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Imperfect detection alters the outcome of management strategies for protected areas

Edd Hammill[1] and Christopher F. Clements[2]

[1]Department of Watershed Sciences and the Ecology Center, Utah State University, 5210 Old Main Hill, Logan, UT, USA

[2]School of Biological Sciences, University of Bristol, Bristol, BS8 1TQ, UK

Statement of Authorship. The experiment was conceived by EH following multiple conversations with CFC. EH conducted the experiment and ran the analyses relating to species richness, probability of predators, and number of extinctions. CFC designed and conducted all analyses relating to sampling protocols. EH wrote the first draft of the MS, both authors contributed substantially to editing the MS.

Running title: Patch configuration versus sampling regime

Correspondence to Edd Hammill - edd.hammill@usu.edu, 435 265 5946, Department of Watershed Sciences, 5210 Old Main Hill, Logan, UT, USA, 84322

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Statement on data accessibility

Data have been submitted to the Dryad Data repository, DOI https://doi.org/10.5061/dryad.stqjq2c0d

Abstract

Designing protected areas configurations to maximize biodiversity is a critical conservation goal. The configuration of protected areas can significantly impact the richness and identity of the species found there; one large patch supports larger populations but can facilitate competitive exclusion. Conversely, many small habitats spreads risk but may exclude predators that typically require large home ranges. Identifying how best to design protected areas is further complicated by monitoring programs failing to detect species. Here we test the consequences of different protected area configurations using multi-trophic level experimental microcosms. We demonstrate that for a given total size, many small patches generate higher species richness, are more likely to contain predators, and have fewer extinctions compared to single large patches. However, the relationship between the size and number of patches and species richness was greatly affected by insufficient monitoring, and could lead to incorrect conservation decisions, especially for higher trophic levels.

Introduction

The designation of protected areas remains at the heart of conservation efforts (Watson *et al.* 2016). When designing protected areas, managers are tasked with deciding whether greater outcomes may be achieved by investing in a small number of large, or a large number of small protected areas (Diamond 1975). A small number of large protected areas may allow species to exist at higher population abundances, thereby reducing extinction risk (Mccarthy *et al.* 2011). This issue of home range may be especially true of apex predators, which may require larger home ranges than species in lower trophic levels (Mcnab 1963; Fauvelle *et al.* 2017). However, having a small number of protected areas may increase stochasticity, demographic and otherwise, potentially decreasing the number of species protected through extinctions (Whittaker & Fernández-Palacios 2007). In addition, a small number of large protected areas may increase the chance that the whole network becomes dominated by a few highly competitive species (Schippers *et al.* 2015). Given the potentially negative outcomes for a small number of large protected areas, managers may instead opt for a large number of smaller protected areas (Higgs & Usher 1980; Simberloff & Abele 1982; Wintle *et al.* 2019). However, a large number of smaller locations may be unable to support species with large resource requirements, and runs contrary to principles from species-area relationships and the theory of island biogeography (Diamond 1975; Tjørve 2010). The question of “single/few large” or “several/many small” (The SLOSS or FLOMS debate) has received a substantial amount of attention in the decades since its inception, but whether greater biodiversity outcomes are achieved through a small number of large protected areas, or a large number of small protected areas remains unresolved. It therefore becomes critical to test the consequences of different protected area configurations using a replicated, manipulable system that contains a suite of species embedded in a complex, multitrophic foodweb.

Having sufficient information relating to the distribution of species is critical when making any decisions about the importance of different habitat patches (Maxwell *et al.* 2015). Insufficient sampling may fail to detect species when they are present, leading to biased estimates of site occupancy (Mackenzie *et al.* 2003; Loehle & Weatherford 2017). In addition, monitoring efforts should be designed so as to achieve sufficient temporal and spatial coverage to understand the state of the system (Legg & Nagy 2006; Rhodes & Jonzén 2011). For example, while targeted monitoring may provide information as to whether or not a particular species of interest is present, it may fail to detect species that exist in non-targeted locations, leading to an underestimate of total biodiversity (Nichols & Williams 2006; Nielsen *et al.* 2009). Given the difficulties associated with monitoring wild systems, understanding whether better conservation outcomes are achieved through the designation of few large or several small protected areas becomes difficult. It therefore becomes necessary to test how allocating protected areas using imperfect information affects conservation outcomes compared to those generated using perfect information.

Laboratory-based systems provide an ideal arena to test questions relating to the SLOSS/FLOMS debate, and the issue of monitoring, as populations of multiple interacting species can be assembled and closely monitored. One system used to test broad ecological concepts is that based on freshwater ciliate protozoa due to their small size, ease of manipulation, and high replicability (Drake & Kramer 2012; Altermatt *et al.* 2015). In addition, the global biomass of protists has been estimated to be double that of the animal kingdom (Bar-On *et al.* 2018), highlighting their ecological relevance. The short generation times of the species used means that multi-generational data can be rapidly collected, making the system ideally suited for studies on population biology (Lawler & Morin 1993; Hammill *et al.* 2015), community ecology (Clements *et al.* 2013a; Carrara *et al.* 2015), metacommunity dynamics (Holyoak & Lawler 2005; Resetarits *et al.* 2018), biodiversity-ecosystem function experiments (DeLong & Gibert 2019), and conservation issues (Fryxell *et al.* 2006; Benton *et al.* 2007). The protist system can also contain complex food webs, consisting of up to four trophic levels that incorporate omnivory and specialization (Forbes and Hammill 2013, Fig 1). Protist microcosms represent an excellent system to address the SLOSS/FLOMS question as customized microcosms of a range of different sizes can be easily and accurately produced. The individual microcosms can then be combined to produce portfolios of microcosms that are representative of suites of protected areas that have no connectivity, a situation present in ~50% of global protected areas (Santini *et al.* 2016; Saura *et al.* 2018).

