RESEARCH ARTICLE

Seed plant features, distribution patterns, diversity hotspots, and conservation gaps in Xinjiang, China

Jihong Huang[1,2], Canran Liu[3], Zhongjun Guo[4], Keping Ma[5], Runguo Zang[1,2], Yi Ding[1,2], Xinghui Lu[1,2], Jiping Wang[6], Ruoyun Yu[1]

I *Key Laboratory of Forest Ecology and Environment, the State Forestry and Grassland Administration, Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, #2 Dongxiaofu, Xiangshan Road, Haidian District, Beijing, China 100091, China* **2** *Co-Innovation Centre for Sustainable Forestry in Southern China, Nanjing Forestry University, #159 Longpan Road, Nanjing 210037, China* **3** *Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria 3084, Australia* **4** *Institute of Forest Ecology, Xinjiang Forestry Academy, #191 Anjunnan Road, Shuimogou District, Urumqi 830000, China* **5** *State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, #20 Nanxincun, Xiangshan, Haidian District, Beijing 100093, China* **6** *Research Center of Saline and Alkali Land of State Forestry and Grassland Administration, #2 Dongxiaofu, Xiangshan Road, Haidian District, Beijing 100091, China*

Corresponding author: *Jihong Huang* (northalluvion@caf.ac.cn)

Abstract

The flora in Xinjiang is unique. Decisions about biodiversity conservation and management based on seed plant diversity hotspots and conservation gaps in Xinjiang are essential to maintain this unique flora. Based on a species distribution dataset of seed plants, we measured seed plant diversity using species richness and phylogenetic diversity indices. Five percent of Xinjiang's total land area with the highest biodiversity was used to identify hotspots for each index. In total, eight hotspots were identified. Most hotspots were located in mountainous areas, mainly in the Tianshan Mountains and Altai Mountains. Furthermore, we detected conservation gaps for Xinjiang's seed flora hotspots by overlaying nature reserve maps on to maps of identified hotspots and we designated priority conservation gaps for hotspots by overlaying global biodiversity hotspot maps on to hotspot conservation gaps maps. Most of Xinjiang's seed plant hotspots are poorly protected; only 10.45% of these hotspots were covered by nature reserves. We suggest that it is essential to promote network function of nature reserves within these hotspots in Xinjiang to conserve this unique flora.

Introduction

Biodiversity is unevenly distributed; therefore, prioritisation is essential to minimise biodiversity loss (Brooks et al. 2006). Identifying priority areas or hotspots where biodiversity is most threatened is critical for effective conservation (Myers et al. 2000; Olson and Dinerstein 1998). Biodiversity hotspots are characterised by exceptional concentrations of endemic species that experience relatively high rates of habitat loss (Myers et al. 2000; Prendergast et al. 1999). This approach to defining hotspots has been used in many studies (Mittermeier et al. 2005). To adequately define a hotspot, its boundaries must be delineated. A predefined proportion of areas with large amounts of both endemic species richness and habitat loss was previously considered a hotspot (Orme et al. 2005; Prendergast et al. 1993). Recently, the definition of the term 'hotspot' for biodiversity has been generalised. In addition to the amount of endemic species and habitat loss, other aspects, including total number of species, number of threatened species and evolutionary history, have been proposed to identify hotspots (Cadotte et al. 2010; Forest et al. 2007; Rosauer and Laffan 2008). Researchers previously focused taxonomic diversity (Brooks et al. 2006; Reid 1998). However, more recently, many researchers focused on the evolutionary processes (Cadotte et al. 2010; Huang et al. 2016c; Xu et al. 2017). Phylogenies (evolutionary trees) have become increasingly important for identifying biodiversity hotspots. Phylogenetic diversity is often favoured because it shows relationships between extant species and provides more information about evolution over a long time-scale (Omland et al. 2008).

Xinjiang Uygur Autonomous Region (Xinjiang hereafter) is a large (1.64 million km²), topographically varied inland province in northwest China that extends from high mountains (8611 m above sea level) to lowland basins (156 m below sea level) and humid forest to grasslands and dry desert. Consequently, this region has an extreme arid and cold climate and exhibits striking climate, soil and vegetation patterns. Species richness patterns in Xinjiang were previously reported for woody plants (Zhang and Zhang 2014). The vegetation types in Xinjiang are mainly desert and grassland (including steppe and meadow) and forest covers relatively small areas. The constructive species of the grassland are Gramineae, Compositae and Leguminosae, whereas the constructive species of the forest include the coniferous *Larix*, *Picea*, *Abies*, and *Pinus*; broadleaved *Betula* and *Populus*; and the native fruit species of *Malus*, *Armeniaca* and *Juglans*, which are endemic to Ily Valley (Xinjiang Investigation Group of Chinese Academy of Sciences 1978). Although many studies have been conducted on Xinjiang's flora, data are still not sufficient to provide systematic guidance for regional biodiversity conservation.

In this paper, based on native seed plant species in Xinjiang, we identified seed plant hotspots using both species richness and phylogenetic diversity indices. Moreover, Xinjiang's seed plants conservation gaps, which are hotspot areas that are not covered by China's nature reserves, were detected by overlaying the seed plant hotspot map

on a map of Xinjiang's nature reserves; and Xinjiang seed plant priority conservation gaps, which are areas within both Xinjiang seed plant hotspots and global biodiversity hotspots that are not covered by Xinjiang's nature reserves, were detected by overlaying the Xinjiang seed plant conservation gap map on a global biodiversity hotspot map. Finally, we discussed the potential causes of the patterns revealed in this study and proposed suggestions for Xinjiang flora protection.

Methods

Data sets

We compiled a species distribution database that included all seed plant species distributed in Xinjiang based on a large number of herbarium specimens (http://www.cvh.org.cn/), a list of Chinese seed plant species and distribution information at provincial level (Wu et al. 1994–2012) and other literature sources that mainly included Flora Xinjiangensis (Commissione Redactorum Florae Xinjiangensis 1992–2011), Sylva Xinjiangensis (Yang 2012), Desert Plants in China (Lu et al. 2012) and Rare Endangered Endemic Higher Plants in Xinjiang of China (Yin 2006). The county was used as the basic spatial unit for species distributions and geographical coordinates were included for each specimen. To improve the quality of the distribution data at the county level, we overlaid county distributions over altitudinal range distributions for each species. We divided the entire region into 50×50 km grids and then overlaid these grids on to the distribution polygons or points. Finally, we acquired presence/absence data for each species in each grid. In our study, data on 3715 seed plant species were obtained based on a total of 145781 occurrence records.

A georeferenced or spatially referenced dataset of 33 nature reserves in Xinjiang was constructed. The main body of nature reserves was downloaded from the World Database on Protected Areas (http://www.protectedplanet.net/). The global biodiversity hotspots dataset was downloaded from Conservation International (http://www.conservation.org/How/Pages/Hotspots.aspx).

Diversity measures and phylogenetic tree construction

To detect the distribution patterns of Xinjiang seed flora, we calculated seed species richness and phylogenetic diversity of seed plants. Phylogenetic diversity is a simple measure of evolutionary diversity that is determined based on the sum of the lengths of all branches that are members of the corresponding minimum spanning path (Faith 1992). The phylogenetic diversity equation is:

$$PD = \sum_{\{c \in C\}} L_c ,$$

where *C* indicates the set of branches in the minimum spanning path joining the species to the root of the tree, *c* indicates a branch in the spanning path, and L_c indicates the length of branch *c*. Phylogenetic diversities were calculated by using the phylogenetic diversity algorithm in PHYLOCOM (Webb et al. 2008).

PHYLOMATIC (Webb and Donoghue 2005) was used to construct a phylogenetic supertree for Xinjiang's seed plants. The backbone of the supertree in PHYLOMATIC was based on the latest plant phylogeny group classification. The BLADJ algorithm (Webb et al. 2008) was used to adjust the branch lengths of our phylogenetic tree by using known molecular and fossil dates (Wikstrom et al. 2001).

Hotspot identification and ranking and conservation gap analysis

Both diversity indices were calculated using two packages, Ape and Picante, in R 3.3.2 (R Core Team 2016) and the resulting maps were generated with ARCGIS 9.0 (ESRI, Redlands, CA, USA). The relationship between the patterns of diversity characterised with the different indices was calculated using Spearman's correlation coefficient for each pair of indices. Hotspots were identified with each diversity index as indicated hereafter. We accumulated all of the cells with the highest values of the two diversity indices until the total area reached 5% of Xinjiang's total land area. It was well known that complementarity (Vane-Wright et al. 1991) plays an important role in biodiversity hotspot identification (Zhang and Ma 2008). Here, we did not use complementarity (Vane-Wright et al. 1991) to identify hotspots, because our method ensures that all local areas (i.e. cells) within the hotspots have more species than any other areas outside the hotspots. Additionally, our grid cell size was 50km × 50km, which is large enough for local authorities to manage.

We then identified 'conservation gaps' (Huang et al. 2016a) and 'priority conservation gaps' (Huang et al. 2016a) for Xinjiang seed plants. We synthesised species richness, hotspot size, the proportion of protected areas that overlap with hotspots and global role in biodiversity conservation to rank all hotspots identified in our study for conservation. Species richness was calculated by species number in each hotspot; hotspot size was determined by the area of each hotspot; the proportion of protected areas that overlap with hotspots was calculated based on the each hotspot divided by the area covered by nature reverses; and global role in biodiversity conservation was calculated based on the area of each hotspot divided by the area covered by global biodiversity hotspots. These four indices were ranked and divided by total number of hotspots then summed to produce a total value. All hotspots were ranked according to their total value.

Results

Xinjiang seed plant features

Based on our data, Xinjiang has 3715 seed plant species (including 66 subspecies and 224 varieties), which account for 12.71% of all seed plant species in China. These seed

Table 1. Numbers of seed plant orders, families, genera and species in each geographical region of Xinjiang. Regional division of Xinjiang is shown in Fig. 1.

	Geographical regions										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Overall**
No. of orders	40	43	42	38	36	39	38	35	26	25	45
No. of families	92	104	95	76	72	79	83	64	42	40	113
No. of genera	572	629	569	365	315	378	403	251	130	153	767
No. of species	2291	2445	2110	1102	824	1163	1398	618	264	343	3716
Gymnosperms	22	30	25	17	12	17	18	9	2	2	41
Dicots	1820	1963	1708	870	630	881	1081	472	198	235	2994
Monocots	449	452	377	215	182	265	299	137	64	106	681

Figure 1. Map showing the 10 geographical regions of Xinjiang. Black number identifies geographical regions: **1** North of Junggar Basin **2** South of Junggar Basin **3** Ily Valley **4** Eastern zone of Xinjiang **5** West-North Tarim Basin **6** East-North Tarim Basin **7** West Tarim Basin **8** West-Central Tarim Basin **9** South-Central Tarim Basin **10** East-Central Tarim Basin.

plants belong to 767 genera and 113 families. Of these species, gymnosperms, dicots and monocots accounted for 1.10%, 80.57% and 18.33%, respectively, of all Xinjiang seed plant species (Table 1).

Xinjiang seed plants distribution and similarity at the regional level

Species richness at the regional scale generally increased with decreasing latitude in Xinjiang. In the 10 geographical regions (Fig. 1), region 9 (South-Central Tarim Basin) had
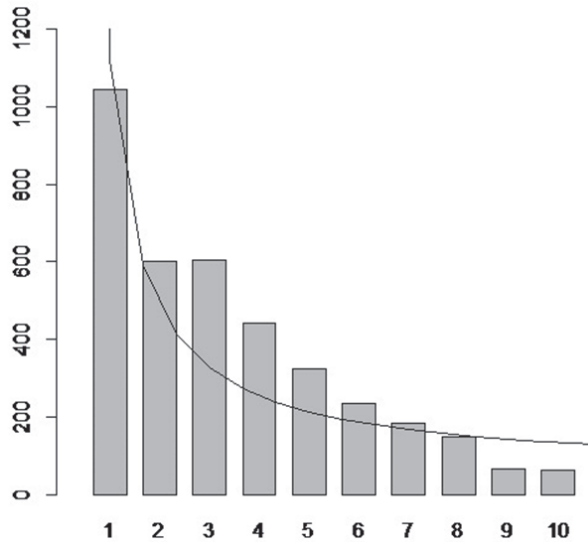
Figu 2. Species richness distribution for 3715 species in Xinjiang across the 10 spatial range classes. Spatial range was measured based on the number of geographical regions, without specific reference to particular regions. The line represents the least-squares regression using a hyperbola model with 61.07 and 1060.20 as the a and b parameters, respectively (R^2=0.89, P<0.001).

the lowest species richness (only 264 species) and region 2 (South of Junggar Basin) had the highest species richness (2445 species) (Table 1). In terms of regional occurrence, only 61 species (1.64% of total species) were found in each of the 10 geographical regions. By comparison, 1046 species (28.15% of total species) occurred in only one of the 10 geographical regions. The number of species that were present in a given number of regions decreased as the number of regions increased, which demonstrates that seed plants species with narrow spatial ranges were more common than those with wide or transcontinental spatial regions (Fig. 2). With the hyperbola model ($G=61.07+1060.20/RT$), where G was the number of species and RT was the total number of regions (Fig. 2), the spatial range of the genetic distributions of seed plants explained 89% of the variation in the number of species with varied distribution ranges.

The similarity indices for pairwise comparisons of regional species composition ranged from 0.07 to 0.58 (Table 2). In general, region 9 was the least similar to all other regions. This trend was, in part, due to the low number of species in this region. The lowest similarity indices were found between the regions 9 and 1 and 9 and 3, whereas the highest was found between the regions 1 and 2 (Table 2).

Diversity hotspots of Xinjiang seed plants

Distributions of Xinjiang seed plant species richness and phylogenetic diversity were uneven and concentrated in the northern regions of Xinjiang (i.e. north of the Tianshan

Table 2. Jaccard similarity indices for pairwise comparisons of geographical regions using all Xinjiang seed plant species. Regional division of Xinjiang is shown in Fig. 1.

	1	2	3	4	5	6	7	8	9	10
1										
2	0.58									
3	0.55	0.58								
4	0.35	0.38	0.34							
5	0.26	0.28	0.28	0.36						
6	0.36	0.38	0.38	0.41	0.38					
7	0.33	0.36	0.34	0.35	0.38	0.40				
8	0.15	0.18	0.17	0.25	0.30	0.27	0.33			
9	0.07	0.08	0.07	0.13	0.17	0.14	0.15	0.27		
10	0.09	0.09	0.08	0.14	0.17	0.15	0.18	0.26	0.30	

Table 3. Seed plant diversity hotspots and conservation ranks in Xinjiang.

Code	Plant diversity hotspots	Number of species	Area of hotspots (km²)	Proportion of area uncovered with nature reserves (%)	Proportion of area covered with global biodiversity hotspots (%)	Conservation rank
H1	Mountain areas in and around Altay city-Burqin County	1406	32500	94.21	0.00	2
H2	Mountainous areas in and around Urumqi city	1627	30000	88.5	63.06	3
H3	Mountainous areas in and around Yining city-Gongliu county	1084	15000	98.48	99.29	1
H4	Fuyun-Qinghe-Qitai-Jimsar Counties boundaries	969	10000	47.66	0.00	7
H5	Shihezi City and Shawan county	751	7500	100.00	42.39	4
H6	Tacheng City and Yumin county and Ermin county boundary	618	5000	100.00	0.00	5
H7	Bole City-Huocheng County and Wenquan County boundary	698	5000	94.68	99.08	6
H8	Karamay City-Hoboksar County-Shawan County boundaries	481	2500	92.19	0.00	8

Mountains; Fig. 3a–c). Both species richness and phylogenetic diversity indices calculated for each spatial unit across Xinjiang were highly significantly correlated with each other (R=0.99, P<0.0001). The diversity hotspots identified based on Xinjiang seed plant species richness were distributed in seven distinct centres: H1-H7 (Table 3, Fig. 3d). The diversity hotspots identified based on phylogenetic diversity included eight centres, which included all of the above seven hotspots and H8 (Table 3, Fig. 3e). All hotspots were located north of the Tianshan Mountains (Fig. 3f).
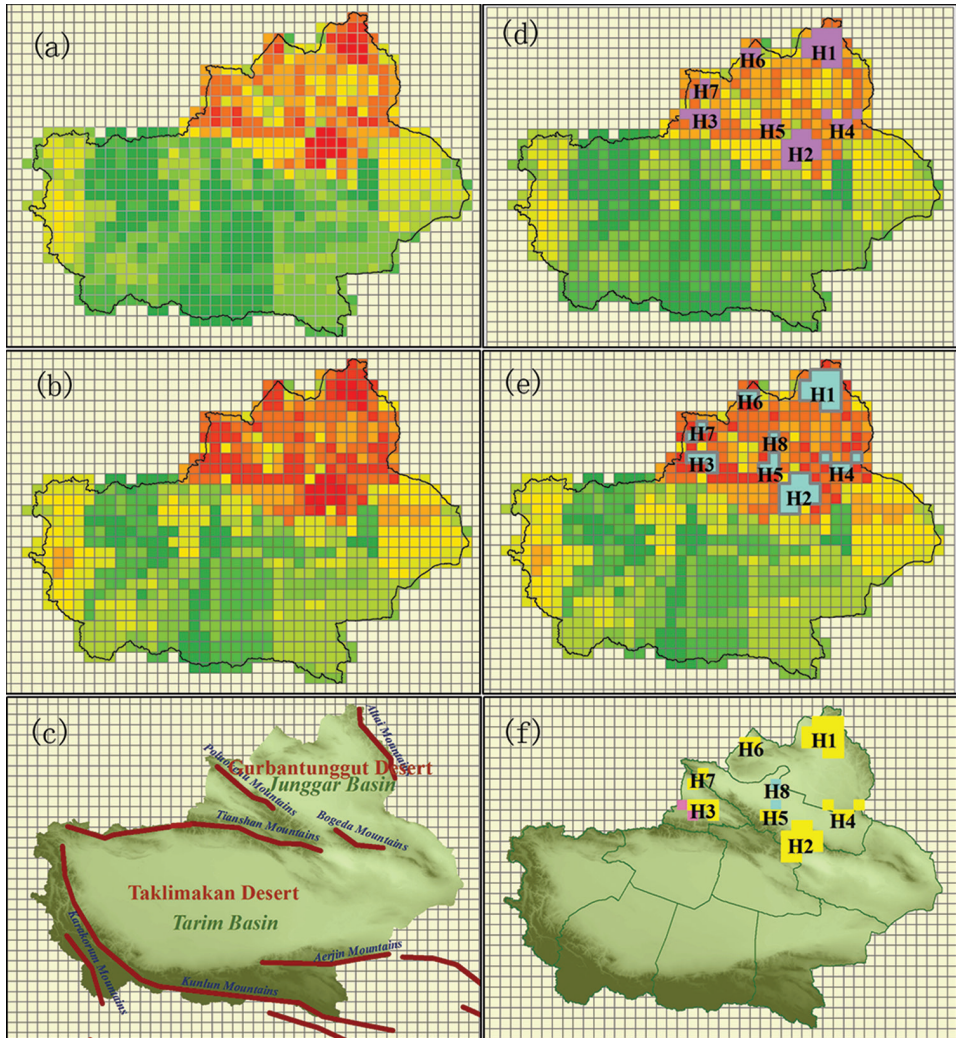
Figure 3. Geographic distribution of Xinjiang seed plant diversity patterns and hotspots and major mountains ranges in northwest China. **a** Geographic distribution of species richness **b** geographic distribution of phylogenetic diversity **c** major mountains ranges **d** diversity hotspots identified by species richness **e** diversity hotspots identified by phylogenetic diversity and **f** diversity hotspots identified by both species richness and phylogenetic diversity. Albers projection. Hotspot centre codes are consistent with those listed in Table 3.

Priority conservation gap areas for Xinjiang seed plant hotspots

The spatial distributions of 33 nature reserves and eight hotspots of Xinjiang seed plant species significantly differed (Fig. 4). These reserves and hotspots overlapped across 11238.63 km², which represented 4.18% of the total area of Xinjiang's nature reserves; 89.55% of the hotspot areas were not covered by nature reserves. Therefore, most of the seed plant hotspots are not well protected and thus represent conservation gaps (Fig. 4).
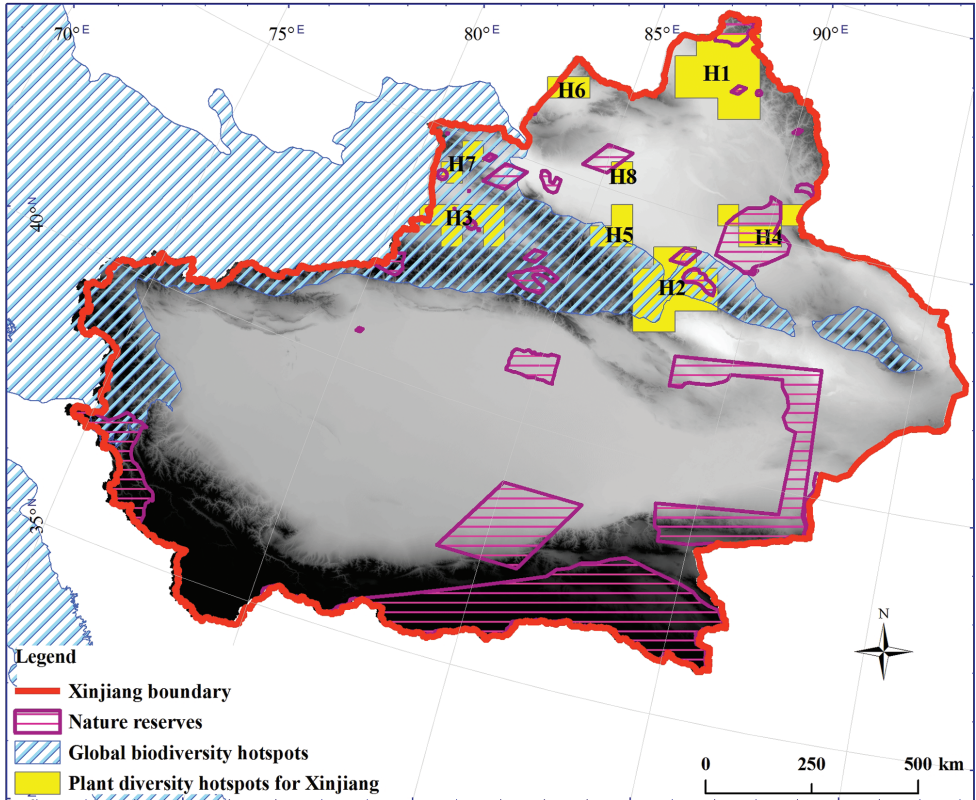
Figure 4. Geographic distributions of seed plant conservation and priority conservation gaps in Xinjiang, northwest China. Albers projection. Hotspot centre codes are consistent with those listed in Table 3.

Four of the eight hotspots overlapped with global biodiversity hotspots (H2, H3, H5 and H7; 60000.00 km²); these overlapping areas accounted for 55.81% of the total area of all hotspots and contained 59.11% (2192 species) of all Xinjiang seed plant species. The remaining four hotspots harboured 1836 seed plant species, which accounted for 49.42% of all Xinjiang seed plant species. A priority conservation area that could alleviate Xinjiang seed plant conservation gaps is centre H5 (Fig. 4). Xinjiang's nature reserves, seed plant hotspots and global biodiversity hotspots overlap across 3319.79 km² and only account for 1.23% of the total area of Xinjiang's nature reserves.

Discussion

Xinjiang flora uniqueness and complexity

Xinjiang is located in the contact area of several natural geographical units, including the Altai Mountains, Tianshan Mountains, Pamirs, Kunlun Mountains, Altun Mountains

and Northern Tibetan Plateau. Moreover, phytogeographically, Xinjiang also has intersections of Eurasian Forest, Eurasian steppe, Central Asian desert and Himalayan Chinese plant sub-regions. These characteristics have led to floral uniqueness and vegetation complexity in Xinjiang (Xinjiang Investigation Group of Chinese Academy of Sciences 1978). It was reported that there are 4081 species of vascular plants in Xinjiang (including subspecies), which account for 13.4% of the total number of plants in China (Yin 2006). Although, the number of species in Xinjiang is relatively low, the proportion of species that are locally endemic to Xinjiang is high (Huang et al. 2016b; Huang et al. 2014).

Xinjiang seed plant hotspots

Species richness is higher in Xinjiang mountains than basins (Li et al. 2013). The eight Xinjiang seed plant hotspots identified in this study cover key mountain ranges in Xinjiang, including both the Tianshan Mountains and Altai Mountains. Wang et al. (1993) proposed that these two terrestrial areas are key for conservation of China's biodiversity and these areas represent two of the 32 terrestrial priority areas for biodiversity conservation in China developed by the Ministry of Environmental Protection (Editorial Committee of China National Biodiversity Conservation Strategy and Action Plan 2011) and one of 19 centres of Chinese endemic seed plant hotspots (Huang et al. 2016a). Thus, Xinjiang seed plant hotspots encompass key areas for biodiversity conservation in China. This finding indicates that Xinjiang's seed plants represent a valuable group for identifying hotspots or priority areas that can be used to aid Xinjiang biodiversity conservation.

Hotspots in major mountain ranges of Xinjiang

Seed plant species are unevenly distributed across Xinjiang; all their hotspots are located in mountain areas mainly within the Tianshan Mountains, Altai Mountains and western mountainous areas in Junggar. These areas approximately correspond to the montane forest and cold temperate coniferous forest in temperate steppe and temperate desert regions (Chen 2014). Mountainous areas were potentially important for shaping the distribution of Xinjiang seed plant species; the high level of heterogeneity in the physiognomy could have offered a diversity of environments and thus promoted formation of the substantial species diversity (Qian and Ricklefs 1999) and caused aggregation of seed plants in the mountain regions of Xinjiang. The Tianshan Mountains and Altai Mountains are two natural physical barriers that alter atmospheric movement and consequently cause changes in precipitation and temperature. These conditions maintain a greater variety of habitats and thus probably enhance speciation, differentiation and preservation of the species that live in these areas, because species diversity increases with habitat variation (Latham and Ricklefs 1993; Rosenzweig 1995). These natural physical barriers also restrict north-south and east-west plant migration and

interchange by forming a geographically isolated area (Wu and Wang 1983), which thereby facilitates the speciation and differentiation of endemics. Alternatively, other factors may play prominent but not decisive roles in some local regions, such as transitional areas (Axelrod et al. 1996; Wu 1980; Wu and Wang 1983) and islands isolated from the continents (Lomolino et al. 2006). Consequently, the abundance of seed plant species in mountainous forests results from a combination of effects and is mainly attributable to the heterogeneous terrain, geological history and contemporary environments in this region.

Suggestions for Xinjiang flora protection

Xinjiang is the largest province in China and accounts for one-sixth of China's total areas (Xinjiang Investigation Group of Chinese Academy of Sciences 1978). Xinjiang has extensively varied geographical and topographical features and a complex and ancient geographical history. Although plants are not abundant (Yin 2006), Xinjiang has unique flora and complex vegetation. Therefore, it is necessary and urgent to protect plants in Xinjiang. Based on the results of this study, some suggestions for conservation of the Xinjiang flora are proposed.

Firstly, we need to create a unified conservation plan for the Xinjiang flora. Conservation biologists have proposed many conservation suggestions and schemes across China (Li et al. 1993; Liu et al. 2003; Sang et al. 2011; Wu et al. 2011). However, these suggestions have unfortunately not been perfected and implemented at the provincial level, mainly because solid data support for plant diversity conservation in Xinjiang is lacking. Although research on species diversity in Xinjiang is very limited, researchers have conducted a large number of taxonomic investigations on plants in Xinjiang (CRFX 1992 –2011).

