed us to assess if the observed effect of increased genetic diversity differed depending upon the genotype used as the low diversity comparison. To answer our final question, we determined if the effects of increased quantity or genetic diversity across all genotypes were related to measured genotype life-history traits, or the similarity of genotype source habitat to the experimental habitat. The effects of both manipulations could vary depending upon phenotypic traits related to performance (e.g., body size, which affects predator vulnerability in *Daphnia*, Riessen and Young 2005). If this is the case, then the observed magnitude of the effect of increased quantity or genetic diversity would correlate to one or more of our measured life-history traits. Additionally, the *D. pulex* involved in our experiment were sourced from two regions in Ontario, Canada that differ in their underlying geology, and 9 lakes that differ in water chemistry (see Appendix C: Table C1). Colonists sourced from the same region as our experimental habitat, or from lakes more similar to it, could be more likely to succeed in the recipient environment (i.e. habitat matching). If this is the case, then the effect of the quantity and/or genetic diversity treatments would correlate to region of origin or habitat similarity.

Methods

Candidate *Daphnia pulex* genotypes were selected for use in our experiment based on unique

differences in both genotype and phenotype to ensure that genotypic differences also corresponded to measurable differences in relevant ecological traits. Genotypes for our experiment were selected from a pool of 54 *D. pulex* individuals based on maximal differences in life-history traits (determined using a pilot experiment, see Appendix C1), and genetic differences determined using 12 microsatellite markers (for detailed genomic methods see Appendix C2). A total of nine distinct genotypes referred to as the ‘experimental genotypes’ (Table 4.1) were chosen for use in our experiment: six sourced from lakes in central Ontario, Canada (the Muskoka region) and three from lakes in southern Ontario, Canada (the region surrounding the Queen’s University Biology Station or QUBS).

Life-history trials

To obtain detailed phenotypic data we conducted 21-day life-history trials on each of the nine pre-selected experimental genotypes. Life-history trials were conducted from 31-May-2016 until 26-June-2016. Twenty newly released neonates were obtained from each of the nine experimental genotypes from the pilot experiment (totaling 180 neonates). These neonates were isolated and maintained following the same trial procedures as detailed in Appendix C1, and were checked daily for 21 days. Data was collected on age (in days), day of first reproduction, body size (measured from the top to the base of the carapace) at first reproduction, number of offspring released in all broods over the course of the 21-day trials, and daily survivorship. These traits were found in the pilot study to differ the most between genotypes. The intrinsic rate of increase (r) was calculated for each genotype by iteratively solving for r in the equation $1 = \sum e^{-rx} l_x m_x$ where l_x is the survivorship of *D. pulex* females from birth to age x , and m_x is female offspring per female of age x .

Experiment

The experiment was conducted at the Queen’s University Biology Station, ON, Canada from 22-June-2016 until 2-Sept-2016. We employed an unbalanced design for the experiment in which individual

field mesocosms were inoculated by either one colonist (a ‘low quantity/low diversity’ treatment) or by nine colonists of the same genotype (‘high quantity/low diversity’), or a mixture comprised of one individual from each of the nine genotypes (‘high quantity/high diversity’). The low quantity/low diversity introductions were replicated 10 times per genotype (90 mesocosms total), while the high quantity/low diversity introductions were replicated five times per genotype (45 mesocosms total). Higher replication was employed for the low quantity treatments to better capture the higher variation of outcomes likely to occur in populations founded by a single individual. High quantity/high diversity treatments were replicated seven times.

The experiment was set up in two periods, controlled for using blocking variables in our analysis, wherein 109 mesocosms (cylindrical tanks 84 cm diameter × 53 cm high) were set up in the first block on 22-June-2016. Six replicates of the low quantity/low diversity treatments (54 mesocosms), and five replicates for each of the high quantity/low diversity (45 mesocosms) and high quantity/high diversity (five mesocosms) treatments were haphazardly assigned to this block, along with five mesocosms assigned to a control treatment that received no experimental introductions to control for possible natural colonization. Mesocosms were filled with 180L of water from nearby Lake Opinicon (water unfamiliar to all genotypes, Appendix C: Table C1) which was filtered through a 50µm mesh to remove zooplankton while allowing for edible phytoplankton to pass through, and covered with 1mm mesh to protect from insects, debris, and litter. Mesocosms were then inoculated with a diverse, native zooplankton community filtered from 18,720L of lake water from the pelagic region of nearby Buck Lake (Appendix C: Table C1) using an 80µm net. All mesocosms were inoculated with the equivalent of 180L of Buck Lake zooplankton (Buck Lake does not contain *D. pulex*) from a condensed and constantly mixed pool, with this inoculum divided evenly over two aliquots. Following inoculation, zooplankton acclimated to the mesocosms for one week.

Between 25-28 June 2016, 72 female neonates were collected from each experimental genotype. All neonates for each genotype were divided evenly between six 120mL glass containers and maintained

until the time of introduction in 100mL of filtered Elbow Lake water and fed 30µg of carbon per container per day. On 29-June-2016, all individuals for introduction were transported in their cooled containers to the field site. For each genotype, individuals were haphazardly captured from their containers and released below the water line into their respective mesocosms until all introductions necessary to create each treatment had been completed.

On 6-July-2016, the second block of 38 mesocosms were set up and haphazardly assigned to either the remaining four replicates for each of the low quantity/low diversity introductions for each genotype (36 mesocosms), or the two remaining replicates in the high quantity/high diversity treatment (two mesocosms). These tanks were filled, covered, and inoculated with a native zooplankton community collected from 6,480L of lake water from Buck Lake on 7-July-2016 following the same procedures as the first block. Neonates for introduction were collected between 9-12 July 2016 and introduced on 13-July-2016 following the same procedures detailed for the first block of mesocosms.

Sampling protocol

Each block of the experiment concluded seven weeks following initial introductions. This time frame was chosen as it allowed us to assess whether populations had persisted for about 4 to 5 generations (average time to first reproduction in our experimental genotypes was ~11 days, Table 4.1). Sampling of the first block was conducted on 18-Aug-2016 and the second was sampled on 1-Sept-2016. Mesocosm sub-samples were collected using an 8cm diameter tube sampler that captured most of the depth of the water column. Mesocosm water was gently stirred prior to sampling and 2L samples were taken from five different points in each mesocosm (creating a total sample of 10L), passed through a 50µm filter, then preserved in 95% ethanol. All sampling equipment was carefully rinsed between mesocosms to prevent the transfer of organisms. Following the collection of sub-samples, all water within each mesocosm was then siphoned through a 50µm filter and all filtered material was preserved in 95% ethanol. Enumeration of all samples was performed using a Leica MZ16 dissecting scope, with all abundance counts converted

to number per litre. Additionally, 20 *D. pulex* were collected from each of the low quantity/low diversity and high quantity/low diversity mesocosms, or as many as possible in cases where densities were too low to obtain 20, along with 50 *D. pulex* from each of the high quantity/high diversity mesocosms. These individuals were preserved in 95% ethanol and genotyped at McGill University (see Appendix C2).

Statistical analysis

Population success was quantified by measuring the presence/absence and density of *D. pulex* from the final sampling date. However, *Daphnia* can produce diapausing eggs when conditions are unfavorable, which can hatch months or years later to augment the current population or re-colonize following extirpation. Therefore, our measurements of population success are applicable only to the currently active population, and likely underrepresent the true population size, which could potentially include both active and dormant individuals.

To determine the effects of our colonist quantity treatments, we compared *D. pulex* abundance between the low and high quantity treatments for each of our experimental genotypes using a Hurdle model with a binomial distribution (logit link function) to model presence/absence and a negative binomial distribution (log link function) to model non-zero abundances. *D. pulex* abundance in the final week was the response variable, along with categorical predictor variables representing each genotype, the low/high quantity treatments, and the two experimental blocks. We also calculated the effect sizes of increasing colonist quantity using Cliff's δ ($\delta = \Pr(x_{i1} > x_{j2}) - \Pr(x_{i1} < x_{j2})$, where x_{i1} is an observation from group 1 and x_{j2} is an observation from group 2; Cliff 1993). It measures the degree to which one group of observations overlaps another (e.g. *D. pulex* abundance in low versus high quantity mesocosms). Cliff's δ is more appropriate for our data than other effect size measurements because it is not affected by non-normality, unequal variance, or extreme outliers. The sign of the effect size for each genotype was determined to be positive if *D. pulex* abundance in the high quantity mesocosms tended to be higher (i.e. performed better) than the low quantity mesocosms, and negative if abundance in the high

quantity mesocosms tended to be lower than the low quantity mesocosms. Confidence bands for Cliff's δ were calculated using the consistent estimate of the variance (Cliff 2014).

To determine the effects of our colonist genetic diversity treatments, we compared *D. pulex* abundance from each of the low diversity treatments (nine colonist monoclonal populations) to the high diversity treatment. This was done using generalized linear models (GLMs) with a negative binomial distribution (log link function). Each of these models used *D. pulex* abundance in the final week as the response variable, along with a categorical variable representing whether the treatment was low or high diversity. Benjamini-Hochberg False Discovery Rate *P*-values corrected for multiple comparisons are reported for these results. Effect sizes for increasing colonist genetic diversity (measured as Cliff's δ) were calculated based on the difference in abundance measurements between the low diversity treatments for each genotype and the high diversity treatment. The sign of the effect size was determined to be positive if *D. pulex* abundance in the high diversity treatment tended to be higher than a particular low diversity treatment, and negative if abundance tended to be lower.

Linear models were used to test our initial predictions of relationships between the effect sizes of quantity/genetic diversity and habitat similarity or life-history traits. Cliff's δ for the effect sizes of the quantity and genetic diversity treatments were the continuous response variables in all models. Predictor variables for region of origin and habitat similarity were, respectively, a categorical variable for our two lake regions (Muskoka versus QUBS) and a continuous variable for Euclidean distance between the water chemistry of each source lake to Opinicon Lake. Euclidean distances were calculated by using water chemistry variables (scaled to a mean of zero and standard deviation of one) sampled from all lakes involved (see Appendix C: Table C1) to produce a Euclidean distance matrix. The Euclidean distance between each source lake to Opinicon Lake was then extracted from this matrix to represent their similarity, with lower values of Euclidean distance indicating source lakes with water chemistry similar to that of Opinicon Lake. Predictor variables for life-history traits were the continuous variables for *r* and body size at first reproduction for each genotype.

To assess which genotypes persisted in the high diversity treatment, and to determine if the same genotypes persisted among replicates, we estimated the presence of each genotype based on the detection of unique alleles (see Appendix C2) using five independent DNA extractions per mesocosm and two PCR replicates. If an allele unique to a genotype was detected, then we considered that particular genotype to be present in a mesocosm, and absent if no unique alleles were detected. Differences in presence/absence between genotypes was assessed using a GLM with a binomial distribution (logit link function). Presence/absence of each genotype in each mesocosm replicate was the response variable, along with categorical predictor variables representing the genotype to which each score belonged and the experimental block of each replicate.

Selection of predictor variables for all models detailed above was performed following Crawley (2005). Beginning with the full model for each metric, fixed predictors and interactions that explained significant deviance in the model were determined by comparing the deviance of successively reduced models using likelihood ratio tests. Fixed terms were discarded if the deviance of a simpler model versus a more complex model was not significantly different ($P > 0.05$). Removal of terms continued until a higher order term was retained, or the null model was determined to be the minimum adequate model. Significance of fixed terms in LMs was assessed using ANOVA or linear regression ($P < 0.05$). Significance of fixed terms in GLMs was assessed by dropping terms from the minimum adequate model, then comparing the deviance of models with and without the dropped term using likelihood ratio tests (LRTs, $P < 0.05$). Assumptions of LMs and LMMs was assessed using plots of residual versus fitted values, normal quantile-quantile, and scale-location plots, along with plots of model residuals against the random effect if present. Log-transformation was used if necessary to meet model assumptions. Assumptions of GLMs were assessed using plots of Pearson residuals versus fitted values, scale-location plots, and dispersion (with ϕ in the results indicating the dispersion of each model). All statistical analyses were performed using $\alpha = 0.05$ in R 3.3.2 (R Core Team 2016), along with the “pscl” (version 1.4.9; Zeileis et al. 2008, Jackman 2015) and “glmmADMB” (version 0.8.3.2; Fournier et al. 2012, Skaug et al.

2015) packages.

Results

No *D. pulex* were observed in any of our control mesocosms that did not receive colonists. Additionally, based on genetic analysis, the only genotypes present in their respective mesocosms were those that had been purposefully introduced, indicating no occurrences of natural colonization or cross-contamination by other *D. pulex* genotypes.

Effect of quantity on population success

To determine if the observed effect of increasing colonist quantity depended upon colonist identity, we assessed the effect of our quantity treatment on *D. pulex* abundance in each of our experimental genotypes. *D. pulex* presence/absence was not significantly different between genotypes or quantity treatments. None of the nine-colonist treatments were extinct by the conclusion of the experiment, and only 12% of all one-colonist replicates were extinct. *D. pulex* densities achieved by the end of the experiment were significantly different between genotypes (LRT, $n = 135$, Deviance_{16,23} = 46.8, $\phi = 1.2$, $P < 0.0001$), and quantity treatments (LRT, $n = 135$, Deviance_{2,23} = 12.7, $\phi = 1.2$, $P = 0.0018$), with high quantity treatments generally achieving higher average densities (~8 individuals L⁻¹) compared to low quantity treatments (~5.7 individuals L⁻¹, Fig. 4.1). There was no significant interaction between the genotype and quantity treatments.

Based on effect sizes, however, there were differences in the magnitude of the effect of increased colonist quantity among genotypes (Fig. 4.2a). Genotypes C, E, F, L, M, and R exhibited weak to medium positive effects of increased colonist quantity, while genotypes D, G, and O exhibited no effect of increased colonist quantity. Increasing colonist quantity also had similarly variable effects on the direction and magnitude of the change in average *D. pulex* density between genotypes (C: +0.35; D: -0.6; E: +4.5; F: +1.3; G: -3.3; L: +1.5; M: +6.6; O: +2.1; R: +8.6 individuals L⁻¹). Given that our mesocosms

contained 180L of water, these changes in average density ranged from increasing total population abundance by ~1200 individuals, to decreasing total population abundance by ~600 individuals.

Effect of genetic diversity on population success

To determine if the observed effect of increasing colonist genetic diversity depended upon colonist identity, we compared *D. pulex* abundance in our high diversity treatment to each of the high quantity/low diversity (monoclonal) treatments. After adjustments for multiple comparisons, *D. pulex* abundances were only significantly higher in the high diversity treatment when compared to the C genotype populations (LRT, $n = 12$, $\text{Deviance}_{1,3} = 8.06$, $\phi = 0.99$, $P = 0.04$), with no significant differences detected between the high diversity treatment and any of the other low diversity treatments (see Appendix C: Table C2). Average *D. pulex* density in the high diversity treatment was 10.6 individuals L^{-1} , while in the C genotype treatment it was 2.1 individuals L^{-1} .

There were also differences in the effect size of increasing colonist genetic diversity among genotypes (Fig. 4.2b). Genotypes C, F, G, and L exhibited weak to medium positive effects of increased genetic diversity, while genotypes D, E, M, O, and R exhibited no effects. Average population density in the high diversity treatment was much higher when compared to densities in the C and F genotypes (+8.2 and 7.5 more individuals L^{-1} in the high diversity treatment compared to the low diversity treatments), moderately higher compared to the D, E, G, and L genotypes (+2.2, +0.9, +4.0, +3.7 individuals L^{-1} respectively), and slightly lower compared to the M, O, and R genotypes (-2.0, -0.3, -0.9 individuals L^{-1} respectively). Translating these per litre differences into total population abundances, *D. pulex* abundances in the high genetic diversity treatment ranged from being ~1400 individuals higher to ~360 individuals lower when compared to respective low diversity treatments.

Correlations with source habitat characteristics and life-history traits

The effect sizes of increased colonist quantity for each genotype were significantly, negatively

correlated to body size at first reproduction (Linear regression, $n = 9$, $R^2 = 0.46$, $P = 0.043$; Fig. 4.3b), with no significant relationship to genotype intrinsic rate of increase (Linear regression, $n = 9$, $R^2 = 0.004$, $P = 0.87$), habitat similarity of the genotype source lake (Linear regression, $n = 9$, $R^2 = 0.19$, $P = 0.24$), or genotype region of origin (ANOVA, $n = 9$, $R^2 = 0.003$, $P = 0.88$) (Fig. 4.3; see Appendix C: Table C3 and Fig. C1). The negative relationship between the effect size of increased quantity and body size was driven by the low effect of increased colonist quantity in genotype G, our largest genotype (Table 4.1). Removal of this observation rendered the relationship non-significant (Linear regression, $n = 8$, $R^2 = 0.12$, $P = 0.40$), but still negative ($r = -0.34$).