We performed an experiment to test how the configuration of simulated protected areas performs in terms of the amount of species represented, and how different monitoring protocols affect our ability to detect what species were present. The experiment utilized custom designed protist microcosms of varying volumes that were inoculated with nine species. Each microcosm (or patch) was disconnected from each other, and is intended to represent a single, isolated protected area. Individual patches were periodically sampled to assess the abundances of the species present. These patch-level data were then used to construct protected area portfolios (analogous to "experimental landscapes" used in Pasari *et al.* 2013; Hammill *et al.* 2018) that allowed us to investigate whether for a given total area, better biodiversity outcomes were achieved through few large or many small patches. Protected area portfolios were produced by combining (without replication) the abundance data for between 1 and 8 of the patches, and the number of patches and total volume was recorded. To these portfolios with perfect knowledge of the community composition we then applied various imperfect sampling protocols. Using this set up we were then able to ascertain how increasing the number of patches vs. increasing the overall size of the portfolio 1) affects the number of species present in the system, 2) affects the presence or absence of apex predators, 3) alters the number of extinctions, and 4) how imperfect sampling changes our ability to discern between optimal and suboptimal management options.

Methods

Experimental microcosms

The experiment was conducted using custom-designed, 3D-printed microcosms, produced at GoR3 Printing Services (Logan, UT, USA) on a Formlabs© Form 2 printer using Formlabs© standard resin in white. The 3D printer was used to create 9 (160mm * 140mm * 14mm) plates, each of which had 5 unconnected patches, one each of 4, 8, 16, 32 and 64ml, for a total of 45 patches. Patches were designed in such a manner that as overall ecosystem size increased, surface area to volume ratio, and edge to volume ratio both remained constant (supplementary material).

Experimental media consisted of 0.4 g/L crushed protozoa pellets (no. 13-2360; Carolina Biological Supply, Burlington, NC, USA) dissolved in Purelife™ mineral water (Nestle, USA). Plates were covered by Perspex lids to reduce evaporation and were topped up to their original level every 3.5 days with deionized water. Following top up, 10% of the media was removed from each ecosystem and replaced in order to ensure some nutrients were available for bacterial growth (Forbes & Hammill 2013; Hammill *et al.* 2015). All patches were initially inoculated with the following (values in parentheses give the initial inoculation densities) *Paramecium* *multimicronucleatum* (50 ind.ml^{-1}), *Paramecium aurelia* (100 ind.ml^{-1}), *Blepharisma sp* (50 ind.ml^{-1})*, Euplotes sp* (50 ind.ml^{-1})*, Tetrahymena sp* (200 ind.ml^{-1}) and *Didinium nasutum* (5 ind.ml^{-1})*,* the algae *Volvox* (20 ind.ml^{-1})*,* the Turbellarian flatworm *Stenostomum virginianum* (2 ind.ml^{-1})*,* and the rotifer *Philodina* (10 ind.ml^{-1}). The community was supported by a bacterial basal resource consisting of *Serratia* and *Bacillus subtilis*. The food web consisted of three trophic levels, with intraguild predation occurring (Fig 1). We defined *Stenostomum* and *Didinium* as apex predators as they are not consumed by any species, and do not consume the basal resource. All taxa were obtained from Carolina Biological Supply (Burlington NC) with the exception of *S. virginianum*, which were raised from a single individual isolated from a pool on the Logan river, UT.

The experiment was conducted at 19°C on a 16hr:8hr light:dark cycle and ran for 21 days. On day 21, the entire media in each patch was analyzed using a Bogorov counting chamber and we recorded total number of taxa present (species richness), whether the ecosystem contained apex predators, the total abundance of each taxa in the ecosystem, and whether any extinctions had taken place. As all taxa were inoculated into each patch, an extinction was deemed to have occurred if a taxa was not detected in the total sample on day 21.

Protected area portfolios

We combined the abundance data from each patch to produce 21045 portfolios of patches by sampling without replacement a specified number of patches (from 1 to 8). It is critical to note that within these portfolios, there was no connectivity among the patches, meaning they represent an extreme condition as connectivity represents a critical landscape characteristic (Taylor *et al.* 1993). However, this lack of connectivity is considered representative of 50% of protected area networks in the field, (Santini *et al.* 2016; Saura *et al.* 2018). Within our portfolios there was therefore no potential for some processes associated with reducing extinctions in natural populations, such as rescue effects (Ferraz *et al.* 2007). In total we had abundance data for 45 patches, meaning the total number of potential portfolios was greater than $1.6e^{56}$, forcing us to use a subsample. We used data from all single-patch portfolios (45 portfolios). The remaining 21000 portfolios were produced using a stratified sampling protocol to produce a balanced number of portfolios across different combinations of patch number (3000 for each patch number) and total volume. Some configurations were not possible given the physical characteristics of the patches (i.e. maximum volume of a single patch is 64ml, therefore a portfolio consisting of 2 patches cannot have a volume of 150ml). We used the following parameters as descriptive variables in all analyses - the number of patches within the portfolio and the total volume. To compare how these descriptive variables affected diversity, we calculated (i) portfolio level species richness (ii) whether the portfolio contained apex predators, and (iii) number of extinctions from whole portfolios. Each response variable was analyzed using a generalized linear model, and we used hierarchical partitioning (Chevan & Sutherland 1991) to estimate the proportion of the total variation in each response variable attributable to patch number and total portfolio volume. We opted to use hierarchical partitioning in place of reporting p-values for the portfolio-level analysis as hierarchical partitioning demonstrates the importance of each response variable, and the large sample sizes used in our experiment are always likely to generate significant p-values. To specifically look at effect sizes and address the SLOSS/FLOMS question, we also looked at the difference in each biodiversity metric between portfolios containing 1 patch of 64ml (single large), and 8 patches that total 64ml (several small). To tease apart how the number of patches in a portfolio and total portfolio volume influenced our response variables, we split the data and ran individual models looking at total portfolio volume for each number of patches.