Secondly, we need to establish a provincial georeferenced (spatially referenced) database for biodiversity that includes plant, animal and nature reserve distributions. In particular, efficient conservation of Xinjiang's plant resources requires accurate data on the current distribution and threat status of plant species in the country (Sang et al. 2011). A large amount of plant diversity information has been digitised in China, including through the National Specimen Information Infrastructure (http://www.nsii.org.cn/), Chinese Virtual Herbarium (http://www.cvh.org.cn/), Chinese Field Herbarium (http://www.cfh.ac.cn/), the Flora of China project (http://hua.huh.harvard.edu/china), Scientific Database of China Plant Species (http://db.kib.ac.cn/eflora/Default.aspx). Some datasets have also been published with Chinese administrative counties as the spatial units, such as in studies on wood plants in China (Fang et al. 2009) and Chinese endemic seed plants (Huang et al. 2014). Additionally, by the end of 2014, China established 2729 nature reserves (Ministry of Environmental Protection the People's Republic of China 2015). Although we produced a spatially referenced dataset of 2139 nature reserves (the World Database on Protected Areas: http://www.protectedplanet.net/), the boundaries of many nature reserves were not accurate, because they did not

have clear boundaries. Moreover, although a vegetation map of China was published in 2008 (Editorial Committee of Vegetation Map China 2008), many vegetation field survey projects were completed 30–50 years ago. All of the described studies provide an important data for research on biodiversity conservation across China. However, the data provided by those projects could not meet the precision requirements for biodiversity conservation research at the provincial level; therefore, it is necessary to develop additional solid sources of information for Xinjiang plants.

Conclusions

Different diversity indices revealed similar distribution patterns of plant diversity in Xinjiang Province. A total of eight seed plant hotspots in Xinjiang were identified by species richness and phylogenetic diversity. All of these hotspots are located in the mountainous areas within the Tianshan Mountains and Altai Mountains. Most centres of Xinjiang seed plant hotspots are not covered by the current nature reserve system.

Acknowledgements

References

Axelrod DI, Al-Shehbaz I, Raven PH (1996) History of the modern flora of China. In: Zhang AL, Wu SG (Eds) Floristic Characteristics and Diversity of East Asian Plants. China Higher Education Press, Beijing, 43–55.

Brooks TM, Mittermeier RA, da Fonseca GAB, Gerlach J, Hoffmann M, Lamoreux JF, Mittermeier CG, Pilgrim JD, Rodrigues ASL (2006) Global biodiversity conservation priorities. Science 313(5783): 58–61. https://doi.org/10.1126/science.1127609

Cadotte MW, Jonathan Davies T, Regetz J, Kembel SW, Cleland E, Oakley TH (2010) Phylogenetic diversity metrics for ecological communities: Integrating species richness, abundance and evolutionary history. Ecology Letters 13(1): 96–105. https://doi.org/10.1111/j.1461-0248.2009.01405.x

Chen L (2014) Flora and Vegetational Geography of China. Science Press, Beijing.

CRFX (1992–2011) Flora Xinjiangensis. Xinjiang Science & Technology & Hygiene Publishing House, Urumqi.

Editorial Committee of China National Biodiversity Conservation Strategy and Action Plan (2011) China National Biodiversity Conservation Strategy and Action Plan. China Environmental Science Press, Beijing

Editorial Committee of Flora of Xinjiang (1992) Flora of Xinjiang, Vol. 1. Xinjiang Science, Technology and Sanitation Press, Urumqi.

Editorial Committee of Vegetation Map China (2008) Vegetation Map of China (1:1000 000). Geology Press, Beijing.

Faith DP (1992) Conservation Evaluation and Phylogenetic Diversity Biol Conserv 61: 1–10.

Fang J, Wang Z, Tao Z (2009) Atlas of Woody Plants in China:Distribution and Climate. Higher Education Press, Beijing.

Forest F, Grenyer R, Rouget M, Davies TJ, Cowling RM, Faith DP, Balmford A, Manning JC, Procheş Ş, van der Bank M, Reeves G, Hedderson TAJ, Savolainen V (2007) Preserving the evolutionary potential of floras in biodiversity hotspots. Nature 445(7129): 757–760. https://doi.org/10.1038/nature05587

Huang J, Huang J, Liu C, Zhang J, Lu X, Ma K (2016a) Diversity hotspots and conservation gaps for the Chinese endemic seed flora. Biological Conservation 198: 104–112. https://doi.org/10.1016/j.biocon.2016.04.007

Huang J, Huang J, Lu X, Ma K (2016b) Diversity distribution patterns of Chinese endemic seed plant species and their implications for conservation planning. Scientific Reports 6(1): 33913. https://doi.org/10.1038/srep33913

Huang J, Lu X, Huang J, Ma K (2016c) Conservation priority of endemic Chinese flora at family and genus levels. Biodiversity and Conservation 25(1): 23–35. https://doi.org/10.1007/s10531-015-1027-0

Huang J, Ma K, Chen B (2014) Diversity and Geographic Distribution of Endemic Species of Seed Plants in China. Higher Education Press, Beijing.

Latham RE, Ricklefs RE (1993) Continental comparisons of temperate-zone tree species diversity. In: Ricklefs RE, Schluter D (Eds) Species Diversity in Ecological Communities: Historical and Geographical Perspectives. University of Chicago Press, Chicago, 294–314.

Li L, Wang Z, Zerbe S, Abdusalih N, Tang Z, Ma M, Yin L, Mohammat A, Han W, Fang J (2013) Species Richness Patterns and Water-Energy Dynamics in the Drylands of Northwest China Plos One 8: e66450. https://doi.org/10.1371/journal.pone.0066450

Li W, Wang X, Wang S (1993) Conservation stragety for biodiversity of China. In: Chen L, Wang X, Wang S (Eds) Biodiversity in China: Status and Conservation Strategy. Science Press, Beijing, 164–205.

Liu J, Ouyang Z, Pimm SL, Raven PH, Wang X, Miao H, Han N (2003) Protecting China's biodiversity. Science 300(5623): 1240–1241. https://doi.org/10.1126/science.1078868

Lomolino MV, Riddle BR, Brown JH (2006) Biogeography (3rd edn). Sinauer Associates, Inc., Sunderland, Massachusetts.

Lu Q, Wang J, Chu J (2012) Desert Plants in China. China Forestry Publishing House, Beijing.

Ministry of Environmental Protection the People's Republic of China (2015) The 2014 Report on the State of Environment in China. Beijing.

Mittermeier RA, Gil PR, Hoffman M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, da Fonseca GAB, Seligmann PA, Ford H (2005) Hotspots Revisited: Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions. The University of Chicago Press, Chicago.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. https://doi.org/10.1038/35002501

Olson DM, Dinerstein E (1998) The global 200: A representation approach to conserving the Earth's most biologically valuable ecoregions. Conservation Biology 12(3): 502–515. https://doi.org/10.1046/j.1523-1739.1998.012003502.x

Omland KE, Cook LG, Crisp MD (2008) Tree thinking for all biology: The problem with reading phylogenies as ladders of progress. BioEssays 30(9): 854–867. https://doi.org/10.1002/bies.20794

Orme CDL, Davies RG, Burgess M, Eigenbrod F, Pickup N, Olson VA, Webster AJ, Ding T-S, Rasmussen PC, Ridgely RS, Stattersfield AJ, Bennett PM, Blackburn TM, Gaston KJ, Owens IPF (2005) Global hotspots of species richness are not congruent with endemism or threat. Nature 436(7053): 1016–1019. https://doi.org/10.1038/nature03850

Prendergast JR, Quinn RM, Lawton JH (1999) The gaps between theory and practice in selecting nature reserves. Conservation Biology 13(3): 484–492. https://doi.org/10.1046/j.1523-1739.1999.97428.x

Prendergast JR, Quinn RM, Lawton JH, Eversham BC, Gibbons DW (1993) Rare Species, the Coincidence of Diversity Hotspots and Conservation Strategies Nature 365: 335–337.

Qian H, Ricklefs RE (1999) A comparison of the taxonomic richness of vascular plants in China and the United States Am Nat 154:160-181.

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Reid WV (1998) Biodiversity hotspots. Trends in Ecology & Evolution 13(7): 275–280. https://doi.org/10.1016/S0169-5347(98)01363-9

Rosauer DF, Laffan SW (2008) Linking phylogenetic trees, taxonomy & geography to map phylogeography using Biodiverse. Taxonomic Data Working Group 2008, Perth, Australia.

Rosenzweig ML (1995) Species diversity in space and time. Cambridge University Press, Cambridge.

Sang W, Ma K, Axmacher JC (2011) Securing a future for China's wild plant resources. BioScience 61: 720–725. https://doi.org/10.1525/bio.2011.61.9.11

Vane-Wright RI, Humphries CJ, Williams PH (1991) What to protect? Systematics and the agony of choice. Biological Conservation 55(3): 235–254. https://doi.org/10.1016/0006-3207(91)90030-D

Wang X, Feng Z, Fu L, Wu P, Xing G (1993) Key taxa and biodiversity areas in China. In: Chen L, Wang X, Wang S (Eds) Biodiversity in China: status and conservation strategy. Science Press, Beijing, 164–205.

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: Software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics (Oxford, England) 2418): 2098–2100. https://doi.org/10.1093/bioinformatics/btn358

Webb CO, Donoghue MJ (2005) Phylomatic: Tree assembly for applied phylogenetics. Molecular Ecology Notes 5(1): 181–183. https://doi.org/10.1111/j.1471-8286.2004.00829.x

Wikstrom N, Savolainen V, Chase MW (2001) Evolution of the angiosperms: Calibrating the family tree. Proceedings. Biological Sciences 268(1482): 2211–2220. https://doi.org/10.1098/rspb.2001.1782

Wu R, Zhang S, Yu DW, Zhao P, Li X, Wang L, Yu Q, Ma J, Chen A, Long Y (2011) Effectiveness of China's nature reserves in representing ecological diversity. Frontiers in Ecology and the Environment 9(7): 383–389. https://doi.org/10.1890/100093

Wu Z (1980) The Vegetation of China. Science Press, Beijing.

Wu Z, Raven PH, Hong D (1994–2012) Flora of China. Science Press or Missouri Botanical Garden Press, Beijing or St. Louis.

Wu Z, Wang H (1983) Physical Geography of China: phytogeography (I). Science Press, Beijing.

Xinjiang Investigation Group of Chinese Academy of Sciences (1978) The vegetation of Xinjiang and its application. Science Press, Beijing.

Xu Y, Shen Z, Ying L, Wang Z, Huang J, Zang R, Jiang Y (2017) Hotspot analyses indicate significant conservation gaps for evergreen broadleaved woody plants in China. Scientific Reports 7(1): 1859. https://doi.org/10.1038/s41598-017-02098-0

Yang C (2012) Sylva Xinjiangensis. China Forestry Publishing House, Beijing.

Yin L (2006) Rare Endangered Endemic Higher Plants in Xinjiang of China. Xinjiang Science & Technology Publishing House, Urumqi.

Zhang H-X, Zhang M-L (2014) Insight into distribution patterns and conservation planning in relation to woody species diversity in Xinjiang, arid northwestern China. Biological Conservation 177: 165–173. https://doi.org/10.1016/j.biocon.2014.07.005

Zhang Y, Ma K (2008) Geographic distribution patterns and status assessment of threatened plants in China. Biodiversity and Conservation 17(7): 1783–1798. https://doi.org/10.1007/s10531-008-9384-6

RESEARCH ARTICLE

How large spatially-explicit optimal reserve design models can we solve now? An exploration of current models' computational efficiency

Yicheng Wang[1], Hayri Önal[2], Qiaoling Fang[3]

1 *College of Resources and Environment, Qingdao Agricultural University, 700 Changcheng Road, Chengyang District, Qingdao 266109, China* **2** *Department of Agricultural and Consumer Economics, University of Illinois at Urbana-Champaign, 1301 West Gregory Drive, Urbana, IL 61801, USA* **3** *College of Management, Ocean University of China, 238 Songling Road, Laoshan District, Qingdao 266100, China*

Corresponding author: *Yicheng Wang* (ywangqau@163.com)

Citation: Wang Y, Önal H, Fang W (2018) How large spatially-explicit optimal reserve design models can we solve now? An exploration of current models' computational efficiency. Nature Conservation 27: 17–34. https://doi.org/10.3897/natureconservation.27.21642

Abstract

Spatially-explicit optimal reserve design models select best sites from a set of candidate sites to assemble nature reserves to protect species (or habitats) and these reserves display certain spatial attributes which are desirable for species. These models are formulated with linear 0–1 programming and solved using standard optimisation software, but they were run on different platforms, resulting in discrepant or even conflicting messages with regard to their computational efficiency. A fair and accurate comparison of the convenience of these models would be important for conservation planners who use these models. In this article, we considered eight models presented in literature and tested their computational efficiency using randomly generated data sets containing up to 2000 sites. We focused on reserve contiguity and compactness which are considered crucial to species persistence. Our results showed that two of these models, namely Williams (2002) and Önal et al. (2016), stand out as the most efficient models. We also found that the relative efficiency of these models depends on the scope of analysis. Specifically, the Williams (2002) model solves more of the test problems when contiguity is the only spatial attribute and a large subset of the candidate sites needs to be selected. When compactness is considered also, the Önal et al. (2016) model generally performs better. Large scale models are found to be difficult to solve in a reasonable period of time. We discussed factors that may affect those models' computational efficiency, including model size, share of selected sites, model structure and input data. These results provide useful insight and guidance to conservation practitioners and researchers who focus on spatial aspects and work with large-scale data sets.

Introduction

Anthropogenic activities have caused tremendous impacts on ecosystems all over the world. These impacts include but are not limited to environmental pollution, habitat loss and fragmentation, invasion of exotic species and climate change (e.g. Aplet and McKinley 2017). These impacts led to an increased rate of extinction of species and impaired the services that ecosystems have been providing to humans. Establishing nature conservation reserves have been adopted across the globe as a direct way to restore ecosystems and protect species. However, resources devoted to this purpose such as land and budget have always been scarce and they often face conflicting demands from other sectors of society such as housing and industry. With the aim of designing ecologically effective and economically efficient nature reserves comes the science of reserve design (Kingsland 2002).

The reserve design problem is defined as selecting a subset from a larger set of candidate sites to assemble nature conservation reserves to provide adequate protection to targeted species or habitats. This problem has been studied using various approaches, including site scoring (e.g. Pressey and Nicholls 1989), gap analysis (e.g. Scott et al. 1993), heuristics (e.g. Pressey et al. 1997) and formal optimisation (e.g. Possingham et al. 2000, Williams et al. 2004). Gap analysis and heuristics have been widely used both in academic research and conservation practices, primarily because they are intuitive, easy to use and computationally convenient. Some popular nature reserve design software such as Marxan and C-plan use heuristics as their major solution algorithm (Ball et al. 2009, Sarkar 2012). However, it has long been demonstrated that these techniques find optimal solutions only occasionally and often generate solutions which significantly deviate from the optimal ones (Önal 2003, Williams et al. 2005). This means that they may not be allocating scarce conservation resources in the best possible manner. The problem can also be formulated as an optimisation model, specifically as a linear mixed integer programme (MIP), to determine the best selection of sites while meeting the conservation goals. The resulting model can be solved using MIP solvers such as CPLEX and GUROBI integrated in off-the-shelf mathematical modelling software such as GAMS and AMPL (AMPL 2016, GAMS 2016). Finding the best possible solution helps guarantee efficient allocation of conservation resources. Due to this advantage, optimal reserve design models have received substantial attention since the 1990s.

The early optimisation formulations of the reserve design problem adapted the set covering and maximal covering frameworks well known in the operations research literature (Cocks and Baird 1989, Underhill 1994, Camm et al. 1996, Church et al.

1996, Ando et al. 1998). These initial formulations neglected spatial attributes of the reserve, usually ending up with a solution where selected sites are highly scattered over the landscape and far apart from each other. A reserve with such a spatial configuration is not very meaningful in practice because both species survival and reserve management benefit from a spatially coherent reserve. The second generation optimum reserve design models incorporated spatial attributes to improve the coherence and functionality of the reserves (Williams et al. 2005). Amongst the spatial attributes discussed in literature, the most important ones are spatial contiguity (or connectivity) and compactness of the selected sites. Spatial contiguity means that any two sites in the reserve can be linked by a path of selected sites. Compactness is a more difficult concept to define, but, in general, it means that the selected sites must be close to each other (or grouped together) to the greatest possible extent. Some studies used the total distance between pairs of selected sites and the boundary length of the reserve as proxy measures for compactness. These two spatial attributes have been successfully modelled using graph theory, network flow theory and mathematical techniques (see Williams et al. 2005, Moilanen et al. 2009, Billionnet 2013 for reviews of those studies). Incorporating these spatial attributes into the optimisation frameworks requires more complex formulations, however, including a large number of additional variables and constraints. Thus, the reserve selection problem gets computationally more difficult when spatial configuration is of concern. For instance, a set covering formulation involving thousands of candidate sites could be solved in seconds (Rodrigues and Gaston 2002), but a compactness model involving about 700 sites could not be solved to optimality within 10 hours (Fischer and Church 2003).

Several modelling approaches have been introduced in the reserve design literature to incorporate spatial aspects in site selection. Due to their differences in structure and size, those models differ in their computational performance. Moreover, individual models were run on different platforms with different CPU speeds and solved using different MIP solvers and, therefore, it is not clear how those models compare to each other in terms of their computational efficiency. A fair and accurate comparison of the convenience of these models would be important for on-the-ground conservation practitioners. This is the main motivation for this article. For this purpose, we selected eight optimisation models incorporating (or could incorporate easily by proper modification) contiguity and compactness and conducted computational experiments with these models by running them on the same computer and using the same data sets that were generated randomly. We aimed to answer two questions: i) how do these models perform in solving contiguity and compactness problems with different sizes and ii) what are the likely factors affecting their computational efficiencies?

We carried out the tests on a Lenovo desktop computer with Intel Pentium (R) CPU of 2.8 gigahertz and RAM of 8.0 gigabyte and the operation system was Windows 64-bit. As the MIP solver, GUROBI 5.0 integrated in GAMS 23.9 was used.

Material and methods

Contiguity models

Table 1 summarises the eight models we tested. The 'Algebraic formula' column of the table presents the basic algebraic formula used to ensure spatial contiguity.

Some of the models were not originally proposed for reserve design purposes or did not consider the cost of site selection. We made necessary modifications to those models so that they are applicable to the reserve design problem we address here. The objective of all the models is stated as the minimisation of the total cost of site selection, namely:

Minimize $\sum_i c_i U_i$ (1)

where U_i is a binary variable which equals 1 if site i is selected and 0 otherwise and c_i is the cost of selecting site i.

Those models, not originally proposed for reserve design problems, are extended by adding the species coverage constraint described below:

$\sum_i e_i U_i \geq p$ (2)

where e_i is the population size of target species in site i; and p is the targeted (minimum viable) population of target species. This constraint is supposed to ensure a long-term survival chance for target species.

The Miller et al. (1960) model is modified as below to control the formation of arcs between selected sites.

$X_{ij} + X_{ji} \leq U_i$ for all $i<j, j \in N_i$ (3)

$X_{ij} + X_{ji} \leq U_j$ for all $i<j, j \in N_i$ (4)

$\sum_{j \in N_i} X_{ij} \leq U_i$ for all i (5)

$\sum_i \sum_{j \in N_i} X_{ij} = \sum_i U_i - 1$ (6)

where X_{ij} is a binary variable which equals 1 if an arc directs from i to neighbour j and 0 otherwise and N_i is the set of i's neighbour sites. For details, see Williams (2002).

We first generated ten hypothetical data sets. Each data set considers a 10*10 grid partition including one hundred sites. The distribution of species over these sites was generated by using a uniform distribution function with the range of [0, 5], which means that the population size (e_i) in each site takes an integer value between 0 and 5. We generated the selection cost (c_i) similarly using the range of real numbers [1, 10]. We assigned a different value to the target population size (p) in each data set, where p is calculated by $p=a*n$ where $n=100$ is the number of candidate sites and a is a popula-

Table 1. Mechanisms used to ensure contiguity and algebraic formulae of the eight models used in the computational efficiency tests.

Model number	Mechanism to ensure contiguity	Algebraic formula	References
1	An arc can direct from node i to neighbour node j only if the value assigned to i is less than the value assigned to j, so an arc directing away from a node will not return to it and therefore loops are prevented.	(1)-(6); $$Y_i - Y_j + nX_{ij} \leq n - 1 \; i{\neq}j, j{\in}N_i$$	Miller et al. (1960); Duque et al. (2011)'s Tree^{PRM} model.
2	Two trees are formed, one in primal graph, the other in dual graph, the interwoven structure of these two trees prevents the formation of loops.	(1)-(6); (2)-(4) in Williams (2002).	Williams (2002)
3	One selected site serves as a sink, every other selected site provides one unit of supply to the flow and all flows finally reach the sink.	(1) and (2); (1)-(3) in Shirabe (2005).	Shirabe (2005, 2009); Duque et al. (2011)'s Flow^{PRM} model
4	The tail length of a selected site equals the number of arcs linking to it and that length is strictly larger than that of the site's preceding sites.	(1)-(2); (3)-(7) in Önal and Briers (2006).	Önal and Briers (2006)
5	Solve a relaxed problem first with no contiguity constraints. If a cycle occurs, apply an inequality (a cut) to that cycle and solve again until there is no cycle in the solution.	(1)-(6); (8) in Önal and Wang (2008).	Önal and Wang (2008)
6	An amount of flow is injected into the partition from an outside node, each selected site consumes one unit of flow, residual flow is absorbed by a predefined variable.	(1) and (2); (5)-(8) in Conrad et al. (2012).	Conrad et al. (2012)
7	Capital flows from the supply node to trans-shipment nodes and for each selected site, capital can come into it from only one of its adjacent sites.	(1) and (2); (2)-(7) in Jafari and Hearne (2013).	Jafari and Hearne (2013); Jafari et al. (2017)
8	If a site is selected then at least one of its adjacent sites, which is nearer (structurally or functionally) to the centre, must also be selected.	Minimize $\sum_k\sum_i c_i X_{ki}$; (2),(4),(6) and (8) in Önal et al. (2016).	Önal et al. (2016); Wang and Önal (2016)

tion coefficient ranging from 0.25 to 2.5 with an increment of 0.25 (thus, a population between 25 and 250 individuals must be protected in the reserve and a larger a implies a larger reserve area). By designing reserves with different areas, we intend to test the impact of the number of selected sites on computational efficiency.

To eliminate the impact of input data on the test result, for each value of a we generated 50 data sets, each with a different distribution of species and selection cost across the 100 sites and used in a different run. To save time in the test and avoid tying the computer to a long computational time, in each run we imposed a one-hour maxi-

mum processing time limit. The total time limit for all runs was limited to 2 hours (i.e. the programme was terminated if it took more than 2 hours to complete the 50 runs). Average CPU times in completed runs were reported.

Contiguity and compactness models

The Önal et al. (2016) model and the Williams (2002) model were modified and used in the contiguity and compactness test because these two models were found to be the most efficient models in the above contiguity test. We modified the objective function of the Önal et al. (2016) model as below so it incorporated selection cost which is an important factor in reserve design practices:

$$\text{Minimize} \sum_{k}\sum_{i} d_{ki} X_{ki} + \sum_{k}\sum_{i} c_{i} X_{ki} \quad (7)$$

where X_{ki} is a binary variable which equals 1 if site i is selected into the reserve which is centred at site k and d_{ki} is the distance between centre site k and selected site i. The first part of (7) is the total distance between the cluster centres and selected sites and the second part is selection cost. These two components can be given different weights to reflect their importance in site selection; for computational purposes, we used the same weights (=1.0).

In the Williams (2002) model, we made the following modifications to incorporate compactness assuming that compactness is measured by total pair-wise distance between selected sites (Önal and Briers 2002).

$$\text{Min} \sum_{i}\sum_{j>i} d_{ij} W_{ij} + \sum_{i} c_{i} U_{i} \quad (8)$$

s.t.:

$$W_{ij} \geq U_{i} + U_{j} - 1 \text{ for all } i{<}j \quad (9)$$

where W_{ij} is a binary variable which equals 1 when sites i and j are both selected and 0 otherwise. The first part of (8) represents reserve compactness while the second part is the cost of site selection. Constraint (9) relates W_{ij} to the site selection variables.

Results

Contiguity: the 100-site problem

The computational results of the 100-site contiguity problem are summarised in Table 2 (for clarity reasons, we only report results with populations (p) of 50 to 250 with an increment of 50). Except Williams (2002) and Önal et al. (2016), all models encountered

Table 2. Computational efficiency of alternative contiguity models in solving a 100-site problem.

Models	Population size to be covered in the reserve				
	50	100	150	200	250
Miller et al. (1960)	136.9	>3600.0 [a]	>3600.0 [a]	>3600.0 [a]	>2400.0 [b]
Williams (2002)	9.0	50.8	20.2	3.0	0.1[c]
Shirabe (2005)	64.2	>969.1[a]	>477.1[a]	47.0	0.1[c]
Önal and Briers (2006)	>2024.3 [a]	>3600.0 [a]	>3600.0 [a]	>3600.0 [a]	>2400.6 [b]
Önal and Wang (2008)	>505.6 [a]	>944.4 [a]	>824.2 [a]	>2405.3 [a]	>1224.9 [b]
Conrad et al. (2012)	>1854.8 [a]	>2551.1 [a]	>2026.5 [a]	>123.5 [a]	0.2 [c]
Jafari and Hearne (2013)	>1905.5 [a]	>3600.0 [a]	>3600.0 [a]	>311.8 [a]	0.9 [c]
Önal et al. (2016)	12.0	5.3	3.3	4.2	6.6 [c]

a: 2 to 50 runs were completed within the total time limit (2 hours). These values are averages of the CPU times used in completed runs.

b: 3 to 11 runs were completed within the total time limit (2 hours), amongst which 1 to 5 runs were infeasible due to high coverage requirement and/or inadequate species occurrence. The reported values are averages of the CPU times used in feasible runs. The ">" indicates that at least one run could not be completed within 1 hour.

c: 22 runs were infeasible. These values are averages of the other feasible 28 runs.

computational difficulty when solving the problem. That is, at least in one run, they could not find the optimal solution within an hour. The Miller et al. (1960) model completed only two runs in 2 hours in most population settings. The Shirabe (2005) model encountered difficulty in two population settings (*p*=100 and 150), in which most other models also encountered computational difficulty. These results are consistent with the findings of Duque et al. (2011) who applied these methods in a districting problem and found that the model adapted from Shirabe (2005) was fastest while their own models could not solve a 49-site districting problem in 3 hours.

The models of Önal and Briers (2006) and Jafari and Hearne (2013) completed only two runs in 2 hours in, respectively, three and two population settings (for Önal and Briers (2006) model, these settings are *p*=100, 150 and 200 and for Jafari and Hearne (2013) model, the settings are *p*=100 and 150). The models of Önal and Wang (2008) and Conrad et al. (2012) are better in the sense that they completed more runs in most population settings.

The Williams (2002) and Önal et al. (2016) models stand out as the fastest amongst the eight models and completed all 50 runs within the allowed time limits (each run was completed in less than one minute of processing time). The Williams (2002) model took 0.1 second to about 50 seconds to solve those problems, while the model of Önal et al. (2016) seems a little faster, in most settings using less than 10 seconds except one case where it used an average of 12.0 seconds.

All models, except the Önal et al. (2016) model, solved the problem relatively easily when a very large or very small population was to be covered (equivalently, a very large or very small number of sites need to be selected). A similar result was

reported by Williams (2002) also. The Önal et al. (2016) model was computationally steady regardless of the population size specification (the run times were in the range of 3.3–12.0 seconds).

Contiguity: larger size problems

Having observed that, in the 100-site test problems, the Williams (2002) and Önal et al. (2016) models are substantially more efficient than the other models, in the remaining test runs we compared these two models against each other in solving larger problems that involved 200 to 2000 sites. We relaxed the optimality requirement by setting the relative gap (percentage difference between the incumbent solution and the best possible solution) at 1 percent level (instead of 0 percent, no gap) to avoid long processing times that might possibly be needed to solve large problems to exact optimality (this is done in GAMS by writing a simple code *option optcr=0.01*). Table 3 reports the results for different model sizes.