The effect sizes of increased colonist genetic diversity were significantly, negatively correlated to the intrinsic rate of increase for each genotype (Linear regression, $n = 9$, $t_{1,7} = 2.97$, $R^2 = 0.56$, $P = 0.021$; Fig. 4.3d), and this relationship was not driven by any particular genotype. There were no significant relationships between the effect sizes of increased genetic diversity and body size at first reproduction (Linear regression, $n = 9$, $R^2 = 0.02$, $P = 0.72$), habitat similarity (Linear regression, $n = 9$, $R^2 = 0.1$, $P = 0.4$), or region of origin (ANOVA, $n = 9$, $R^2 = 0.08$, $P = 0.45$).

Genotype persistence in high diversity populations

We detected significant differences among the genotypes in their persistence in high diversity populations (LRT, $n = 56$, $\text{Deviance}_{7,10} = 23.04$, $\phi = 0.71$, $P = 0.0017$). This result should, however, be interpreted with caution as the model was slightly underdispersed. Genotypes E, G and M were detected in all high diversity replicates (Table 4.2), with genotypes D, L, and O present in at least five out of seven replicates. The difference in presence/absence between genotypes seemed to be primarily driven by genotypes C and R. Both were present in less than half of the replicates (Table 4.2), suggesting a higher rate of extinction for these genotypes when in the high diversity treatments, compared to genotypes D, E, F, G, L, M, and O.

Discussion

Increased colonist quantity and genetic diversity had a generally positive effect on the success of *Daphnia pulex* in our experiment. Populations founded by more colonists, or by a greater diversity of genotypes, tended to have higher abundances than those founded by smaller or less diverse introductions. However, this effect varied among experimental genotypes, which exhibited a range of positive and neutral effects of increased quantity and genetic diversity. These results suggest that the importance of population size, and the mechanisms of quantity and genetic diversity through which it operates, could vary between populations and colonist pools. Some populations may benefit from a greater number of individuals, or greater genotypic diversity, while others may readily succeed even at extremely small population sizes. This potential for colonist identity to determine the value of quantity and genetic diversity must be taken into account both when experimentally assessing the importance of both factors for population success, and when determining how responsive a natural population or group of colonists might be to changes in their size.

Although increased colonist quantity had an overall positive influence on *D. pulex* success, the effect of this treatment appeared to differ between genotypes, and was related to differences in body size among genotypes (Fig. 4.3b). Genotypes that achieved higher body sizes at the time of first reproduction exhibited a lower effect of increased colonist quantity. This was potentially due to a size-dependent predator Allee effect. Our mesocosms were naturally colonized by *Chaoborus* (the larvae of the phantom midge), which is a gape-limited predator of *Daphnia*. Predators with a type II functional response, such as *Chaoborus*, can create an Allee effect because consumption asymptotes as prey density increases (Sinclair et al. 1998), creating a positive relationship between prey population growth and prey density (Gascoigne and Lipcius 2004). This has been experimentally shown to occur in *Daphnia-Chaoborus* systems (Kramer and Drake 2010). However, the strength of this Allee effect could vary with prey body size given that *Chaoborus* is gape-limited, and because larger-bodied *Daphnia* can be less vulnerable to *Chaoborus* predation (Pastorok 1981, Riessen and Young 2005). This could produce a reduced effect of increased

colonist quantity in larger-bodied *D. pulex* genotypes because they are less vulnerable to *Chaoborus* predation, and therefore less reliant on higher quantities for population success.

This potential for intraspecific variation in the benefits of increasing conspecific density has important experimental and practical applications. Firstly, in terms of experiments, a study that concludes that increasing quantity benefits population success may only have observed this result because of the particular individuals involved. There are already several examples that suggest the experimental conclusions of the importance of colonist quantity could differ between individuals and studies of the same species (e.g., Maron 2006, or Drake et al. 2005 versus Kramer and Drake 2010; discussed in the introduction). An additional example can be found by comparing two studies on introductions of *Tribolium castaneum* by Szűcs et al. (2014, 2017). Both experiments show that higher colonist quantities benefit establishment, but the strength of this effect differs between them. In the earlier experiment, all populations founded by two outbred colonists had ~50% establishment, and increasing quantity improved establishment to 100%. In the later study of the same species, the establishment success of two outbred colonists was much higher at ~75%. The benefits of increased quantity are therefore much greater in the earlier compared to the later study, even though both examine the establishment of the same species in a similar experimental environment. Similarly, one of our earlier studies (Sinclair and Arnott 2017) into the effects of colonist quantity on *Daphnia pulicaria* (the same species complex as *D. pulex*) found no influence of colonist quantity on abundance when comparing introductions of two versus eight colonists (though genotype/phenotype was not controlled in this experiment). However, in our present study we observed a generally positive effect when *D. pulex* quantities were increased. There are likely numerous factors that differ between these experiments, but their results, in combination with those from this study, indicate that the particular set of individuals introduced could be partly responsible for whether an experiment does or does not observe an effect of colonist quantity. Secondly, in terms of practical applications, the potential for intraspecific variation in the importance of quantity, the control of which is central to many population management strategies (Shaffer 1981, Montalvo et al. 1997, Blackburn et al.

2015), has implications for how populations or introductions are managed. Depending upon the particular individuals in the population, one group might readily succeed with few conspecifics, while a different group from the same species might fail. Thus, depending upon the population involved, management efforts to control the quantity of individuals may be much less or more effective than expected, and the optimal management strategy may also vary from one population to another.

The effect of increased colonist genetic diversity differed between genotypes, and appeared to be related to differences in their intrinsic rates of increase (Fig. 4.3d). The size of the genetic diversity effect was likely driven by differences between the r of a genotype in isolation compared to the average r of all genotypes in mixture. Monoclonal populations founded by genotypes with a high r (e.g., as observed in genotypes M or R from our life-history trials, Table 4.1) tended to perform equally to, or better than, the more diverse introductions, and thus did not benefit from mixture with other genotypes. Conversely, genotypes with a low r (e.g., C or F) tended to perform comparatively poorly in isolation, and thus overall performance of the species improved when the colonist pool was instead composed of multiple genotypes. These results are similar to other studies that have indicated that the effect of increasing genetic diversity could depend upon the performance of particular genotypes (e.g., Vellend et al. 2010, Erfmeier et al. 2013), highlighting how the identity of the individuals in a small or low diversity population could determine its likelihood of success. If the population is composed of high-performing individuals (in the case of our experiment those with a high r), then higher genetic diversity is not necessarily required for success, and including other, poor-performing genotypes may actually reduce performance.

Interestingly, there was some evidence for negative interactions between our genotypes in the high diversity treatment. While genotypes C and R persisted in all single colonist and nine colonist monoclonal populations, both went extinct in over 50% of the high diversity replicates (Table 4.2). Competitive exclusion between *Daphnia* clones can be driven by differences, for example, in their growth rates (Loaring and Hebert 1981), behaviour (Reede 1997), or resource specialization (Weider et al. 2005). Genotype C exhibited the lowest growth rates in our life-history trials, which indicates potential

competitive exclusion when mixed with higher growth rate genotypes, but this does not explain the higher rate of extinction in genotype R (one of our highest growth rate genotypes). While the exact reason driving these extinctions is unclear, these results highlight that although higher genetic diversity may have overall positive effects on population success, it can simultaneously negatively impact the persistence of particular genotypes due to intraspecific competition. Higher diversity in our experiment had generally beneficial effects because it ensured the highest performing genotype was always present in each introduction, but was also detrimental to the survival of genotypes C and R.

Our experiment potentially underrepresents the value of colonist quantity and genetic diversity for buffering against extinction. Our conclusions regarding the effects of both are based only on density because extinction was generally low in our experiment. This is not unexpected for *Daphnia*, which can colonize natural environments with just 1 or 2 individuals of a single genotype (e.g., Hairston et al. 1999, Haag et al. 2005), but the benefits of increased quantity or genetic diversity for extinction may have been more apparent with a different study design. For example, colonist quantity may have little effect on extinction if all environments are highly suitable (Duncan 2016). Our mesocosms experience natural daily fluctuations in temperature, light, and weather, but our experiment only spanned a single season, and the mesocosms are spatially homogeneous by design. Introduced *D. pulex* may therefore have had little trouble establishing and persisting in our experiment, and a more heterogeneous environment may have been required to better reveal the value of colonist quantity for buffering against extinction. Similarly, a longer duration experiment may be required to properly assess the benefits of increased genetic diversity. The relative abundance of *Daphnia* genotypes can change seasonally as local abiotic and biotic conditions change (e.g., Stibor and Lampert 2000), indicating that certain genotypes might not be suited to surviving at certain times of year. Some of our monoclonal populations may have therefore been unable to persist across seasons, and a longer-term experiment would have potentially provided a more comprehensive assessment of the value provided by higher diversity introductions. However, altering our experimental design may also have done little to influence our results. Effects of quantity on *Daphnia* extinction have

been observed in laboratory microcosms (Kramer and Drake 2010), which are undoubtedly more homogeneous and suitable for establishment than field mesocosms with a resident community of competitors and predators. A multi-season experiment may also have done little to change our observed effects of genetic diversity as single genotype *Daphnia* populations in natural systems are able to persist across seasons and even years (Hairston et al. 1999, Haag et al. 2005). Additionally, although our results are limited only to density, our broader conclusions regarding the role played by identity in determining the importance of quantity or genetic diversity are still applicable to other metrics of successful colonization, such as establishment and extinction. Establishment, spread, and persistence are all affected by mechanisms, such as Allee effects (e.g., Kadam and Velicer 2006), whose severity can vary intraspecifically and thus could depend upon the identity of the individuals present in a population or colonist pool. It would be beneficial to have more experiments that investigate the potential influence of identity in determining the importance of increased colonist quantity or genetic diversity for different metrics of population success, and how this influence may vary depending upon habitat heterogeneity and temporal scale.

Implications

Management efforts to promote or prevent population success commonly focus on manipulating population size. Research on the role of quantity and genetic diversity in population success is therefore key for informing practical control efforts. Our results shed light on how the identity and the traits of the individuals present in a population or colonist pool could, at least partly, determine how important population size is for mediating the demographic and genetic factors involved in population success. While, in general, populations founded by a higher quantity or genetic diversity of colonists performed better than smaller or less diverse populations, this effect varied widely between colonist genotypes. Additionally, our results suggest, along with other similar studies (e.g., Vellend et al. 2010), that the benefits provided by increased quantity and genetic diversity could be determined by the traits of the

particular individuals involved. These conclusions regarding the role of identity are not limited to asexual aquatic zooplankton, and are potentially applicable to any aquatic, terrestrial, asexual, or sexual species. We tested the role of identity in *D. pulex* by manipulating the set of traits possessed by different colonising individuals. Individuals from any species and study system can be characterized by a unique phenotype, which we show could determine their reliance on higher quantity or genetic diversity for population success. Future work determining the potential variation in individual performance that may exist within any population, and that manipulates the inclusion of such individuals in future research on colonization and extinction, will be crucial both to our search for general principles about the value of quantity and genetic diversity, and to efforts to control species establishment, persistence, and spread.

Table 4.1 Origin and trait values from the 21-day life-history trials for each *Daphnia pulex* genotype involved in the experiment. Trait values were determined based on body size (mm) at which each genotype released their first brood, age class (in days) at which each genotype released their first brood, the average number of offspring produced per day, and survivorship per age class.

Genotype	Source lake	Origin region	Size at first reproduction (mm)	Age at first reproduction (days)	Net reproductive rate (R_0)	Intrinsic rate of increase (r)
C	Crozier	Muskoka	1.97	12.4	8.4	0.15
D	Dyson	Muskoka	1.96	11.8	18.0	0.20
E	Elbow	QUBS	1.83	10.3	12.1	0.18
F	Fifteen Mile	Muskoka	1.88	11.9	11.7	0.16
G	Grandview	Muskoka	2.25	11.2	31.7	0.23
L	Lindsay	QUBS	1.81	10.0	15.8	0.21
M	McKay	Muskoka	2.11	9.2	40.6	0.28
O	Round	QUBS	2.01	9.9	25.0	0.24
R	Ridout	Muskoka	1.96	10.8	20.4	0.21

Table 4.2 Presence/absence (1/0) of unique alleles for each *Daphnia pulex* genotype determined across 12 microsatellite loci (see Appendix C: Table C4) in each high-diversity mesocosm (replicates 1-7), along with the proportion of all replicates in which each genotype was determined to be present.

Genotype	Unique alleles	Presence/absence of unique alleles (1/0)							Proportion present
		1	2	3	4	5	6	7	
C	1	0	0	0	1	0	1	0	0.29
D	1	1	1	1	1	0	1	1	0.86
E	1	1	1	1	1	1	1	1	1.0
F	0	-	-	-	-	-	-	-	-
G	1	1	1	1	1	1	1	1	1.0
L	1	1	0	1	0	1	1	1	0.71
M	9	1	1	1	1	1	1	1	1.0
O	1	0	1	1	1	1	1	1	0.86
R	2	0	0	1	1	1	0	0	0.43

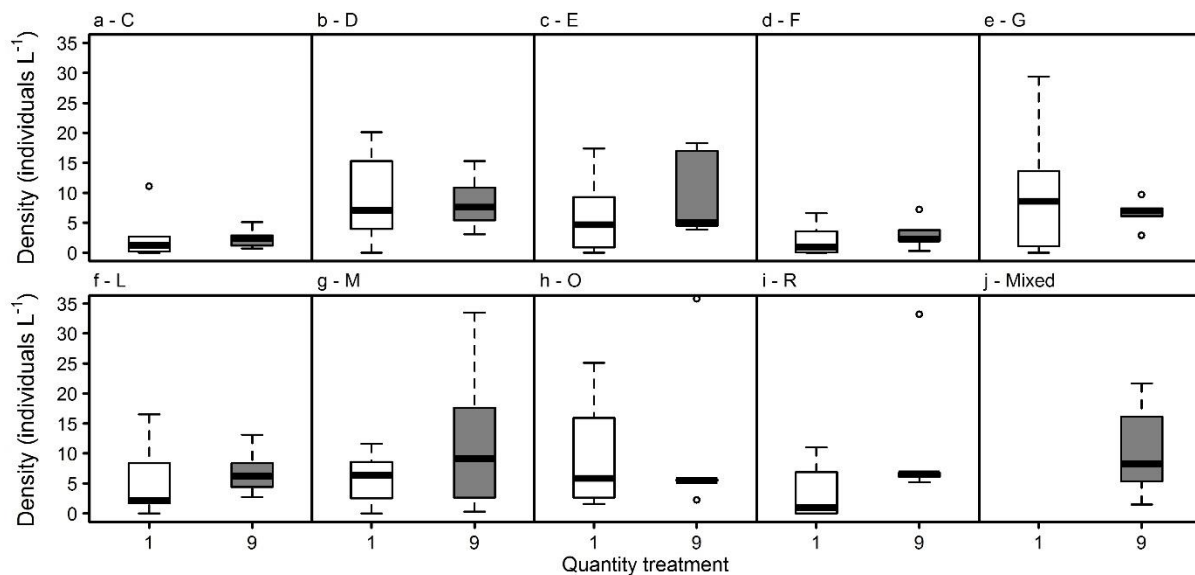


Figure 4.1 Density (individuals L^{-1}) of (a-i) single *Daphnia pulex* genotypes (low diversity) and (j) mixed genotype (high diversity) populations for introductions of 1 colonist (unshaded, low quantity) or 9 colonists (shaded, high quantity) at the conclusion of the experiment (Low quantity/low diversity: $n = 10$; High quantity/low diversity: $n = 5$; High diversity: $n = 7$). Bottom and top edges of boxes represent the first and third quartiles respectively, with a dark, horizontal line as the median. Whiskers encompass data points that lie within 1.5 times the inner quartile range, data outside this range are shown as separate points.