Imperfect sampling protocols

We applied imperfect sampling to understand how different search efforts and protocols provide information about diversity measures. Following the method of Clements et al. (2013, 2015) we implemented four search efforts (“SE”) – 0.1ml, 1ml, 10ml, and 100ml. In addition, we simulated four search regimes (“SR”) that determined how search effort was split across patches within the portfolio – (i) from the smallest to largest patch, (ii) from the largest to smallest patch, (iii) uniformly across all patches, and (iv) randomly allocating which patch was searched first. For the smallest to largest, largest to smallest, and random search regimes the search effort was allocated to a single patch, then any remaining search effort was reallocated to the next patch and so on. For uniform sampling SE was divided amongst the patches in the portfolio, regardless of the patch size. To assess whether a species was observed in a patch under a given SE, the SE allocated to that patch was converted to a percentage of the patch sampled. The number of individuals of each species observed was then calculated by drawing a binomial random variate with size equal to the known abundance of the species in the patch and probability set to the percentage search effort. All analyses were conducted using the R statistical programming language (R Core Team 2018).

Results

Patch volume

Patch volume accounted for 30.04% of the variation in species richness, with increased volume being associated with increased richness ($z = 2.12$, P = 0.034, Fig 2a). Increased volume also increased the chance of apex predators being present ($z = 2.163$, P = 0.031, Fig 2b), accounting for 26.63% of the total variation. Increased volume also all decreased the number of extinctions (Fig 2c), accounting for 30.20% of the variation in extinction number ($z = -2.65$, P = 0.0081, Fig 2c).

Portfolio configuration

Increasing the number of patches represented a significantly better option when aiming to increase species richness and reduce the number of extinctions than increasing portfolio number (GLM with Poisson distribution Fig 3). Overall species richness within a portfolio increased as patch number and total volume increased (Fig 3a), accounting for 25.47% and 15.11% of the variation in species richness respectively (hierarchical partitioning). With respect to SLOSS/FLOMS, GLM outputs predicted that for portfolios of 64ml, species richness increased 39.20% ± 1.71% from 6.64 ± 0.43 species in a one patch portfolio to 9.01 ± 0.32 species in an eight patch portfolio. When the data were split on the basis of patch number and individual models run, model outputs revealed that at any given volume, to increase species richness to the same extent as adding a patch to the portfolio would require a volumetric increase of 25.12ml ± 7.41ml (Fig 3a). The probability that all nine species were present on day 21 increased with patch number and portfolio volume (GLM with binomial distribution Fig 3b). Patch number accounted for 14.59% of the total variation in the probability a portfolio contained all nine species, while total portfolio volume accounted for 9.94%. GLM model outputs predicted that the probability a 64ml portfolio contained all nine species increased by 15.36 ± 0.25 times from

0.049 ± 0.002 for a single large patch portfolio to 0.76 ± 0.010 for an eight patch portfolio. GLM outputs also revealed that total portfolio volume would need to increase by 61.77ml ± 1.66ml (Fig 3b) in order to achieve the same benefit as increasing the number of patches by one while keeping volume constant.

The probability that a portfolio contained apex predators increased as both the number of patches and total portfolio volume increased (Fig 3c). The number of patches within the portfolio and total portfolio volume accounted for 6.04% and 4.13% of the total variation in apex predator probability respectively. In terms of the SLOSS/FLOMS question, GLM model outputs predicted that the probability a 64ml portfolio contained predators increased by 2.02% ± 1.30 % from 0.97 ± 0.007 when the portfolio contained a single large patch to 0.99 ± 0.06 when the portfolio contained 8 patches. To further tease apart how patch number and portfolio volume affect predator probability we again split the data and ran separate models. We constrained out analysis to portfolios containing < 5 patches due to the very high probability of predators occurring in all portfolios with 5 or more patches. We found the total volume of the portfolio would need to increase by 2.53ml ± 0.36ml to increase the probability of apex predators being present to the same extent as keeping volume constant but increasing the number of patches by 1 (Fig 3c).

The number of extinctions observed at the portfolio level decreased as both patch number and total portfolio volume increased (Fig 3d). Patch number and total portfolio volume accounted for 25.24% and 23.33% of the variation in patch number respectively. GLM outputs indicated the number of extinctions being reduced by 32.18% ± 2.38% from 1.61 ± 0.024 to 0.52 ± 0.015 as the number of patches increased from one to eight in a 64ml portfolio. When the portfolios were split on the basis of patch number, to reduce the number of extinctions to the same extent as increasing the number of patches in a portfolio would require increasing overall portfolio volume by 15.56ml ± 0.25ml (Fig 3d).

Imperfect sampling and optimal portfolio configurations

Imperfect sampling significantly altered the probability of observing all nine taxa (Fig. 4), with low search efforts (0.1ml) typically detecting around 1/3 of the species observed with high search efforts (100ml). We however found a significant interaction between search effort and the search regime (how search effort was allocated across patches). At high search efforts (100ml) the search regime had little effect on the number of species detected (Fig. 4), whilst at low search efforts choosing the right search regime had a significant impact on the ability to determine the species richness of the portfolios (Fig. 4). Of the four search regimes, uniform sampling detected the highest number of species when search efforts were low to moderate (0.1ml to 10ml). Where search effort was directional (allocated either from large to small patches, or from small to large) and low (0.1ml and 1ml) there was an interaction between the volume of the portfolio and patch number, making it possible to significantly underestimate the species richness of large portfolios with many patches (which in reality contained the most species) (Fig. 4).