   The Williams (2002) model solved the problem for almost all sizes when the population coefficient a was high (2.5 and 2.0), although the processing times increased as the number of candidate sites (n) was increased. For instance, when $n=200$ and $a=2.5$, the model completed all feasible runs (in some cases the model was infeasible due to high population requirement and/or inadequate population distribution in the candidate sites) with an average processing time of 0.2 second. For $n=2000$, the average processing time increased to 4.2 seconds. A similar pattern is observed when the population coefficient was decreased to $a=2.0$. For $n=200$, an average of 4.0 seconds was used, while for $n= 2000$, only 14 runs were completed within the 2 hour total time limit. For other population settings, the model encountered computational difficulty more often and could not find a solution after running 1 hour in at least one run (indicated in Table 3 with ">"). The computational difficulty has become more severe as the problem size increased. For the setting $a=1.5$ and $n=200$, for example, the model completed 14 runs (average processing time =552.3 seconds). For $a=1.5$ and $n=500$, the model completed only 4 runs (average processing time = 2124.9 seconds). The model completed only 2 runs when $n=1000$, 1500 and 2000. The worst performance was observed when $a=1.0$ and 0.5, where the model ran out of the 1 hour time limit almost in each run even with the smallest test problem ($n=200$).

   The Önal et al. (2016) model displayed a relatively steady computational performance as in the 100-site problem. In general, the model used less time when a smaller number of sites was selected (Table 3, $n=200$). However, it encountered more difficulties than the Williams (2002) model in solving large problems even when the population settings were high. For instance, when $n=500$, the model completed only two runs for each population setting; and when $n=1000$, 1500 and 2000, it stopped without finding any solution within the 1 hour time limit. A more serious problem was encountered in some cases where the computer ran out of memory due to the large size of the model (millions of variables and equations, see Table 3).

Table 3. Computational efficiency of Williams (2002) and Önal et al. (2016) models in solving large-scale contiguity problems.

Models	α_a	n=200 (10*20)		n=500 (20*25)		n=1000 (25*40)		n=1500 (30*50)		n=2000 (40*50)	
		Model size [b]	Time (s)	Model size [b]	Time (s)	Model size [b]	Time (s)	Model size [b]	Time (s)	Model size [b]	Time (s)
Williams (2002)	2.5	2,361 1,682	0.2 [c]	6,141 4,322	0.5 [c]	12,481 8,742	1.1 [c]	18,831 13,142	2.4 [c]	25,244 17,594	4.2 [c]
	2.0		4.0		26.5		108.3		383.5 [d]		e
	1.5		>552.3 [d]		>2124.9 [d]		>3600.0 [d]		>3600.0 [d]		e
	1.0		>3600.0 [d]		>3600.0 [d]		>3600.0 [d]		>3600.0 [d]		e
	0.5		>1822.0 [d]		>3600.0 [d]		e		e		e
Önal et al. (2016)	2.5	40,001 39,263	>3278.5 [d]	250,001 248,093	>3600.0 [d]	1,000,001 996,133	e	2,250,001 2,244,163	e	4,000,001 3,992,183	e
	2.0		2851.4 [d]		>3600.0 [d]		>3600.0 [d]		e		e
	1.5		2608.3 [d]		>3600.0 [d]		>3600.0 [d]		e		e
	1.0		>2613.6 [d]		>3600.0 [d]		>3600.0 [d]		f		g
	0.5		1238.5 [d]		>3600.0 [d]		>3600.0 [d]		f		g

a: Population coefficient. The size of population to be covered in the reserve equals to *a* times the number of candidate sites (*n*).

b: The first number is the number of variables, the second number is the number of equations in the model.

c: 17 to 29 runs are infeasible due to high coverage requirement and/or inadequate species occurrence. Values are average of times used in feasible runs.

d: 2 to 19 runs were completed in 2 hours. The ">" indicates at least one run was out of time limit (1 hour).

e: 2 or more runs were completed, at least one of which terminated with no solution after running 1 hour or with integer solution but gap not available.

f: Only 1 run was completed which took more than 7 hours and found integer solution with gap over 80 percent or no solution returned.

g: Out of memory after running about 2000 seconds or integer solution found after running nearly 3 hours but with gap greater than 70 percent.

Contiguity and compactness

In this section, we compare the computational efficiency of the Williams (2002) and Önal et al. (2016) models in solving contiguity and compactness problems. We called the model based on Williams (2002) but with (8) as the objective function and (9) as an additional constraint "W-CM" (CM for contiguity and compactness) and the model based on Önal et al. (2016) but with (7) as the objective function "Ö-CM". The number of sites in the test runs was set as *n*=100, 200 and 500 and we defined the population coefficient *a* as in the contiguity tests. When *n*=500, both models encountered severe computational difficulty for most population coefficient settings. Table 4 summarises the results.

Table 4. Comparison of W-CM model and Ö-CM model in solving contiguity and compactness problems with various sizes.

Models	α[a]	n=100 (10*10)		n=200 (10*20)		n=500 (20*25)	
		Model size [a]	Time (s)	Model size [a]	Time (s)	Model size [a]	Time (s)
W-CM	2.5	6,091; 5,772	1.4[b]	22,261; 21,582	4.8[b]	130,891; 129,072	78.3[b]
	2.0		178.2[c]		>2401.6[d]		>3600.0[c]
	1.5		1692.3[c]		>3600.0[d]		>3600.0[c]
	1.0		>3318.8[c]		>3600.0[d]		>3600.0[c]
	0.5		90.6		>3600.0[d]		>3600.0[c]
Ö-CM	2.5	10,001; 19,443	2.4[b]	40,001; 39,263	1474.4[b]	250,001; 248,093	>3600.0[c]
	2.0		2.0		1360.7[c]		>3600.0[c]
	1.5		1.9		1135.4[c]		>3600.0[c]
	1.0		1.9		1073.4[c]		>3600.0[c]
	0.5		1.7		767.6[c]		>3600.0[c]

a: See Table 3 for the explanations of *a*, model size and symbol ">".
b: Some runs were infeasible due to high population requirement and/or inadequate species occurrence. Values are averages of times used in feasible runs.
c: 2 to 41 runs were completed in 2 hours. Values are averages of times used in completed runs.

The W-CM model encountered difficulty for some population settings (*a*) when solving the 100-site problem. For *a*=2.5, the solution time was only 1.4 second, but for the settings *a*=2.0 and 1.5, it jumped to 178.2 and 1692.3 seconds. For *a*=1.0, only 3 runs were completed within the 2 hour total time limit. However, for *a*=0.5, the solution time was decreased dramatically to 90.6 seconds, showing the fast-slow-fast pattern observed when solving the contiguity problems. For *n*=200 and 500, the model completed only 2 runs within the 2 hour total time limit for most population settings. In general, larger models require more solution times. However, raising the population requirement level improved the computational efficiency. For example, for *a*=2.5, the average solution times were only 4.8 and 78.3 seconds for *n*=200 and *n*=500, respectively.

The Ö-CM model used only about 2 seconds in the 100-site problem for all population coefficient settings. When *n*=200, the model used about 20 minutes or less for all population settings, again better than the W-CM model. When *n* was increased to 500, this model encountered difficulty too, where only 2 runs could be completed within the 2 hour total time limit.

It is interesting that the Ö-CM model is computationally more efficient than the W-CM model although it has a larger size both in terms of the number of variables and

the number of equations (see Table 4). This indicates that model size may not be the only factor determining the computational efficiency of MIP models.

Finally, we note that the Ö-CM model (which includes an additional distance component

$$\sum_{k}\sum_{i}d_{ki}X_{ki}$$

in the objective function, see (7)) is computationally more efficient than the Önal et al. (2016) model used in this test, although the size of these two models are exactly the same. For instance, for a=1.5 and n=200, the Önal et al. (2016) model used an average of 2608.3 seconds of processing time (see Table 3), while the Ö-CM model used only 1135.4 seconds (see Table 4). This, again, indicates that the model size is not the only factor that may affect computational efficiency of MIP models. We discuss these factors in the next section.

Discussion

Based on the computational experiments we conducted, we identified four important factors that affect the computational efficiency of MIP models in nature reserve design problems with contiguity and compactness considerations: 1) Model size, 2) The share of selected sites, 3) Model structure and 4) Input data. We discuss these factors below.

Model size is the most important factor affecting the computational efficiency of these models. As the model size (especially the number of binary variables) increases, solution time may increase substantially. Figure 1 depicts an example using the Williams (2002) model. As the number of candidate sites n increased from 100 to 1500, which gradually increased the number of variables from 6,091 to 18,831 and the number of constraints from 5,772 to 13,142, the solution time increased exponentially from 3 seconds to nearly 400 seconds. This is largely due to the number of nodes solved during the branch and bound procedure used by most MIP solvers. In general more nodes have to be solved when the model has a larger size.

Our test runs showed that the solution times also depend significantly on the share of selected sites in the optimal solution, which in turn depends on the specification of the population coefficient (a). This can be seen clearly, for instance, in the results obtained with the Shirabe (2005), Conrad et al. (2012) and Jafari and Hearne (2013) models for n=100 (see Table 2) and in the Williams (2002) model results for n=200 (see Table 3). Generally, these models follow a fast-slow-fast pattern, that is, as the share of selected sites increases, the solution time increases first, reaches a peak (where the share of selected sites is about 20–30 percent of all the candidate sites) and then decreases. This may be explained by the number of binary variables that have to be fixed at the values of 0 or 1 during the solution process. When the share of selected sites is small (or large), a large portion of binary variables will be fixed at 0 (or 1), leaving fewer combinations (a smaller branch and bound tree) that need to be searched for improving the
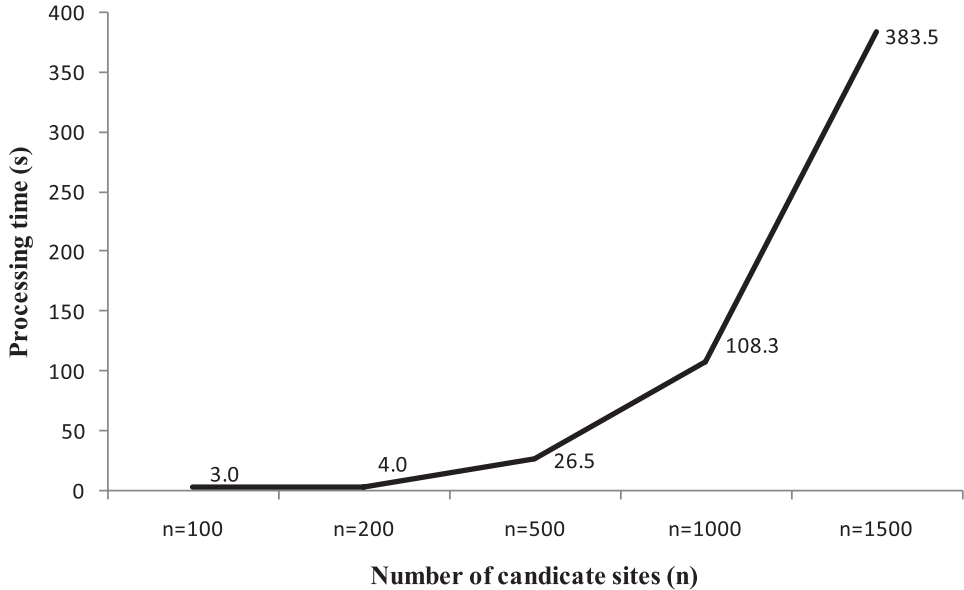
Figure 1. Relationship between processing time and the number of candidate sites (Williams (2002) model was used. population coefficient *a*=2.0. See text for detailed explanation of *a*).

objective function value (A brief description of the branch and bound algorithm can be found in Önal 2003). Similar results have been reported earlier by Williams (2002).

The Önal et al. (2016) model seems to be an exception. As noted above, it showed a relatively steady pattern in solution times for all population coefficient settings. The solution time tends to decrease only when a very small number of sites are to be selected.

Model structure is the third important factor that affects computational performance of the models we tested. Although the number of variables and constraints in a model may be substantial, due to the algebraic model structure, a large number of those variables and constraints may actually be redundant. Those redundant elements are eliminated during the preliminary solution procedure (Presolve in some GAMS solvers such as CPLEX and GUROBI) and the model sizes are much smaller after the Presolve. This is one of the main reasons for the superior computational advantage of the Önal et al. (2016) model. This situation was also observed in empirical applications of the Önal et al. (2016) model.

In addition, some algebraic forms may be integer friendly and some may not. For example, the graph-theoretic contiguity model introduced by Önal and Briers (2006) has the following constraint:

$$\sum_{i\in N_j} X_{ij} \leq 4\,U_j \text{ for all } j \text{ (10)}$$

where X_{ij} and U_j are binary variables as defined before and N_j is the set of *j*'s neighbour sites. This constraint states that if site *j* is selected, then at most four arcs can
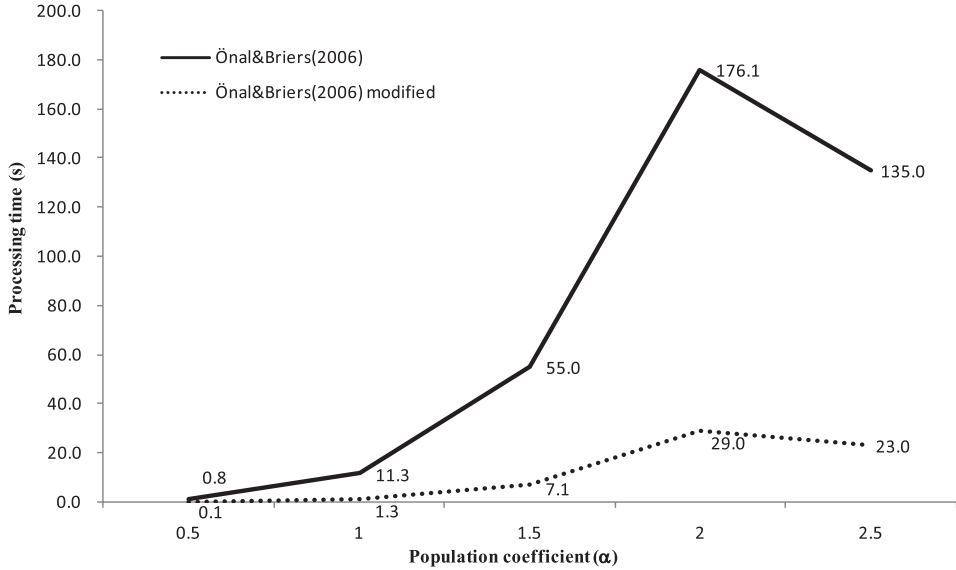
Figure 2. Significance of model structure in affecting the model's computational efficiency (number of sites *n*=36. The size of population to be covered equals to *a* times *n*.).

direct to it from its neighbour sites. The computational efficiency of the model can be improved substantially by modifying this constraint as in constraints (3) and (4). A comparison of the original Önal and Briers (2006) model and the modified model is depicted in Figure 2.

It is clear that replacing constraint (10) with constraints (3) and (4) slashes the solution time. We performed a paired samples t-test for each value of *a* to test the significance of the difference between these two models' processing times. The result reported a *p* value less than 0.05 for each *a* value, indicating that the difference between the processing times is statistically significant. This result should be attributed to the fact that a constraint like (10) is integer unfriendly while the constraints (3) and (4) improve the model's computational efficiency by helping construct a unimodular constraint matrix (ReVelle 1993, Williams 2002). Indeed, having a unimodular constraint matrix is one of the main reasons for the Williams (2002) model's good efficiency.

Input data such as land price and species distribution across candidate sites are also crucial factors that can affect the model's computational efficiency. Figure 3 depicts the processing times used by the models of Önal et al. (2016) and Williams (2002) when solving a 100-site problem with population coefficient *a*=0.5. The problem size remained unchanged across the 50 runs while species population and site costs in each run were varied.

The shortest time used by the Önal et al. (2016) model is only about 1.2 seconds, while the longest time is 131.2 seconds. For the Williams (2002) model, the shortest and longest times are 0.3 seconds and 84.5 seconds, respectively. These results
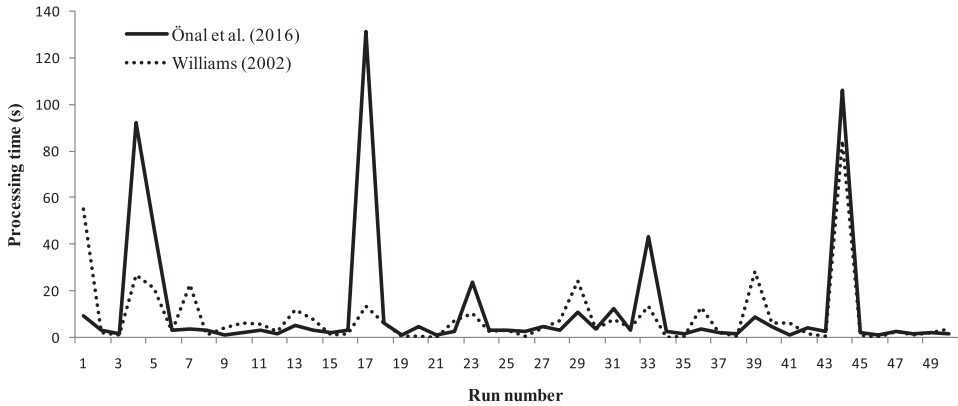
Figure 3. Computational efficiency of the Önal et al. (2016) and Williams (2002) models in solving a 100-site problem with various species distributions and site costs data.

indicate that alterations in the input data may increase or decrease the solution time substantially even if the model size remains the same. This can also be explained by the workings of the branch and bound procedure. One data set may yield a 'good' integer solution in an early iteration and eliminate a large portion of the branch and bound tree; therefore, a fewer number of nodes needs to be solved (or just the opposite may occur). This seems to have happened in runs 4, 17, 33 and 44 with the Önal et al. (2016) model and in runs 1, 4, 7, 29, 39 and 44 with the Williams (2002) model.

We should note that none of these factors alone can determine the performance of a MIP model, instead they affect the model's performance together. Solution techniques have been designed to speed up the computational process, such as supplying a cut value (Önal 2003, Cerdeira et al. 2005, Carvajal et al. 2013) or using a hybrid two-phase solution method (Conrad et al. 2012). We did not explore the effects of these techniques on the reserve design models tested here.

Conclusions

We have compared the computational efficiencies of eight representative optimal reserve design models that explicitly incorporate contiguity and compactness considerations. We identified the models presented by Williams (2002) and Önal et al. (2016) as the most efficient models. They both solved a 100-site contiguity problem easily, but encountered difficulty in solving the 200-site and larger contiguity problems. When compactness is also included, the Williams (2002) model had difficulty in solving a 100-site problem, while the Önal et al. (2016) model could solve a 200-site problem in a reasonable processing time (about 20 minutes). The Williams (2002) model could solve contiguity problems and some contiguity and compactness problems with up to 2000 sites when a large fraction of the candidate sites (such as 75 percent or more)

needs to be selected. The Önal et al. (2016) model displayed a steady performance and outperformed the Williams (2002) model when both contiguity and compactness are considered, but still the model could not be solved within 1 hour when 500 sites are involved. We identified model size, share of selected sites, model structure and input data as the four important factors affecting the computational efficiency of spatially-explicit optimal reserve design models. When developing such a model, one should try to define as few variables and equations as possible and to construct the model in such a way that its constraint matrix is unimodular (ReVelle 1993). These findings may be useful to conservation planners who select and use models in reserve design or management practices. This work may also provide guidance to academic researchers who try to develop computationally efficient optimal reserve design models.

The current nature reserves, whether terrestrial or marine, may not be adequate in providing sufficient protection to biodiversity. For instance, only over 1.89% of the marine protected areas (MPAs) worldwide is covered by exclusively no-take MPAs, far from the Convention on Biological Diversity's (CBD) Aichi Target 11 of 10% MPA coverage by 2020 and even further from the 30% target recommended by the IUCN World Parks Congress 2014 (IUCN 2018). It is almost certain that the current systems of nature reserves should be expanded. Acquiring more protected areas, however, would have to be done in a world where the human economy struggles to grow and the ecosystems and nonhuman species demands protection. Indeed, there is a conflict between economic growth and wildlife conservation (Czech 2014). Various disciplines such as ecological economics and environmental philosophy discussed this conflict and offered principles and guidelines to mediate the conflict and achieve sustainability. The science of nature reserve design or, more specifically, the optimal reserve design models, can contribute too. These models aim to solve practical conservation problems by incorporating ecological and socioeconomic considerations and help best prioritise conservation effort by guaranteeing optimal solutions. With the remarkable improvements in optimisation methods and computational power, it is reasonable to anticipate that spatial optimisation models will likely play a more significant role in the future than it does today in analysing and acquiring new protected areas.

Acknowledgments

References

Ando A, Camm JD, Polasky S, Solow A (1998) Species distribution, land values and efficient conservation. Science 279(5359): 2126–2128. https://doi.org/10.1126/science.279.5359.2126

Aplet GH, McKinley PS (2017) A portfolio approach to managing ecological risks of global change. Ecosystem Health and Sustainability 3(2): 1–15. https://doi.org/10.1002/ehs2.1261

Ball IR, Possingham HP, Watts ME (2009) Marxan and relatives: software for spatial conservation prioritization. In: Moilanen A, Wilson KA, Possingham HP (Eds) Spatial Conservation Prioritisation: Quantitative Methods and Computational Tools. Oxford University Press, Oxford, 185–195.

Billionnet A (2013) Mathematical optimization ideas for biodiversity conservation. European Journal of Operational Research 231(3): 514–534. https://doi.org/10.1016/j.ejor.2013.03.025

Camm JD, Polasky S, Solow A, Csuti B (1996) A note on optimal algorithms for reserve site selection. Biological Conservation 78(3): 353–355. https://doi.org/10.1016/0006-3207(95)00132-8

Carvajal R, Constantino M, Goycoolea M, Vielma JP, Weintraub A (2013) Imposing Connectivity Constraints in Forest Planning Models. Operations Research 61(4): 824–836. https://doi.org/10.1287/opre.2013.1183

Cerdeira JO, Gaston KJ, Pinto LS (2005) Connectivity in priority area selection for conservation. Environmental Modeling and Assessment 10(3): 183–192. https://doi.org/10.1007/s10666-005-9008-4

Church RL, Stoms DM, Davis FW (1996) Reserve selection as a maximal covering location problem. Biological Conservation 76(2): 105–112. https://doi.org/10.1016/0006-3207(95)00102-6

Cocks KD, Baird IA (1989) Using mathematical programming to address the multiple reserve selection problem: An example from the Eyre Peninsula, South Australia. Biological Conservation 49(2): 113–130. https://doi.org/10.1016/0006-3207(89)90083-9

Conrad JM, Gomes CP, vanHoeve WJ, Sabharwal A, Suter JF (2012) Wildlife corridors as a connected subgraph problem. Journal of Environmental Economics and Management 63(1): 1–18. https://doi.org/10.1016/j.jeem.2011.08.001

Czech B (2014) The conflict between economic growth and wildlife conservation, In: Gates JE, Trauger DL, Czech B (Eds) Peak Oil, Economic Growth, and Wildlife Conservation. Springer, New York, 99–117. https://doi.org/10.1007/978-1-4939-1954-3_5

Duque JC, Church RL, Middleton RS (2011) The p-regions problem. Geographical Analysis 43(1): 104–126. https://doi.org/10.1111/j.1538-4632.2010.00810.x

Fischer DT, Church RL (2003) Clustering and compactness in reserve site selection: An extension of the biodiversity management area selection model. Forest Science 49: 555–565.

Jafari N, Hearne J (2013) A new method to solve the fully connected reserve network design problem. European Journal of Operational Research 231(1): 202–209. https://doi.org/10.1016/j.ejor.2013.05.015

Jafari N, Nuse BL, Moore CT, Dilkina B, Hepinstall-Cymerman J (2017) Achieving full connectivity of sites in the multiperiod reserve network design problem. Computers & Operations Research 81: 119–127. https://doi.org/10.1016/j.cor.2016.12.017

Kingsland SE (2002) Creating a science of nature reserve design: Perspectives from history. Environmental Modeling and Assessment 7(2): 61–69. https://doi.org/10.1023/A:1015633830223

Miller CE, Tucker AW, Zemlin RA (1960) Integer Programming Formulation of Traveling Salesman Problems. Journal of the Association for Computing Machinery 7(4): 326–329. https://doi.org/10.1145/321043.321046

Moilanen A, Wilson KA, Possingham HP (2009) Spatial Conservation Prioritization: Quantitative Methods and Computational Tools.Oxford University Press, Oxford and New York.

Önal H (2003) First-best, second-best, and heuristic solutions in conservation reserve selection. Biological Conservation 115(1): 55–62. https://doi.org/10.1016/S0006-3207(03)00093-4

Önal H, Briers RA (2002) Incorporating spatial criteria in optimum reserve network selection. Proceedings. Biological Sciences 269(1508): 2437–2441. https://doi.org/10.1098/rspb.2002.2183

Önal H, Briers RA (2006) Optimum selection of a connected reserve network. Operations Research 54(2): 379–388. https://doi.org/10.1287/opre.1060.0272

Önal H, Wang Y (2008) A graph theory approach for designing conservation reserve networks with minimal fragmentation. Networks 52(2): 142–152. https://doi.org/10.1002/net.20211

Önal H, Wang Y, Dissanayake ST, Westervelt JD (2016) Optimal design of compact and functionally contiguous conservation management areas. European Journal of Operational Research 251(3): 957–968. https://doi.org/10.1016/j.ejor.2015.12.005

Possingham H, Ball I, Andelman S (2000) Mathematical methods for identifying representative reserve networks. In: Ferson S, Burgman M (Eds) Quantitative Methods for Conservation Biology. Springer, New York, 291–305. https://doi.org/10.1007/0-387-22648-6_17

Pressey RL, Nicholls AO (1989) Efficiency in conservation evaluation: Scoring versus iterative approaches. Biological Conservation 50(1–4): 199–218. https://doi.org/10.1016/0006-3207(89)90010-4

Pressey RL, Possingham HP, Day JR (1997) Effectiveness of alternative heuristic algorithms for identifying indicative minimum requirements for conservation reserves. Biological Conservation 80(2): 207–219. https://doi.org/10.1016/S0006-3207(96)00045-6

ReVelle C, ReVelle CS (1993) Facility siting and integer-friendly programming. European Journal of Operational Research 65(2): 147–158. https://doi.org/10.1016/0377-2217(93)90329-L

Rodrigues ASL, Gaston KJ (2002) Optimisation in reserve selection procedures – why not? Biological Conservation 107(1): 123–129. https://doi.org/10.1016/S0006-3207(02)00042-3

Sarkar S (2012) Complementarity and the selection of nature reserves: Algorithms and the origins of conservation planning, 1980–1995. Archive for History of Exact Sciences 66(4): 397–426. https://doi.org/10.1007/s00407-012-0097-6

Scott JM, Davis F, Csuti B, Noss R, Butterfield B, Groves C, Anderson H, Caicco S, D'Erchia F, Edwards TC, Ulliman J, Wright RG (1993) Gap Analysis: A Geographic Approach to Protection of Biological Diversity. Wildlife Monographs 123: 3–41.