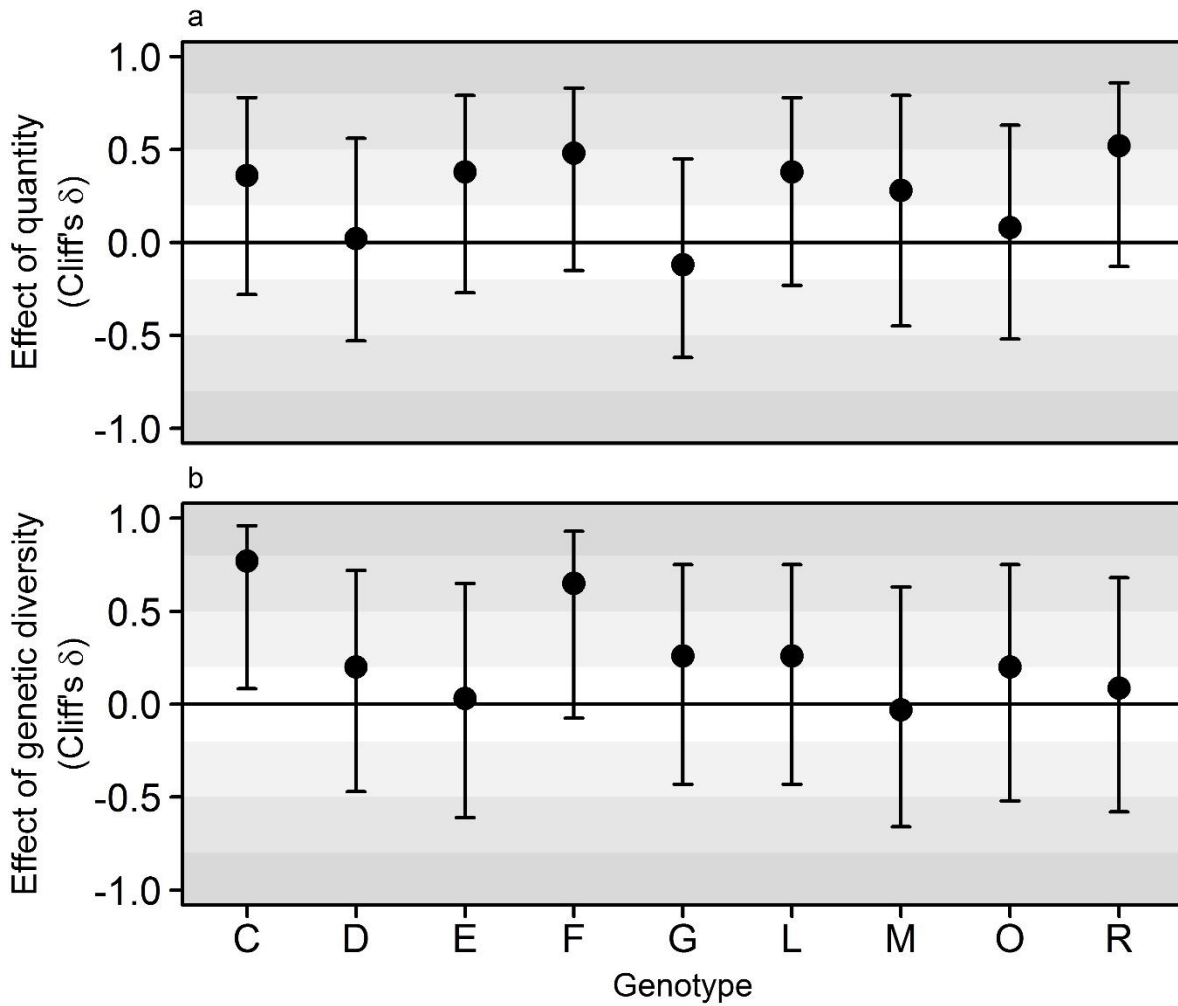


Figure 4.2 Cliff's δ (value \pm CI) for the treatment effects of (a) quantity and (b) genetic diversity within each genotype. Blocks of grayscale background color are used to indicate the rule of thumb effect size threshold regions. White background indicates regions of no treatment effects, and background colors become progressively darker at the effect size thresholds for small (Cliff's $\delta = 0.2$ or -0.2), medium (Cliff's $\delta = 0.5$ or -0.5), and large (Cliff's $\delta = 0.8$) effects.

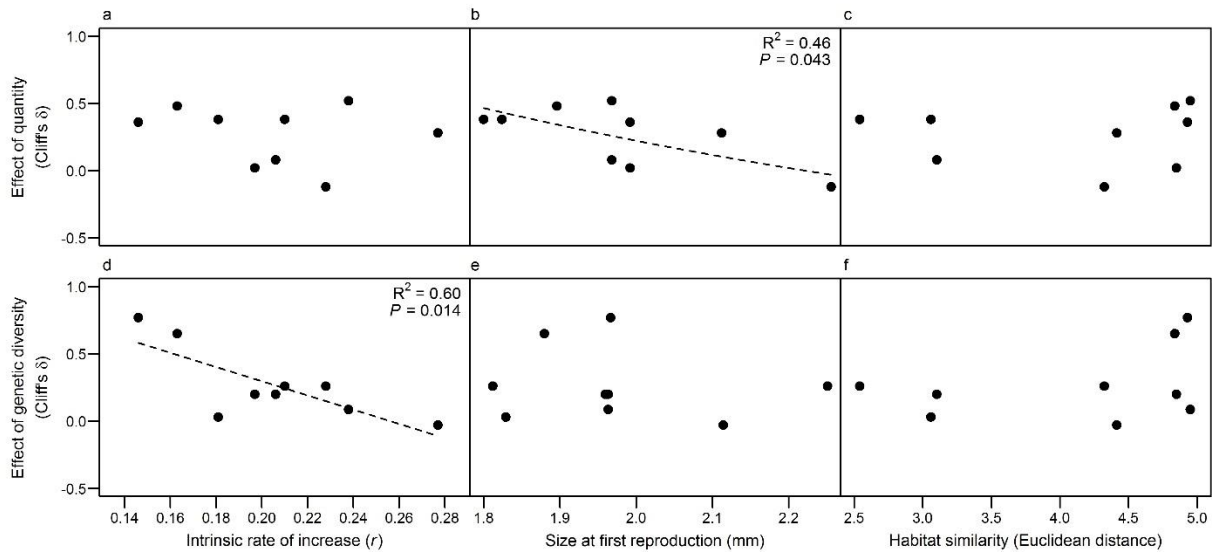


Figure 4.3 Relationships between the effect sizes (Cliff's δ) of (abc) quantity and (def) genetic diversity for each genotype (Fig. 4.2) and their respective intrinsic rates of increase (r), mean body sizes at first reproduction (mm, Table 4.1), and habitat similarities (Euclidean distance). In (c and f), higher Euclidean distances represent greater dissimilarity between the water chemistries of the genotype's source lake and Opinicon Lake (see Appendix C: Table C1). Note the dotted lines in (b) and (d), which represent the predicted linear relationship between their respective effect sizes and life-history traits.

Chapter 5

A framework for predicting which individuals and species are introduced as non-natives

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Formatted for

Diversity and Distributions

Abstract

Aim

Understanding the processes that determine which non-native organisms are likely to be introduced is essential for assessing the risk of invasion, and for predicting the quantity and identity of individuals that will arrive. However, little is known of the mechanisms acting to filter non-native organisms prior to arrival. This manuscript outlines a framework that can be used to understand and investigate the unique, pre-introduction processes of human-mediated transport that determine which subset of individuals are likely to enter, survive, and exit human vectors.

Location

Global

Methods

Concept and literature review

Results

We propose that the subset of non-natives eventually introduced are determined by two primary filtering mechanisms: i) the characteristics of organisms, and the way in which these characteristics are shaped by and interact with their environment; and ii) the properties, movement, and behaviour of human vectors.

We review how species traits, environmental conditions, and ecological interactions can filter organisms between each pre-introduction stage of non-native transport. Additionally, we apply a modified version of the movement ecology framework to human movements, and review the mechanisms involved in how human behaviour and movement patterns determine which subset of individuals are likely to transition from one pre-introduction stage to the next.

Main conclusion

Conceptualising the mechanisms involved in non-native transport must, by nature, integrate the biological- and human-based mechanisms that act in concert to filter non-native individuals between each stage of the movement process. The stage-and-filter based framework we outline and review serves to distill down the human-mediated transport process to only its most critical components, and provides a simple approach to elucidate the relevant mechanisms involved, and to provide initial estimates of introduction risk in any given system.

Introduction

An integral component in the prevention and management of biological invasions is the assessment of whether a non-native species will become invasive (Andersen, Adams, Hope, & Powell, 2004). High-risk invaders are those deemed likely to be introduced, to establish, spread, and have damaging impacts, and resources are primarily allocated towards their control. Determining which mechanisms allow non-natives to pass through the stages of the invasion process, from initial transport to subsequent impact, is therefore a central theme throughout invasion ecology. However, much of invasion research (Floerl & Inglis, 2005; Puth & Post, 2005) and most major theories of invasibility (e.g. ‘fluctuating resources’, ‘enemy release’, ‘biotic resistance’; Catford, Jansson, & Nilsson, 2009; Jeschke, 2014) focus on uncovering the mechanisms at work after the transport and introduction of non-natives has occurred. While this post-arrival focus has provided valuable insight into the factors that can drive successful invasions, it also hampers efforts to predict invaders, and our general understanding of invasion dynamics, as it ignores key mechanisms occurring prior to non-native arrival that lead to successful invasion. For example, the number of arriving species (colonisation pressure) and individuals (propagule pressure), along with their respective traits and standing genetic diversity, are all useful indicators of eventual non-native establishment and spread (Rejmánek & Richardson, 1996; Lockwood, Cassey, & Blackburn, 2009; Forsman, 2014; Blackburn, Lockwood, & Cassey, 2015). Each of these

factors is, however, an end-product of the *pre*-introduction stages of invasion. Therefore, they cannot be predicted without examining how the pre-introduction filters of the invasion process determine which species and individuals ('propagule bias'; Colautti, Grigorovich, & MacIsaac, 2006) are likely to be introduced (Fig. 5.1). Improving our ability to assess invasion risk, and our overall grasp of why and how species establish non-native populations, necessitates a deeper understanding of the mechanisms involved in the pre-introduction stages of invasion.

The call for greater focus on the pre-arrival invasion process is not new (e.g. Carlton, 1996; Ruiz & Carlton, 2003; Colautti & MacIsaac, 2004; Puth & Post, 2005), and efforts to incorporate information on what occurs prior to non-native introduction have been growing over the last two decades (e.g. Colautti & MacIsaac, 2004; Carlton & Ruiz, 2005; Hulme et al., 2008; Wilson, Dormontt, Prentis, Lowe, & Richardson, 2009; Essl et al., 2015; Hulme, 2015). This has added substantially to our ability to prevent species invasions, such as enabling the categorisation of certain transport pathways as 'riskier' than others based on known differences in the quantity and identity of species and individuals they transport. But it does not explicitly address the mechanisms driving these differences. Which subset of species or individuals are likely to be transported and why? What determines who does and does not survive? How do we predict which of the surviving individuals will be introduced? These are key questions that we currently cannot answer, but that must be addressed to better predict introduction risk and further our understanding of the drivers of species invasions.

An integrated framework for the pre-introduction invasion stages

We seek to build on past research on the early stages of the invasion process, and to further the predictive capabilities of invasion science, by providing an integrative framework of the mechanisms that determine the likely quantity and identity of non-natives introduced to any one location, given a particular invasion pathway and transport vector. We propose that the subset of non-natives eventually introduced are determined by two primary filtering mechanisms: i) the characteristics of organisms, and the way in

which these characteristics are shaped by and interact with their environment; and ii) the properties, movement, and behaviour of human vectors. In reviewing the first mechanism, we discuss how species traits, environmental conditions, and ecological interactions contribute to the pre-introduction filters of uptake, survival, and release developed by Colautti and MacIsaac (2004) (the biological side of Fig. 5.2). For the second mechanism, we extend a well-accepted framework on movement ecology to human vector movements (the human vector side of Fig. 5.2), which affect the quantity and identity of transported non-natives. We advocate that human vector movement patterns can be understood by investigating *why* a vector is moving, *how* it is moving, and *when* and *where* is it moving (similar to Carlton & Ruiz, 2005), along with the factors external to the vector that might be affecting the movement process itself. Notably for invasion scientists, these questions can be asked of each pre-introduction filter to determine which individuals are likely to successfully transition through, and exit from these vectors. This approach provides simple questions for uncovering the mechanisms driving human movements that can be easily understood and applied by students, researchers, or managers in any scientific discipline. It also integrates new research on non-native introduction with similar work in the fields of movement ecology (e.g. Tsoar, Shohami, & Nathan, 2010), transport geography (e.g. Auffret, Berg, & Cousins, 2014), and human mobility (Meekan et al., 2017). By combining these biological and human movement frameworks, we can better understand the primary mechanisms acting to filter non-native introduction, and improve our ability to predict invaders and assess risk.

Intentional and unintentional pathways

Mechanisms by which non-natives are introduced can be divided into two broad types: ‘intentional’ and ‘unintentional’ (Alpert, 2006; Hulme et al., 2008). Intentional pathways are responsible for the movement of intentionally collected organisms, such as pets, fishing bait, game animals, biocontrol organisms, agricultural crops, and decorative plants. Conversely, unintentional vectors are responsible for the inadvertent collection and movement of organisms. This occurs, for example, when

propagules are accidentally stowed in truck or airplane cargo; are moved within water flowing within canals and waterways; or attach to clothing, cars, livestock, bikes, recreational boats, or fishing gear. These pathways can be further subdivided into six categories (release, escape, contaminant, stowaway, corridor and unaided; Hulme et al., 2008) based on the method by which transported non-native individuals are eventually introduced. Our framework focuses on how biological- and human-based processes interact to bias non-native introduction, and therefore applies only to the release, escape, contaminant, and stowaway pathways as these involve associated human vectors (e.g. clothing, cars, boats, airplanes).

Part I – The biological processes that influence introduction

Entrance into, survival within, and exit from a human vector is influenced by the characteristics of individuals, populations, and species, and how these characteristics are affected by their abiotic and biotic environment. A key component of our framework is therefore recognising that transported non-natives can be equally responsible for driving differences in which species or individuals are likely to transition through the uptake, survival and release filters of non-native transport.

Uptake filter

The likelihood that a species or individual enters a vector is affected by its distribution and phenotype. Species, populations, and individuals with distributions that overlap human movement networks, or that closely associate with urban habitats, are more likely to enter human vectors (Carlton, 1996; Floerl & Inglis, 2005; Liebhold et al., 2016). Distribution can also bias uptake geographically and taxonomically towards species that are, for example, abundant and widespread (Cassey, Blackburn, Russell, Jones, & Lockwood, 2004; Gravuer, Sullivan, Williams, & Duncan, 2008).

Uptake can be influenced by phenotype, including morphological, behavioral, and life-history traits. For example, individuals and species with seed structures that can attach to clothing, those that are

bold or explorative, and those that show high reproductive output or small body size could have a higher probability of uptake (Colautti et al., 2006; Chapple, Simmonds, & Wong, 2012; Auffret & Cousins, 2013). Similarly, species intentionally transported and introduced as non-natives often have specific traits that increase their probability of uptake. These traits are tightly associated with aesthetics, utility, novelty, cultural value, reproductive capabilities, susceptibility to capture, or the suitability of a species for an intended recipient environment (Ruiz & Carlton, 2003; Cassey et al., 2004; Alcaraz, Vila-Gispert, & García-Berthou, 2005; Mack, 2005; Thuiller, Richardson, Rouget, Procheş, & Wilson, 2006; Theoharides & Dukes, 2007).

Distribution and phenotype are not constant. Predicting the quantity and identity of non-natives likely to enter a vector requires assessing the abiotic and biotic context in the source environment. Organisms from source locations where the abiotic and biotic conditions increase their propensity or ability to enter human vectors, which can occur due to variation in source climate and weather (e.g. Enge, 2005; Ansong & Pickering, 2014), will have a higher probability of uptake. Source environments also vary spatially and temporally in their habitat quality and conspecific density. Quality and density can influence the expression of individual phenotypes, such as attachment structures, specific body sizes, and bolder behaviours (Matthysen, 2005; Benard & McCauley, 2008; Cote, Clobert, Brodin, Fogarty, & Sih, 2010), that are potentially associated with a higher likelihood of uptake.

Survival filter

The survival filter weeds out organisms that are unable to tolerate conditions experienced during transport. The chance of surviving transport therefore depends upon individual phenotype, which can also be affected by abiotic and biotic conditions in the natal habitat (Bell & Stamps, 2004; Daws et al., 2006; Benard & McCauley, 2008). Examples of phenotypic traits that could be advantageous for surviving challenging abiotic vector conditions, or avoiding methods employed to kill transported non-natives, include morphological structures that reduce desiccation (Franchi et al., 2011), behavioral flexibility

(Mason, 2010; Chapple et al., 2012), and the ability to outlast harsh environmental conditions through dormancy, possessing heat-shock proteins, or having a large enough mass to sustain life functions for long stretches of deprivation (Kobelt & Nentwig, 2008; Franchi et al., 2011; Zerebecki & Sorte, 2011). Individuals that possess morphological, behavioral, life-history, or physiological traits that confer ‘hardiness’, or that reduce sensitivity to the stress of movement, handling, and captivity will have a higher likelihood of persisting within harsh vector conditions.