The probability of detecting apex predators was determined by the search regime implemented, the search effort, and the number of patches within a portfolio (Fig. 5). Again, uniform sampling produced the least biased estimates of apex predator presence regardless of search effort, whilst other search regimes typically overestimated the importance of small, low volume portfolios at low search efforts (0.1-10ml). However, uniform sampling showed a significant decline in the probability of observing apex predators with increasing portfolio volume at low search efforts, whereas in reality patch volume and the number of patches had a significant positive effect on the number of predators present (Fig. 3c). At high search efforts both small to large and large to small search regimes underestimated the importance of high-volume portfolios with high numbers of patches (Fig. 5).

Discussion

The question of whether conservation managers should invest in a few large, or many small protected areas has resurfaced multiple times since its inception (Diamond 1975; Higgs & Usher 1980; Simberloff & Abele 1982; Virolainen *et al.* 1998; Whittaker & Fernández-Palacios 2007; Lindenmayer *et al.* 2015; DeLong & Gibert 2019). Our results reveal that with perfect information about the system (i.e. the entire portfolio is sampled), increasing the number of patches within a portfolio increases species richness, increases the likelihood of apex predators being present, and decreases extinctions. However, our analyses also demonstrate that "incorrect" results (i.e. deducing that fewer patches increase the probability of maximizing species richness) may be observed if the amount of the portfolio sampled is insufficient, and/or an incorrect sampling protocol is used.

At the single patch level, we found that higher levels of species richness and lower levels of extinction were associated with greater patch volumes. These results agree with classic concepts such as the species area relationship (Preston 1962; Connor & McCoy 1979) and the theory of island biogeography (MacArthur & Wilson 1967), which state that as patch area increases, richness should increase through reduced extinction rates. As all species were initially inoculated into all patches, extinction represents the only way richness can decline, via either competitive exclusion (Hardin 1960; Johnson & Bronstein 2019), over-exploitation by predators (Delong & Vasseur 2013) or stochasticity (Melbourne & Hastings 2008). With respect to higher trophic levels, we found that greater patch volume increased the probability of apex predators, agreeing with previous studies describing how larger patches provide sufficient resources to support predator populations (Post *et al.* 2000). In our present study apex predators were found in all patches greater than 30ml in volume, however they were also present in five of the nine smallest patches (4ml). As all species were inoculated in all patches, the lack of apex predators in some smaller patches suggests stochastic extinctions potentially due to small population sizes in the small patches. Given recent work showing the importance of apex predators as indicators of healthy patches (Atwood & Hammill 2018) and drivers of resilience (Llope *et al.* 2011), one key management goal might be to increase the probability of apex predators persisting. Our results suggest that if this is to be achieved, portfolios should contain at least some large habitats.

With respect to portfolio-level species richness, we found that to increase the probability of all nine species being present to the same extent as adding a single patch would require a volumetric increase of over 60ml, a volume greater than 4 of the 5 patches used in the study. This requirement of a large increase in volume suggests that partitioning a portfolio into a greater number of patches, rather than just increasing size, may be a more efficient process to yield biodiversity gains (Oertli *et al.* 2002; Rösch *et al.* 2015). The importance of multiple patches for species richness is further highlighted by the amount of variation in species richness associated with changes in patch number, and the substantial increase in richness observed between a 1-patch an 8-patch portfolio of 64ml total volume. However, the factors determining whether a portfolio contained apex predators were less clear, with an increase of just 2.53ml being required to achieve the same benefit as increasing patch number. Given that the smallest patch used in the study was 4ml, a volume greater than 2.53ml, increasing patch number may not be the best option to increase apex predator probability. Total portfolio volume also accounted for a greater proportion of the variation in the probability a portfolio contained apex predators, and the difference in the probability of predator presence between a 1-patch and an 8-patch 64ml portfolio was very small. These modest increases associated with increased patch number suggest it may be better to incrementally increase the size of patches rather than add another. This process of incrementally increasing the amount of habitat may also be cheaper than establishing new areas, due to previously demonstrated economies of scale (Balmford *et al.* 2004; Bruner *et al.* 2004). Our results therefore suggest that the next step to be taken to increase biodiversity outcomes may depend on management goals. If the primary goal is to increase the chance that the portfolio contains the maximum number of species, then adding a new patch to the portfolio would generate the best potential outcomes. Conversely, if the main goal is to increase the chance that a portfolio contains apex predators (Macdonald *et al.* 2015), then incrementally increasing the size of existing protected areas within the portfolio may be a better option, highlighting the need to identify clear, quantifiable goals to guide management (Moilanen *et al.* 2009).

Imperfect sampling of the portfolios generated qualitatively different outcomes than when portfolios were perfectly sampled. Most critically, when search effort was low (i.e. 0.1ml or 1ml) and total patch volume was > 100ml, the probability of observing all nine species was higher when patch number was lower for all but the uniform sampling regime. This result is the opposite of that obtained when the entire portfolio was perfectly sampled, and highlights the need for monitoring programs to have sufficient power to understand the overall system (Legg & Nagy 2006; Rhodes & Jonzén 2011). For portfolios with many patches, the probability that at least one of the patches would be small (i.e. 4ml) is increased. The increased chance of there being one small patch would mean that under a “small to large” search protocol, it is likely that all the search effort would be put into sampling a small patch, which were never observed to contain all nine species. Therefore, under a "small to large" search protocol with a large number of patches, it becomes difficult to observe all nine species, even though other patches in the portfolio may contain other species. When patch number is small, the chance of there being at least one small patch is reduced, increasing the probability that a larger patch is sampled under a "small to large" protocol. Under a "large to small" search protocol, the chance that very large (i.e. 64ml) patches were selected for search would increase as patch number increased. For these large patches, a small search effort appears insufficient to detect all nine species. The insufficient effort dedicated to a large patch may explain why in a "large to small" search protocol, the probability of detecting all nine species decreases as patch number increases. However, with sufficient search effort (i.e. >=10ml sampled) or under a "uniform" sampling regime, qualitatively similar results are obtained under imperfect sampling as for perfect, highlighting the effectiveness of proper monitoring protocols (Lindenmayer & Likens 2010). The negative consequences of insufficient sampling and/or an inappropriate protocol become more apparent with respect to predator detection. Due to their lower population densities and wide ranges, predators may require either more search effort, or the importance of correct monitoring protocols may be increased (Johnson *et al.* 2019). In the present study when search effort is 0.1ml – 10ml, both a small to large protocol and a large to small protocol always produce incorrect results (i.e. predators are more likely in low volume portfolios that contain few patches). In addition, even a uniform sampling protocol may produce incorrect results if the search effort is 1ml and portfolio volume is less than ~25ml, highlighting the need for sufficient power and an appropriate sampling design (Lindenmayer & Likens 2010; Rhodes & Jonzén 2011). These results have significant implications for the monitoring of protected areas, and implies that when dividing limited search effort across multiple habitats, the same amount of absolute effort should be invested to each patch regardless of their size, as doing so reduces the chances of biodiversity estimates being different to what is taking place in the system.