Shirabe T (2005) A model of contiguity for spatial unit allocation. Geographical Analysis 37(1): 2–16. https://doi.org/10.1111/j.1538-4632.2005.00605.x

Shirabe T (2009) Districting modeling with exact contiguity constraints. Environment and Planning. B, Planning & Design 36(6): 1053–1066. https://doi.org/10.1068/b34104

Underhill LG (1994) Optimal and suboptimal reserve selection algorithms. Biological Conservation 70(1): 85–87. https://doi.org/10.1016/0006-3207(94)90302-6

Wang Y, Önal H (2016) Optimal design of compact and connected nature reserves for multiple species. Conservation Biology 30(2): 413–424. https://doi.org/10.1111/cobi.12629

Williams JC (2002) A zero-one programming model for contiguous land acquisition. Geographical Analysis 34(4): 330–349. https://doi.org/10.1111/j.1538-4632.2002.tb01093.x

Williams JC, ReVelle CS, Levin SA (2004) Using mathematical optimization models to design nature reserves. Frontiers in Ecology and the Environment 2(2): 98–105. https://doi.org/10.1890/1540-9295(2004)002[0098:UMOMTD]2.0.CO;2

Williams JC, ReVelle CS, Levin SA (2005) Spatial attributes and reserve design models: A review. Environmental Modeling and Assessment 10(3): 163–181. https://doi.org/10.1007/s10666-005-9007-5

RESEARCH ARTICLE

Rediscovery of the endemic gastropod *Dianella schlickumi* (Gastropoda, Hydrobiidae) and its discrimination from *Dianella thiesseana*: environmental correlates and implications for their conservation

Chrysoula Ntislidou[1,2], Canella Radea[3], Sinos Giokas[4], Martin T. Pusch[2],
Maria Lazaridou[1], Dimitra C. Bobori[1]

1 *Department of Zoology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece* **2** *Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), 12587 Berlin, Germany* **3** *Department of Ecology and Systematics, Faculty of Biology, School of Sciences, National and Kapodistrian University of Athens, 15784, Panepistimioupolis, Greece* **4** *Department of Biology, University of Patras, 26500, Patra, Greece*

Corresponding author: *Chrysoula Ntislidou* (ntislidou@bio.auth.gr)

Citation: Ntislidou C, Radea C, Giokas S, Pusch MT, Lazaridou M, Bobori DC (2018) Rediscovery of the endemic gastropod *Dianella schlickumi* (Gastropoda, Hydrobiidae) and its discrimination from *Dianella thiesseana*: environmental correlates and implications for their conservation. Nature Conservation 27: 35–58. https://doi.org/10.3897/natureconservation.27.23289

Abstract

The aquatic snail genus *Dianella* (Gastropoda: Hydrobiidae) has only two representatives in Greece: *Dianella schlickumi* Schütt, 1962 and *Dianella thiesseana* (Kobelt, 1878). *D. schlickumi*, a narrow endemic species to Lake Amvrakia (in Aitoloakarnania, western-central Greece), is considered as Critically Endangered (Possibly Extinct, sensu IUCN 2017). Our study confirmed its presence in Lake Amvrakia, where it had not been detected for more than 30 years. We document the unknown anatomical characters based on the *D. schlickumi* specimens. Moreover, the presence of *D. thiesseana* in the nearby lakes Trichonis and Lysimachia was also confirmed, while morphometric analyses enabled the discrimination between the two species. Redundancy Analysis revealed conductivity, dissolved oxygen and pH as the main environmental variables related to the above species' distribution, shaping their community structure. Both *Dianella* species require urgent conservation measures to be enforced, due to their habitat degradation from human activities, which are limiting and fragmenting their range. For that purpose, effective management plans have to be elaborated and implemented at the mentioned lakes, focusing on the reduction of human pressures and on the improvement of their habitats.

Introduction

Freshwater habitats cover less than 1% of the earth's surface, but they support almost 10% of the known species on the planet (Strayer and Dudgeon 2010). However, freshwater species are in a worldwide decline (Howard et al. 2015), resulting thus in the decrease of freshwater biodiversity, which is mainly threatened by over-exploitation, water pollution, hydrological alterations, destruction and degradation of habitats and invasion by exotic species (Dudgeon et al. 2006).

The Balkan region is rich in freshwater fauna due to high endemism (Griffith et al. 2004, Glöer et al. 2007). The rich fauna of freshwater molluscs is considerably threatened (Albrecht and Wilke 2008) and 29 species of the Balkan Peninsula have already become extinct (Régnier et al. 2009), representing 21% of the 140 known extinctions of freshwater mollusc species worldwide. In this region, many endemic gastropod species exist with a restricted range only extending to small hydrographic systems (rivers, lakes and springs). The karst landscape, which occurs in a large proportion of the Balkan Peninsula, also contributes to this high degree of endemism (Régnier et al. 2009). These species are vulnerable not only due to habitat degradation caused by human activities, but also by their very limited range, which increases the risk of accidental extinction (Régnier et al. 2009). For Greece, a similar crucial decline of population densities and losses of some endemic mollusc species have been reported (Albrecht et al. 2006), suggesting the necessity for urgent actions for their conservation.

The distribution of gastropod species is often associated with biotic and abiotic habitat parameters (e.g. Covich 2010), which are related to specific geographical characteristics, physicochemical conditions, biological interactions and historical and random factors (Lodge et al. 1987). Thus, the distributional patterns of endemics can be often better explained based on records of key environmental parameters, which drive the structure of biological communities (Pérez-Quintero 2012). Such knowledge may also help to identify and protect specific regions for the conservation of biodiversity of little-known freshwater bodies (Abell et al. 2007), especially in the Mediterranean region which has been neglected.

The species of the subfamily Pyrgulinae (Caenogastropoda: Truncatelloidea: Hydrobiidae) are widely distributed in western Asia as well as in central and south-eastern Europe (Wilke et al. 2007). In the latter area, the subfamily consists of ten genera, namely *Chilopyrgula* Brusina, 1896, *Dianella* Gude, 1913, *Ginaia* Brusina, 1896, *Neofossarulus* Polinski, 1929, *Ochridopyrgula* Radoman, 1955, *Pyrgohydrobia* Radoman, 1955, *Pyrgula* De Cristofori & Jan, 1832, *Stankovicia* Polinski 1929, *Trachyochridia* Polinski, 1929 and *Xestopyrgula* Polinski, 1929, comprising 17 species (Bank 2017).

The pyrgulinids are mostly lacustrine snails, colonising the littoral and sublittoral zones of ancient deep freshwater lakes (Radoman 1983, Wilke et al. 2007, Schreiber et al. 2012). Only one species, *Chilopyrgula sturanyi* Brusina, 1896, has been recorded in springs (Radoman 1983).

Out of those, two pyrgulinid species, belonging to the genus *Dianella* Gude, 1913, are known to be present in lentic systems of Greece: *Dianella schlickumi* Schütt, 1962 and *Dianella thiesseana* (Kobelt, 1878). The first species is known from Lake Amvrakia and the latter from lakes Trichonis and Lysimachia (Aitoloakarnania, western-central Greece) (Schütt 1962, Radoman 1973, 1983, Szarowska et al. 2005, Szarowska 2006). Both species are narrow endemic species, with major gaps in historical records, while no information is available about their abundance, population structure, life cycle or genetic diversity. In the IUCN Red List of Threatened Species, *Dianella thiesseana* appears under the status "Critically Endangered", while *D. schlickumi* has been declared as "Possibly Extinct" (IUCN 2017). Apart from *Dianella*, the aquatic gastropod communities of these lakes include several other endemic hydrobiids, such as *Islamia graeca* Radoman, 1973, *Islamia trichoniana* Radoman, 1979, *Pseudoislamia balcanica* Radoman, 1979, *Trichonia trichonica* Radoman, 1973 (Radoman 1983). *D. schlickumi* has not been observed for more than 30 years, despite several sampling efforts performed in Lake Amvrakia (Reischütz and Reischütz 2002, Szarowska et al. 2005, Albrecht et al. 2006, 2011a), which is considered as the type locality of this species. Only fresh empty shells of this species were recorded in an irrigation channel, near Acheloos River (close to Lake Amvrakia) by Reischütz et al. (2008). *D. thiesseana* has only been found at the northeast bank of Lake Trichonis during the last decade (Albrecht et al. 2009, 2011b).

The external morphology, the anatomy and the phylogenetic position of the majority of Pyrgulinae have been extensively studied and discussed (Schütt 1962, Radoman 1983, Riedel et al. 2001, Szarowska et al. 2005, Szarowska 2006, Anistratenko 2008, Wilke et al. 2013). The combination of anatomical and phylogenetic data suggests that the Pyrgulinae subfamily has a very close (possibly even sister-group) systematic relationship with Hydrobiinae (Szarowska et al. 2005). Concerning the two species of *Dianella,* it has been reported that they have similar external morphology and soft body anatomy, but different body size (Radoman 1983). However, the inter-specific differences have been questioned by Szarowska et al. (2005), which has made the taxonomic distinction between *D. thiesseana* and *D. schlickumi* unclear and questionable. Although the soft body anatomy of *D. thiesseana* is described and/or depicted in detail (Radoman 1983, Szarowska et al. 2005, Szarowska 2006), various anatomical characters of *D. schlickumi* remain unknown, un-described or unpublished.

Hence, the aims of the present study were to: (a) report the rediscovery of the seemingly extinct pyrgulinid *D. schlickumi*, (b) describe its unknown anatomical characters, (c) examine its morphometric discrimination from *D. thiesseana* and (d) reveal the environmental parameters that probably drive the distribution of both species.

Material and methods

Study area

We studied three natural lakes located in the western-central part of Greece (Figure 1). These lakes are considered as the last remains of a former big lake, which by several processes, has been split into four smaller lakes (Albanakis et al. 1995). The Acheloos River separates Amvrakia and Ozeros lakes from Trichonis and Lysimachia lakes (Figure 1). Lake Amvrakia (Table 1) is a semipolje karst lake of tectonic origin with high sulphate concentrations, situated in Mesozoic limestone (Verginis and Leontaris 1978, Overbeck et al. 1982). Its water level shows significant seasonal fluctuations, which may be attributed to various factors such as the hydraulic communication with karst aquifers, high evaporation rates, especially during summer and intensive use of water for agricultural purposes (Danielidis et al. 1996). Lake Trichonis (Table 1) is the deepest and the largest natural lake in Greece, which is also a karst water body (Zacharias et al. 2002), situated in a tectonically active area (Poulimenos and Doutsos 1997). It is supplied by precipitation, approximately 30 intermittent streams and sub-aquatic karst springs (Dimitriou and Zacharias 2006). At its western end, a narrow canal connects Lake Trichonis with Lake Lysimachia (Table 1), which is shallower (Figure 1) and it was receiving the untreated urban wastewaters from the city of Agrinio until the year 2000. At its western end, Lake Lysimachia drains to the Acheloos River via an artificial canal (Figure 1).

Field sampling

Macroinvertebrate samplings were conducted in spring and autumn 2014 by boat, at the sublittoral and profundal zones of each lake, using an Ekman-Birge grab (three replicates per station, 225 cm² sampling area). A total of 66 samples were collected in the studied lakes [Amvrakia: 14 stations (7 stations at each zone), Trichonis: 13 (5 and 7 stations at the sublittoral and profundal zones, respectively) and Lysimachia: 6 (3 stations at each zone); Figure 1]. Samples were sieved with a 500 μm mesh and preserved

Table 1. Geographical position, morphological and limnological features of the studied lakes. Alt: altitude; Z_{mean}: mean depth; Z_{max}: max depth; L_a: lake surface area; TL: trophic level, ET: eutrophic, MT: mesotrophic, OL: oligotrophic; WMM: warm monomictic.

Lake	Latitude	Longititude	Alt (m.a.s.l)	Z_{mean} (m)	Z_{max} (m)	L_a (km²)	TL	Lake type
Trichonis	38.573333	21.552222	18[†]	30[†]	57[‡]	96.5	OL/MT[$]	WMM[†]
Lysimachia	38.558597	21.376873	16[†]	3[l]	9[l]	13.1	ET[l]	WMM[l]
Amvrakia	38.652150	21.219867	16[†]	23	53[¶]	11.8	MT[#]	WMM[#]

[†]Zacharias et al. (2002), [‡]Skoulikidis et al. (1998), [$]Doulka and Kehayias (2008), [l]Leonardos (2004), [¶]Albanakis et al. (1995), [#]Danielidis et al. (1996)
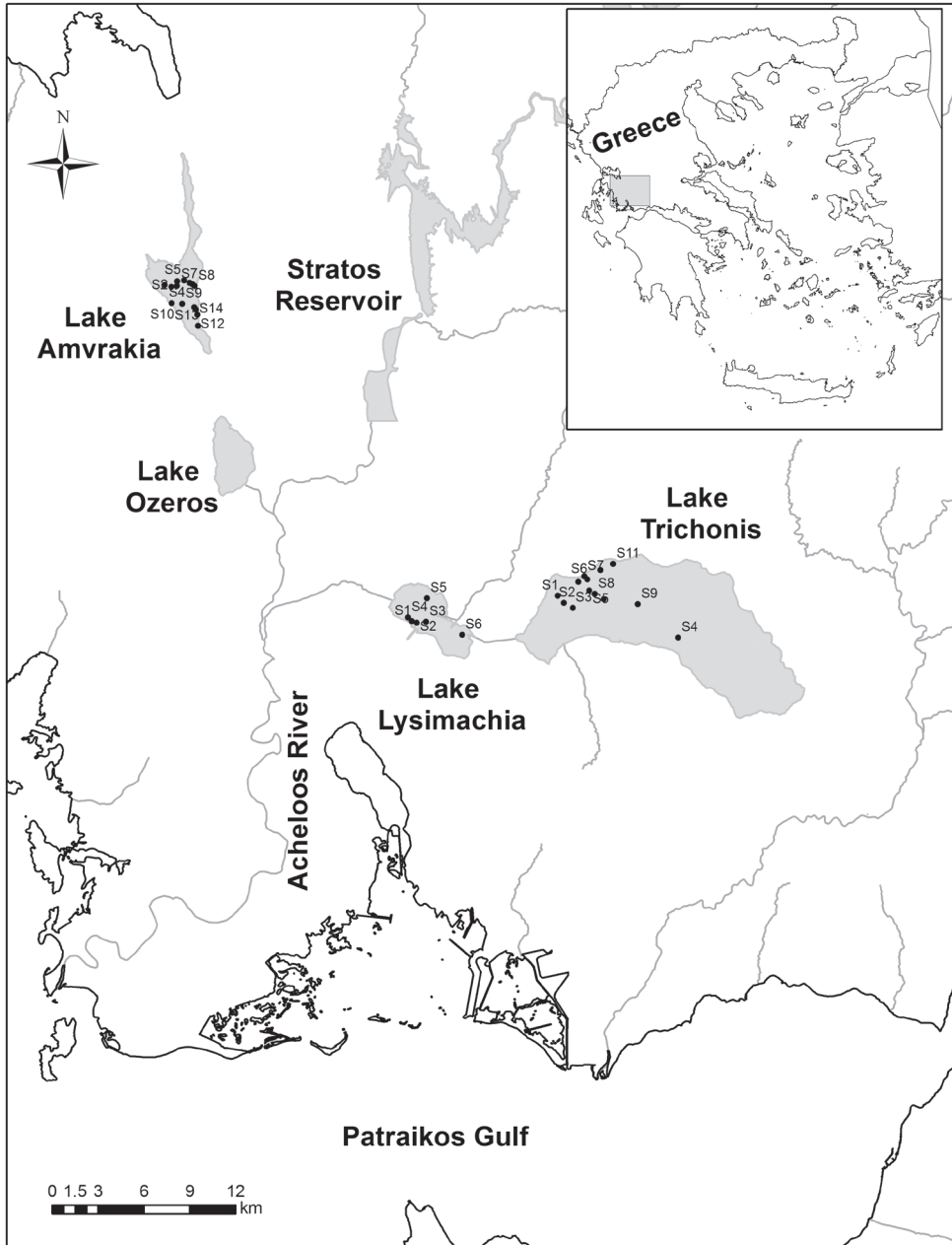
Figure 1. Geographical location of the studied lakes and sampling stations.

in 70% ethanol. After sorting, zoobenthos was identified to the lowest possible taxon and abundance was converted to density (ind./m^2). Additionally, at each station, water samples from 1 m above the bottom of each lake were collected, using a Niskin-type sampler. Environmental parameters such as water temperature (WT, ^{0}C), pH, conduc-

tivity (Cond, μS/cm) and dissolved oxygen concentration (DO, mg/l) were measured *in situ* just above the bottom sediment of each station using the Aqua Read AP-2000 probe (Kent, GB). Secchi depth (Secchi, cm) was also recorded.

Anatomy

Genitalia of male and female specimens and other soft body characteristics *of D. schlickumi* from Lake Amvrakia were studied and compared with those of *D. thiesseana* collected from Lake Trichonis during the present study and with some additional individuals collected in 2016 (Figure 2). Before dissection, the shells of the specimens studied were removed by soaking in Perenyi solution, following Hershler and Ponder's (1998) morphological terminology. The characterisation of the nervous system was based on Davis et al. (1986) and its concentration was measured as the RPG ratio (Davis et al. 1976).

Morphometry

Shell morphometry was recorded in both *D. thiesseana* (Trichonis: 33 individuals and Lysimachia: 5 individuals) and *D. schlickumi* (Amvrakia: 44 individuals) in well preserved shells of adult specimens, using the software ImageFocus v3.0.0.1. Seven linear measurements were taken, specifically: shell height (H), shell width (W), shell aperture height (Ha), shell aperture width (Wa), spire height (SH), body whorl height (BWH) and penultimate whorl height (PWH) (Figure 3). Measurements were then expressed as ratios: shell height/shell width (H/W); shell aperture height/shell aperture width (Ha/Wa); spire height/body whorl height (SH/BWH), penultimate whorl height/body whorl height (PWH/BWH) and body whorl height/shell width (BWH/W). Prior to further analyses, all the linear measurements and the ratios were logarithmically transformed for normalisation. Subsequently, the t-test for independent samples was performed for detecting significant differences ($p < 0.05$) between the two species using the statistical software SPSS v22.

The studied populations were also tested for morphological diversification using a landmark-based method of acquiring geometrical data of shape and size. Shell variation has been traditionally quantified through linear measurements and ratios to distinguish between individuals and populations, amongst and within snail species. Recently, geometric morphometrics (GMs) have been employed for examining shells, both to provide direct size-free analyses of shell shape (Conde-Padin et al. 2007b, Hayes et al. 2007) and to answer broader evolutionary questions (Pfenninger and Magnin 2001, Conde-Padin et al. 2007a, Haase and Misof 2009, Páll-Gergely et al. 2012, Giokas et al. 2014). GMs have the advantage over traditional morphometrics in being, theoretically, free of effects due to size, position, rotation and scale (Rohlf and Marcus 1993).
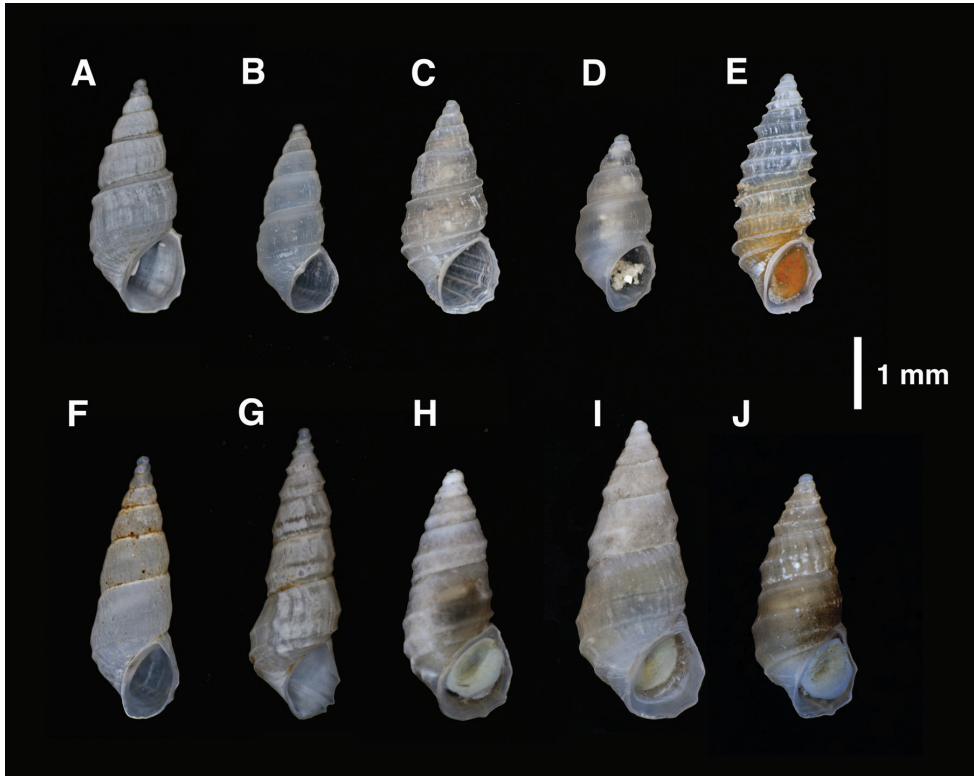
Figure 2. Specimens of: *Dianella schlickumi* from Lake Amvrakia **(A–E)** and *Dianella thiesseana* from Lake Trichonis **(F–J)**, demonstrating their large inter-specific variation of shell morphology.

For that purpose, we used sub-samples of the undamaged and well preserved shells of adult specimens (i.e. 15 *D. schlickumi* and 9 *D. thiesseana* specimens), using the aperture features. Specimens were set down on the same plane, with the aperture facing up and we took digital photographs of them. Geometric morphometric variables of the shells were obtained with 18 landmarks (LM) representing the outline of the shell and of the aperture as shown in Figure 4. Landmarks were digitised using tpsDig (Rohlf 2006) and occupied the same positions over the sum of the specimens. Two of these (LM1 and LM18) were of type I (developmentally homologous), 12 landmarks (LM2-LM13) represented suture lines and were considered type II (geometrically homologous). The remaining landmarks were either extreme points on surfaces or were placed halfway between other landmarks, making them type III (Bookstein 1991, Zelditch et al. 2004).

All geometric morphometric analyses were performed with MorphoJ (Klingenberg 2011). The coordinates of shape to be used for further statistical analysis were obtained with Procrustes generalised least squares superimposition. This method excludes the impact of size on the shape of the shell and, theoretically, variations independent of shape are removed using this analysis (Zelditch et al. 2004). Size variables
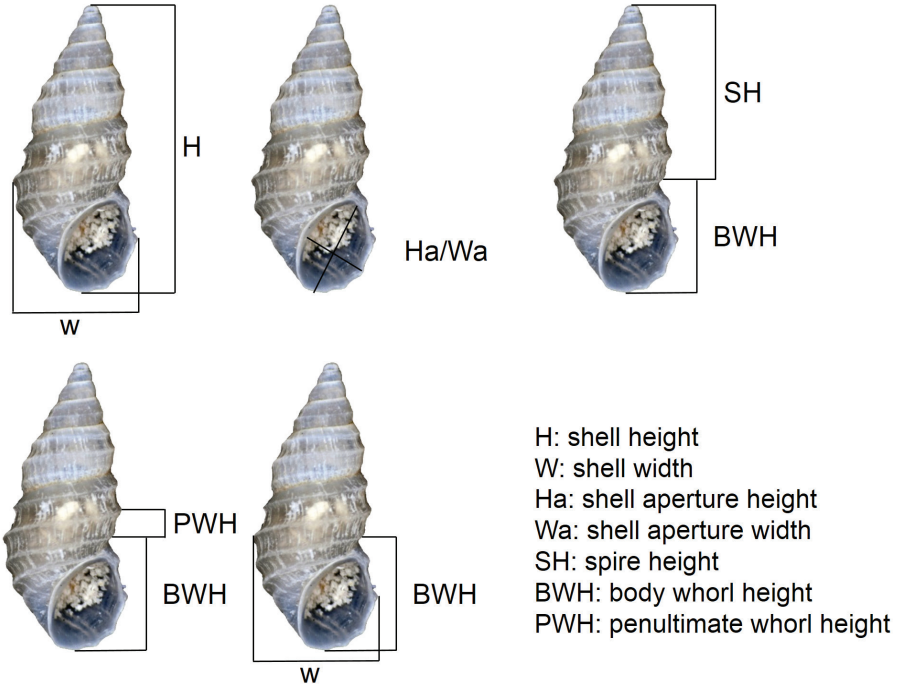
Figure 3. External morphological measurements taken in specimens of *Dianella schlickumi* and *Dianella thiesseana*.
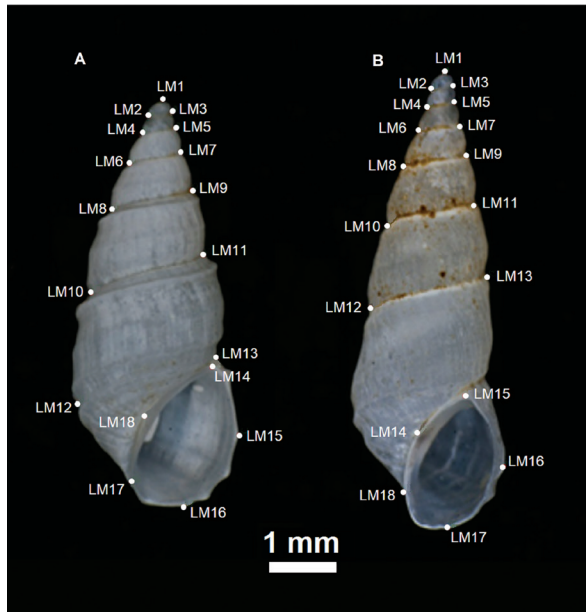

Figure 4. *Dianella schlickumi* and *Dianella thiesseana* specimens positioned with the axis of the shell on the y-axis and the aperture in the same plane as the objective, showing the location of the 18 landmarks (LM1-LM18) used. **A** *D. schlickumi* **B** *D. thiesseana*.

(centroid size) for each specimen were also generated. We examined, using pooled within-group regression analysis, the effect of centroid size on shape, regressing to the resulting Procrustes coordinates on centroid size. That regression score was not signifi-cant (Permutation test, 10,000 rounds against the null hypothesis of independence, % predicted = 6.170, *p = 0.2183*). Therefore, we eventually used the Procrustes coordi-nates, which were analysed with Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) in order to explore the morphological variation between the two *Dianella* species. The reliability of the discrimination was assessed by leave-one-out cross-validation.

Analyses of abundance and environmental data

Detrended Correspondence Analysis (DCA) (indirect gradient analysis) (programme CANOCO version 4.5.1; Ter Braak and Šmilauer 1998) was performed to abundance data in order to verify if they corresponded linearly to the environmental gradient or if abundances peaked around an environmental optimum (unimodal response) (Ter Braak and Šmilauer 1998). In this study, the species abundance data corresponded roughly linearly to the gradient, because the length of the gradient of the first axis was three times less than the range of the within-sample standard deviation (Ter Braak and Šmilauer 1998). Thus, Redundancy Analysis (RDA) was further applied to link the en-vironmental parameters to species abundance data. The Monte Carlo permutation test was performed to select the statistically significant ($p < 0.05$) environmental parameters that explain most of the variance of species abundance, while the variation inflation factor (>20) was used to assess the interrelation of the parameters.

Results

Species distribution

Dianella schlickumi was found at the south-east part of Lake Amvrakia, in 5 (S7, S11, S12, S13, S14; Figure 1) out of the 14 stations (36%) surveyed, at depths ranging from 5 m to 13 m. No individuals were recorded at its western part (S9 and S10, Figure 1), not even empty shells. The highest abundance was recorded at station S15 (607 ind./m^2) at depth 5 m (Table 2). *D. thiesseana* was found in 2 (S6 and S13) out of the 13 stations (15%) in Lake Trichonis, located on the northeast side of the lake, at depths of 13 m to 18 m (Figure 1, Table 2). During the current study, only empty shells from *D. thiesseana* were collected in Lake Lysimachia. However, the presence of a live *D. thiesseana* was confirmed in the east side of Lake Lysimachia by surveys executed dur-ing the National Water Monitoring Programme in spring 2015 (Mavromati, personal communication). Both species were present during the spring surveys while none was found during autumn.

Table 2. *Dianella schlickumi* and *Dianella thiesseana* abundances (number of individuals sampled by Ekman-Birge grab, 225 cm^2 sampling area) per sampling station and environmental parameters measured in lakes Trichonis, Lysimachia and Amvrakia.

Lake	Station	Abundance (ind./m^2)		Environmental parameters					
		Dianella schlickumi	*Dianella thiesseana*	Depth (m)	Secchi (m)	DO (mg/l)	pH	Cond (µS/cm)	WT (°C)
Trichonis	S6		59	18.0	6.4	9.05	10.04	319	18.8
	S13		44	13.0	5.6	8.92	10.73	320	15.0
Lysimachia	LYS		15	3.8	1.7	9.77	8.13	363	11.9
Amvrakia	S7	15		13.0	5.2	8.87	9.74	982	21.2
	S11	15		8.0	4.7	8.38	10.10	921	20.5
	S12	44		11.5	4.8	8.48	10.48	922	20.5
	S13	44		9.0	5.1	8.66	10.42	916	21.6
	S14	607		5.0	3.2	8.47	10.62	898	20.9

Soft body anatomy of *D. schlickumi*

Ctenidium-Osphradium: Ctenidial filaments broader than high; osphradium elongate, approximately opposite to the middle of ctenidium.

Nervous system (Figure 5A): Cerebral ganglia similarly sized, white; supraoesophageal and suboesophageal ganglia white; supraoesophageal connective much longer than the suboesophageal. Nervous system extremely elongate, RPG ratio 0.75.