Biotic interactions affecting transport conditions are not commonly studied, but negative interactions, such as predation, parasitism, and competition, are all likely to occur in vectors that transport multiple organisms (e.g. ballast water; Galil & Hülsmann, 1997). Shared captivity can also cause crowding stress, a primary source of mortality in intentionally transported species (Teixeira, de Azevedo, Mendl, Cipreste, & Young, 2007). Conversely, positive biotic interactions, such as mutualism and facilitation, might improve survival during transit. For example, hull fouling species that are resistant to toxic paints can facilitate the attachment and survival of other, less tolerant hull fouling organisms (Floerl, Pool, & Inglis, 2004). Phenotypes that can withstand the negative biotic interactions, or that are better able to take advantage of the positive ones, are therefore more likely to survive.

Release filter

The likelihood that an individual exits a vector is also affected by its phenotype. In the case of unintentional transport, the point of exit could potentially occur anywhere along a vector’s route, and the subset of organisms that exit can be filtered by both active or passive mechanisms. An organism not trapped within a vector can actively choose when and where it leaves. This decision can be affected by behavior (boldness or exploration, Chapple et al., 2012) or habitat selectivity (Clobert, Le Galliard, Cote, Meylan, & Massot, 2009). Similarly, an individual can affect its likelihood of departure passively through morphology (e.g. seed structure; Ansong & Pickering, 2014) and life-history (e.g. timing of larval release; Acosta & Forrest, 2009).

For intentional transport, phenotype can also affect the likelihood of release or escape. For example, life-history traits are related to the escape of plants from cultivation (Dehnen-Schmutz, Touza, Perrings, & Williamson, 2007). Behavior and stress physiology also relate to the probability of escape. Individuals that show greater fear and stress responses towards humans could be more likely to escape captivity than those that are more docile (e.g. Cabezas, Carrete, Tella, Marchant, & Bortolotti, 2013). How phenotypes are influenced by their environment can similarly affect the likelihood of departure, escape, or release. The development of phenotypes associated with the ability or propensity to exit, such as body size or settlement preferences, is influenced by the quality and conditions in the source habitat from which individuals originated (Matthysen, 2005; Stamps, 2006; Benard & McCauley, 2008; Bonte et al., 2012). Extended periods of transport and captivity in the recipient environment can also acclimate individuals to stress, reducing their likelihood of expressing behaviours related to escape (Cabezas et al., 2013).

The framework so far

We have outlined the first set of mechanisms structuring the pre-arrival filters of the invasion process. Predictions of which, and how many, individuals will be introduced are based on individual distribution, phenotype, and the ways in which these attributes interact with the abiotic and biotic environment of the source region, transport vector, and destination habitat. The second part of our framework compliments these ideas and must also be considered to accurately predict the likelihood of non-native introduction. Here we describe mechanisms of human movement and behaviour that also contribute to the filters of the pre-introduction process.

Part II - human movement ecology

Anthropogenic species invasions are inextricably linked with human movements. Therefore, a mechanistic understanding of the drivers and patterns of human movement is necessary to predict how

many and which non-natives are likely to be introduced. We suggest that the movement ecology framework of Nathan et al. (2008) can be modified for use in the context of human movements, and provides an easily understood, generally applicable, and targeted approach for assessing human-based mechanisms that filter organisms in each stage of non-native transport. This framework employs four broad questions to predict individual non-human movement and behavior. First, an individual must initiate movement (why move?), a process controlled by its 'internal state'. Second, an individual must have some method of movement (how to move?), termed 'motion capacity'. Third, an individual can select or influence the timing and location of movement (when and where to move?), described as its 'navigation capacity'. And fourth is the recognition that numerous factors external to an individual, such as its environment, can affect movement, termed 'external factors' by Nathan et al. (2008).

Applying the movement ecology paradigm to human vectors requires a consideration of why, how, where, and when these vectors move, and the external factors affecting their movements. For 'why', human movements are largely motivated by needs for work, travel, recreation, or visitation (Bell & Ward, 2000), and by consumer demand and cultural connections (Perrings, Dehnen-Schmutz, Touza, & Williamson, 2005; Dehnen-Schmutz et al., 2007; Hulme, 2009; Helmus, Maler, & Losos, 2014). How vectors move varies widely, and can refer to individual people (clothing and footwear), vehicles (boats, cars, airplanes), or human-associated vectors, such as livestock and equipment (Wichmann et al., 2009; Wilson et al., 2009; Auffret et al., 2014). Vectors can also be divided into subcomponents (e.g. outer surfaces versus interior compartments), each of which can transport a particular subset of organisms (Carlton & Ruiz, 2005). Where and when vectors move depend on routes and timings of human movements, largely following movement networks between locations. For example, movements associated with trade follow routes dictated by trade networks and connections between metropolitan areas (Ruiz & Carlton, 2003; Kaluza, Kölzsch, Gastner, & Blasius, 2010; Colunga-Garcia, Haack, Magarey, & Borchert, 2013), while non-trade movements, such as for travel or work, are associated with airline, road, and street networks (Guimerà, Mossa, Turtschi, & Amaral, 2005; Hillier & Iida, 2005).

Some external factors will mirror those for non-human individuals, such as weather, which influences motivations for movement, how people move, and when and where they go (Cools & Creemers, 2013 and references therein). However, human vectors also have unique external factors, such as (1) changes in technology, which affects the methods and speed of movement (Carlton, 1996; Carlton & Ruiz, 2005); (2) economics, which can change vector movement patterns at the whims of the market (Perrings et al., 2002; Duggan, Rixon, & MacIsaac, 2006; Dehnen-Schmutz et al., 2007; Hulme, 2009); and (3) policy and regulations, which influence transport methods and directionality (Perrings et al., 2005; Chivers, Drake, & Leung, 2017). Changes in these external factors can affect why humans are moving, how they move, and where and when they move, acting to close old transport pathways and open new ones.

Adapting movement ecology to invasion vectors allows us to clearly define and describe human movement. It is, however, not enough to simply recognise that humans can move for different reasons, via different methods, following different routes and timings, all of which are influenced by external forces. Crucially, we must also consider how each aspect of human movement ecology interacts with species biology to filter organisms at each pre-introduction stage of the invasion process.

Uptake filter

Human movement influences species uptake by biasing which species and individuals have the opportunity to enter human vectors. Firstly, our motivations for movement (why move?) can create uptake bias if vectors are moving to obtain particular species. Our desires to enhance our environment, recreate, or consume particular foods (Mack et al., 2000) result in a specific subset of global species diversity that are utilised to satisfy these motivations. For example, intentional pathways are often responsible for transporting freshwater fish, amphibians, reptiles, and plants, whereas arthropods and marine invertebrates are more likely to move within unintentional pathways (Lockwood, Hoopes, & Marchetti, 2013). There are often clear biases for intentionally transporting species within specific

Families or Orders that are, for example, hunted as game, used as bait, or are attractive within home aquaria (Lockwood, 1999; Duggan et al., 2006; Drake & Mandrak, 2014). Human preferences based on utility, desired traits, or market value can also create an uptake bias when heavily advertised species become more valued by consumers and are thus imported at higher volumes (Duggan et al., 2006; Drew, Anderson, & Andow, 2010).

The vector responsible for transporting non-native species (how to move?) can also bias which individuals and species are entrained, allowing us to predict which organisms are likely to be collected, such as aquatic vectors probably collecting aquatic organisms. However, more refined predictions can be made for how the method of movement might bias collection by dividing vectors into subcomponents. Ship ballast, for example, can be divided into the ballast tank walls, the ballast water column, the sediment that settles to the bottom of the tank, and the biofilm that forms on all surfaces, with the species likely to be collected differing between each subcomponent (Carlton & Ruiz, 2005).

Finally, the quantity and subset of species potentially encountered by a vector can be estimated by predicting when and where vectors tend to move, in what temporal window they tend to be active, and their probable destination. Sources, routes, and destinations with a higher volume of use, such as commonly used movement networks or popular destinations, are more likely to transport a greater quantity and diversity of propagules (Floerl, Inglis, Dey, & Smith, 2009; Hulme, 2009; Auffret & Cousins, 2013). Predicting the timing and routes of vector movement, and thus which organisms are likely to overlap with and become entrained within a vector, can be accomplished through a variety of tools. One well-studied predictive tool in this regard is a gravity model, whereby the probability of human movement between two locations is determined by the distance (represented by geographic distance or cost of movement) and size (based on GDP, population, attractiveness) of the sites (e.g. Leung, Bossenbroek, & Lodge, 2006). The likelihoods of when and where a vector will move can also be influenced by the number of commercial opportunities available along a particular travel route (the intervening opportunities model; Stouffer, 1940), or by a diverse set of factors that fully encapsulate the

‘attractiveness’ of a destination beyond that of simple size (often described using a random utility model; Block & Marschak, 1960). Research on the predictability of human movements has progressed rapidly thanks to the recent explosion of trackable data, such as from mobile phones (e.g. González, Hidalgo, & Barabási, 2008; de Montjoye, Hidalgo, Verleysen, & Blondel, 2013). The ecological applications of big data on human movements are only just beginning (Meekan et al., 2017), but could be applied to predicting not only where and when transport vectors tend to move, but also the set of species, and numbers of individuals, these vectors are likely to collect along their way.

Survival filter

Human movement ecology plays an integral role in which individuals are likely to survive transport by dictating the abiotic and biotic conditions they experience and, in the case of captive organisms, the duration of the transport process itself. Survival may be easier if movement is motivated by the intentional desire to collect organisms, as intentional vectors often provide conditions that are as optimal for survival as possible. The likelihood that an individual survives could thus depend upon whether vectors are driven by motivations for intentional collection, or if transport is an unintended consequence of other motivations driving vector movements. However, both intentional (e.g., Teixeira et al., 2007; Carrete et al., 2012) and unintentional (e.g., Wonham, Walton, Ruiz, Frese, & Galil, 2001) motivations can also produce high mortality in transported organisms.

Human movement ecology also plays an integral role in which individuals are likely to survive transport by dictating the conditions, directionality, and tempo of the transport process itself. Vector types and their subcomponents can differ widely in their transport conditions (e.g. Verling et al., 2005; McNeill et al., 2011), ranging from harsh to mild. Vectors, and vector subcomponents, that inflict more difficult transport conditions are likely to have lower propagule pressure and higher bias when compared to milder ones. Vectors that follow a more direct or shorter transit route will also tend to successfully introduce more organisms because individuals do not have to survive stressful conditions for long periods (Carlton

& Ruiz, 2005; Teixeira et al., 2007). Similarly, faster conveyance methods will likely improve survival (Carlton, 1996; Carlton & Ruiz, 2005). The directionality of vector movement also determines the conditions transported organisms are exposed to, and the duration of transport. Localities differ in their climatic conditions, which affects the environment organisms must be capable of surviving during transport, and regulatory methods to remove or kill transported organisms (e.g. variation in border inspection strategies between European countries; Bacon, Bacher, & Aebi, 2012). Movement pathways that travel through milder regions, at more benign times of year, or through areas with fewer regulations for transported organisms will have a weaker survival filter, and a subsequently greater chance of introducing non-natives.

Release filter

Vector movement ecology influences the likelihood of release through its influence on the ease with which individuals can exit. Firstly, the reasons driving movement can determine the strength of the release filter. For example, purposeful release is the most common way that individuals transported to become game animals or for biocontrol exit their transport vector (Hulme et al., 2008), resulting in a weak release filter, while species transported for agriculture, ornamentation or the pet trade exit human control primarily via escape (Hulme et al., 2008), leading to a stronger release filter.

The method selected for movement can also affect the risk of introduction by determining how far from the source location individuals are likely to be released, and the regulations that a route may be subjected to. Some vector types are slow, or move shorter distances, depositing most transported individuals closer to their source environment (e.g. footwear or clothing, Fig. 5.3a), while others travel quickly over longer distances, increasing the probability that individuals are deposited outside of their native range (e.g. vehicle vectors, Fig. 5.3b). If most propagules are deposited close to the source habitat, then non-native introduction is unlikely. This likelihood increases as vector speed and distance traveled increases. Some vectors may even trap transported individuals until the destination has been reached, such

as may occur for air or ship cargo. In such cases, the likelihood of introduction would be low close to the source, when transported organisms are confined and have few opportunities for release, but would dramatically increase once the vector reaches its destination (Fig. 5.4). In addition to deposition patterns, methods of movement also differ in their regulations. Searches for non-native species are becoming more common at airports, ship ports, and borders, along with inspections of intentional pet and horticultural pathways (Hulme, 2011). Organisms transported via movement methods that are subject to strong regulation and inspection might be capable of surviving transport (overcoming the survival filter), but are intercepted upon arrival and thus never successfully exit human control (failing at the exit filter). Movement methods that are more regulated may thus have a lower likelihood of release, compared to less regulated methods such as local vehicle or foot traffic.

In terms of where and when vectors move, the routes via which vectors travel vary in their conditions and regulations, which in turn affects the probability of release. For example, wet weather can increase the propensity with which seeds are deposited from vehicles (Taylor, Brummer, Taper, Wing, & Rew, 2012), and extreme weather events can be responsible for the escape of confined species (as is suspected to have occurred with *Python bivittatus*, the Burmese python; Engeman, Jacobson, Avery, & Meshaka, 2011). Routes through or into locations in which the conditions promoting release are more common, such as wetter regions or those with a higher frequency of extreme weather events, will subsequently have a higher likelihood of releasing transported organisms. Similarly, transport routes through regions with little or no regulations prohibiting release of non-native species, or during times that inspections do not occur (e.g. outside peak activity periods), will have a weaker release filter, compared to routes that encounter more intensive quarantine or inspection.

The framework so far, part II

We have now outlined the second set of broad, human-based mechanics that contribute to the uptake, survival, and release filters. The reasons why people move, how they move, where/when they

move, and the external factors that affect each, combine to drive the patterns and behaviours of human movements. By questioning the motivations, methods, directionality, and timing of human movements, we can improve predictions of which subset of organisms are likely to be moved, the conditions experienced during transport, and the probability of release.

Using the framework

The key to applying our framework is to use its various sections as a guide to profiling which organisms and vectors likely pose the highest risk in a given pathway. To illustrate this process, we apply our framework to two example invasion pathways, the first intentional and the second unintentional, that involve vector-mediated transport.

Example 1 – Intentional transport of pet reptiles, Fig. 5.5a

The trade in pet reptiles has grown exponentially since the 1920s (Kraus, 2008). Reptiles sold as pets are initially either captured in the wild, bred in captivity, or ‘ranching’ (first caught in the wild, then maintained in captivity). At first glance this intentional transport for the pet trade seems to be a predominantly human-controlled process, with no role for the organisms involved. People collect or rear reptiles, transport them for sale, and release is either purposeful or due to accidental escape. However, conceptualising this process as a filter-based interaction between organisms and their human vectors can help to elucidate not only the human elements that drive this process, but also the biological mechanisms that are at work behind the scenes.

Biological processes

Distribution, phenotype, and the ways in which both are shaped by source abiotic and biotic conditions, can all contribute to filtering the uptake, survival, and release of pet reptiles. Colorful, patterned species and those with attractive genetic variants are popular (Auliya, 2003; Van Wilgen,

Wilson, Elith, Wintle, & Richardson, 2010). Species that are abundant in their native range, or whose native range overlaps with that of humans, are also more readily captured by locals for inclusion in the pet trade (e.g. Ceballos & Fitzgerald, 2004; Reed, 2005). Behaviour may also play a role as bolder species or individuals could be more easily approached by trappers (Biro & Dingemanse, 2009). Other behaviours, such as movement between ponds for reproduction, can also drive reptiles out into the open where they are more readily captured (Enge, 2005). Survival during transport or in captivity can vary among species (Auliya, 2003; Robinson, St. John, Griffiths, & Roberts, 2015) or individuals (e.g. those that are timider, Carrete et al., 2012). Reptiles that were reared in captivity may exhibit higher survival than those caught in the wild (Robinson et al., 2015). Finally, the eventual release of reptiles can be related to biological differences between species and individuals. For example, less effort might be put into confining common, abundant species (Cassey et al., 2004; Van Wilgen et al., 2010), or release could be more likely for reptiles that reach a high maximum body size (Snow et al., 2007; Stringham & Lockwood, in review). Phenotypes related to capture, survival, and release, such as individual behaviour (Cote & Clobert, 2007), body size (Ashton & Feldman, 2003), and coloration (Broennimann et al., 2014), can also vary between source habitats. Reptiles from habitats that tend to promote the development of phenotypes associated with uptake, survival, and release will subsequently have a greater risk of introduction.