In conclusion, our results show that for a given total size, increasing the number of patches appears to generate better biodiversity outcomes than increasing patch size if the goal is to maximize diversity. However, our results highlight the importance of appropriate monitoring schemes, as insufficient effort or incorrect protocols generate estimates of species richness that are the inverse of those generated with perfect information. This is particularly true when conserving higher trophic level species such as apex predators, which are both of critical importance for the functioning of ecosystems (Atwood *et al.* 2015), and difficult to conserve due to their range requirements.

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Figure Legends

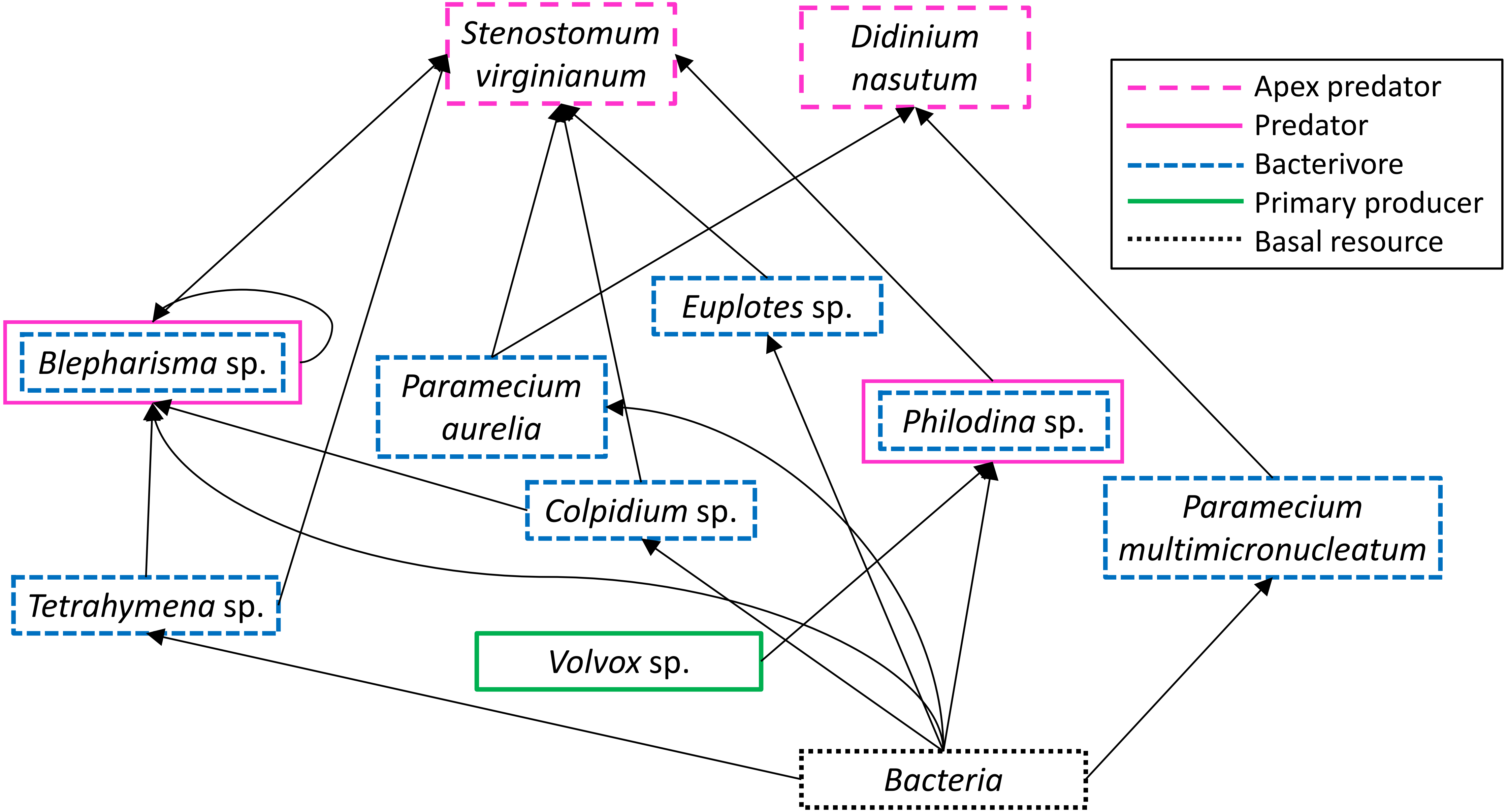
Fig. 1. Proposed food web of the communities used in the experiment. The food web consists of multiple trophic levels and includes a high degree of intraguild predation. UV lighting was provided to facilitate primary production. Consumptive links were obtained from (Lawler & Morin 1993; Spencer & Warren 1996; Worsfold *et al.* 2009; Forbes & Hammill 2013).