Gastric caecum: Large and elongate gastric caecum on the posterior stomach chamber.

Rectum: The U-shaped intestine loop in the pallial cavity roof was wide and the faecal pellets were packed sideways (Figure 5B).

Female reproductive system (Figure 6A): Pallial oviduct glands with straight border; bursa copulatrix medium-sized, ovoid-globular, positioned posteriorly to the albumen gland; bursal duct long; renal oviduct unpigmented, coiled tightly with more than two bends; typical seminal receptacles absent; a beak-shaped dilatation of oviduct in the position of seminal receptacle rs2, anteriorly to the bursa.

Penis (Figure 6B): Penis large, gradually tapered, proximal part folded, penial base of medium width, its attachment area central and well behind the head, penial duct almost straight located at the outer edge of the penis.

Egg capsule (Figure 6C–D): Several lenticular and transparent egg capsules (each one containing a single egg) in various growth stages were found on the external shell surface of two specimens.

Morphometry

Seven out of the 12 morphometric measurements taken, differed statistically (*p < 0.001*) between the two species. Generally, individuals of *D. thiesseana* exhibited higher mean values compared to *D. schlickumi*, except for SH/BWH ratio, which was higher in *D. schlickumi* (Table 3).
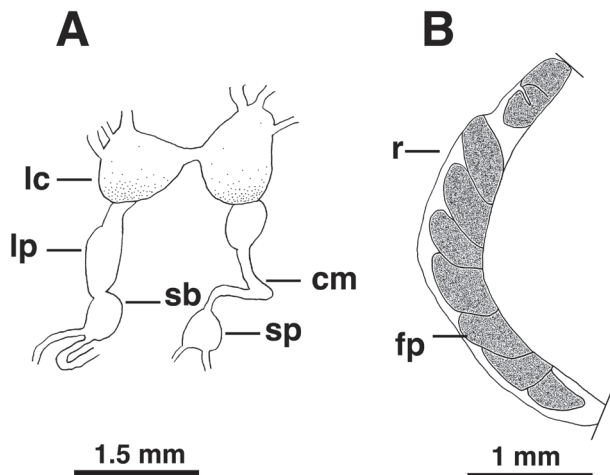
Figure 5. Non-genital anatomy of *Dianella schlickumi*. **A** Nervous system **B** Rectum full of faecal pellets. Abbreviations: cm -commissure; fp -faecal pellets; lc -left cerebral ganglion; lp -left pleural ganglion; sb - suboesophageal ganglion; sp -supraoesophageal ganglion; r -rectum.

Table 3. Average (±standard error) and range (minimum and maximum values) of the morphometric measurements (in mm) taken in *Dianella thiesseana* and *Dianella schlickumi* specimens. The results of t-test are also provided (* indicates the significant differences). n = number of individuals used: H: shell height; W: shell width; Ha: shell aperture height; Wa: shell aperture width; SH: spire height; BWH: body whorl height; PWH: penultimate whorl height.

Variables	*Dianella thiesseana* (n=38)	*Dianella schlickumi* (n=44)	t-test	
			F	*p*
H	6.79 ± 0.25 (4.16–9.78)	6.17 ± 0.21 (4.34–10.89)	3.95	0.063
W*	3.01 ± 0.07 (2.20–3.86)	2.65 ± 0.06 (1.93–3.75)	0.40	<0.001
Ha*	2.45 ± 0.06 (1.58–3.16)	2.07 ± 0.04 (1.61–2.92)	1.45	<0.001
Wa*	1.67 ± 0.04 (1.18–2.22)	1.45 ± 0.03 (1.05–1.91)	0.04	<0.001
SH	4.10 ± 0.19 (2.05–6.38)	3.97 ± 0.17 (2.50–7.70)	8.87	0.797
BWH*	2.69 ± 0.07 (2.00–3.77)	2.20 ± 0.05 (1.64–3.25)	0.86	<0.001
PWH*	0.67 ± 0.02 (0.34–0.92)	0.54 ± 0.01 (0.34–0.78)	5.23	<0.001
H/W	2.24 ± 0.06 (1.69–3.14)	2.31 ± 0.04 (1.99–3.29)	3.09	0.250
Ha/Wa	1.47 ± 0.02 (1.19–1.82)	1.44 ± 0.02 (1.16–1.93)	0.85	0.255
SH/BWH*	1.52 ± 0.06 (0.87–2.37)	1.80 ± 0.04 (1.36–2.89)	12.14	<0.001
PWH/BWH	0.25 ± 0.01 (0.16–0.38)	0.25 ± 0.00 (0.17–0.32)	0.62	0.731
BWH/W*	0.89 ± 0.01 (0.77–1.09)	0.83 ± 0.01 (0.73–0.98)	0.15	<0.001

Geometric morphometric analysis revealed a clear size and shape distinction between the two *Dianella* species. Shells of *D. thiesseana* were significantly larger (Centroid Size) than those of *D. schlickumi* ($F_{1, 22}$ = 38.01, *p* < 0.0001, Figure 8). The landmark-based analyses of individual shells also confirmed a shape distinction between the two species.
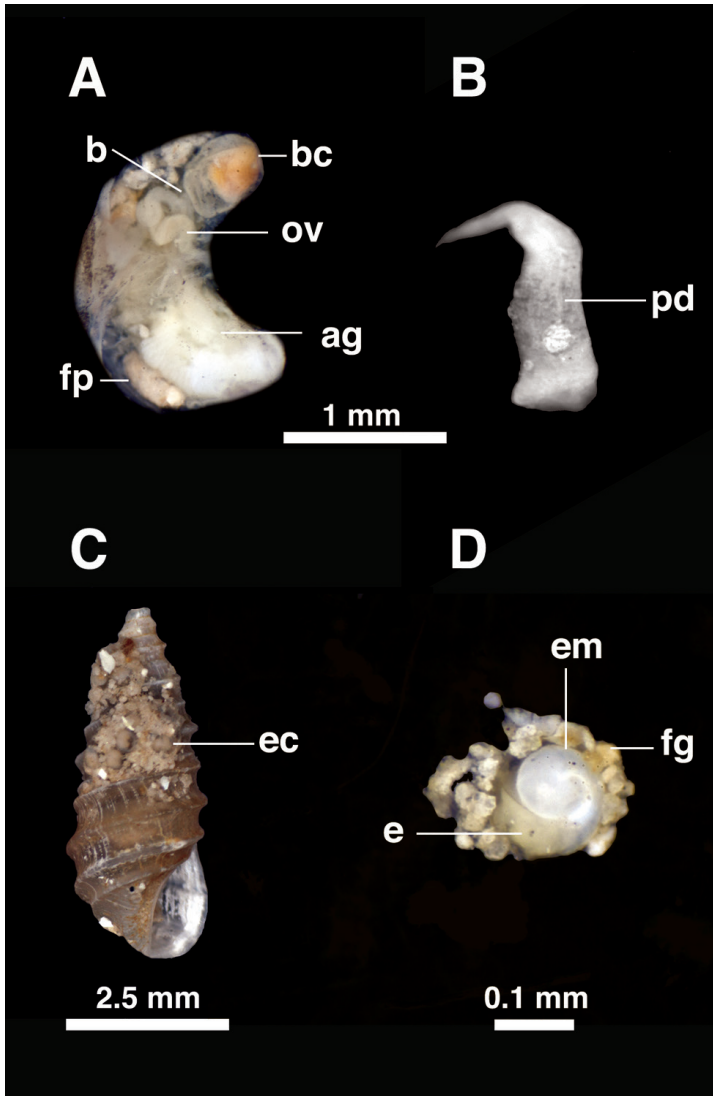
Figure 6. Genital anatomy and egg capsules of *Dianella schlickumi*. **A** Female genitalia **B** Penis **C** Shell bearing egg capsules covered by fine-grained material **D** Egg capsule with embryo. Abbreviations: ag–albumen gland; b–beak-shape dilatation of the oviduct; bc–bursa copulatrix; fp–faecal pellets; fg–fine-grained material; e–eye; ec–egg capsules; em–embryo; ov: renal oviduct; pd–penial duct.

ANOVA of Procrustes coordinates also showed a clear shape difference between the two species ($F_{32, 704}$ = *20.78, p < 0.0001*). PCA of Procrustes coordinates, which aimed at finding linear combinations maximising the total variance, revealed three principal components (PC1-PC3) explaining 88.1% of the total variance. The distinction between the two species was quite evident along the PC1 axis (Figure 9). In the CVA, which maximised differentiation amongst species (or predefined groups), the first and only extracted canonical variate
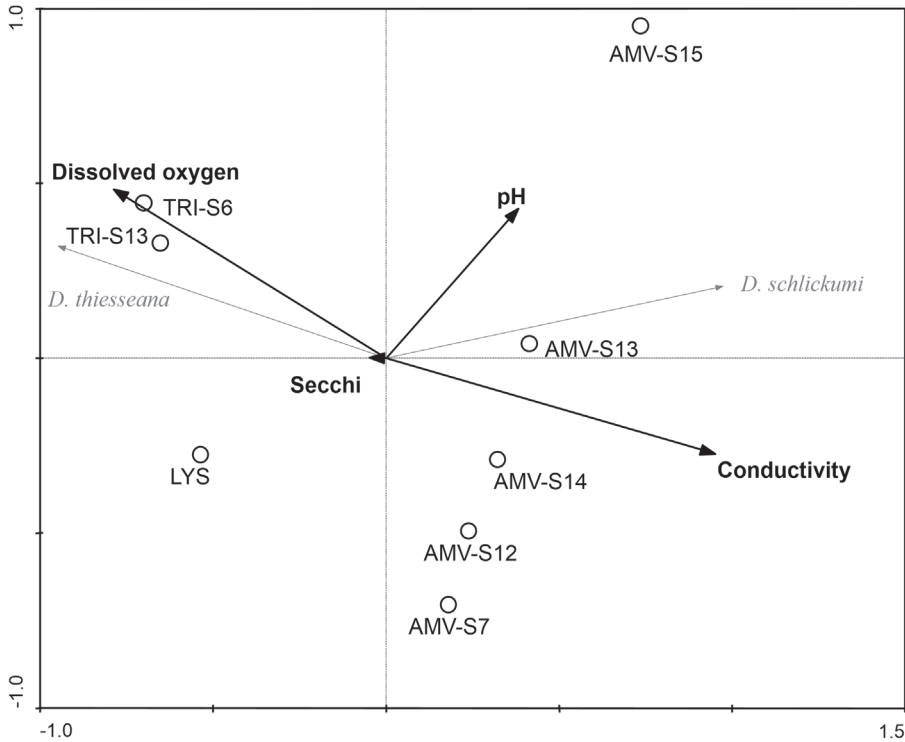
Figure 7. RDA ordination diagram of *Dianella thiesseana* and *Dianella schlickumi* abundances at sampling sites (circles) in relation to environmental parameters in lakes Trichonis (TRI), Lysimachia (LYS) and Amvrakia (AMV) in spring 2014 and 2015.
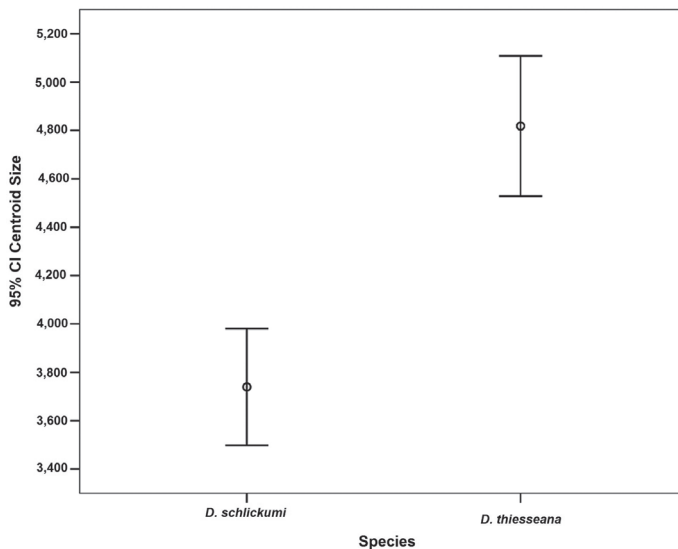

Figure 8. Means ± 95% Confidence Intervals of centroid size for the two *Dianella* species and their ordinations based on landmark data.
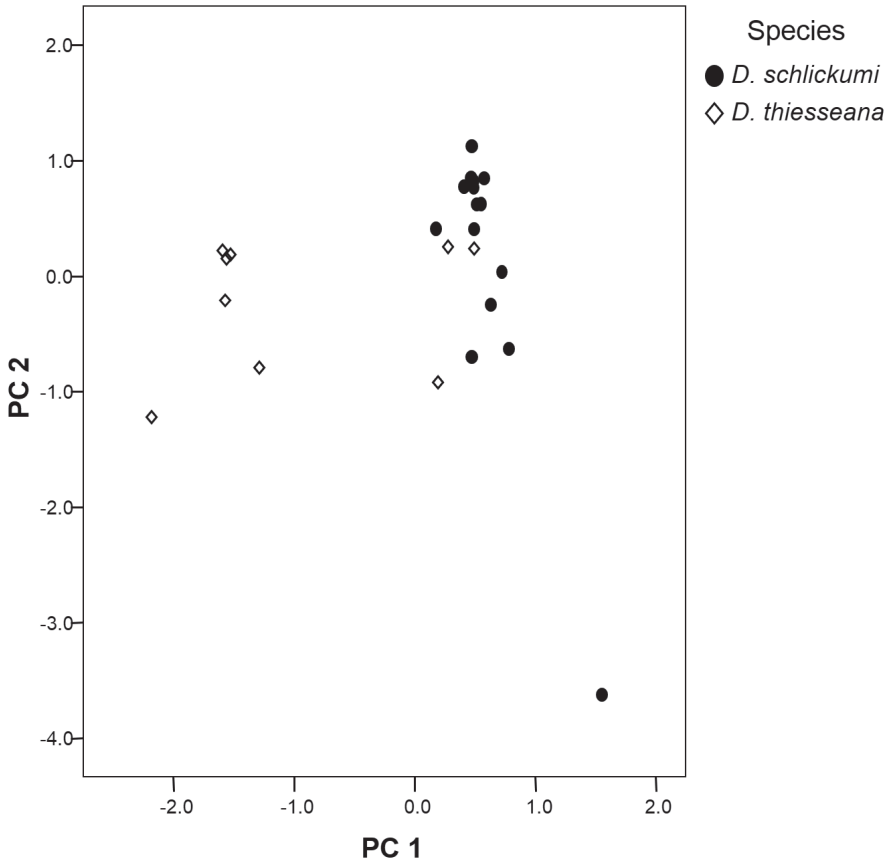
Figure 9. Principal component analysis of *Procrustes coordinates* for *Dianella schlickumi* (black circles) and for *Dianella thiesseana* (open diamonds).

(CV1) explained 100% of total variation and showed a much clearer separation than PCA between the two morphotypes along the CV axis (Figure 10). Clear shape discrimination was also found between *D. thiesseana* and *D. schlickumi* shells after a permutation Discriminant Function Analysis (T-squared = 236.3713, p (parametric) = 0.8335, *p (permutation) < 0.0001*). However, after a permutation test with 1,000 replicates, only 54.2% of "unknown" individuals (leave-one-out cross-validation) were correctly classified. Shape changes along the CV1 are shown in Figure 11 and portray the tendency for *D. thiesseana* to be slimmer compared to *D. schlickumi* and also to have a larger, i.e. elongated, aperture.

Environmental parameters

The Monte Carlo test in RDA analysis revealed two out of the six environmental parameters examined as significantly different between the two species ($p < 0.05$): conductivity and Secchi depth. Depth and water temperature were excluded from the analysis
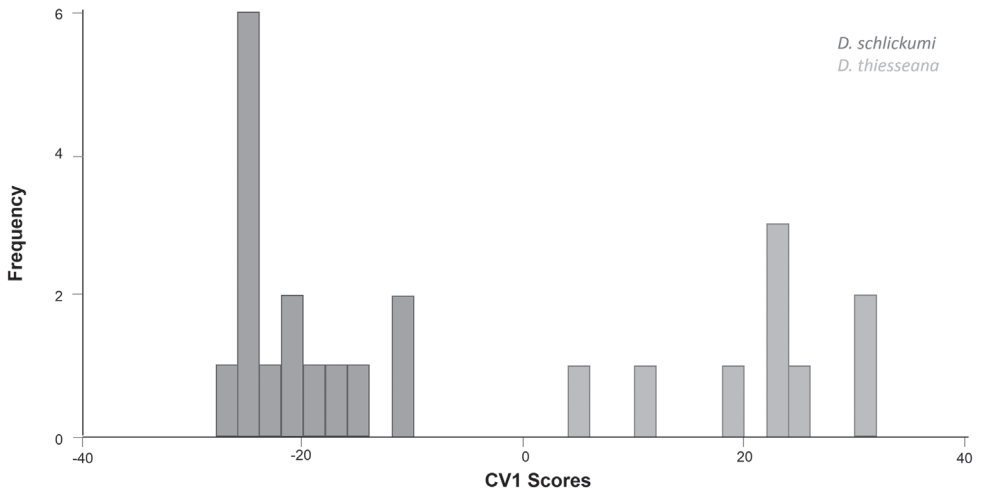
Figure 10. Canonical Variate Analysis: Dark-grey bins stand for *Dianella schlickumi* specimens and light-grey bins for *Dianella thiesseana* specimens.
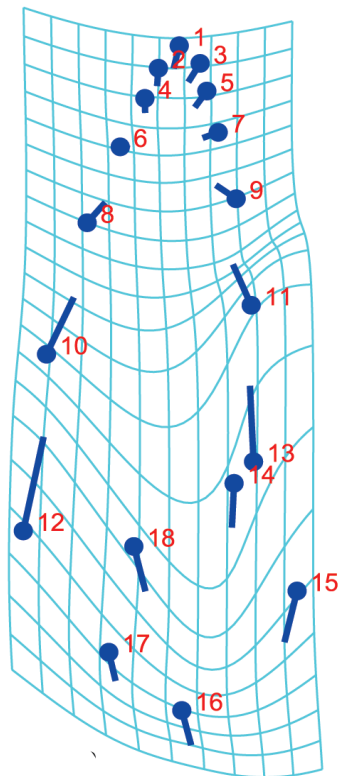

Figure 11. Shape changes along the CV1: the shifts of landmark positions are indicated by straight lines. Each line starts with a dot at the location of the landmark in the starting shape (i.e. *Dianella schlickumi*). The length and direction of the line indicate the movement of the respective landmark towards the final shape (i.e. *Dianella thiesseana*).

due to their high inflation factor (> 20). The first and all canonical axes were statistically significant (*p = 0.044* and *p = 0.002* respectively). The first two ordination axes of RDA explained 99.5% of the total species variance (Monte Carlo test, *p < 0.05*). Axis I (eigenvalue 0.929, *p < 0.05*) was related to conductivity and DO (intra-set correlation values 0.952 and -0.788 respectively) and Axis II (eigenvalue 0.066) to pH (intra-set correlation value 0.428). *D. schlickumi* specimens from Lake Amvrakia were ordered at the positive side of Axis I, having the highest conductivity values while *D. thiesseana* specimens from the other lakes were ordered at the negative side of Axis I and correlated with lower values of conductivity and higher values of DO (Figure 7, Table 2).

Discussion

Dianella schlickumi was recorded in the sublittoral zone of Lake Amvrakia in 1962 (Schütt 1962), but no exact details about this location were provided. The latest record confirming the presence of the species in the lake was before 1983 (Radoman 1983). After that, *D. schlickumi* was assumed to be extinct, as reported by Reischütz and Reischütz (2002), Szarowska et al. (2005) and Albrecht et al. (2006), based on sampling efforts in 1983, 2002 and 2003 respectively. Albrecht et al. (2011a) mentioned that the originally recorded area of *D. schlickumi* is the northern extent of Lake Amvrakia. At this part of the lake water, level fluctuations were high during the last years, due to insufficient connectivity with the main body of the lake. In fact, during extremely dry summers, this area is completely dry or water remains only in small pools. In such cases, the predation pressure (mainly from fish) is intense, resulting in local extinction of molluscs (Nilsson et al. 2008, Downing et al. 2010). Such rough conditions have probably negatively affected the population size and the distribution of *D. schlickumi* in this lake. Nevertheless, in our study, we were able to collect specimens of various ages at several sampling sites, indicating that this snail is still extant and thriving in its type locality.

Under the IUCN Red List guidelines (IUCN 2017), *D. schlickumi* is considered as "Critically Endangered (Possibly Extinct)" [following the criteria B1ab (i, iii)] (Albrecht et al. 2011a). However, since a viable population of this species was demonstrated in the present study, the tag "Possibly Extinct" should be removed from its assessment. The rediscovery of the species does not however improve its conservation status, as its extent of occurrence is estimated to be less than 100 km^2, restricted to the sublittoral zone of Lake Amvrakia (<10 km^2). Moreover, *D. schlickumi* is known to exist only in a single location, where habitat degradation was also noticed due to agricultural practices and pesticide contamination (Thomatou et al. 2013 a, b). Consequently, the classification of Critically Endangered should be maintained, as the IUCN criteria continue to be met. As for *D. thiesseana*, it should also continue to be considered as Critically Endangered [B1ab (ii, iii) and 2ab (ii, iii)] since it has an extent of occurrence of 94 km^2 and an area of occupancy of 2 km^2. Its habitat is also degraded because of intensive agricultural practices, urban sewage, stock grazing land and small industries (Bertahas et al. 2006).

Both species thrive on soft substrate (Albrecht et al. 2011a, b). *D. schlickumi* seemed to prefer slightly shallower habitats, from 3 m to 15 m, while *D. thiesseana* had a range of distribution from 2.5 m to 27 m. The species absence from autumn surveys may be due to depth migrations often observed in gastropods (Brown 2001). Even though this study has confirmed the rediscovery of *D. schlickumi*, it does not clarify in detail the accurate distribution of the species, which might be the subject of future research.

The anatomical characters of *D. schlickumi* known from literature (Schütt 1962) (i.e. the radula) and those described in the present paper (i.e. the ctenidium and osphradium, the central nervous system, the gastric caecum, the rectum and the male and female reproductive organs) are similar to those reported by Radoman (1983) in the diagnosis of the family Pyrgulidae Brusina 1881 (nominal subfamily Pyrgulinae) and of the genus *Dianella* Gude, 1913 [(type species *Dianella thiesseana* (Kobelt, 1878)]. Similarities have also been found between *D. schlickumi* and *D. thiesseana* in the nervous system, the gastric caecum and the female reproductive organs as they are described for the latter species by Szarowska et al. (2005, pp. 22–31 and related figures). However, a notable difference exists in the shape of the penis between the two species: the penis of *D. thiesseana* is wide, with a triangular shape due to its expanded base and has a clear outgrowth on its left side (Szarowska et al. 2005, p. 29, figs 32–37). The penis of *D. schlickumi* does not have an expanded base and is gradually tapering without any distinct outgrowth.

D. schlickumi and *D. thiesseana* are discriminated due to their shell dimensions; the first one is smaller than the second (Radoman 1983). In our study, traditional and geometric morphometric analyses support the assumption that *D. thiesseana* and *D. schlickumi* are quite distinguishable in terms of external morphology. However, neither in the original description of the two taxa (Kobelt 1878 and Schütt 1962 respectively) nor in the subsequent studies (Radoman 1983, Szarowska et al. 2005), shell shape appears to be significantly different between the two taxa. Moreover, Szarowska et al. (2005) reported that the shell characters and dimensions of *D. schlickumi*, described in the literature and those of *D. thiesseana* from Lake Lysimachia, lie within the range observed for *D. thiesseana* from Lake Trichonis. Even though, the intra-subspecific morphometric variation is quite high, the two species are actually distinct in size and shape (Figures 8–10). Therefore, in the "*Dianella*" case, geometric morphometric analysis has been proved to be a useful tool for exploring patterns of species diversity and seems promising for examining morphological adaptations of these species. Specifically, it is clear that the Centroid Size, derived from Geometric Morphometric Analysis, can be used safely to distinguish those two species.

In some cases, the environmental parameters set limits (Pyron and Brown 2015) and may influence (Økland 1983, Pip 1986, Falniowski 1987) the distribution of freshwater snails. The analysis performed revealed conductivity, DO and pH as the most significant parameters explaining the current species variance. *D. thiesseana,* according to RDA, seems to prefer more oxygenated waters with lower conductivity values and pH than *D. schlickumi*. Conductivity, dissolved oxygen (e.g. Çabuk et al. 2004, Kazibwe et al. 2006, Cloherty and Rachlin 2011) and pH (Økland 1983, Pip 1986, Çabuk et al. 2004) are also referenced as key environmental proxies explaining

gastropods distribution in water bodies. Biological interactions and/or historical factors, which need further surveys and analyses, may also be responsible.

Both *Dianella* species require urgent conservation measures to be enforced and suitable management plans to be implemented in the whole studied area, focusing on the protection of these species and the improvement of their habitats. The studied lakes form an ecologically important complex as Special Conservation areas (Council Directive 92/43/EEC), listed in the NATURA 2000 network. However, the integrity of local mollusc populations in these ecosystems is threatened due to the presence of invasives; *Ferrissia fragilis* (Tryon, 1863) and *Physa acuta* Draparnaud, 1805 in lakes Trichonis and Lysimachia (Albrecht et al. 2014) and of *Potamopyrgus antipodarum* (J.E. Gray, 1843) in Lake Trichonis (Radea et al. 2008). The invasive impacts upon native molluscs may be direct, through competition for food and space (Carlsson et al. 2004) or indirect, through changes in ecosystem function and/or parasitism (Brown et al. 2008). The antagonistic behaviour of *P. antipodarum* and *P. acuta* is already well-known (e.g. Schreiber et al. 2003, Zukowski and Walker 2009), although no such outcomes have been documented for *F. fragilis* until now (Albrecht et al. 2014). Available knowledge on the effects of invasive species on native mollusc species and especially on *D. thiesseana*, is insufficient to estimate the possible threats.

One of the main pressures identified in lakes Amvrakia, Trichonis and Lysimachia is eutrophication from agriculture run-off and wastewaters (Bertahas et al. 2006, Thomatou et al. 2013 a, b). *Dianella* inhabits the sublittoral zone of these lakes, which is mainly affected by eutrophication (Pilotto et al. 2012). The development of water quality indices based on zoobenthic communities and related to eutrophication will help to assess the ecological status of these water bodies and prevent their further deterioration. The impacts could be minimised by preserving natural vegetation or establishing buffer zones. Only low-impact activities should be allowed near the lakes and harmful practices should be prohibited or regulated to a more distant zone. Furthermore, it is crucial to restore the type locality of *D. schlickumi* by maintaining the connectivity of the northern extension of Lake Amvrakia with the main lake water body, thus preventing the species' historical distribution area to become dry. Additional measures to reduce water abstraction could help the maintenance of the ecological water level in the lakes, securing slight seasonal fluctuations and preventing aquatic biodiversity in general (Zohary and Ostrovsky 2011). Further temporal and spatial surveys which will identify the ecological features, the ecosystem functions and the habitat preferences of the studied species could significantly support conservation efforts. Environmental education and citizen awareness could additionally support conservation efforts for both endemic gastropods.

Conclusion

Our results confirm the rediscovery of the narrow endemic species *Dianella schlickumi* in Lake Amvrakia since past sampling efforts did not detect it for more than

30 years. Moreover, they provide basic knowledge on its anatomical, morphological and distributional patterns and its discrimination from *Dianella thiesseana*. The two species are discriminated by their shell size and shape. Moreover, the presence of *Dianella thiesseana* is confirmed in lakes Trichonis and Lysimachia. We conclude that further conservation measures have to be implemented for effective protection of both species.

Acknowledgements

References

Abell R, Allan JD, Lehner B (2007) Unlocking the potential of protected areas for freshwaters. Biological Conservation 134(1): 48–63. https://doi.org/10.1016/j.biocon.2006.08.017

Albanakis C, Vouvalidis K, Palikaridis Ch, Psilovikos AA (1995) Phenomena of anoxic conditions in karstic Lake Amvrakia. Proceedings of 4th Pan-Hellenic Geographical Congress of the Hellenic Geographical Society, Athens (Greece), October 1995, 54-63. http://geolib. geo.auth.gr/index.php/pgc/article/view/9098/8850

Albrecht C, Lohfink D, Schultheiss R (2006) Dramatic decline and loss of mollusk diversity in long-lived lakes in Greece. Tentacle 14: 11–13.