Human movement ecology

The intentional import of pets is driven by human desire for entertainment and companionship (Bush, Baker, & MacDonald, 2014), and pathways often target species that best satisfy these motivations, such as those deemed to be ‘attractive’ (e.g. Vall-Iloera & Cassey, 2017). Additionally, these pathways often gather species from particular areas. For example, many non-native reptiles are sourced from Indonesia due to its large diversity of species and few regulations (Natusch & Lyons, 2012). The strength of the survival filter could differ widely depending upon the motivations, methods, directionality, and timing of human movements. Highly valued species may be transported in better conditions (Cassey et

al., 2004). Reptile trade routes can also vary in length and quality. Many routes involve several temporary housing facilities as organisms are transferred between collectors, breeders, suppliers, exporters and dealers (Auliya, 2003), and vectors with transparent boundaries, variable temperature, or that crowd transported individuals are particularly stressful for reptiles (Warwick, Arena, Lindley, Jessop, & Steedman, 2013). Transport conditions can also depend upon the chosen transport route and destination because the provision of conditions promoting pet welfare, and even the concept of animal welfare, differs between regions (Baker et al., 2013). In terms of release, the quantity, diversity, and probability of pet release/escape can relate to species desirability, transport conditions, and the eventual destination. Highly desired pets may be more carefully confined and more likely to be kept, while cheap, or more commonly traded pets may be more prone to release or escape (Cassey et al., 2004; Warwick et al., 2013).

Lastly, every aspect of human movement ecology that relates to pet reptile transport could change depending upon shifts in external factors. Regulations, for example, determine the likelihood and quantity of organisms collected (Natusch & Lyons, 2012), the welfare provided during transport (Baker et al., 2013), and which species are acceptable for export and import (Bush et al., 2014). Changes in pet collection and transport regulations can therefore determine why particular pathways exist, how they move, and their routes and timings of travel.

Example 2 – Unintentional dispersal of plant seeds on clothing, Fig. 5.5b

Humans are capable long- and short-distance dispersers of plant seeds attached to clothing and footwear ('anthropochory'; Hodkinson & Thompson, 1997), and are emerging as a major global dispersal vector due to globalisation and the expansion of the human population (Mack & Lonsdale, 2001; Hodkinson & Thompson, 1997). Plants have not evolved to disperse via humans, but some traits can pre-adapt certain species and individuals for this process (Hodkinson & Thompson, 1997). Additionally, the patterns and behaviour of individual human movements can bias which seeds are likely to be moved, and how far they might be deposited from their source habitat.

Biological processes

Plant distribution, plant phenotype, seed phenotype, and life history can all determine which seeds are likely to be collected by human vectors. Uptake into human vectors can be greater in plants that are widespread and that thrive in human-disturbed habitats (e.g. Ansong & Pickering, 2013). The position of seeds on plants, the quantity produced, seed size, and seed morphology can all influence attachment to clothing (Mount & Pickering, 2009; Pickering, Mount, Wichmann, & Bullock 2011; Auffret & Cousins, 2013; Ansong & Pickering, 2014). Plant distribution, seed quantity, and the expression of traits that potentially influence uptake can also vary based on season, source habitat conditions, and weather (Mount & Pickering, 2009; Pickering et al., 2011; Ansong & Pickering, 2014). Some places and times could therefore produce seeds with a greater ability to attach to clothing vectors. Survival during transit is not often studied in human-dispersed plant seeds, but there are some indications that species could differ in post-transport seed viability (e.g. McNeill et al., 2011). There is also evidence that seed release can be affected by phenotype. Seeds with morphological adaptations that enhance attachment, such as hooks or appendages, remain attached to clothing for longer periods (Pickering et al., 2011), increasing the probability of late release and long-distance dispersal.

Human movement ecology

Unintentional human-mediated seed dispersal is an incidental consequence of many human movements for daily needs, work, travel and recreation. These motivations can, in turn, determine the quantity and identity of seeds collected. Motivations that drive vectors through vegetated routes (e.g. recreation, tourism), and movement during seeding seasons, can increase the amount and diversity of collected seeds (McNeill et al., 2011; Ware, Bergstrom, Müller, & Alsos, 2012; Huiskes et al., 2014). The structure of clothing and footwear vectors can similarly bias seed collection. More seeds have been detected on footwear and bags compared to other vector types (Huiskes et al., 2014). Clothing made with adhesive blends of cloth, pockets, cuffs, and Velcro®, or shoes made from leather, can also collect a

higher quantity and diversity of seeds (Whinam, Chilcott, & Bergstrom, 2005; Wichmann et al., 2009; McNeill et al., 2011; Pickering et al., 2011). In terms of survival, there are almost no studies that have compared seed viability between different human vectors (but see Hughes, Lee, Ware, Kiefer, & Bergstrom, 2010). There are some indications that the timing of travel could affect survival, such as improved seed viability in vectors that operate during cooler, moister conditions (McNeill et al., 2011). For release, most human movements cover short distances (tens of kilometers; González et al., 2008), driven by daily needs for work and necessities. Such movements will tend to release seeds within, or close, to the source habitat. Longer-distance movements occur less often, driven by motivations for travel and recreation, but are more likely to deposit seeds outside their native range, such as in long-distance air and ship travel (Whinam et al., 2005; McNeill et al., 2011; Ware et al., 2012). The type of vector, directionality, and timing of movement also influences seed deposition. Clothing and footwear to which seeds strongly attach can retain seeds for longer periods, and seed detachment may occur more readily in wetter areas or seasons (Ansong & Pickering, 2014).

Finally, changes in factors external to human-mediated seed dispersal can influence the uptake, survival, and release filters. Climate, weather, and economics influences the desire for travel and recreation, along with where and when people tend to move. Outdoor activities are associated with a greater quantity of transported seeds, and the propensity for such activities to occur changes depending upon expense and environmental conditions. Some countries also have more stringent regulations regarding the unintentional transport of seeds on human clothing and footwear (e.g. biosecurity regulations in New Zealand; McNeill et al., 2011), and increased implementation of such regulations would result in an overall decline in the global transport of non-native seeds.

Estimating introduction risk

Introduction risk can be estimated for both examples above using their respective assessments of how biological characteristics and human movement ecology could bias the uptake, survival, and release

filters. A 'high-risk' profile is created based on the worst-case combination of factors that would likely produce a high-risk introduction (Fig. 5.5), and different species, individuals, and vectors can be ranked based on how well they match this profile. Species with biological characteristics that confer a high likelihood of transitioning successfully through each filter will subsequently have a higher introduction risk, higher propagule pressure, and a higher bias at the individual-level for those with the favoured suite of traits. Conversely, species that do not fit some, or many, aspects of the profile could still be transported, but likely with a lower introduction risk and subsequently lower propagule pressure. This same process can be performed for human movement ecology. Propagule pressure and bias is highest for targeted species, or those that tend to overlap the movement route, and lower for non-target species, or those distributed outside the route. Similarly, vectors that collect a large quantity and diversity of propagules, that inflict relatively mild transport conditions, and that readily release propagules outside their native range will tend to be higher risk, with higher propagule and colonisation pressure. Conversely, harsher vectors with stronger limitations on release or escape, or that release propagules close to their source habitat, will tend to be lower risk.

Pathways with both high-risk species, based on the biological profile, and high-risk vectors, based on the movement ecology profile, are the most likely to introduce non-native organisms. Additionally, the profile can be used to make initial estimations of the probable traits of successfully transported non-natives (i.e. phenotype), along with their potential genetic and species diversity (i.e. Is uptake diversity likely high or low? Is survival and release bias high or low?). As the degree of match to either the biological and movement ecology profile declines, so too does the overall risk of introduction. For example, even though an organism may closely match the biological profile of a high-risk introduction, if it interacts with low-risk vectors then it is unlikely to enter, survive, or exit transport, with the same true for high-risk vectors that encounter low-risk species or individuals.

Different sections of the profile can also produce conflicting conclusions. For example, in the case of the intentional transport of reptile pets (Fig. 5.5a), desirable species are predicted to be the most

likely to be collected, suggesting higher introduction risk. However, higher desirability is also associated with a lower likelihood of release or escape, suggesting lower introduction risk. Our reptile pet example would therefore classify desirable species as simultaneously likely and unlikely to be introduced, making an overall assessment of risk difficult. Such conflicts are probably due to the coarse degree with which we explored our two examples. A deeper examination of, for example, ‘desirability’ could further divide this human motivation into different subcategories, each with their own level of introduction risk. Pathways transporting desirable species because they are expensive and rare would not transport many propagules, and release or escape would be unlikely, producing a low risk of introduction. However, pathways moving species desired because they are popular and common would transport a large number of individuals, with a higher chance of release or escape, and thus a higher risk of introduction. Resolving conflicts between different aspects of the framework, and further refining its sections to the pathway of interest, will be important to address in future studies and experiments on the pre-introduction filters of the invasion process.

Conclusions

The ecological and economic damage caused by invasive species has inspired and driven research into the causes of non-native establishment and spread. While this work has provided insight into invasibility and impact, prediction remains elusive. A major gap preventing the continued development of an overall predictive framework for invasion is our understanding of the processes that occur before non-natives even arrive in the habitats which they invade. These introduced organisms are not random, as they are the subset of individuals that are able to successfully enter, survive, and exit human transport vectors. Predicting the amount and identity of introduced non-natives therefore requires a deeper examination of the mechanisms driving these initial biases. Here, we have outlined how to improve assessments of which broad, biological- and human-based mechanisms interact to create the uptake, survival, and release filters of the human-mediated transport process. By questioning how organism traits, abiotic/biotic conditions,

and human movements may structure these filters, we can better estimate the likelihood of introduction, and the probable quantity, traits, genetic diversity, and species diversity of the propagules eventually introduced, which could then be applied to predicting establishment (e.g. Bradie, Chivers, & Leung, 2013). Not all aspects of this framework are equally relevant to every type of human vectored movement, but all should be considered when developing testable hypotheses about the mechanisms determining which non-native organisms will eventually be introduced, and for informing how best to prevent or control these introductions.

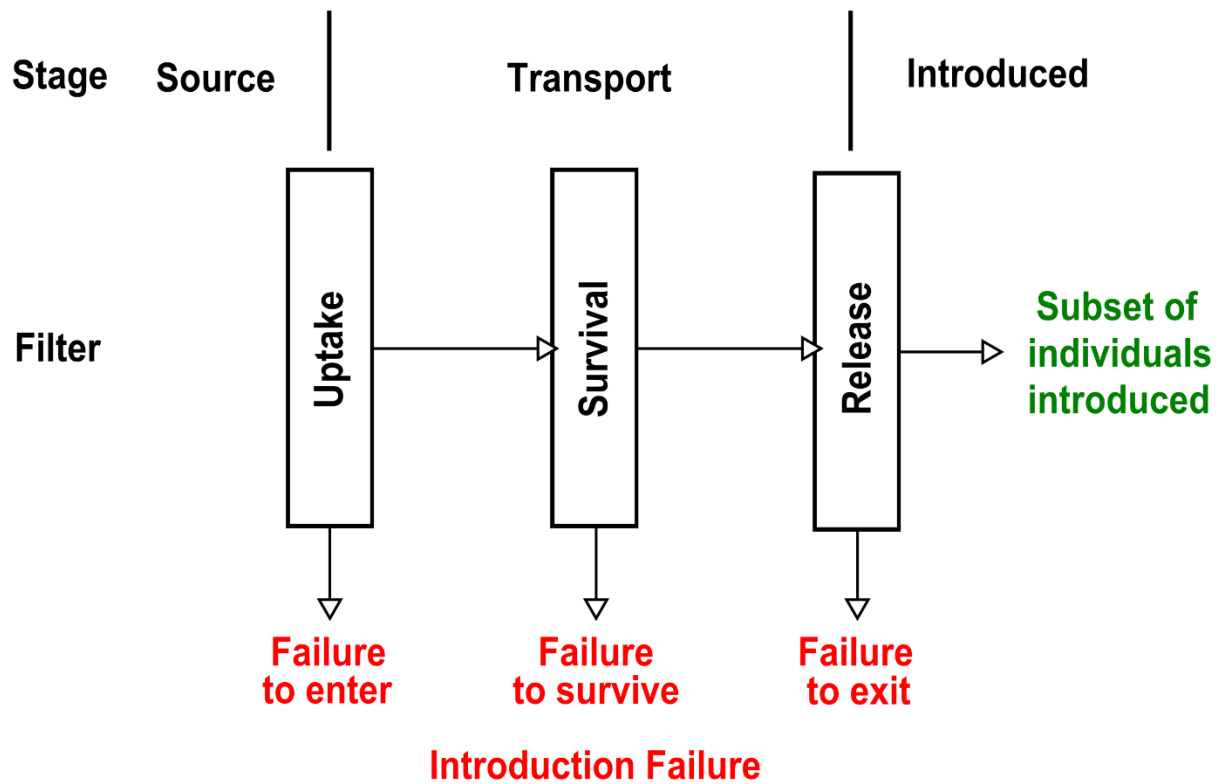


Figure 5.1 Stage- and filter-based invasion framework of the first three stages of the invasion process (source, transport, and introduced). Framework structure and terminology has been adapted from those detailed in Colautti and MacIsaac (2004), Catford et al. (2009) and Blackburn et al. (2011).

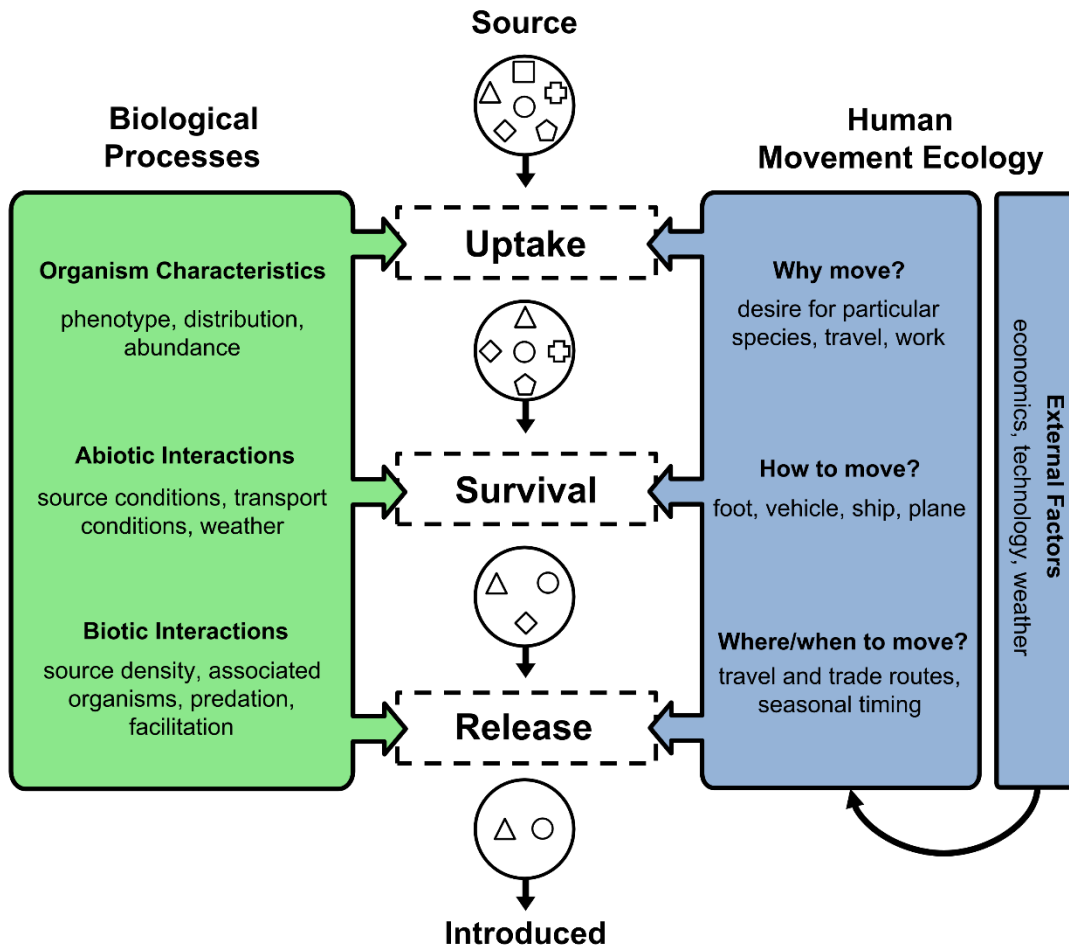


Figure 5.2 Conceptual interaction between the biological processes and aspects of human movement ecology that determine which individuals (represented by different shapes) will successfully pass through the uptake, survival, and release filters of the human mediated dispersal process. The non-bolded text in the figure details a few examples for each sub-section of the framework. Each section of the framework does not necessarily apply to all possible situations and types of vectors (e.g. for some vectors there will be few or no biotic interactions), but all should be considered when evaluating potential mechanisms structuring the filters. This framework structure has been modeled after other similar stage-based invasion (particularly Colautti et al., 2004, Catford et al., 2009, and Blackburn et al., 2011) and movement ecology (particularly Viana, Santamaría, and Figuerola, 2016) frameworks.