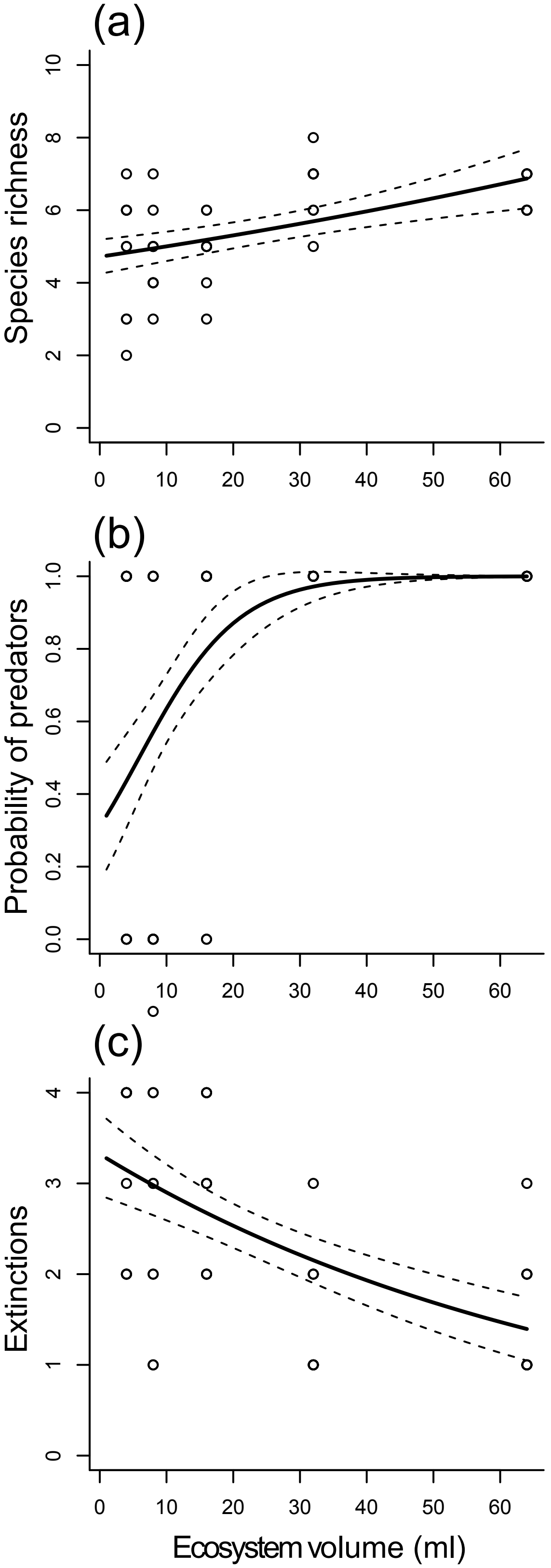
Fig. 2. Effects of increasing the volume of a single ecosystem on (a) species richness, (b) the probability of predators being present in an ecosystem, and (c) the number of observed extinctions. Solid lines represent individual model outputs for each ecosystem number, dashed lines represent standard errors.

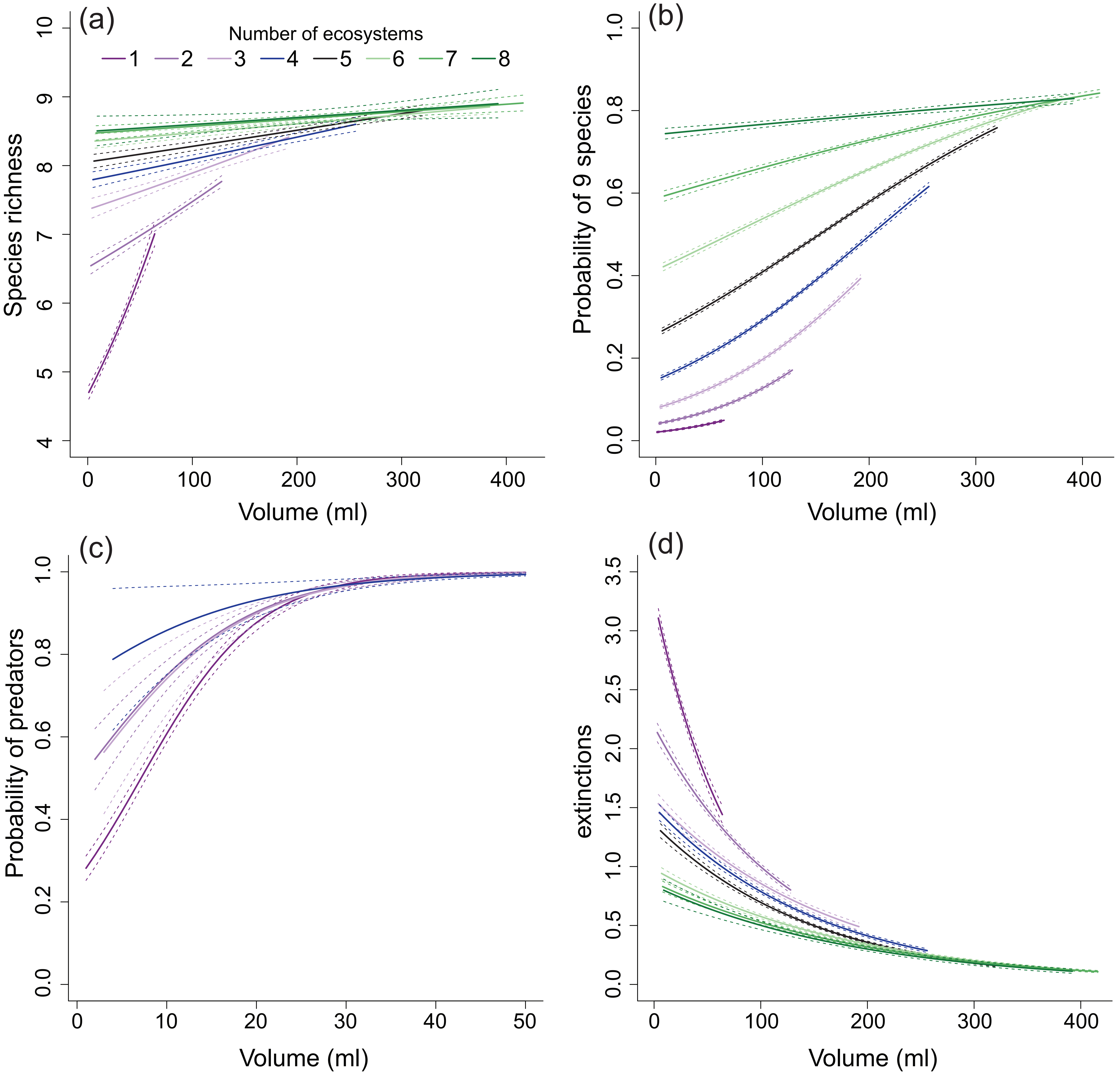
Fig. 3. Relationship between the number of patches in a landscape and total landscape volume on (a) landscape level species richness and (b) the probability a landscape contained all nine species. Effects of increasing total landscape volume and the number of patches in a landscape on (c) the probability a landscape contains predators and (d) the number of extinctions in a landscape. in the case of predator probability (c), all landscapes with 5 or more patches or greater than 50ml in volume contained predators, so we limited the analysis to a subset of the data. Solid lines represent model output for individual models ran for each ecosystem number, dashed lines represent standard errors.