Albrecht C, Wilke T (2008) Ancient Lake Ohrid: Biodiversity and evolution. Hydrobiologia 615(1): 103–140. https://doi.org/10.1007/s10750-008-9558-y

Albrecht C, Hauffe T, Schreiber K, Trajanovski S, Wilke T (2009) Mollusc biodiversity and endemism in the putative ancient Lake Trichonis (Greece). Malacologia 51(2): 357–375. https://doi.org/10.4002/040.051.0209

Albrecht C, Hauffe T, Schreiber K (2011a) *Dianella schlickumi*. The IUCN Red List of Threatened Species 2011. e.T156088A4891185: https://doi.org/10.2305/IUCN.UK.2011-1.RLTS.T156088A4891185.en

Albrecht C, Hauffe T, Schreiber K (2011b) *Dianella thiesseana*. The IUCN Red List of Threatened Species 2011. e.T155578A4802619: https://doi.org/10.2305/IUCN.UK.2011-1.RLTS.T155578A4802619.en

Albrecht C, Föller K, Clewing C, Hauffe T, Wilke T (2014) Invaders versus endemics: Alien gastropod species in ancient Lake Ohrid. Hydrobiologia 739(1): 163–174. https://doi.org/10.1007/s10750-013-1724-1

Anistratenko VV (2008) Evolutionary trends and relationships in hydrobiids (Mollusca, Caenogastropoda) of the Azov-Black Sea Basin in the light of their comparative morphology and paleozoogeography. Zoosystematics and Evolution 84(2): 129–142. https://doi.org/10.1002/zoos.200800001

Bank R (2017) Fauna Europaea: Pyrgulinae. Fauna Europaea version 2.6. Available online at http://www.fauna-eu.org [Accessed on 20 June 2017]

Bertahas I, Dimitriou E, Karaouzas I, Laschou S, Zacharias I (2006) Climate change and agricultural pollution effects on the trophic status of a Mediterranean lake. CLEAN–Soil, Air. Water (Basel) 34: 349–359. https://doi.org/10.1002/aheh.200500637

Bookstein FL (1991) Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, 1–512. https://doi.org/10.1002/bimj.4710350416

Brown K (2001) Mollusca: Gastropoda. In: Thorp JH, Covich AP (Eds) Ecology and classification of North American freshwater invertebrates. Academic press, California, 297–329.

Brown KM, Lang B, Perez KE (2008) The conservation ecology of North American pleurocerid and hydrobiid gastropods. Journal of the North American Benthological Society 27(2): 484–495. https://doi.org/10.1899/07-062.1

Çabuk Y, Arslan N, Yılmaz V (2004) Species composition and seasonal variations of the Gastropoda in Upper Sakarya River System (Turkey) in relation to water quality. CLEAN–Soil, Air. Water (Basel) 32: 393–400. https://doi.org/10.1002/aheh.200300544

Carlsson NO, Brönmark C, Hansson LA (2004) Invading herbivory: The golden apple snail alters ecosystem functioning in Asian wetlands. Ecology 85(6): 1575–1580. https://doi.org/10.1890/03-3146

Cloherty TM, Rachlin JW (2011) Physicochemical and shoreline development factors affecting lake littoral benthic macroinvertebrates. Journal of Freshwater Ecology 26: 517–525. https://doi.org/10.1080/02705060.2011.588435

Conde-Padin P, Carvajal-Rodriguez A, Carballo M, Caballero A, Rolán-Alvarez E (2007a) Genetic variation for shell traits in a direct-developing marine snail involved in a putative sympatric ecological speciation process. Evolutionary Ecology 21(5): 635–650. https://doi.org/10.1007/s10682-006-9142-8

Conde-Padin P, Grahame JW, Rolán-Alvarez E (2007b) Detecting shape differences in species of the *Littorina saxatilis* complex by morphometric analysis. The Journal of Molluscan Studies 73(2): 147–154. https://doi.org/10.1093/mollus/eym009

Council Directive (1992) 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Union 206: 7–50. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31992L0043&from=EN

Covich AP (2010) Winning the biodiversity arms race among freshwater gastropods: Competition and coexistence through shell variability and predator avoidance. Hydrobiologia 653(1): 191–215. https://doi.org/10.1007/s10750-010-0354-0

Danielidis DB, Spartinou M, Economou-Amilli A (1996) Limnological survey of Lake Amvrakia, western Greece. Hydrobiologia 318(3): 207–218. https://doi.org/10.1007/BF00016682

Davis GM, Kitikoon V, Temcharoen P (1976) Monograph on "*Lithoglyphopsis*" *aperta*, the snail host of Mekong River schistosomiasis. Malacologia 15: 241–287.

Davis GM, Guo YH, Hoagland KE, Chen PL, Zheng LC, Yang HM, Chen DJ, Zhou YF (1986) Anatomy and Systematics of Triculini (Prosobranchia: Pomatiopsidae: Triculinae), Freshwater Snails from Yunnan, China, with Descriptions of New Species. Proceedings of the Academy of Natural Sciences of Philadelphia Natural Sciences of Philadelphia 138: 466–575. http://www.jstor.org/stable/4064917

Dimitriou E, Zacharias I (2006) Quantifying the rainfall-water level fluctuation process in a geologically complex lake catchment. Environmental Monitoring and Assessment 119(1-3): 491–506. https://doi.org/10.1007/s10661-005-9039-y

Downing JA, Van Meter P, Woolnough DA (2010) Suspects and evidence: A review of the causes of extirpation and decline in freshwater mussels. Animal Biodiversity and Conservation 33: 151–185. http://www.raco.cat/index.php/ABC/article/view/214978/285495

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan CA (2006) Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews of the Cambridge Philosophical Society 81(02): 163–182. https://doi.org/10.1017/S1464793105006950

Falniowski A (1987) Hydrobioidea of Poland (Prosobranchia: Gastropoda). Folia Malacologica 1: 1–119. https://doi.org/10.12657/folmal.001.001

Glöer P, Albrecht C, Wilke T (2007) Enigmatic distribution patterns of the Bithyniidae in the Balkan Region (Gastropoda: Rissooidea). Mollusca 25: 13–22. http://www.malaco.de/Sonderdrucke/03_Gloeer_Albrecht_Wilke.pdf

Giokas S, Páll-Gergely B, Mettouris O (2014) Nonrandom variation of morphological traits across environmental gradients in a land snail. Evolutionary Ecology 28(2): 323–340. https://doi.org/10.1007/s10682-013-9676-5

Griffith HI, Kryštufek B, Reed JM (2004) Balkan biodiversity: pattern and process in the European hotspot. Kluwer Academic Publishers, Dordrecht, 1–358. https://doi.org/10.1007/978-1-4020-2854-0

Haase M, Misof B (2009) Dynamic gastropods: Stable shell polymorphism despite gene flow in the land snail *Arianta arbustorum.* Journal of Zoological Systematics and Evolutionary Research 47(2): 105–114. https://doi.org/10.1111/j.1439-0469.2008.00488.x

Hayes DM, Minton RL, Perez KE (2007) *Elimia comalensis* (Gastropoda: Pleuroceridae) from the Edwards Plateau, Texas: Multiple unrecognized endemics or native exotic? American Midland Naturalist 158(1): 97–112. http://www.jstor.org/stable/4500639. https://doi.org/10.1674/0003-0031(2007)158[97:ECGPFT]2.0.CO;2

Hershler R, Ponder WF (1998) A review of morphological characters of hydrobioid snails. Smithsonian Contributions to Zoology 600(600): 1–55. https://doi.org/10.5479/si.00810282.600

Howard JK, Klausmeyer KR, Fesenmyer KA, Furnish J, Gardali T, Grantham T, Katz JVE, Kupferberg S, McIntyre P, Moyle PB, Ode PR, Peek R, Quiñones RM, Rehn AC, Santos N, Schoenig S, Serpa L, Shedd JD, Slusark J, Viers JH, Wright A, Morrison SA (2015) Patterns of Freshwater Species Richness, Endemism, and Vulnerability in California. PLoS One 10(7): e0130710. https://doi.org/10.1371/journal.pone.0130710

Kazibwe F, Makanga B, Rubaire-Akiiki C, Ouma J, Kariuki C, Kabatereine NB, Booth M, Vennervald BJ, Sturrock RF, Stothard JR (2006) Ecology of *Biomphalaria* (Gastropoda: Planorbidae) in Lake Albert, Western Uganda: snail distributions, infection with schistosomes and temporal associations with environmental dynamics. Hydrobiologia 568(1): 433–444. https://doi.org/10.1007/s10750-006-0224-y

Klingenberg CP (2011) MorphoJ: An integrated software package for geometric morphometrics. Molecular Ecology Resources 11(2): 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Kobelt W (1878) Diagnosen neuer Arten. Jahrbuch der Deutschen Malakozoologischen Gesellschaft 5: 319–321.

Lodge DM, Brown KM, Klosiewski SP, Stein RA, Covich AP, Leathers BK, Brönmark C (1987) Distribution of freshwater snails: Spatial scale and the relative importance of physicochemical and biotic factors. American Malacological Bulletin 5: 73–84.

Nilsson E, Olsson K, Persson A, Nyström P, Svensson G, Nilsson U (2008) Effects of stream predator richness on the prey community and ecosystem attributes. Oecologia 157(4): 641–651. https://doi.org/10.1007/s00442-008-1097-8

Økland J (1983) Factors regulating the distribution of freshwater snails (Gastropoda) in Norway. Malacologia 24: 277–288.

Overbeck J, Anagnostidis K, Economou-Amilli A (1982) A limnological survey of three Greek lakes: Trichonis, Lyssimachia and Amvrakia. Archiv für Hydrobiologie 95: 365–394.

Páll-Gergely B, Kornilios P, Giokas S (2012) Higher than anticipated diversity within an *Albinaria* species (Gastropoda, Pulmonata, Clausiliidae) in Southern Turkey. Journal of Biological Research (Thessaloniki) 18: 345–352.

Pérez-Quintero JC (2012) Environmental determinants of freshwater mollusc biodiversity and identification of priority areas for conservation in Mediterranean water courses. Biodiversity and Conservation 21(12): 3001–3016. https://doi.org/10.1007/s10531-012-0351-x

Pfenninger M, Magnin F (2001) Phenotypic evolution and hidden speciation in *Candidula unifasciata ssp* (Helicellinae, Gastropoda) inferred by 16S variation and quantitative shell traits. Molecular Ecology 10(10): 2541–2554. https://doi.org/10.1046/j.0962-1083.2001.01389.x

Pilotto F, Free G, Cardoso AC, Wolfram G, Solimini AG (2012) Spatial variance of profundal and sublittoral invertebrate benthic communities in response to eutrophication and morphological pressures. Fundamental and Applied Limnology. Archiv für Hydrobiologie 180(2): 101–110. https://doi.org/10.1127/1863-9135/2012/0206

Pip E (1986) The ecology of freshwater gastropods in the central Canadian region. The Nautilus 100: 56–66.

Poulimenos G, Doutsos T (1997) Flexural uplift of rift flanks in central Greece. Tectonics 16(6): 912–923. https://doi.org/10.1029/97TC01658

Pyron M, Brown KM (2015) Introduction to mollusca and the class Gastropoda. In: Thorp JH, Rogers DC (Eds) Covich's Freshwater Invertebrates: Ecology and General Biology. Elsevier, 383–421. https://doi.org/10.1016/B978-0-12-385026-3.00018-8</edb>

Radea C, Louvrou I, Economou-Amilli A (2008) First record of the New Zealand mud snail *Potamopyrgus antipodarum* JE Gray 1843 (Mollusca: Hydrobiidae) in Greece – Notes on its population structure and associated microalgae. Aquatic Invasions 3(3): 341–344. https://doi.org/10.3391/ai.2008.3.3.10

Radoman P (1973) On the relations of some freshwater Mollusca of the Balkan Peninsula and Asia Minor. Basteria 37: 77–84. http://natuurtijdschriften.nl/download?type=document;docid=596599

Radoman P (1983) Hydrobioidea a superfamily of Prosobranchia (Gastropoda) 1. Systematics. Monographs 547/57. Srpska Akademija Nauka i Umetnosti Posebna Izdanja, 1–256.

Régnier C, Fonatine B, Bouchet P (2009) Not knowing, not recording, not listing: Numerous unnoticed mollusc extinctions. Conservation Biology 23(5): 1214–1221. https://doi.org/10.1111/j.1523-1739.2009.01245.x

Reischütz A, Reischütz PL (2002) Helleniká pantoía, 2: Limni Amvrakia – vom raschen Sterben eines Langzeitsees (Aitolien/Akarnanien, Griechenland). Nachrichtenblatt der Ersten Vorarlberger Malakologischen Gesellschaft 10: 59-60. https://www.zobodat.at/pdf/NachErstMalaGes_10_0059-0060.pdf

Reischütz A, Reischütz PL, Fischer W (2008) Helleniká pantoía, 17: Zur Kenntnis der Molluskenfauna des Acheloos (Aitolien/Akarnanien, Griechenland). Nachrichtenblatt der Ersten Vorarlberger Malakologischen Gesellschaft 15: 17–20. http://ipp.boku.ac.at/private/wf/Publikationen/N_15_17-20.pdf

Riedel F, Healy JM, Röpstorf P, Sitnikova TY (2001) Ecology, shell morphology, anatomy and sperm ultrastructure of the caenogastropod *Pyrgula annulata*, with a discussion of the relationship between the 'Pyrgulidae' and Caspian and Baikalian rissooideans. Limnologica 31(4): 289–302. https://doi.org/10.1016/S0075-9511(01)80031-1

Rohlf FJ (2006) tpsDig, Digitize landmarks and outlines, version 2.0. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf FJ, Marcus LF (1993) A revolution in morphometrics. Trends in Ecology & Evolution 8(4): 129–132. https://doi.org/10.1016/0169-5347(93)90024-J

Schreiber ESG, Quinn GP, Lake PS (2003) Distribution of an alien aquatic snail in relation to flow variability, human activities and water quality. Freshwater Biology 48(6): 951–961. https://doi.org/10.1046/j.1365-2427.2003.01070.x

Schreiber K, Hauffe T, Albrecht C, Wilke T (2012) The role of barriers and gradients in differentiation processes of pyrgulinid microgastropods of Lake Ohrid. Hydrobiologia 682(1): 61–73. https://doi.org/10.1007/s10750-011-0864-4

Schütt H (1962) Neue Süsswasser-Prosobranchier Griechenlands. Archiv für Molluskenkunde 91: 157–166.

Strayer DL, Dudgeon D (2010) Freshwater biodiversity conservation: Recent progress and future challenges. Journal of the North American Benthological Society 29(1): 344–358. https://doi.org/10.1899/08-171.1

Szarowska M (2006) Molecular phylogeny, systematics and morphological character evolution in the Balkan Rissooidea (Caenogastropoda). Folia Malacologica 14(3): 99–168. https://doi.org/10.12657/folmal.014.014

Szarowska M, Falniowski A, Riedel F, Wilke T (2005) Phylogenetic relationships of the subfamily Pyrgulinae (Gastropoda: Caenogastropoda: Hydrobiidae) with emphasis on the genus *Dianella* Gude, 1913. Zootaxa 891(1): 1–32. https://doi.org/10.11646/zootaxa.891.1.1

Ter Braak CJF, Šmilauer P (1998) CANOCO Reference manual and User's Guide to CANOCO for Windows. Software for Canonical Community Ordination (version 4). Centre for Biometry,Wageningen, 1–352.

Thomatou AA, Zacharias I, Hela D, Konstantinou I (2013a) Determination and risk assessment of pesticide residues in lake Amvrakia (W. Greece) after agricultural land use changes in the lake's drainage basin. International Journal of Environmental Analytical Chemistry 93(7): 780–799. https://doi.org/10.1080/03067319.2012.656099

Thomatou AA, Triantafyllidou M, Chalkia E, Kehayias G, Konstantinou I, Zacharias I (2013b) Land Use Changes Do Not Rapidly Change the Trophic State of a Deep Lake. Amvrakia

Lake, Greece. Journal of Environmental Protection 4(05): 426–434. https://doi.org/10.4236/jep.2013.45051

Verginis S, Leontaris S (1978) Beiträge zur Morphologie und Entwicklung des Semipoljes Amvrakia (Limni Amvrakia), West-Griecheland. Internationale Revue der Gesamten Hydrobiologie und Hydrographie 63: 831–839. https://doi.org/10.1002/iroh.19780630615

Wilke T, Albrecht C, Anistratenko VV, Sahin SK, Yıldırım MZ (2007) Testing biogeographical hypotheses in space and time: Faunal relationships of the putative ancient Lake Eğirdir in Asia Minor. Journal of Biogeography 34(10): 1807–1821. https://doi.org/10.1111/j.1365-2699.2007.01727.x

Wilke T, Haase M, Hershler R, Liu HP, Misof B, Ponder W (2013) Pushing short DNA fragments to the limit: Phylogenetic relationships of 'hydrobioid' gastropods (Caenogastropoda: Rissooidea). Molecular Phylogenetics and Evolution 66(3): 715–766. https://doi.org/10.1016/j.ympev.2012.10.025

Zacharias I, Bertachas I, Skoulikidis N, Koussouris T (2002) Greek lakes: Limnological overview. Lakes and Reservoirs: Research and Management 7(1): 55–62. https://doi.org/10.1046/j.1440-1770.2002.00171.x

Zelditch ML, Swiderski DL, Sheets HD, Fink WL (2004) Geometric morphometrics for biologists. A Primer. Elsevier Academic Press, Amsterdam, 1–488.

Zohary T, Ostrovsky I (2011) Ecological impacts of excessive water level fluctuations in stratified freshwater lakes. Inland Waters 1(1): 47–59. https://doi.org/10.5268/IW-1.1.406

Zukowski S, Walker KF (2009) Freshwater snails in competition: Alien *Physa acuta* (Physidae) and native *Glyptophysa gibbosa* (Planorbidae) in the River Murray, South Australia. Marine & Freshwater Research 60(10): 999–1005. https://doi.org/10.1071/MF08183

RESEARCH ARTICLE

Breeding site fidelity, and breeding pair infidelity in the endangered Carnaby's Cockatoo *Calyptorhynchus latirostris*

Denis A. Saunders[1], Nicole E. White[2], Rick Dawson[3], Peter R. M. Mawson[4]

1 *CSIRO Land & Water, GPO Box 1700, Canberra ACT 2601, Australia* **2** *Trace and Environmental DNA Laboratory, School of Molecular and Life Sciences, Curtin University, Perth WA 6102, Australia* **3** *Waikiki, WA 6169, Australia* **4** *Perth Zoo, Department of Biodiversity, Conservation and Attractions, P.O. Box 489, South Perth, WA 6951, Australia*

Corresponding author: *Denis A. Saunders* (denis.saunders@csiro.au)

Abstract

The ecology and behaviour of the endangered Carnaby's Cockatoo *Calyptorhynchus latirostris* have been studied in detail at Coomallo Creek in the northern wheatbelt of Western Australia from 1969 until the present. Results of research on this breeding population conducted on individually marked birds from 1970 to 1990 were compared with results from analyses of DNA taken from nestlings in the study area from 2003, 2005, and each year from 2009 to 2013. Analyses of DNA confirmed earlier findings about the stability of adult breeding pairs, and that females used the same breeding hollow they used previously, provided the hollow was not occupied when they returned to breed. When moving to another hollow, they chose a hollow in the same vicinity of the previous hollow. Analyses in 22 cases where DNA was obtained from both nestlings of a breeding attempt revealed that in six (27.3%) cases, the second egg was fertilised by a male not paired with the female. These extra-pair copulations were not suspected during the earlier study based on observations of individually marked birds.

Keywords

Carnaby's Cockatoo, *Calyptorhynchus latirostris*, nestling DNA, extra-pair copulations, pair bond stability, nest site fidelity

Introduction

Carnaby's Cockatoo *Calyptorhynchus latirostris* is endemic to south-western Australia. Formally common throughout its range, over the second half of the last century, extensive clearing of native vegetation for the development of broadscale agriculture and urban development saw the species contract significantly in range and abundance. As a result of the loss of breeding and foraging habitat (Fig. 1), the status of the species changed from being classified legally up to the 1960s as vermin with a bounty on its bill, to being listed as endangered (Saunders 1990) by three authorities in the late 1980s. Currently Carnaby's Cockatoo is listed: as "Fauna that is rare or likely to become extinct" in Schedule 1 of the Western Australian *Wildlife Conservation Specially Protected Fauna Notice 2016* under the *Wildlife Conservation Act 1950*; endangered under the Australian Federal Government's *Environment Protection and Biodiversity Conservation Act* 1999; and endangered under IUCN's Red List category and criteria (IUCN 2014). IUCN recently changed the scientific name of the species to *Zanda latirostris*, but we retain the name *Calyptorhynchus latirostris* as specified in Western Australian Government notices. Since 2002 the species has been the subject of two recovery plans (Cale 2003, Department of Environment and Conservation 2012).

Beginning in 1968 and continuing, the ecology and behaviour of the species has been the focus of one of the longest running vertebrate studies in Australia (Saunders 1982, Saunders and Ingram 1998, Saunders and Dawson 2017). One breeding population of the species at Coomallo Creek (Fig. 1) was studied intensively from 1969 to 1977, then monitored intermittently using the same protocols until 1996, and then every year from 2009 (Saunders and Dawson 2017) until the present. As a result of this study, the breeding biology of the species is well known. Once the birds form pairs and reach breeding age (usually at four years), the pairs remain together until one of the partners dies. They return to the same breeding area each year in winter, with rainfall in the first half of autumn influencing the commencement of egg-laying; the wetter the period, the earlier breeding commences. The female selects a nesting hollow in a large eucalypt, usually lays two eggs, on average up to eight days apart. Both eggs usually hatch with the younger nestling dying within 48 hours of hatching. In around six per cent of cases both nestlings are successfully fledged. The female nests in the same hollow used the previous breeding season, provided she successfully fledged a young the previous year, and the hollow is not already occupied when she returns to breed. If she has an unsuccessful breeding attempt she usually moves to another hollow nearby, and makes another breeding attempt, either during the same breeding season or next season (information from Saunders 1982, Saunders et al. 2013, 2014a).

The fact that Carnaby's Cockatoo is rare, and difficult to breed in captivity (Saunders 1976) made it a lucrative target for poachers who took nestlings illegally from hollows used by wild breeding pairs. In accessing the hollow floor, poachers often cut a large hole in the side of the nesting tree rendering the hollow unsuitable for Carnaby's Cockatoo. In dealing with the problem posed by poachers, the Western Australian Government authority responsible for conservation, in conjunction with staff
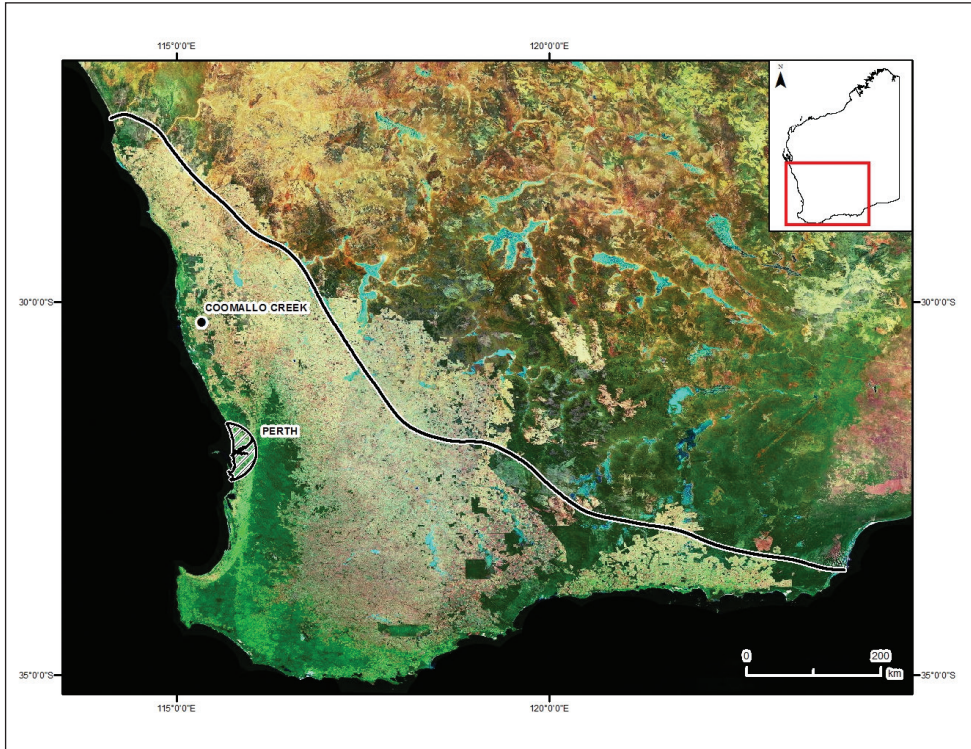
Figure 1. Location of Coomallo Creek study area, and distribution of Carnaby's Cockatoo (between the coast and the black line). Carnaby's Cockatoo has significantly decreased in range and abundance as a result of loss of breeding and foraging habitat. The extent of the loss of habitat (native vegetation indicated in pale yellow) is illustrated clearly in this satellite image.

at Curtin University, investigated the efficacy of using analysis of DNA to establish relationships between putative parents and the nestlings poachers claimed they had bred in captivity. White et al. (2009) developed 20 microsatellite markers for population and forensic applications suitable for Carnaby's Cockatoo. White et al. (2012) demonstrated the successful application of these markers in three case studies, one of which demonstrated clearly that a Carnaby's Cockatoo nestling claimed to have been bred in captivity bore no relationship to the pair the breeder claimed had produced the nestling, and further that the nestling was a full sibling of a nestling known to have been fledged successfully from a nest hollow monitored in the wild. This unequivocal result was used in a successful prosecution for poaching.

White et al. (2012) demonstrated that analyses of DNA provided reliable discriminatory power for identification of individuals, testing kinship and identification of breeding populations, making possible the determination of illegal trade or harvest of live animals from the wild, and providing a method to monitor both wild and aviary populations. They pointed out that use of DNA is a minimally-invasive technique, even less so when moulted feathers are used as described by Hou et al. (2018). White

et al. (2012) also noted that the black cockatoo database they described constituted one of the most extensive Australian wildlife forensic databases.

In this paper we examine the use of DNA taken from nestlings of one breeding population over seven breeding seasons (2003, 2005, 2009-2013) to investigate aspects of the breeding biology of Carnaby's Cockatoo. We compare the results obtained from DNA analyses with earlier research (1969-1996) on the population using observations of individually marked birds to test the efficacy of analyses of nestling DNA for studies of breeding behaviour.

Methods

The Coomallo Creek (Fig. 1) study area is described in detail in Saunders (1982), Saunders and Ingram (1998), and Saunders et al. (2015). The cockatoos nest in hollows in large eucalypt trees that are distributed along a 9km narrow belt of woodland surrounded by agricultural land and uncleared Kwongan (native shrub land *sensu* Beard 1984). There was no further clearing of the eucalypt woodland during the study period, but the number of available nest hollows fluctuated as trees were lost through natural attrition (Saunders et al. 2014b), and new hollows entered service, and were located by us.

From 1969 to 1996 the area was visited for 21 of those years for at least one week in early September, and one week in early November. During each visit every known nest hollow was inspected, and the identity of breeding females established if possible by trapping or by using a telescope to read the number on the bird's leg band/ring. Both methods were time consuming, and it was not possible to identify all banded females. Searches were also undertaken to find any nesting hollows in use not registered in the study area. Any nestlings older than three weeks were measured (length of the folded left wing and body mass) and banded with a stainless steel band with a unique number. Nestlings were aged using the methods of Saunders (1986), and laying dates extrapolated from the age of the nestlings.