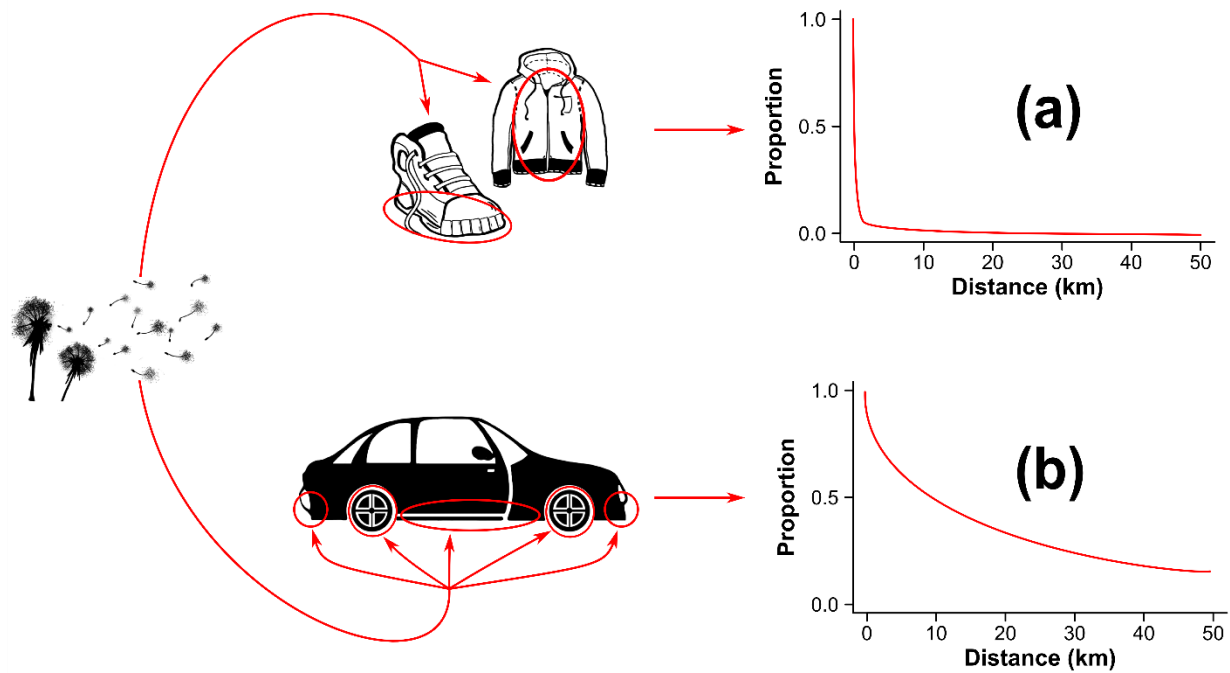


Figure 5.3 Propagule deposition patterns for two types of vectors (clothing vector in red versus vehicle vector in blue) that are interacting with the same organism (plant seeds). In (a), most individuals are deposited within tens of meters of the source environment, while individuals in (b) are deposited over tens of kilometers. Vehicles are therefore the potentially more impactful human vectors in this pathway because they are depositing a larger quantity of transported individuals further from their source region. These example dispersal curves were calculated using averaged coefficients from models of seed dispersal via foot and vehicle vectors from, respectively, Wichmann et al. (2009) and Taylor et al. (2012).

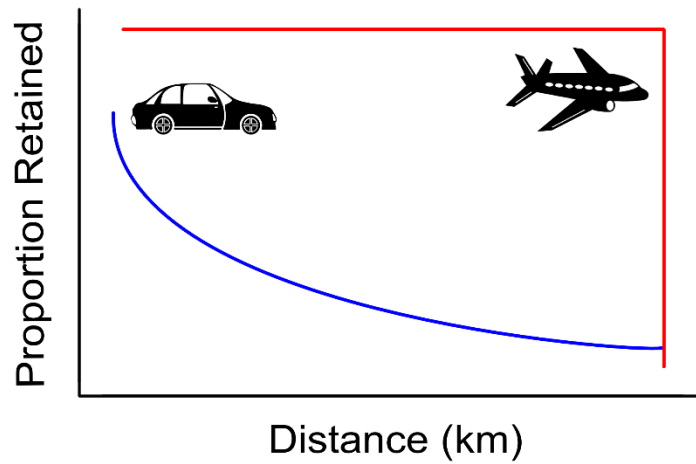
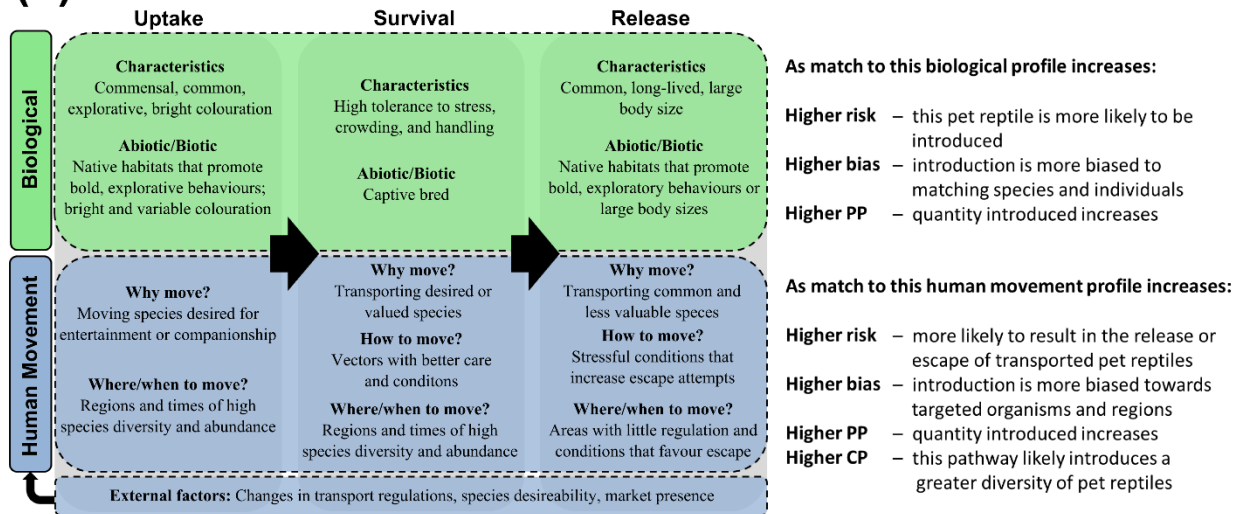


Figure 5.4 Hypothetical differences in propagule deposition patterns between ground (blue curve) versus air (red curve) vectors. The ground vector follows the common dispersal vector pattern of depositing the most propagules closest to the source location. However, the air vector traps collected propagules in its cargo, and all are deposited upon arrival at the intended destination (e.g. when cargo is unpacked). The latter vector is therefore potentially more impactful as the greatest quantity of propagules are most likely to be released the furthest from the source location.

(a) Intentional transport of pet reptiles



(b) Unintentional dispersal of plant seeds on clothing

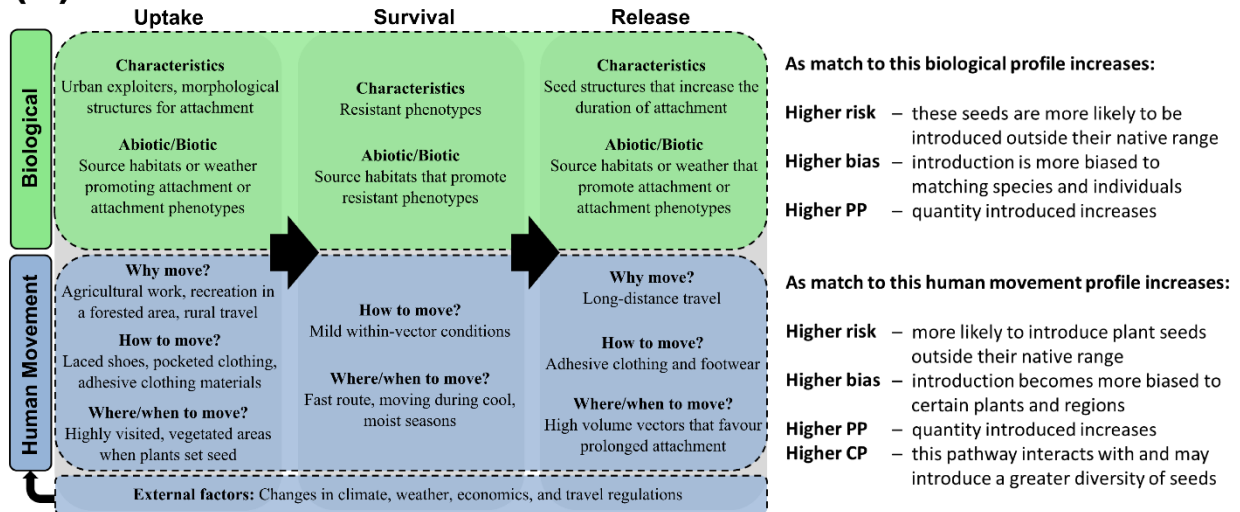


Figure 5.5 High-risk profiles of the biological and human mechanisms that interact in (a) the intentional transport of pet reptiles and (b) the unintentional dispersal of plant seeds on clothing. Examples of changes in relevant external factors that could influence each aspect of human movement ecology are listed in separate blue bubbles below the ‘Human Movement’ sections. Introduction risk, bias, propagule pressure (‘PP’), and colonisation pressure (‘CP’) can be estimated for individuals, species, and pathways by determining how well they match the framework profile of an organism likely to be introduced, and a pathway likely to introduce a high quantity and diversity of non-natives.

Chapter 6

General Conclusions

Summary

Each of the chapters of my thesis has contributed to our understanding of how the importance of population size, quality, and/or arrival frequency can shift between species and individuals, and improved our grasp of the drivers of human-mediated colonization. In chapter 2, I showed that population size, not arrival frequency, was the primary factor determining the survival and performance of introduced populations of *Hemimysis anomala*. Prior to my study, the experimental research in this area indicated that arrival frequency was at least as important as population size for colonist success (e.g. Drake et al. 2005; Hedge et al. 2012). My study therefore acts as an important contrast by showing a case in which the influence of population size dominates over that of arrival frequency.

In chapter 3, I found that population size was not the factor primarily responsible for the success of two zooplankton species, *Daphnia pulicaria* and *Skistodiaptomus oregonensis*. Instead, the population dynamics of *D. pulicaria* were only influenced by their quality, while the establishment of *S. oregonensis* was more strongly influenced by arrival frequency. The literature on the relative importance these colonist characteristics is a small collection of single species studies, in different systems, that examine the interaction between quantity and arrival frequency, or quantity and quality (detailed in Table 3.1). The value of my study is therefore in its simultaneous examination of the relative importance of three colonist characteristics in two different species within the same field study system. Additionally, a broad comparison of the collective results from past work suggests that the value of population size is generally equal to, and sometimes greater than, that of quality and arrival frequency. However, my work indicates that this is not always the case as the relative importance of these three factors can shift between species. Some species may rely more on higher population sizes for success (e.g. *H. anomala*), while others derive

little benefit from increased population size (e.g. *D. pulicaria*), or more benefit from increased arrival frequency (e.g. *S. oregonensis*).

In chapter 4, I showed that the benefits of increasing colonist quantity and genetic diversity, both mechanisms by which population size affects colonization success, can change based on colonist identity. For some individuals, higher quantities or genetic diversities improved their success, while for other colonists it had little effect. This study provided new evidence that the value of population size could vary not just among species, but also among individuals of the same species.

Chapter 5 details the ways in which human-mediated dispersal is driven by the interplay between how human vectors interact with organisms, and how organisms interact with human vectors. Assessing introduction risk, and predicting the quantity and identity of transported organisms, therefore requires a consideration of how each of these processes combine to filter individuals at each stage of transport. This review fills in a missing gap in our understanding of what is occurring prior to non-native arrival, it improves our ability to estimate potential invaders, and it fosters cross-communication between ecological disciplines studying invasive species, dispersal, movement ecology, and human mobility.

Implications and future directions

The results and conclusions of my thesis chapters all serve to further develop our understanding of colonization. Population size is a crucial factor determining colonist success, but research on the drivers of colonization has been gradually focusing on other, less studied, characteristics that could play an equal role, such as quality (e.g. Burgess and Marshall 2011; Lange and Marshall 2016), arrival frequency (e.g. Drake et al. 2005; Hedge et al. 2012), and individual identity (e.g. Vellend et al. 2010). Similarly, efforts to better understand the causes of human-mediated colonization have been focusing more on the earlier stages of the colonization process (e.g. the acknowledgement of the role of pre-arrival processes and propagule pressure in driving species invasions, Floerl and Inglis 2005; Lockwood et al. 2005; Colautti et al. 2006; Simberloff 2009; Blackburn et al. 2015). My work is part of, and provides a

unique contribution to, both of these shifting perspectives. It does this by showing how the value of multiple colonist characteristics can vary between species and individuals, and by outlining which pre-arrival processes determine the quantity and identity of individuals likely to be introduced by human vectors. These contributions also have practical implications. My work on population size suggests that management efforts that commonly target population size (e.g. via population augmentation or eradication) may need to consider the potential role of other, less-studied colonist characteristics. Likewise, my review of pre-arrival filters indicates that the likelihood of species introduction and invasion can be more accurately estimated by accounting for the biological- and human-based processes that interact prior to introduction.

However, we currently cannot predict which colonist characteristics will dominate in a particular species or population of concern, nor can we reliably predict human-mediated species introductions. Moving towards a more predictive understanding of colonization requires that we test the mechanisms potentially responsible for driving observed patterns. Studies by myself, and many other researchers, have identified important patterns (e.g. multiple colonist characteristics can drive success) and potential mechanisms (e.g. Allee effects, individual traits, human behaviour) in the colonization process. The next step is therefore to test these mechanisms that potentially control colonization success in different contexts. What is causing the relative importance of population size, quality, and arrival frequency to vary between species and individuals? What mechanisms determine which factor will dominate under a given set of circumstances? Which filtering mechanisms are primarily at work in certain species and vector interactions? Predicting the value of different colonist characteristics, and which individuals are most likely to enter, survive, and exit human vectors, requires further experimentation designed to develop our mechanistic understanding of what is driving both natural- and human-mediated colonization, and why.

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Appendix A

Lake characteristics, summary statistics, and temperature variability for Chapter 2

Table A1 Information for all lakes involved in the experiment. Water chemistry data was collected between May 2014 to August 2014 (S.E. Arnott, unpublished data) and averaged over this period. Lake Opinicon was the source of all mesocosm water, while zooplankton were collected from the other four lakes.

Lake	Latitude (N) Longitude (W)	Area (ha)	Max Depth (m)	pH	DOC (mg L ⁻¹)	Cond. (μ S cm ⁻¹)	TP (μ g L ⁻¹)
Lindsay	44°32'14.2" -76°23'25.1"	31.5	10.9	8.11	6.02	249.60	10.45
Long	44°31'40.5" -76°24'10.0"	15.5	26.0	8.22	4.04	180.72	10.15
Opinicon	44°33'35.4" -76°19'41.0"	788.0	11.3	8.08	4.74	187.78	18.51
Round	44°32'16.9" -76°24'00.3"	15.0	30.1	8.08	3.54	152.22	8.16
Warner	44°31'41.4" -76°22'53.0"	9.2	6.4	8.07	5.32	266.80	12.81

Table A2 Minimum adequate model (MAM) structure, statistical methodology, descriptive statistics, and R^2 values (calculated using the pseudo- R^2 methods from Zuur et al. 2009) for abundance (through time) and presence/absence (4th week following 2-2.5 average growing weeks) models. ‘Size’, ‘Number’, and ‘Time’ entries under the ‘MAM’ heading represent the predictors that were retained in the MAMs.

Metric	MAM	Descriptive Statistics	Distribution	R^2
Abundance – all weeks	Size*Number*Time	Mean: 2.4 Variance: 22.13	Zero inflated Negative Binomial	0.87
P/A – final week	Number	Minimum success: 0 Minimum failure: 3	Zero inflated Binomial	0.77

Table A3 Abundance through time model coefficients. The propagule number category for the single addition treatments (Number – 1) is used as the base level of ‘Number’ in the model.