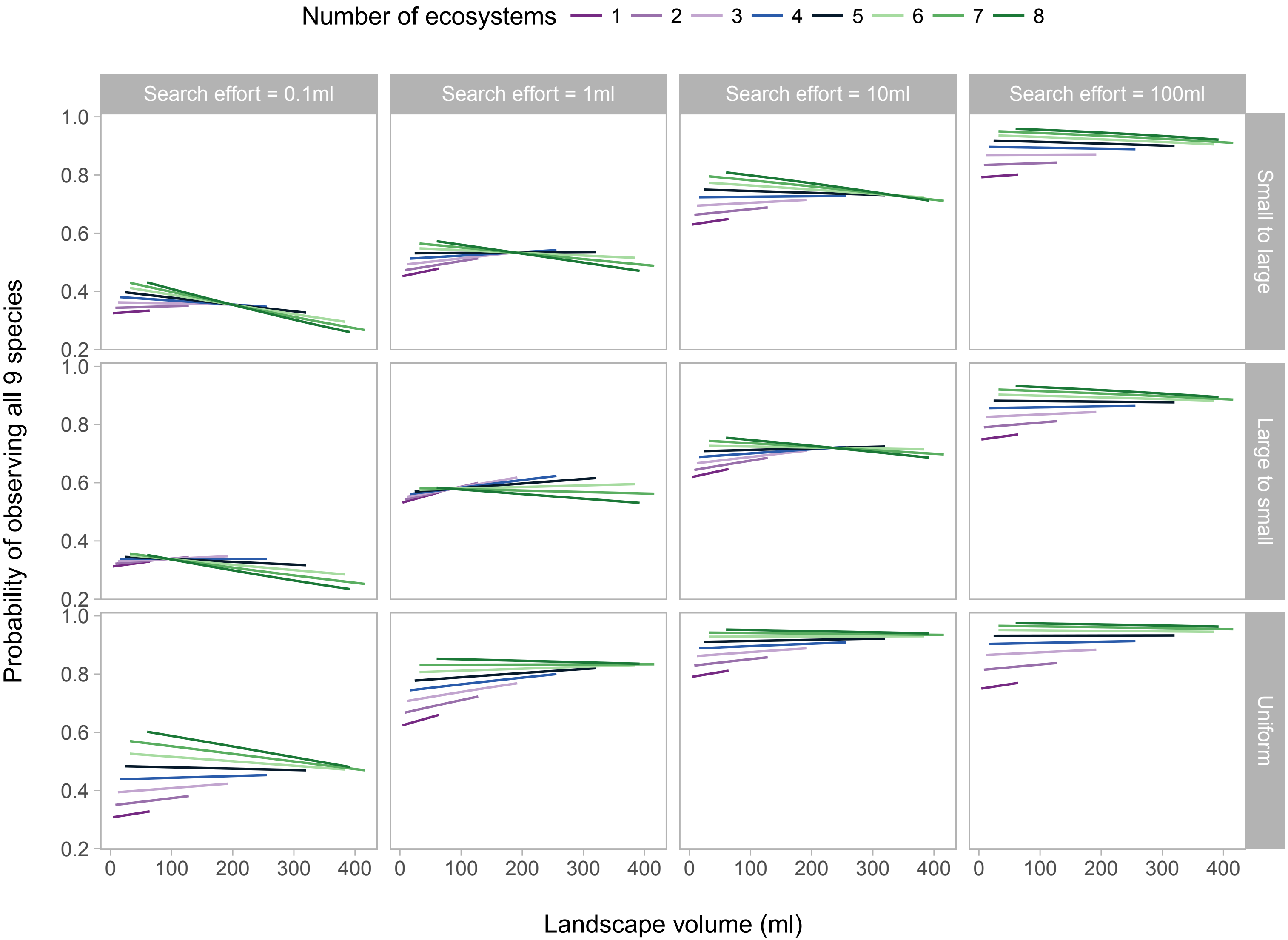
Fig 4. Effects of imperfect sampling and search regimes on the probability of observing all nine species. Uniform sampling consistently detected the greatest number of species regardless of search effort, whilst at low search efforts directional sampling (where the search effort was allocated either from large to small patches, or from small to large patches) suggested that small landscapes with few patches contained more species that large landscapes with many patches, when in fact the opposite was true.