The area was visited once in November 2003 and 2005, and each year from 2009 to 2013 the area was visited for one week in early September and early November, with a three-day visit in early January to establish nesting success, and band any nestlings laid late in the season. The identity of breeding females was established by photographing their legs, and checking for bands using the methods of Saunders et al. (2011). This was a much more successful method for establishing the identity of banded females nesting in the area than was possible in the earlier study. Searches were also undertaken to find any nesting hollows in use not registered in the study area. Nestlings were measured (length of the folded left wing and body mass) and banded, and laying dates extrapolated from nestling age. Nestlings were sexed on shape and colour of their cheek patch, and at least three pin feathers taken from their chests for subsequent DNA analysis. The body of small dead nestlings were taken for DNA analysis, as was at least one toe from larger dead nestlings or dead adults.

Material taken for DNA analysis was plucked with tweezers or cut with scissors, and placed in a vial of preservative (20% dimethylsulphoxide saturated with sodium chloride). The vial was labelled with species identification, collection date, name of collector, name of the study area, nest hollow number, and the description of material taken (e.g., 4 feathers, small dead nestling, toe of dead nestling). All equipment used for handling DNA was washed in disinfectant, 90% ethanol, and rinsed with water between samples to ensure no cross-contamination of DNA.

A total of 309 DNA samples were collected from Coomallo Creek between November 2003 and October 2013 for DNA extraction with Qiagen Blood and tissue kit (Qiagen). Samples were subjected to a real-time quantitative PCR (qPCR) assay to assess the DNA extracts for quality and quantity of nuclear DNA prior to microsatellite genotyping. Genetic profiles were generated from 16 microsatellite markers as previously published by White et al. (2009, 2012, 2014).

The software COANCESTRY 1.0.1.7 (Wang 2011) was used to estimate the pairwise relatedness (r_{xy}) between individual birds. A total of 873 birds were included in the analysis, 587 birds were DNA profiled and published in White et al. (2014), and an additional 286 DNA samples were collected across the breeding range of the species for pairwise relatedness examination. COANCESTRY implements seven methods to estimate the pairwise relatedness between individuals. These methods are: the triadic likelihood estimator (denoted as TrioML; Wang 2007); five moment estimators denoted as Wang (Wang 2002); LynchLi (Lynch 1988, Li et al. 1993); LynchRd (Lynch and Ritland 1999); Ritland (Ritland 1996); QuellerGt (Queller and Goodnight 1989); and a dyadic likelihood estimator (DyadML, Milligan 2003). The relatedness classifications for the genealogical relations were set at r_{xy} = 0.5 for parent-offspring and full siblings, r_{xy} = 0.25 for half siblings, and r_{xy} = 0 for unrelated individuals, with 1000 bootstraps to calculate 95% confidence intervals. For the purposes of this study, we report the genealogical relations of parent-offspring and full siblings from the 309 DNA samples collected at Coomallo Creek.

Distances between nest hollows used successively by the same breeding pair were calculated using the "show distance between waypoints" function of OziExplorer® software. The density of nest hollows in the study area was only calculated for the period 1972-1990 and 2009-2013 due to the fact that nest hollows were being located for the first time at a high rate during the first two years of the study (1970–1971).

Results

Between 1970 and 1990, observations of individually marked females provided data on nest hollow use by 92 breeding pairs, each making two or more breeding attempts. Pairs recorded making two or three breeding attempts provided 68.5% of the data. During that period one female was recorded making 12 breeding attempts (Table 1). Twenty of these breeding pairs had both birds individually marked. In all but one case the cockatoos remained paired with same partner, one pair being together for five years

Table 1. Number of breeding attempts made by individual females 1970–1990 and 2009–2013.

Number of breeding attempts made by individual females	2	3	4	5	6	7	8	9	10	11	12	Total
1970–90	45	18	15	7	2	1	1	0	2	0	1	92
2009–13	24	11	4	2	1	0	0	0	0	0	0	42

Table 2. The breeding outcome from, and location of, successive hollows used for breeding by 92 females 1970–1990 and 34 females 2009–2013. Figures in brackets are percentage of the total.

	# individuals	Successful; same hollow	Successful; different hollow; previous hollow occupied or unusable	Successful; different hollow; no information on hollow use	Unsuccessful; same hollow	Unsuccessful; different hollow	Total
1970–90	92	77 (45)	56 (33)	9 (5)	2 (1)	28 (16)	172
2009–13	34	28 (44)	23 (37)	4 (6)	1 (2)	7 (11)	63

(1972–1977), during which time they made seven breeding attempts. There was only one case of pair separation, where both partners were subsequently seen breeding with other partners. Between 2009 and 2013, DNA taken from nestlings provided data from 42 breeding pairs making two or more breeding attempts (Table 1). Most data (83.3%) were from females making two or three breeding attempts over the five year period, but one female made six attempts, one each year 2009-2013, and a second breeding attempt in 2013 after the first attempt failed. All attempts were with the same partner. One hundred and forty-two relationships between nestlings were established, ranging from two nestlings from the same breeding attempt to nestlings from six breeding attempts.

Data were obtained from 172 successive breeding attempts from the 92 pairs between 1970 and 1990, and 63 successive breeding attempts from 42 pairs between 2009 and 2013 (Table 2). One hundred and thirty-three (93.7%) successful breeding attempts between 1970 and 1990 were followed next season by the pair breeding in the same hollow or a different one if the previous one was not available. Between 2009 and 2013, 51 (92.7%) successful breeding attempts were followed next season by breeding in the same hollow or a different one if the previous one was not available. Between 1970 and 1990, 28 (93.3%) unsuccessful breeding attempts were followed by breeding attempts in a different hollow. Between 2009 and 2013, seven (87.5%) unsuccessful breeding attempts were followed by breeding attempts in a different hollow (Table 2). There was no difference (χ^2=1.21, d.f.= 4, p=0.88) between the relative rates of success or failure of breeding between the two phases of the study.

During the period 1970-1990, data on movements to different hollows between successive breeding attempts were obtained from 60 females that moved 94 times, and 2009-2013 from 30 females that moved 40 times (Fig. 2). The mean distance pairs moved between successive breeding attempts was 0.66±0.74km (n=94; range 0.02-4.05km) for the period between 1970 and 1990 and 1.25±1.31km (n=40, range 0.07 – 5.50km)
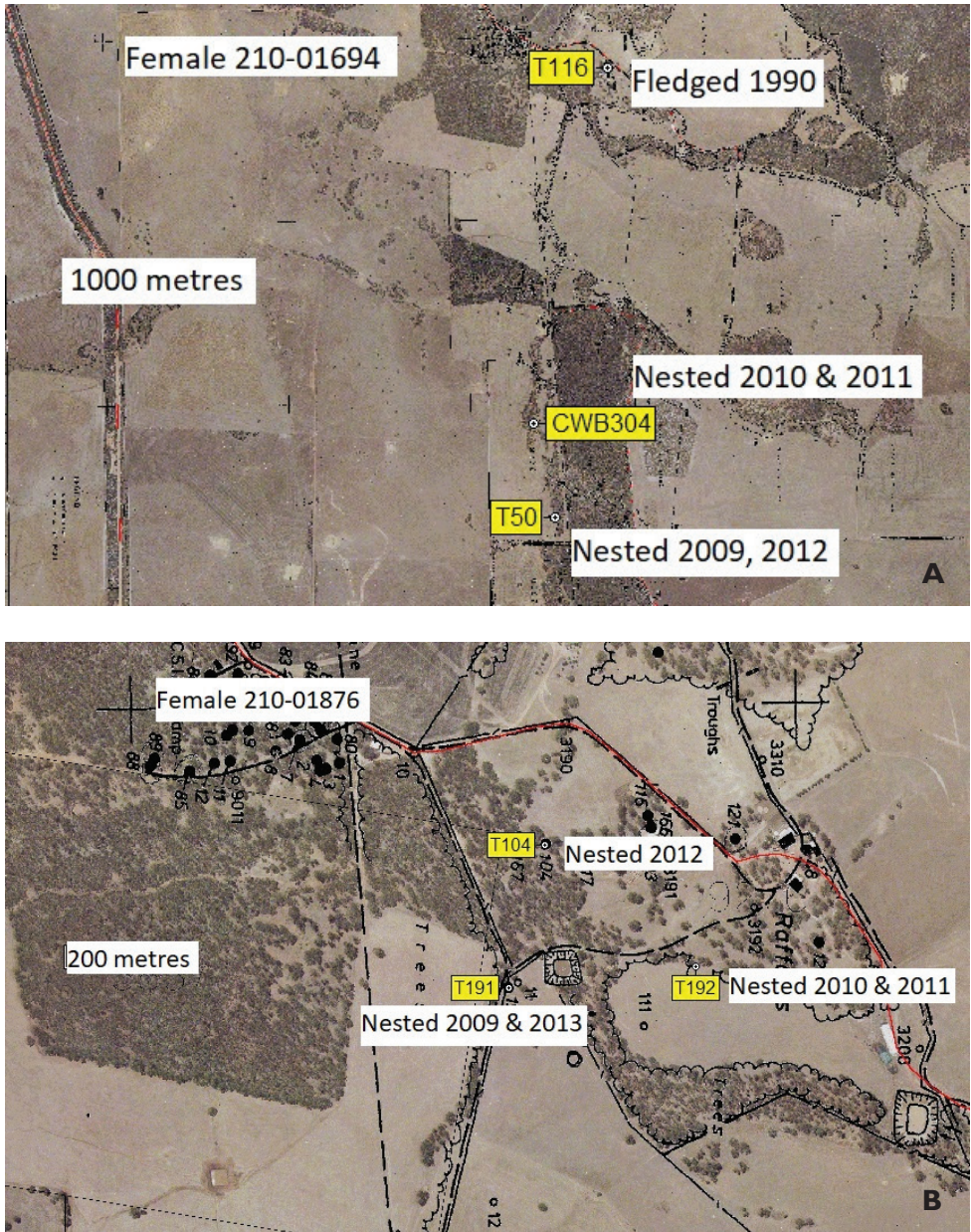
Figure 2. Movements between successive hollows by four females (two banded and two unbanded), confirmed by analysis of nestling DNA; **a** female 210-01694 fledged from T116 in 1990, and nested successfully each year from 2009 to 2012 in the hollows indicated **b** female 210-01876 was banded as an adult, and she nested successfully from 2009 to 2012 in the hollows indicated **c** unbanded Female A nested successfully in T43 from 2009 to 2012, was unsuccessful in T43 in her first breeding attempt in 2013, moved to CWB304 in which she was successful in her second breeding attempt that year **d** unbanded Female B nested successfully from 2009 to 2012 in the hollows indicated. The scale is indicated in the white rectangle with the width of the rectangle indicating the distance.
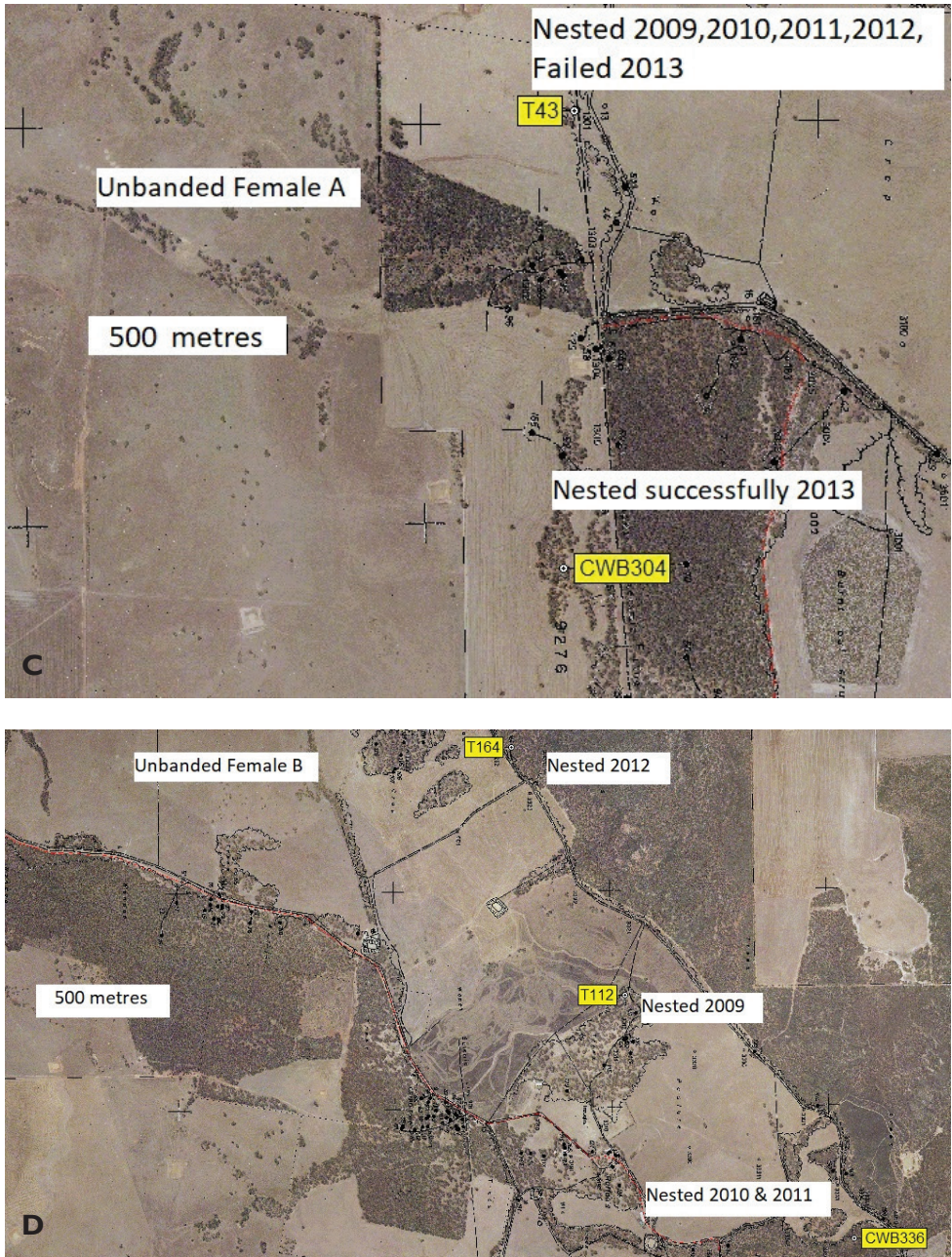
Figure 2. Continued.

between 2009 and 2013 (Table 3). There was a significant difference in the mean distances between successive nest hollows for the periods 1970-1990 and 2009-2013 (Mann-Whitney U-test, one-tailed: Z=-2.81, p=0.002). The distance between nesting hollows used in successive years increased despite the average density of nest hollows remaining

Table 3. Mean distance (km) between nest hollows used by Carnaby's Cockatoo in successive breeding attempts 1970–1990 and 2009–2013.

Years	Mean±s.d. distance moved (km)	Median distance moved (km)	Range	# individuals	# movements
1970–1990	0.66±0.74	0.36	0.02–4.05	60	94
2009–2013	1.25±1.31	0.56	0.07–5.50	30	40

Table 4. Density of nest hollows (km^{-2}) used by Carnaby 's Cockatoos 1972–1990 and 2009–2013.

Years	Mean±s.d. density of nest hollows (km-2)	Range
1972–1990	14.0±4.9	4.5–23.8
2009–2013	16.9±4.8	11.2–23.8

similar between the two phases of the study (Table 4; Mann-Whitney U-test, one-tailed: Z=-1.29, p=0.10).

For the period 2009-2013, DNA was obtained from both nestlings from 22 breeding attempts. Of these, six (27.3%) of the sibling pairs had different males fertilising the two eggs. Both nestlings fledged successfully in 13 cases where both eggs were fertilised by the same male (13/16 = 81.3%), and in four cases where the eggs were fertilised by different males (4/6 = 66.7%). Of the five cases where one or both of the nestlings died, both eggs were fertilised by the same male in three (60%) cases, and two (40%) by different males. The ages of three of the adult females for which there were DNA data on both nestlings from a single breeding attempt were known as the females were banded. Band number 210-03089 was at least 22 (she was banded as an adult), and both eggs were fertilised by the same male; 210-01876 was at least 26, 27 and 28, and the same male fertilised both eggs in all three clutches; and 210-01694 was 20, and the two eggs were fertilised by different males.

The average time between the laying of the two eggs in each case of siblings for which laying dates were known was 10.6±3.9 days (n = 15; range 5-17 days) where the same male fertilised both eggs, compared with 14.8±5.9 days (n = 5; range 9-24 days) where the second egg was fertilised by a different male. There was no significant difference in the mean interval (in days) between the laying of successive eggs in nests fathered by the same male and different males (t-test: t=-1.71, d.f.=17, p=0.053).

Discussion

Nest site fidelity, breeding site tenacity or breeding philopatry is a well-known phenomenon, demonstrated by many vertebrate species (Greenwood 1980). Among birds it is practiced by both migratory and sedentary species, from a wide range of taxa, both passerines and non-passerines, and in most species it is females that display breeding site fidelity (Greenwood 1980). Advantages accruing as a result of breeding site fidelity are

reduction in susceptibility to predation (Burger 1982) and other adverse conditions, such as micro-climatic impacts, and knowledge of rich food resources (Blancher and Robertson 1985, Ehrlich et al. 1994). In such species, breeding success in one season is often followed by use of the same site the next season (Blancher and Robertson 1985). There is tendency for breeding site fidelity to increase with age of individuals, based on the fact that older birds tend to be more experienced breeders, and therefore successful, so moving sites less frequently (McNicholl 1975). McNicholl (1975) pointed out that breeding site fidelity is strongly developed in highly stable habitats (e.g., cliff faces), but greatly reduced in highly unstable habitats (e.g., grasslands, mud beaches or bars in rivers).

Carnaby's Cockatoo breeds in highly stable habitats (*sensu* McNicholl), in that tree hollows are usually available for many years (Saunders et al. 2014b). So it is unsurprising that analysis of successive breeding attempts based on observations of individually marked females at Coomallo Creek between 1970 and 1990 confirmed breeding site fidelity, with 93.7% of successful breeding attempts being followed the next season by the birds breeding in the same hollow, or a hollow nearby if the hollow was in use when they returned to breed. Analysis of nestling DNA found the same result for the period between 2009 and 2013, with 92.7% of successful breeding attempts being followed the next season by the birds breeding in the same hollow, or a hollow nearby if the hollow was in use when they returned to breed. Such breeding site fidelity is shown by other species of Psittaciformes (Rowley 1990, Murphy et al. 2003, Berkunsky and Reboreda 2009). Analyses of data on hollow use following breeding failure from observations of individually marked females and nestling DNA confirmed Saunders' (1982) finding that after a failed breeding attempt, females usually moved to another hollow nearby for their next breeding attempt, either in the same breeding season or the next season.

Saunders (1982) established that when Carnaby's Cockatoo formed breeding pairs, in most cases they were monogamous, pairing for the life of one of the partners. This was based on observations of birds individually marked with patagial tags. Not only did they remain paired during the breeding season, but they remained together throughout the year (Saunders 1980). Analysis of nestling DNA confirmed this fidelity of breeding pairs from 2009 to 2013, but it also revealed extra-pair fertilizations. In 27.3% of the 22 cases where DNA samples were obtained from both nestlings from the same breeding attempt, the second egg was fertilised by a male not paired with the female. While these extra-pair fertilizations were surprising in light of observations of individually marked breeding Carnaby's Cockatoo, this extra-pair fertilization is not uncommon in birds (Petrie and Kempenaers 1998, Forstmeier et al. 2011), and mammals (Crawford et al. 2008). Extra-pair fertilization has been recorded at varying rates in some larger parrot and cockatoo species, for example: 9% extra-pair fertilization in nests of Blue and Yellow Macaw *Ara ararauna* (Caparroz et al. 2011); 20% in nests of Red-tailed Amazona *Amazona brasiliensis* (Fernandes 2015); and 40% in nests of Monk Parakeet *Myiopsitta monachus* (Martinez et al. 2013). In parrot species with multi-male breeding systems, extra-pair fertilizations have been suggested in the Echo Parakeet *Psittacula eques* (Taylor and Parkin 2009), confirmed in Eclectus Parrot *Eclectus roratus* (Heinsohn et al. 2007), and confirmed in 100% of nests of Vasa Parrot *Caracopsis vasa* (Ek-

strom et al. 2007). However, in other species such as the Burrowing Parrot *Cyanoliseus patagonus* (Masello et al. 2002) and Crimson Rosella (*Platycercus elegans*; Eastwood et al. 2018) there is no evidence of extra-pair fertilization.

The chances of observing extra-pair copulations of individually marked birds were low. While many breeding females were individually marked with patagial tags and/or leg bands, far fewer males were individually marked. While copulations were observed, those few cases where both sexes were identified were all with known mates, reinforcing the conclusion of monogamy. In 2010 a documentary about the breeding of Carnaby's Cockatoo was filmed (http://www.abc.net.au/tv/guide/abc1/201203/programs/NH1001W001D2012-03-13T203229.htm accessed 5 April 2018). The documentary was based on one breeding pair, and after the first egg was laid, film was taken of the female leaving the hollow when her unbanded mate was not around. There were two adult males nearby, and both immediately started courtship displays towards the female. One of the males had a leg band, and that could have only been placed on the bird seven years previously or longer. Earlier observations and this film clearly indicate that males will readily court breeding females other than their mates (Saunders 1979), and analysis of nestling DNA indicates that females will accept copulation from males other than their mate. It is highly unlikely that the first egg would result from copulation with a male other than the mate. This is because the pair remain together from the time they return to the breeding area, select and prepare their nesting hollow, and the first egg is laid. At that time the female incubates the egg, and depends on the male for food (Saunders 1982), and males are aggressive to other males approaching their mate (Saunders 1979). During incubation, and until the older nestling is about three weeks old, the female remains either in the hollow or around the nest tree. The male is around the nest tree at dawn, mid-morning when he feeds the female, and again when he feeds her at dusk (Saunders 1982). On occasions the female may be out of the hollow when the mate is not around. At this time extra-pair copulations are possible. The second egg is laid on average eight days after the first, but may be laid up to 24 days later (Saunders 1982, Saunders et al. 2014a). At Coomallo Creek, both nestlings have fledged from hollows when there was 24 days difference in age, with nestlings growing at the same rate; i.e., the younger nestling does not grow faster than the older in order to fledge at the same time. These differences in time between the eggs provide ample opportunity for extra-pair copulations. The limited data available from analysis of nestling DNA indicate that older, more experienced females may indulge in extra-pair copulations, and that second nestlings, the result of such copulations may fledge along with the older nestling, the result of copulation with the male of the pair. These data came from banded females, however nothing is known about such rates in younger breeding females.

Who were the males responsible for these extra-pair copulations? Of the six cases known to us, in three cases, there were no data indicating the second nestling was a half-sibling to any other nestlings from the area that breeding season for whom we had DNA. In each of the other three cases (one in 2009, 2010 and 2012), there was a nestling from three other breeding pairs that indicated a half-sibling relationship. The average distance between any of these hollows and the hollow with the extra-pair copulations was 2.8±1.2km (range 1.7–5.2km). If it was one of the males producing the

half-siblings, they weren't neighbours. Without DNA from the males, unfortunately we cannot identify any of the males responsible.

The conservation implications for such extra-pair fertilizations in Carnaby's Cockatoo are difficult to discern. Extra-pair fertilizations within local breeding populations would result in broader genetic diversity within breeding populations than would result from purely monogamous pairings.

Conclusions

Comparisons of results obtained from long-term studies based observations of individually marked Carnaby's Cockatoo breeding adults, and those from analyses of DNA from Carnaby's Cockatoo nestlings showed that analyses of DNA are useful for establishing stability of breeding pairs and breeding site fidelity. Analyses of nestling DNA demonstrated that aspects of mating behaviour that would be extremely difficult to show using observations of individually marked adults could be revealed by studies of DNA. Analyses of DNA from nestlings over subsequent breeding seasons would be necessary to investigate the identity and ages of the adults involved in extra-pair copulations, and provide evidence of the advantages to the species of breeding pair infidelity.

Acknowledgements

References

Beard JS (1984) Biogeography of the Kwongan. In: Pate JS, Beard JS (Eds) Kwongan: plant life of the sand plain: biology of a south-west Australian shrubland ecosystem. University of Western Australia Press, Nedlands, Western Australia, 1–26.

Berkunsky I, Reboreda JC (2009) Nest-site fidelity and cavity reoccupation by Blue-fronted Parrots *Amazona aestival* in the dry Chaco of Argentina. The Ibis 151(1): 145–150. https://doi.org/10.1111/j.1474-919X.2008.00896.x

Blancher PJ, Robertson RJ (1985) Site consistency in Kingbird breeding performance: Implications for site fidelity. Journal of Animal Ecology 54(3): 1017–1027. https://doi.org/10.2307/4394

Burger J (1982) The role of reproductive success in colony-site selection and abandonment in black skimmers (*Rynchops niger*). The Auk 99(1): 109–115. https://doi.org/10.2307/4086026

Cale B (2003) Carnaby's Black-Cockatoo (*Calyptorhynchus latirostris*) Recovery Plan 2002–2012. Western Australian Department of Conservation and Land Management, Wanneroo, Western Australia, 1–29.

Caparroz R, Miyaki C, Baker AJ (2011) Genetic evaluation of the mating system in the blue-and-yellow macaw (*Ara ararauna*, Aves, Psittacidae) by DNA fingerprinting. Genetics and Molecular Biology 34(1): 161–164. https://doi.org/10.1590/S1415-47572010005000112

Crawford JC, Zhiwei L, Nelson TA, Nielsen CK, Craig K (2008) Microsatellite analysis of mating and kinship in beavers (*Castor canadensis*). Journal of Mammalogy 89(3): 575–581. https://doi.org/10.1644/07-MAMM-A-251R1.1

Department of Environment and Conservation (2012) Carnaby's Cockatoo (*Calyptorhynchus latirostris*) Recovery Plan. Western Australian Wildlife Management Program No. 52. Department of Environment and Conservation, Perth, Western Australia, 1–56.

Eastwood JR, Berg ML, Ribot RFH, Stokes HS, Martens JM, Buchanan KL, Walder K, Bennett ATD (2018) Pair fidelity in long-lived parrots: Genetic and behavioural evidence from the Crimson Rosella (*Platycercus elegans*). The Emu 1–6. https://doi.org/10.1080/01584197.2018.1453304

Ehrlich PR, Dobkin DS, Wheye D, Pimm SL (1994) The Birdwatcher's Handbook. A Guide to the Natural History of the Birds of Britain and Europe. Oxford University Press, United Kingdom.

Ekstrom JMM, Burke T, Randrianaina L, Birkhead TR (2007) Unusual sex roles in a highly promiscuous parrot: The Greater Vasa Parrot *Caracopsis vasa*. The Ibis 149(2): 313–320. https://doi.org/10.1111/j.1474-919X.2006.00632.x

Fernandes GA (2015) Compartamento reproductive do papagaio-de-cara-roxa (*Amazona brasiliensis*): uma abordagem molecular. 51 f., il. Dissertação (Mestrado em Biologia Animal)—Universidade de Brasília, Brasília.

Forstmeier W, Martin K, Bolund E, Schielzeth H, Kempenaers B (2011) Female extrapair mating behavior can evolve via indirect selection on males. Proceedings of the National Academy of Sciences 108(26): 10608–10613. http://doi.org/10.1073/pnas.1103195108

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28(4): 1140–1162. https://doi.org/10.1016/S0003-3472(80)80103-5

Heinsohn R, Ebert D, Legge S, Peakall R (2007) Genetic evidence for cooperative polyandry in reverse dichromatic *Eclectus* parrots. Animal Behaviour 74(4): 1047–1054. https://doi.org/10.1016/j.anbehav.2007.01.026

Hou X, Xu P, Lin Z, d'Urban-Jackson J, Dixon A, Bold B, Xu J, Zhan X, Hou A (2018) An integrated tool for microsatellite isolation and validation from the reference genome and

their application in the study of breeding turnover in an endangered avian population. Integrative Zoology. https://doi.org/10.1111/1749-4877.12305

IUCN (2014) The IUCN Red List of Threatened Species. Version 2014.2. http://www.iucn-redlist.org [accessed: 28 July 2014]

Li CC, Weeks DE, Chakravarti A (1993) Similarity of DNA fingerprints due to chance and relatedness. Human Heredity 43(1): 45–52. https://doi.org/10.1159/000154113

Lynch M (1988) Estimation of relatedness by DNA fingerprinting. Molecular Biology and Evolution 5: 584–599.