Term	Estimate	SE	Z-value	P
Intercept	0.9043	0.4459	2.03	0.04256
Size	0.0989	0.0262	3.78	0.00016
Time	-0.5179	0.2086	-2.48	0.01302
Number – 2	0.6731	0.7507	0.90	0.36986
Number – 4	-0.5378	1.1429	-0.47	0.63795
Size:Time	0.0138	0.0117	1.18	0.23856
Size:Number – 2	-0.0472	0.0440	-1.07	0.28308
Size: Number – 4	0.0497	0.0685	0.73	0.46840
Time:Number – 2	-0.3273	0.3508	-0.93	0.35083
Time:Number – 4	0.3331	0.6062	0.55	0.58262
Size:Time:Number – 2	0.0136	0.0200	0.68	0.49603
Size:Time:Number – 4	-0.0868	0.0384	-2.26	0.02398

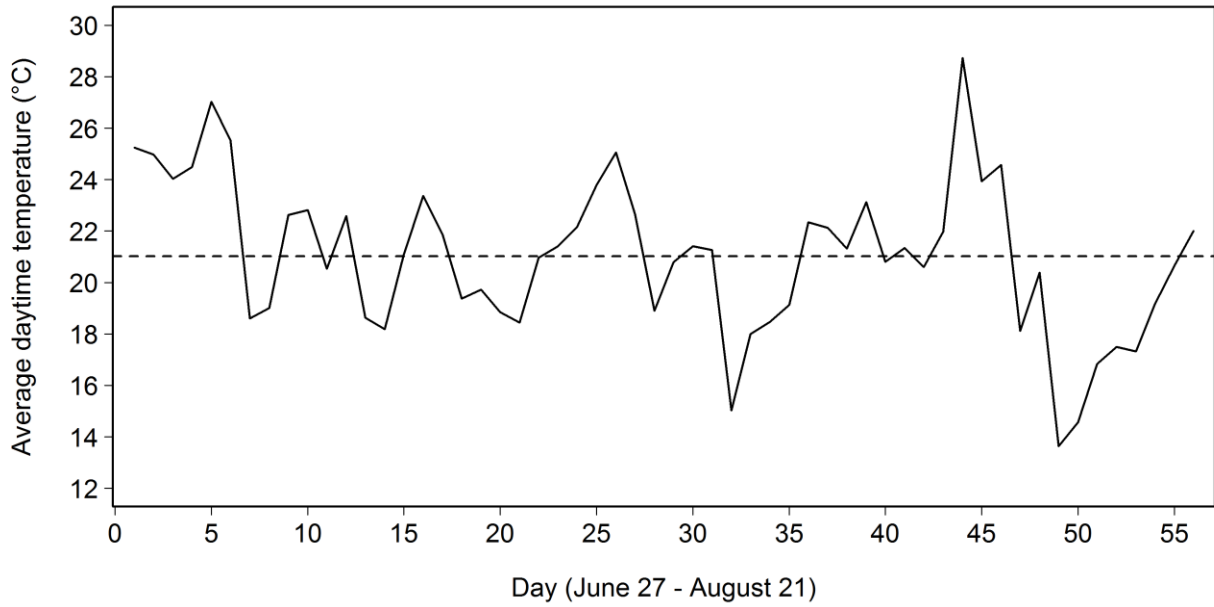


Figure A1 Daytime average temperature (solid line) and overall average (dashed line) for the QUBS area over the period in which the experiment was conducted. Note that the field site was shaded, and cover was provided to all tanks, which would lead to cooler temperatures than those shown and reduce daily fluctuations.

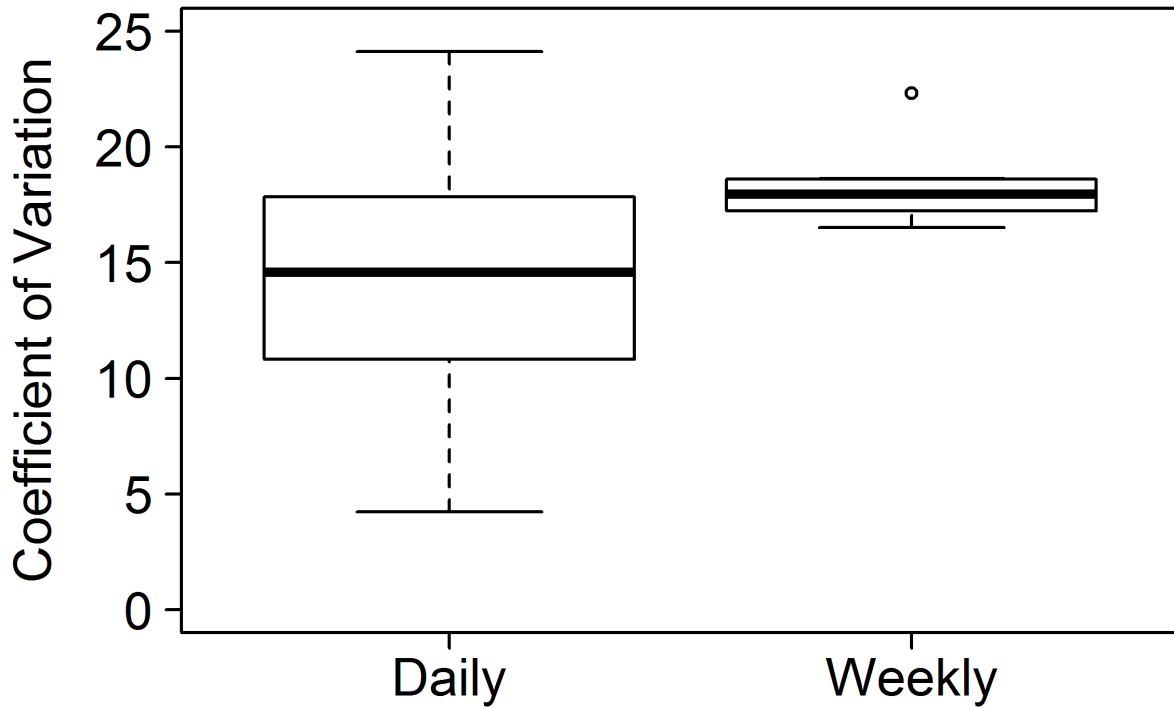


Figure A2 Temperature variability (July 4th – August 31st) at QUBS within each day (measured every 15 minutes) and within each week (8 total weeks) for the period over which the experiment was conducted.

Appendix B

Lake characteristics, summary statistics, and information on *Daphnia* abundance for Chapter 3

Table B1 Information for all lakes involved in the experiment. Water chemistry data was collected between May 2015 to the end of August 2015 (S.E. Arnott, unpublished data) and averaged over this period.

Lake	Latitude (N) Longitude (W)	Area (ha)	Max Depth (m)	pH	DOC (mg L ⁻¹)	Cond. (μ S cm ⁻¹)	TP (μ g L ⁻¹)
Elbow	44°28'37.0" -76°25'40.8"	15.5	10.6	6.6	7.3	70.4	16.0
Opinicon	44°33'35.4" -76°19'41.0"	788.0	11.3	6.9	5.4	180.3	13.2
Warner	44°31'41.4" -76°22'53.0"	9.2	6.4	8.2	6.5	290.7	11.9

Table B2 Estimated coefficients for the minimum adequate models from both experiments. Coefficients for alternate levels of categorical predictor variables are indicated by a dash followed by an identifying label (e.g. ‘Quality-Good’). For linear mixed models, t-values are reported, while z-values are reported for generalized linear models.

Metric	Coefficient	Estimate	SE	t/z
<i>D. pulicaria</i> abundance	Intercept	0.57	0.11	5.16
	Quality-Good	0.25	0.16	1.62
	Week	0.30	0.061	4.90
	Week ²	-0.038	0.0096	-3.93
	Quality-Good:Week	-0.23	0.086	-2.66
	Quality-Good:Week ²	0.030	0.013	2.22
<i>S. oregonensis</i> presence/absence	Intercept	-1.61	0.77	-2.08
	Immigration-LQHF	2.30	0.99	2.33
	Immigration-HQLF	0.51	1.02	0.50

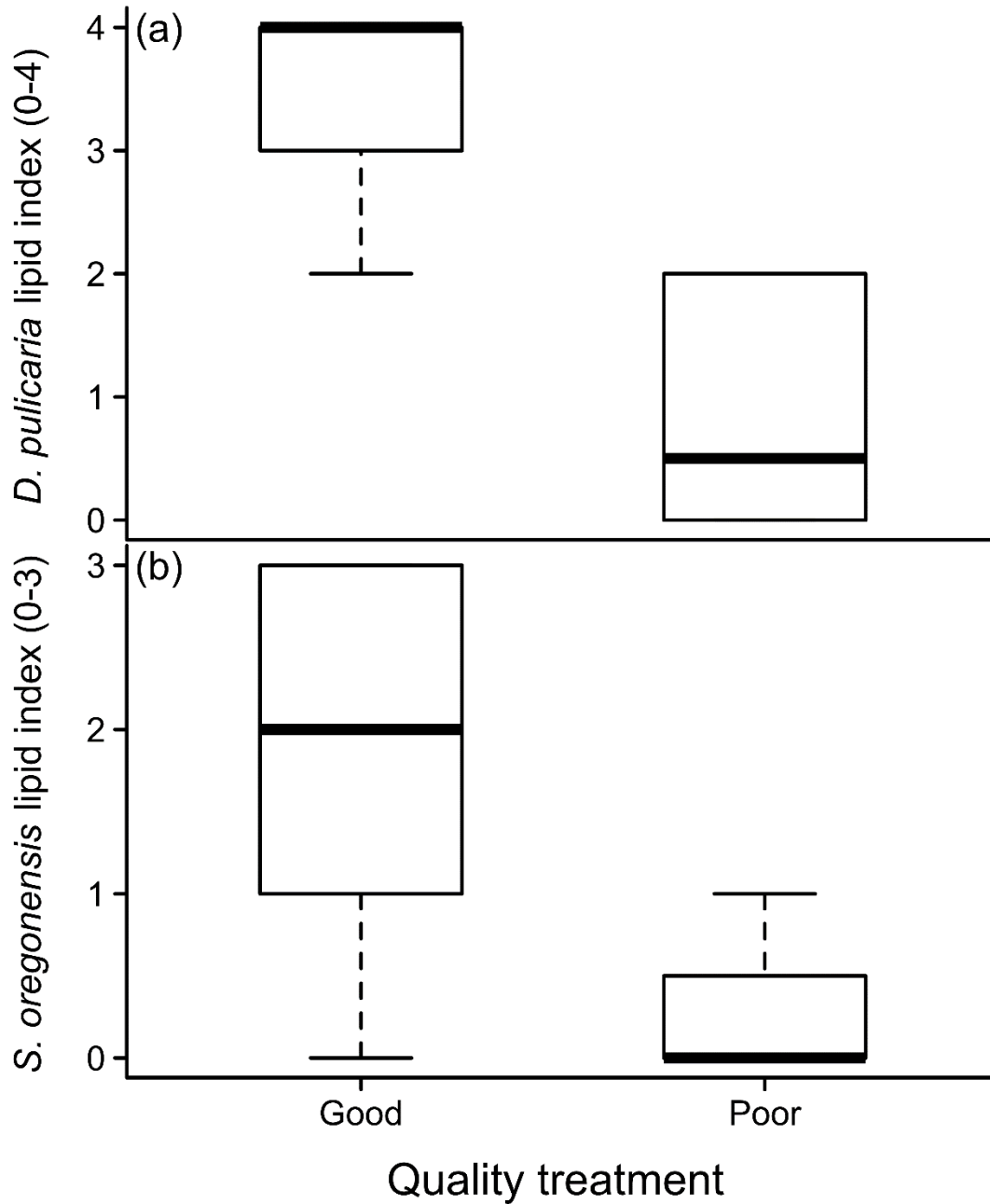


Figure B1 Lipid index values from the pilot experiment for (a) *D. pulicaria* raised in good ($20\mu\text{g C individual}^{-1} \text{ day}^{-1}$, $n=14$) and poor ($2.5\mu\text{g C individual}^{-1} \text{ day}^{-1}$, $n=20$) quality treatments, and (b) *S. oregonensis* raised in good ($20\mu\text{g C individual}^{-1} \text{ day}^{-1}$, $n=13$) and poor ($1\mu\text{g C individual}^{-1} \text{ day}^{-1}$, $n=16$) quality treatments. Differences in sample sizes between groups are due to mortality during the 7 day isolation period; each treatment initially began with 20 individuals.



Figure B2 Example section of one of the macro photographs used to determine abundance of *D. pulicaria* from Experiment 1.

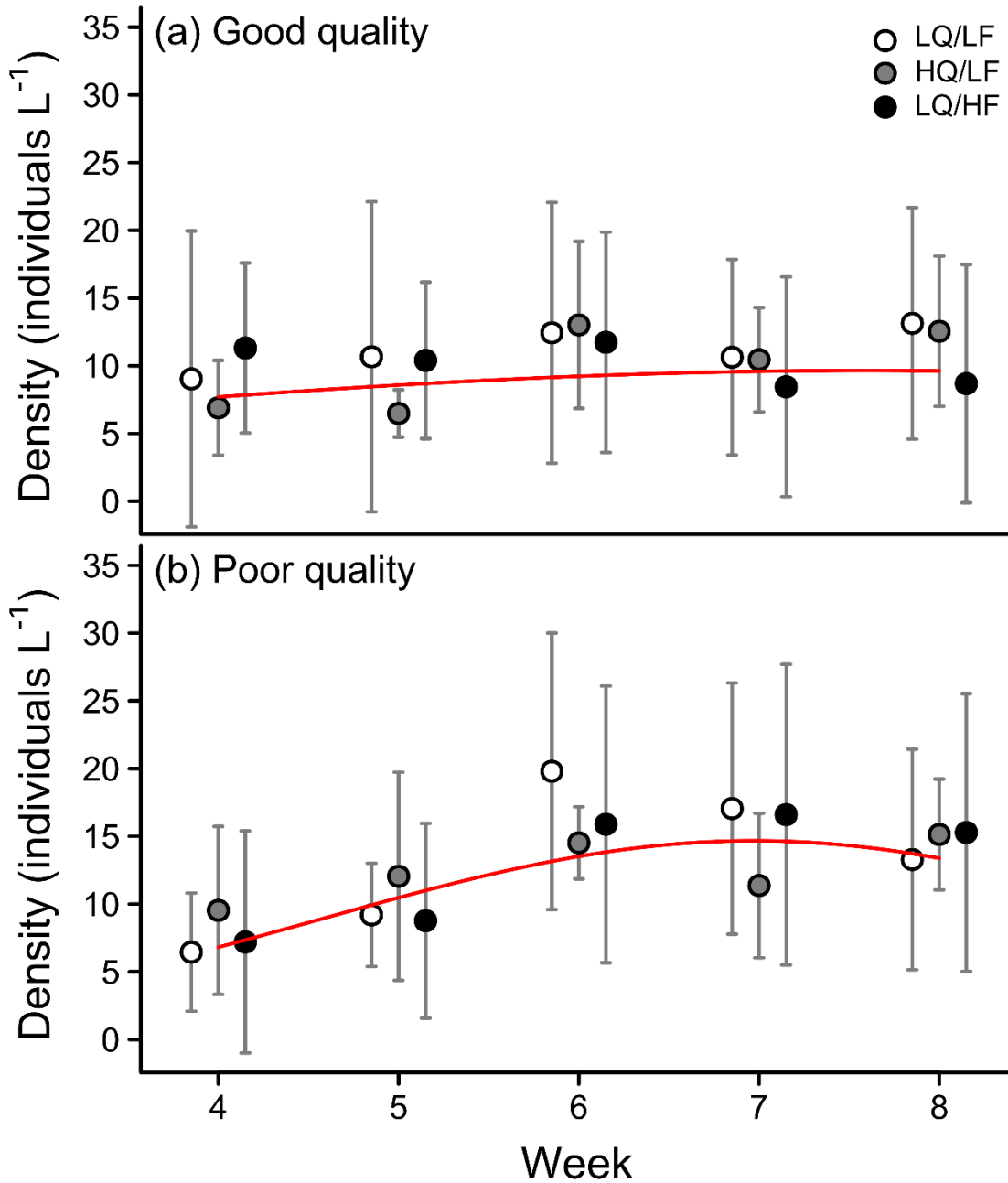


Figure B3 Effects of quantity/frequency treatments (LQ/LF=low quantity, low frequency; HQ/LF=high quantity, low frequency; LQ/HF=low quantity, high frequency) and time on the density (individuals L⁻¹) of (a) good and (b) poor quality *D. pulicaria* across all sampling weeks (mean \pm CI, $n=6$). Best-fit lines represent the best-fit relationships between time and density for good and poor quality treatments.