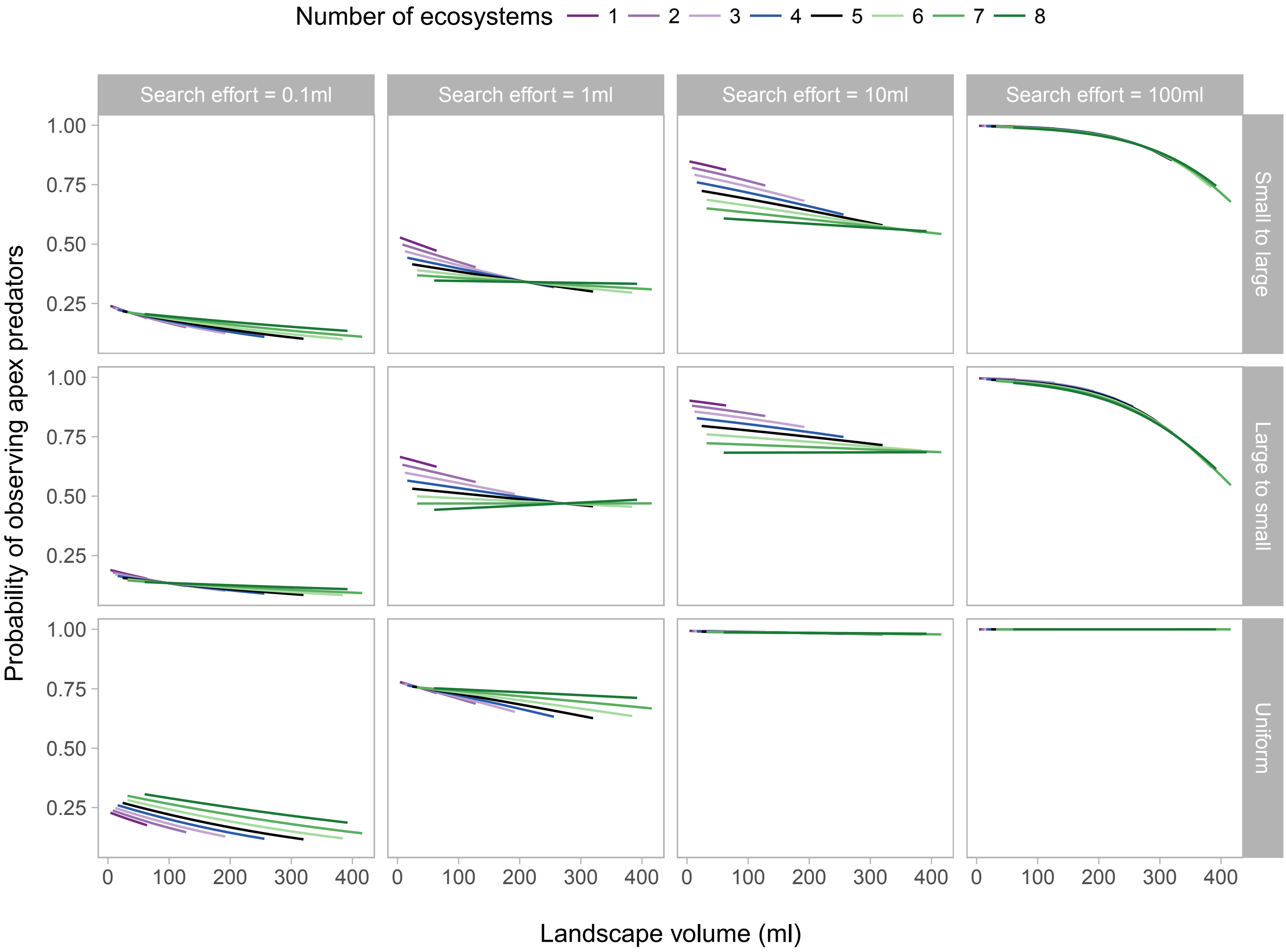
Fig 5. Effects of imperfect sampling and search regimes on the probability of apex predators being observed in a portfolio. Uniform sampling produced the most reliable estimates of apex predator presence regardless of search effort, however at low search efforts the results suggest that a few small patches are more likely to harbor an apex predator than a larger number of patches.


Stenostomum virginianum
Didinium nasutum
Apex predator
Predator
Bacterivore
Primary producer
Basal resource
Blepharisma sp.
Paramecium aurelia
Euplotes sp.
Philodina sp.
Colpidium sp.
Paramecium multimicronucleatum
Tetrahymena sp.
Volvox sp.
Bacteria


(a)
Species richness
0
2
4
6
8
10
0
10
20
30
40
50
60
(b)
Probability of predators
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
(c)
Extinctions
0
1
2
3
4
0
10
20
30
40
50
60
Ecosystem volume (ml)


(a)
Number of ecosystems
1
2
3
4
5
6
7
8
Species richness
4
5
6
7
8
9
10
0
100
200
300
400
Volume (ml)
(b)
Probability of 9 species
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
Volume (ml)
(c)
Probability of predators
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
Volume (ml)
(d)
extinctions
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
100
200
300
400
Volume (ml)


Number of ecosystems
1
2
3
4
5
6
7
8
Search effort = 0.1ml
Search effort = 1ml
Search effort = 10ml
Search effort = 100ml
Small to large
Large to small
Uniform
Probability of observing all 9 species
1.0
0.8
0.6
0.4
0.2
Landscape volume (ml)
0
100
200
300
400


Number of ecosystems
1
2
3
4
5
6
7
8
Search effort = 0.1ml
Search effort = 1ml
Search effort = 10ml
Search effort = 100ml
Small to large
Large to small
Uniform
Probability of observing apex predators
1.00
0.75
0.50
0.25
0
100
200
300
400
Landscape volume (ml)