Appendix C

Lake characteristics, summary statistics, life-history methods, and genotyping methods for Chapter 4

Table C1 Information for all lakes involved in the experiment. Water chemistry data for all Muskoka lakes was collected in summer 2011 by the Canadian Aquatic Invasive Species Network (CAISN), and water chemistry for all Queen’s University Biology Station (QUBS) lakes was collected in summer 2016 by S.E. Arnott. Opinicon was the source of all mesocosm water and Buck was the source of the native zooplankton community. The ‘Region’ column indicates the broad geographic region in which each lake is located. ‘M’ represents the Muskoka region, located in central Ontario, Canada, and ‘Q’ represents the region surrounding the QUBS in southern Ontario, Canada. Water chemistry variables are listed under the column headings ‘Av. TP’ for average total phosphorus ($\mu\text{g L}^{-1}$), ‘Ca’ for calcium (mg L^{-1}), ‘Cond.’ for conductivity ($\mu\text{S cm}^{-1}$), ‘DOC’ for dissolved organic carbon (mg L^{-1}), ‘pH’, and ‘Na’ for sodium (mg L^{-1}).

Lake	Region	Latitude Longitude	Area (ha)	Av. TP	Ca	Cond.	DOC	pH	Na
Buck	Q	44.534001 -76.438326	784.6	7.6	23.3	187.0	4.6	7.6	5.2
Crozier	M	45.334557 -78.849833	56.8	3.3	2.8	31.0	3.1	6.9	1.0
Dyson	M	45.216222 -79.652563	92.4	3.7	3.1	31.0	2.7	7.1	0.9
Elbow	Q	44.475241 -76.428946	27.8	10.2	10.5	77.5	7.0	7.3	1.0
Fifteen Mile	M	45.349633 -78.965305	81.1	4.6	1.9	19.6	3.4	6.9	0.6
Grandview	M	45.202002 -79.051840	64.0	6.9	4.5	75.8	2.7	6.4	6.0
Lindsay	Q	44.536869 -76.390861	13.2	10.7	37.1	241.0	6.6	6.4	1.0

McKay	M	45.058557 -79.172913	132.0	8.5	2.6	22.2	6.1	6.3	1.0
Opinicon	Q	44.558982 -76.328055	792.2	14.9	22.8	187.0	5.8	8.1	4.4
Ridout	M	45.176259 -78.978642	49.9	3.9	1.7	17.6	4.3	6.7	0.7
Round	Q	44.537855 -76.400036	15.0	5.1	25.2	177.0	4.5	8.1	1.5

Table C2 Descriptive statistics and *P*-values for all GLM tests of differences in *D. pulex* densities between each low diversity treatment and the high diversity treatment. Letters under the ‘Genotype’ heading refer to which low diversity genotype treatment is being tested against the high diversity treatment. Descriptive statistics for the models are given under the ‘Mean’ (reported in individuals L⁻¹), ‘Variance’, and ‘Dispersion’ (ϕ) headings, which provide information on how well the negative binomial distribution captures the data. The results of the log likelihood ratio tests are reported under the ‘Deviance’ (difference in deviance explained between models with and without the categorical term for low/high diversity, 1 and 3 degrees of freedom for each), ‘*P* (un. adj.)’ (unadjusted *P*-values from the log likelihood ratio tests), and ‘*P* (adj.)’ (Benjamini-Hochberg False Discovery Rate corrected *P*-values) headings.

Genotype	Mean	Variance	Dispersion	Deviance	<i>P</i> (un. adj.)	<i>P</i> (adj.)
C	2.5	3.0	0.99	8.06	0.0045	0.040
D	8.5	22.9	0.97	0.34	0.56	0.94
E	9.7	52.5	1.02	0.044	0.83	0.94
F	3.1	6.7	0.93	5.26	0.022	0.10
G	6.6	6.1	0.92	1.56	0.21	0.63
L	7.0	16.3	0.99	1.12	0.29	0.65
M	12.6	181.3	0.81	0.094	0.76	0.94
O	10.9	195.5	1.34	0.0030	0.95	0.95
R	11.6	146.4	1.33	0.039	0.84	0.94

Table C3 Regression tables for analyses of relationships between the effect sizes of quantity and genetic diversity to the intrinsic rate of increase (r), body size at first reproduction (mm), and habitat similarity (Euclidean distance).

Metric	Log	Predictor	Estimate	SE	t	df	P	R²
Effect sizes of quantity and r	Yes	Intercept	0.18	0.16	1.16	1,7	0.59	0.043
		r	-0.42	0.75	-0.56			
Effect sizes of quantity and body size	Yes	Intercept	0.87	0.32	2.76	1,7	0.043	0.46
		Body size	-0.39	0.16	-2.46			
Effect sizes of quantity and similarity	No	Intercept	0.22	0.37	0.60	1,7	0.91	0.002
		Similarity	0.01	0.088	0.12			
Effect sizes of genetic diversity and r	No	Intercept	1.35	0.34	4.03	1,7	0.013	0.60
		r	-5.28	1.61	-3.28			
Effect sizes of genetic diversity and body size	No	Intercept	0.84	1.44	0.58	1,7	0.70	0.022
		Body size	-0.29	0.73	-0.40			
Effect sizes of genetic diversity and similarity	No	Intercept	-0.15	0.42	-0.34	1,7	0.74	0.12
		Similarity	0.10	0.10	1.0			

Table C4 The 12 microsatellite loci used to genotype individual clones used in the low diversity and high diversity mesocosm treatments. The linkage group of each primer is documented in Cristescu et al. (2006) and allele fragment size ranges are based on sizes detected in this study.

Primer name	Linkage group	Allele size range
D005	X	302-304
D006	XI	250-270
D009	I	362-363
D010	III	190-198
D012	VI	384-387
D078	III	167-191
D087	V	296-305
D088	IX	115-125
D091	I	262-278
D105	IV	141-151
D145	IX	257-286
D148	I	220-227

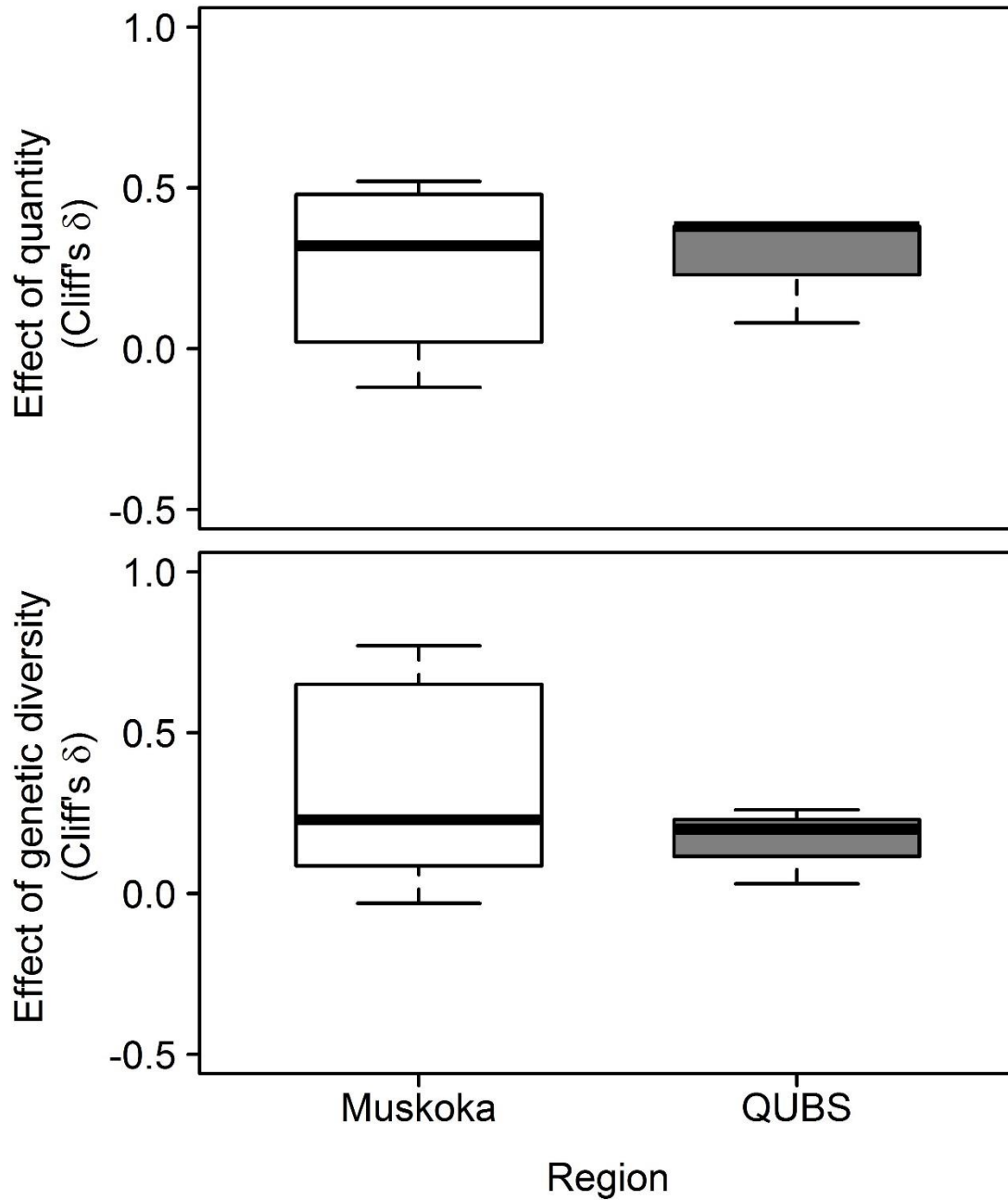


Figure C1 Effect sizes (Cliff's δ) of the (a) quantity and (b) genetic diversity treatments for genotypes from the Muskoka (unshaded, $n = 6$) and QUBS (shaded, $n = 3$) regions. Bottom and top edges of boxes represent the first and third quartiles respectively, with a dark, horizontal line as the median. Whiskers encompass data points that lie within 1.5 times the inner quartile range.

Appendix C1 – Pilot study and life-history trial methods

The pilot experiment occurred from 14-May-2016 until 30-May-2016, the purpose of which was to identify *D. pulex* clone lines that were unique in both genotype and phenotype. Twenty-two candidate adult female *D. pulex* were sourced from lakes from the Muskoka region of central Ontario, Canada and thirty-six from lakes in the region surrounding QUBS in southern Ontario, Canada, to provide a total of 54 candidate individuals. For the Muskoka genotypes, candidate *D. pulex* individuals (originally collected from their source lakes in 2015) were obtained from culture from FLAMES (Field Laboratory for the Assessment of Multiple Ecological Stressors) in Dorset, ON, Canada on 6-Apr-2016. Candidate *D. pulex* from the QUBS region were initially collected on 13-Apr-2016 from the pelagic region of Elbow, Lindsay and Round lakes (Appendix C: Table C1) using vertical tows with an 80µm, 15cm diameter net. Collected *D. pulex* were isolated into separate 120mL glass containers filled with 100mL of Elbow Lake water (filtered through a G4 glass fibre filter with 1.2µm pore size; Fisher Scientific, Waltham, Massachusetts, USA). Lake Opinicon water was not used as our media because we did not want to expose the genotypes to their intended experimental environment prior to introduction. All individuals were kept in environmental chambers at Queen's University, ON, Canada and acclimated to a 16:8h day-night photoperiod, with 20°C daytime and 15°C nighttime temperatures. These environmental conditions were chosen to be similar to conditions their offspring would eventually encounter at the time of experimental introduction (mid-summer) at our intended field site (SE Arnott, unpublished data). For each *D. pulex* individual, upon production of their first brood, 5 female neonates were collected and isolated to create the first generation, with all mothers discarded once the first generation had subsequently produced their own initial brood. This process was repeated to create the subsequent second and third

generations. All isolated *D. pulex* had their water changed weekly, with 50% retained from the previous week and 50% replaced with new, filtered Elbow lake water. They were also fed an amount of *Chlamydomonas reinhardtii* equivalent to 30µg of carbon per individual per day. This amount produced high lipid stores in a closely related *Daphnia* species (*D. pulicaria*, Sinclair and Arnott 2017), ensuring food was not limited.

On 14-May-2016, five newly released female neonates from the 3rd generation for each candidate clone line (54 clone lines with 5 replicates each totaling 270 individuals) were individually isolated in 120mL glass containers filled with 100mL of filtered Elbow lake water, which was fully replaced every day during life-history trials and checked daily for 14 days. Data was collected on body size (measured from the top to the base of the carapace) and tail size (measured from the base of the carapace to the tip of the tail) of individuals upon initial release, upon deposition of eggs into the brood pouch, upon release of the first brood from the pouch and upon any subsequent eggs deposited or broods released over the 14-day duration of the trials. Additionally, the number of neonates released over the course of the 14-day trials was recorded, along with the days on which eggs were deposited into the brood pouch, days on which neonates were released, and adult mortality. Body size of *Daphnia* can determine vulnerability to gape-limited predators, while reproduction and mortality determine the length of time a population might remain small and therefore potentially more vulnerable to extinction.

Only some of the traits calculated from these initial trials, specifically age of first reproduction (in days), body size at first reproduction (measured from the top to the base of the carapace), total number of offspring released over the course of the trials and survivorship were informative of phenotypic differences between candidate clones. Using these morphological and reproductive traits, possible clone lines for use in the experiment were selected from the original

54 for genomic analysis. These clones were selected to be from different source lakes, and selection was also based on which genotypes would represent full coverage of measured life-history trait space (a similar approach to that employed by Vellend et al. 2010). This was determined by analyzing all average life-history trait values (scaled to a mean of 0 and standard deviation of 1) for each genotype in a Principal Components Analysis (PCA). A genotype was selected as a candidate if it occupied a unique location in multivariate PCA space (i.e. no other genotypes were nearby in the PCA), if it was located the furthest along a particular axis (genotypes with the highest/lowest trait values), or if it was the most central in PCA space (a genotype with average values in multiple traits). Of the original 54 clones, we selected 11 that were from unique source lakes and that covered the full range of trait space. Live individuals from each of these 11 candidate clone lines were analyzed at McGill University, QC, Canada (see Appendix C2 for genomic methods), with 9 of these determined to be genetically unique based on 12 microsatellite markers (Appendix C: Table C4). These 9 unique genotypes were the clones used in our experiment.

Appendix C2 – Genetic analysis

Genomic DNA extraction and microsatellite amplification

Genomic DNA was extracted from live samples of the initial nine candidate clones to determine genotypic differences prior to mesocosm inoculation, and from bulk samples collected following the conclusion of the experiment. Bulk samples were obtained from 2 replicates out of 10 for the low quantity/low diversity mesocosms, 2 replicates out of 5 for the high quantity/low diversity mesocosms, and from all 7 replicates in the high quantity/high diversity mesocosms. Bulk samples consisted of 20 total individuals for the low quantity/low diversity and high quantity/high diversity treatments, and 50 total individuals for the high quantity/high diversity treatment. All replicates and a greater number of individuals were sampled from the high quantity/high diversity mesocosms due to the potentially higher variation in genotypic composition between replicates. Extractions followed the cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). DNA extracts were analyzed at 12 previously mapped microsatellite loci (Cristescu et al. 2006; Appendix C: Table C4). The 12.5 μ L polymerase chain reaction (PCR) and thermocycling regimes were conducted independently for each locus and followed that of Cristescu et al. (2006). Amplified product was diluted 20-fold and combined into groups of four according to their fluorescent labels (FAM, NED, PET, VIC). Two microliters of the diluted PCR product was mixed with 8.35 μ L of HiDi formamide (Life Technologies) and 0.15 μ L of GeneScan-500 LIZ size standard (Applied Biosystems, Foster City, CA, USA). Two independent PCRs for each locus on all extracted samples were performed to confirm the genotypes of clones and repeatability of alleles identified in the mesocosms. Electrophoresis was performed using an ABI-3730XL Analyzer and chromatographs were evaluated manually using GeneMapper Software v3.0 (Applied Biosystems).

Microsatellite allele scoring procedure of mesocosm samples

Microsatellite allele bins were set up in GeneMapper based on the fragment sizes of the individually amplified clones. These allele bins were grouped into mesocosm binsets according to the size of the expected alleles in each mesocosm. Through this method we were able to easily identify novel and unexpected alleles, and rule out the possibility of experimental contamination by noting the presence of alleles falling outside the mesocosm binset. Alleles were called manually and blind to the level of genetic diversity present in mesocosm samples. Alleles were considered present regardless of fluorescent peak intensity relative to the LIZ size standard (on average 2,600) since a preliminary test indicates that this approach is likely to correctly identify alleles present at low frequency. Eight of the 9 inoculating clones used in the experiment possessed one or multiple unique alleles that were used to differentiate them from their mesocosm co-habitants. Genotype F had no unique alleles, but had a distinct genotype of shared alleles.

The identity of the persisting clones was estimated by noting the presence and absence of unique alleles. We implemented a scoring procedure based on whether a unique allele was observed in the two PCR replicates of subsample extracts. A genotype with a unique allele detected within a fragment size bin was determined to be present in the replicate (scored as 1), while those with alleles showing no sign of amplification despite being expected in a particular bin (i.e., in mixture treatment) were determined to be absent (scored as 0).