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. Genetics 152: 1753–1766.

Martinez JJ, de Arazamendi MC, Masello JF, Bucher EH (2013) Genetic evidence of extra-pair paternity and intraspecific brood parasitism in the monk parakeet. Frontiers in Zoology 10(1): 68. https://doi.org/10.1186/1742-9994-10-68

Masello JF, Sramkova A, Quillfeldt P, Epplen JT, Lubjuhn T (2002) Genetic monogamy in burrowing parrots *Cyanoliseus patagonus*. Journal of Avian Biology 33: 99–103. doi/abs/10.1034/j.1600-048X.2002.330116.x

McNicholl MK (1975) Larid site tenacity and group adherence in relation to habitat. The Auk 92(1): 98–104. https://doi.org/10.2307/4084420

Milligan BG (2003) Maximum-likelihood estimation of relatedness. Genetics 163: 1153–1167.

Murphy S, Legge S, Heinsohn R (2003) The breeding biology of palm cockatoos (*Probosciger aterrimus*): A case of a slow life history. Journal of Zoology 261(4): 327–339. https://doi.org/10.1017/S0952836903004175

Petrie M, Kempenaers B (1998) Extra-pair paternity in birds: Explaining variation between species and populations. Trends in Ecology & Evolution 13(2): 52–58. https://doi.org/10.1016/S0169-5347(97)01232-9

Queller DC, Goodnight KF (1989) Estimating relatedness using molecular markers. Evolution; International Journal of Organic Evolution 43(2): 258–275. https://doi.org/10.1111/j.1558-5646.1989.tb04226.x

Ritland K (1996) Estimators for pairwise relatedness and inbreeding coefficients. Genetical Research 67(02): 175–186. https://doi.org/10.1017/S0016672300033620

Rowley I (1990) Behavioural Ecology of the Galah *Eolophus roseicapillus* in the Wheatbelt of Western Australia. Surrey Beatty & Sons Pty Ltd, Chipping Norton, New South Wales.

Saunders DA (1976) Breeding of the White-tailed Black Cockatoo in captivity. Western Australian Naturalist (Perth) 13: 171–172.

Saunders DA (1979) The biology of the short-billed form of the White-tailed Black Cockatoo, *Calyptorhynchus funereus latirostris* Carnaby. PhD Thesis. University of Western Australia. https://www.researchgate.net/profile/Denis_Saunders/citations?sorting=recent&page=3

Saunders DA (1980) Food and movements of the short-billed form of the White-tailed Black Cockatoo. Australian Wildlife Research 7(2): 257–269. https://doi.org/10.1071/WR9800257

Saunders DA (1982) The breeding behaviour and biology of the short-billed form of the White-tailed Black Cockatoo *Calyptorhynchus funereus latirostris* Carnaby. The Ibis 124(4): 422–455. https://doi.org/10.1111/j.1474-919X.1982.tb03790.x

Saunders DA (1986) Breeding season, nesting success and nestling growth in the Carnaby's cockatoo *Calyptorhynchus funereus latirostris* over 16 years at Coomallo Creek and a method for assessing the viability of populations in other areas. Australian Wildlife Research 13(2): 261–273. https://doi.org/10.1071/WR9860261

Saunders DA (1990) Problems of survival in an extensively cultivated landscape: The case of Carnaby's cockatoo *Calyptorhynchus funereus latirostris.* Biological Conservation 54(3): 277–290. https://doi.org/10.1016/0006-3207(90)90057-V

Saunders DA, Dawson R (2017) Cumulative learnings and conservation implications of a long-term study of the endangered Carnaby's Cockatoo *Calyptorhynchus latirostris.* Australian Zoologist: 1–15. https://doi.org/10.7882/AZ.2017.010

Saunders DA, Dawson R, Mawson P (2011) Photographic identification of bands confirms age of breeding Carnaby's Black Cockatoo. Corella 35: 52–54.

Saunders DA, Dawson R, Nicholls AO (2015) Aging nestling Carnaby's cockatoo, *Calyptorhynchus latirostris,* and estimating the timing and length of the breeding season. Nature Conservation 12: 27–42. https://doi.org/10.3897/natureconservation.12.4863

Saunders DA, Ingram JA (1998) Twenty-eight years of monitoring a breeding population of Carnaby's Cockatoo. Pacific Conservation Biology 4: 261–270. https://doi.org/10.1071/PC980261

Saunders DA, Mawson PR, Dawson R (2014a) One fledgling or two in the endangered Carnaby's Cockatoo (Calyptorhynchus latirostris) – a strategy for survival or legacy from a bygone era? Conservation Physiology 2(1): cou001. https://doi.org/10.1093/conphys/cou001

Saunders DA, Mawson PR, Dawson R (2014b) Use of tree hollows by Carnaby's Cockatoo and the fate of large hollow-bearing trees at Coomallo Creek, Western Australia 1969–2013. Biological Conservation 177: 185–193. https://doi.org/10.1016/j.biocon.2014.07.002

Saunders DA, Wintle BA, Mawson PR, Dawson R (2013) Egg-laying and rainfall synchrony in an endangered bird species; implications for conservation in a changing climate. Biological Conservation 161: 1–9. https://doi.org/10.1016/j.biocon.2013.02.004

Taylor TD, Parkin DT (2009) Preliminary evidence suggests extra-pair mating in the endangered echo parakeet, *Psittacula eques.* African Zoology 44(1): 71–74. https://doi.org/10.1080/15627020.2009.11407440

Wang J (2002) An estimator for pairwise relatedness using molecular markers. Genetics 160: 1203–1215.

Wang J (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. Genetical Research 89(03): 135–153. https://doi.org/10.1017/S0016672307008798

Wang J (2011) COANCESTRY: A program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources 11(1): 141–145. https://doi.org/10.1111/j.1755-0998.2010.02885.x

White NE, Bunce M, Mawson PR, Dawson R, Saunders DA, Allentoft ME (2014) Identifying conservation units after large-scale land clearing: A spatio-temporal molecular survey of endangered white-tailed black cockatoos (*Calyptorhynchus* spp.). Diversity & Distributions 20(10): 1208–1220. https://doi.org/10.1111/ddi.12202

White NE, Dawson R, Coghlan M, Tridico SR, Mawson PR, Haile J, Bunce M (2012) Application of STR markers in wildlife forensic casework involving Australian black-cockatoos (*Calyptorhynchus* spp.). Forensic Science International. Genetics 6(5): 664–670. https://doi.org/10.1016/j.fsigen.2011.10.003

White NE, Mawson PR, Dawson R, Bunce MA, Spencer PBS (2009) Characterisation and cross-species utility of 20 microsatellite markers for population and forensic application in the endangered Carnaby's Black-cockatoo, *Calyptorhynchus latirostris*. Conservation Genetics Resources 1(1): 341–345. https://doi.org/10.1007/s12686-009-9079-7

RESEARCH ARTICLE

nature conservation

A peer-reviewed open-access journal

Launched to accelerate biodiversity conservation

Stephanopachys linearis (Kugelann, 1792) (Coleoptera, Bostrichidae) in Poland

Jerzy Borowski[1], Jerzy M. Gutowski[2], Marek Sławski[1], Krzysztof Sućko[2], Karol Zub[3]

1 *Department of Forest Protection and Ecology, Warsaw University of Life Sciences, ul. Nowoursynowska 159/34, 02-776 Warsaw, Poland* **2** *Department of Natural Forests, Forest Research Institute, 17-230 Białowieża, Poland* **3** *Mammal Research Institute, Polish Academy of Science, 17-230 Białowieża, Poland*

Corresponding author: *Jerzy Borowski* (jerzy_borowski@sggw.pl)

Citation: Borowski J, Gutowski JM, Sławski M, Sućko K, Zub K (2018) *Stephanopachys linearis* (Kugelann, 1792) (Coleoptera, Bostrichidae) in Poland. Nature Conservation 27: 75–84. https://doi.org/10.3897/natureconservation.27.24977

Abstract

Stephanopachys linearis (Kugelann, 1792) belongs to the family of horned powderpost beetles (Bostrichidae), represented in the fauna of Europe by 29 native species. It is a characteristic element of the northern, boreal zone of the Palaearctic and alpine areas of central and southern Europe. This species as a rare beetle important for the European Union, has been placed in Annex II of the Habitats Directive, on the IUCN Red List of Threatened Species and European Red List of Saproxylic Beetles. *S. linearis* was described from Poland in 1792 and, after 220 years, again encountered in this country. The zoogeographical distribution is shown and elements of its biology and ecology are discussed.

Keywords

Introduction

Stephanopachys linearis (Kugelann, 1792) is a representative of the forest fauna characteristic of boreal and alpine regions of the Palaearctic. It belongs to the family of horned powderpost beetles (Bostrichidae), of which 29 native species occur in Europe

(Borowski 2007), 9 of them – including *S. linearis* – having been reported from Poland (Dominik 1958, Burakowski et al. 1986).

S. linearis was discovered and described in 1792 by the German entomologist J.G. Kugelann, working as an apothecary in Ostróda in the (then) Olsztyn regency of East Prussia (now NE Poland). Besides short Latin and German descriptions of *Apate linearis*, Kugelann gave a diagnosis allowing its distinction it from the now common bark beetle, *Anisandrus dispar* (Fabricius, 1792) (Curculionidae, Scolytinae), with information that the new species had been found only once, on an old hoarding. Kugelann's publication has become the basis of the inclusion – after World War II – of *S. linearis* in the Polish fauna (Dominik 1958, Burakowski et al. 1986, Borowski 2007), but during the 220 years since its description, it has never been reported from this country again and, for example, the renowned German faunist Adolf Horion (1961) considered its occurrence in Poland or Baltic countries as doubtful.

The current area of the distribution of *S. linearis* extends over the boreal zone of the Palaearctic, from Norway and Denmark, through Sweden, Finland and Siberia to the Far East (it was recently discovered in northern Manchuria – Zhang et al. 1995), reaching – through Estonia, Latvia and Lithuania – as far south as NE Poland, Belarus and Ukraine; relict localities are also spread over the mountainous regions of central and southern Europe (France, Switzerland, Germany, Austria, Italy, Slovenia and Bohemia – Borowski 2007), as well as the Caucasus (Horion 1961, Geis 2002) and on Corsica (Sainte-Claire Deville 1902). Its recent discovery in northern Iran (Liu et al. 2016, Nardi and Audisio 2016) seems highly doubtful: the locality, albeit close to the border of Azerbaijan, is practically devoid of vegetation, with the climate unsuitable for the development of this species, so the specimen seems either misidentified or mislabelled; one of us (JB) contacted the Iranian author of the respective publication for possible verification of its taxonomic identity, but the specimen proved unavailable not only for loan but even for taking a photograph.

In the years 2009–2017, in the course of the study of saproxylic insects on a burned site in Białowieża Primaeval Forest, the existence of this species has been discovered. Information concerning this discovery and the data supplementing the current knowledge on the ecology of the development of *S. linearis* are provided below.

Materials and methods

Locality and collection data

In 2009, the research programme was launched in Białowieża Primaeval Forest (Faliński 1986) to study the dynamics of the changes in species composition and abundance of (especially saproxylic) beetles after the disturbance caused by the forest brushwood fire on 28.04.2009. The studied area of the burned site (ca. 7 ha), in the compartment 105B of the Białowieża National Park, included natural stands with high proportion of

old (120 years or more) trees in humid forest (60.5% of the disturbed area), boggy forest (24.5%) and humid mixed forest (13.2%). The site has been left without economic activity, with actions having been restricted to scientific research. The observations started a few days after the fire. Beetles were caught in 2009–2011 and 2015–2017 using 9 non-baited Moericke's and 18 Netocia-type traps (Piętka and Borowski 2015) as well as 3 black funnel traps each baited with α-pinen, ethanol, fuscumol, ipsenol and ketol (https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/forestry/forest-health/forest-health-docs/spruce-beetle-docs/spruce_beetle_funnel__traps.pdf). All traps were suspended 1-2 metres above ground.

In June 2017, we measured the diameter at breast height and extent of trunk scorching (from the ground level) of 140 living pine-trees in the burned area and evaluated (in a three-level scale: slight, medium, strong) the degree of insolation of each trunk. All trees had been carefully examined for the presence of *S. linearis* larval galleries.

Statistical methods

To assess the preferences of *Stephanopachys linearis* for particular types of trees, we used a Generalised Linear Model (GLM), with binomial distribution for response variable (0 = tree not occupied by beetle, 1 = tree occupied by beetle). In the model, we used three explanatory variables: diameter of a tree degree of burning of the trunk (continuous, log-transformed variables) and exposure to the sun (categorical, three-level variable). All analyses were done using R (version 3.2.2) software.

Results

The first specimen of *Stephanopachys linearis* (Fig. 1) was caught between 26.04.2015 and 11.05.2015 in the Netocia-type trap on living but scorched pine; it was identified in autumn 2016 and we decided to look for the species at the same place in 2017.

On 9.05.2017, we searched for the species on living insolated pines with visible scorching of the outer layer of bark (Fig. 2). The trees did not show any signs of weakness and, had well developed crowns with green needles. On the black, scorched surface of bark, we saw small (1.4–2.0 mm in diameter) rounded exit holes of *S. linearis* from the previous year (Fig. 3). Whittling the outer bark layer away exposed galleries in the inner layer or under the bark (Fig. 4). Tortuous, variously directed, frequently inter-crossing or overlapping galleries 1.0–2.2 mm in width, filled with fine brown sawdust, did not enter the xylem. Humidity in the feeding places was moderate with a tendency to low. In the galleries, one dead individual, remnants of five others and a living larva of *S. linearis* were found. Galleries occurred only in the scorched part of the trunk, at a height of 0.3 to 2 m, all around the tree.

Figure 1. *Stephanopachys linearis*; the specimen collected in Białowieża Primaeval Forest in 2015 – dorsal view (photo by M. Sławski, 2017). Scale bar = 1 mm.

Also in 2017, black, baited funnel traps were suspended near the place where *S. linearis* had been previously collected and, in one of them one more specimen (22.05.– 5.06.2017) and remnants of another one (20.06.–3.07.2017) were found.

Figure 2. Scots pines burned by fire – host trees of *Stephanopachys linearis* (photo by J.M. Gutowski, 2017).

Figure 3. Imago outlets of *Stephanopachys linearis* (photo by J. Borowski, 2017).

Figure 4. Larval galleries of *Stephanopachys linearis* in the Scots pine bark (photo by J. Borowski, 2017).

Tree preferences

Amongst the measured and analysed trees, 16, i.e. 11.5%, were infested. Logistic regression analysis showed that the beetles preferred trees significantly thinner (mean diameter at breast height: 28 cm) than the average (32 cm; GLM, $z = -2.303$, $p = 0.021$) and more strongly scorched (mean height of scorching 245 cm for infested trees vs. 195 cm for all trees, GLM, $z = 3.050$, $p = 0.002$), while insolation of trunks was irrelevant (GLM, $z = 0.272$, $p = 0.786$) (Fig. 5).

Thus, it seems that *Stephanopachys linearis* prefers thinner trees (it has not been found on the thickest ones), most frequently those of 15-34 cm diameter at breast height (14 trees) and only once it was found feeding on a pine of 38 cm diameter. On the basis of our observations, this species feeds only on living trees, on small subcortical surfaces, where fire had locally damaged the cambium and phloem; it heavily infests scorched pines (up to the height of 135–350 cm), avoiding those blackened only below 130 cm.

S. linearis was accompanied by the deadwatch beetle *Ernobius mollis mollis* (Linnaeus, 1758) (Ptinidae, Ernobiinae); their galleries were almost identical, but the anobiid prefers those places where the fire had killed the cambium, its larvae feed deeper, frequently damaging the cambium and outer layer of xylem. Its exit holes are somewhat larger and less regular.
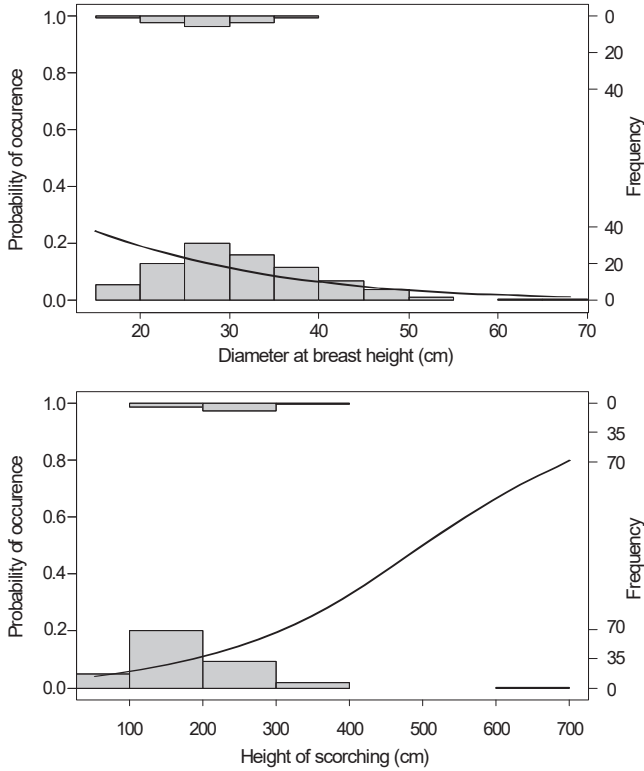
Figure 5. Result of logistic regression in the study of significance between the trees colonised by *Stephanopachys linearis* and the size of breast height and the height of the trunks' blackened by fire.

Discussion

Bionomy of *Stephanopachys linearis*

Stephanopachys linearis is bound biologically to conifers: in the northern zone mainly with Scotch pine (*Pinus sylvestris* L.), in the alpine areas on various species of pines, larches, much less frequently spruces and firs (Brustel et al. 2013, Ranius et al. 2014, Nardi and Audisio 2016). As a pyrophilous species, it primarily infests living trees damaged by fire, being only rarely found on those showing mechanical wounds like bark flayings or local necrosis. Before intensive forest management, the species probably proliferated on areas of natural fires and, when fire did not occur, on lightning-damaged trees. Like the majority of bostrichids, swarming in Poland occurs in spring, from late April to June. As a representative of the subfamily Dinoderinae, females probably gnaw into the bark to lay eggs. Larval galleries, filled with brown cortical sawdust mixed with faeces, terminate with ovate pupal chambers, from which imagines gnaw their round exit holes through bark. The beetles may develop on the infested tree during at least several years (Wikars 2006).

The galleries of *S. linearis* run at the contact surface between bark and phloem, but larvae feed only in bark, not in living phloem, selecting the layer of dead dry phloem previously killed by fire and then overgrown with new, living tissue. The species prefers the unusual environment produced by, for example, fire. As thick bark protects phloem from being killed by fire, the beetles infest mainly thin-barked trees where the subcortical tissues could have been more easily "boiled". Infestation occurs only after partial regeneration of phloem has occurred, usually 2–5 years after the fire (Wikars 2006).

Status of *Stephanopachys linearis* in Europe

Stephanopachys linearis is the 20th – first of the family Bostrichidae (Gutowski and Przewoźny 2013) – of the beetle species listed in Annexes I and II of the Habitats Directive (Council Directive 92/43/EEC), which have been found in Poland. As a rare species, important for the European Union, it has been placed in Annex II of the Habitats Directive (code: 1926) (Gutowski and Przewoźny 2013; Council Directive 97/62/EC), as well as in the *IUCN Red List* and *European red list of saproxylic beetles* (Nieto and Alexander 2010) – in both cases with the category LC – and *Red list of near-extinct and threatened animals in Poland* (Pawłowski et al. 2002) as probably extinct (EX?). *S. linearis* could probably be considered at the Polish regional scale as "Critically Endangered (CR)" following the IUCN criteria B1ab (i,ii,iv) (IUCN 2014).

Apart from *S. linearis*, considered as an obligatory pyrophile, many other beetle species are associated with burned areas, which – as important causes of natural ecological disturbances – make a significant element of the strategy for preservation of biodiversity (Wikars 1992). In the past, ground fires were frequent in the Białowieża Primaeval Forest (Niklasson et al. 2010, Zin et al. 2015), as in Scandinavia (e.g. on the Swedish island Gotska Sandön – Niklasson 2015). The occurrence of many rare pyrophilous beetles (Gutowski and Jaroszewicz 2001, Gutowski et al. 2012, Niklasson 2015) in these areas is probably a consequence of that "igneous" past.

Concluding remarks

Unfortunately, despite its being listed in the EU Habitats Directive, *S. linearis* remains poorly known with regards to its biology of development, and many data from various reports placed from time to time in the internet are contradictory, making them difficult to interpret reliably.

The confirmation of the occurrence of *S. linearis* in Poland will probably stimulate more focused attention to the trees – especially pines – damaged by fire. The authors are convinced that the species can also be found elsewhere in the northeastern parts of Poland, perhaps on larger surfaces with more numerous infested trees (in Białowieża the relevant area was relatively small: 7 ha, 16 pines with galleries of *S. linearis*). This would allow more detailed studies hopefully resulting in the clarification of its bionomy, at the level of other, well recognised species listed on the EU Habitats Directive.

Acknowledgements

References

Borowski J (2007) Bostrichidae. In: Löbl I, Smetana A (Eds) Catalogue of Palearctic Coleoptera. Volume 4. Elateroidea – Derodontoidea – Bostrichoidea – Lymexyloidea – Cleroidea – Cucujoidea. Apollo Books, Stenstrup, 320–328.

Brustel H, Gouix N, Bouyon H, Rogé J (2013) Les *Stephanopachys* de la faune ouest-paléarctique (Coleoptera Bostrichidae): Distribution et reconnaissance des trois espèces françaises au service de l'application de la directive Habitats, Faune, Flore. Entomologiste 69(1): 41–50.

Burakowski B, Mroczkowski M, Stefańska J (1986) Katalog fauny Polski. Część XXIII, t. 11, Chrząszcze, Coleoptera. Dermestoidea, Bostrichoidea, Cleroidea i Lymexyloidea. PWN, Warszawa, 1–243.

Council Directive (1992) 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31992L0043 [accessed 10 December 2017]

Council Directive (1997) 97/62/EC of 27 October 1997 adapting to technical and scientific progress Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora. https://publications.europa.eu/en/publication-detail/-/publication/4cd5bfb6-8e37-46f8-bcf3-78ba2052fe82 [accessed 10 December 2017]

Dominik J (1958) Klucze do oznaczania owadów Polski. Część XIX, Chrząszcze – Coleoptera, z. 39-40, Kapturnikowate – Bostrychidae. PWN, Warszawa, 20 pp.

Fabricius JCh (1792) Entomologia Systematica Emendata et Aucta. Secundum classes, ordines, genera, species, adjectis synonimis, locis observationibus, descriptionibus. Tom 1. Pars II. Hafniae, 538 pp. https://doi.org/10.5962/bhl.title.125869

Faliński JB (1986) Vegetation dynamics in temperate lowland primeval forests. Ecological studies in Białowieża forest. Dr W. Junk Publishers, Dordrecht, 537 pp. https://doi.org/10.1007/978-94-009-4806-8

Geis K-U (2002) Gebietsfremde Splintholz- und Bohrkäfer, nach Mitteleuropa mit Importholz und anderen Gütern eingeschleppt. Eine Bestandsaufnahme (Coleoptera: Lyctidae, Bostrichidae). Mitteilungen des Internationalen Entomologischen Vereins (Supplement 10): 1–106.

Gutowski JM, Jaroszewicz B (Eds) (2001) Katalog fauny Puszczy Białowieskiej. Instytut Badawczy Leśnictwa, Warszawa, 403 pp.

Gutowski JM, Kubisz D, Sućko K (2012) *Nacerdes carniolica* (Gistel, 1834) (Coleoptera: Oedemeridae) – nowy chrząszcz dla polskiej fauny. Wiadomosci Entomologiczne 31(4): 267–273.

Gutowski JM, Przewoźny M (2013) Program NATURA 2000 jako narzędzie ochrony chrząszczy (Coleoptera) w Polsce. Wiadomości Entomologiczne 32(Supl.): 5–40.

Horion A (1961) Faunistik der Mitteleuropäischen Käfer. Band VIII: Clavicornia 2. Teil (Thorictidae bis Cisidae), Teredilia, Coccinellidae. Überlingen – Bodensee, 375 pp.

IUCN (2014) Guidelines for using the IUCN Red List categories and criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. http://www.iucnredlist.org/documents/redlistguidelines.pdf [accessed January 8, 2015]

Kugelann JG (1792) Verzeichniss der in einigen Gegenden Preussens bis jetzt entdeckten Käfer-Arten, nebst kurzen Nachrichten von denselben. Neustes Magazin für Liebhaber der Entomologie 1(4): 477–512.

Linnaeus C (1758) Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Tomus 1. Holmiae, 823 pp.

Liu L-Y, Ghahari H, Beaver RA (2016) An annotated synopsis of the powder post beetles of Iran (Coleoptera: Bostrichoidea: Bostrichidae). Journal of Insect Biodiversity 4(14): 1–22. https://doi.org/10.12976/jib/2016.4.14

Nardi G, Audisio P (2016) Italian account for *Stephanopachys linearis* (Kugelann, 1792), a species listed in Annex II of the Habitats Directive (Coleoptera: Bostrichidae). Fragmenta Entomologica 48(2): 131–136. https://doi.org/10.4081/fe.2016.185

Nieto A, Alexander KNA (Eds) (2010) European Red List of Saproxylic Beetles. Publications Office of the European Union, Luxembourg, VIII+46 pp.

Niklasson M (2015) Skogshistoria och bränder på Gotska Sandön. Länsstyrelsen i Gotlands Lan. Visby, 58 pp.

Niklasson M, Zin E, Zielonka T, Feijen M, Korczyk AF, Churski M, Samojlik T, Jędrzejewska B, Gutowski JM, Brzeziecki B (2010) 350-year tree-ring fire record from Białowieża Primeval Forest, Poland: Implications for Central European lowland fire history. Journal of Ecology 98(6): 1319–1329. https://doi.org/10.1111/j.1365-2745.2010.01710.x

Pawłowski J, Kubisz D, Mazur M (2002) Coleoptera Chrząszcze. In: Głowaciński Z (Ed.) Czerwona lista zwierząt ginących i zagrożonych w Polsce. Polska Akademia Nauk, Instytut Ochrony Przyrody, Kraków, 88–110.

Piętka J, Borowski J (2015) Substrat trocinowy z grzybnią w pułapkach do odłowu chrząszczy mycetobiontycznych. Studia i materiały CEPL w Rogowie 17, 44(3): 165–172.

Ranius T, Bohman P, Hedgren O, Wikars LO, Caruso A (2014) Metapopulation dynamics of a beetle species confined to burned forest sites in a managed forest region. Ecography 37(8): 797–804. https://doi.org/10.1111/ecog.00475

Sainte-Claire Deville J (1902) Sur l'existence en Corse d'espèces actuellement confinees dans la zone arctique (Col.). Bulletin de la Société Entomologique de France 1902: 332.

Wikars L-O (1992) Skogsbränder och insekter. Entomologisk Tidskrift 113(4): 1–11.

Wikars L-O (2006) Åtgärdsprogram för bevarande av brandinsekter i boreal skog. Naturvårdsverket, Stockholm, 77 pp. http://www.naturvardsverket.se/Documents/publikationer/620-5610-7.pdf [accessed 20 February 2018]

Zhang Q, Chu D, Yang Y (1995) A new record genus of Bostrichidae (Coleoptera) from China. Entomotaxonomia 17(1): 75–76.

Zin E, Drobyshev I, Bernacki D, Niklasson M (2015) Dendrochronological reconstruction reveals a mixed-intensity fire regime in *Pinus sylvestris* – dominated stands of Białowieża Forest, Belarus and Poland. Journal of Vegetation Science 26(5): 934–945. https://doi.org/10.1111/jvs.12290

SHORT COMMUNICATION

Different forest cover and its impact on eco-hydrological traits, invertebrate fauna and biodiversity of spring habitats

Martin Reiss[1], Peter Chifflard[1]

1 *Department of Geography, Philipps-Universität Marburg, Deutschhausstr. 10, 35037 Marburg, Germany*

Corresponding author: *Martin Reiss* (reissm@geo.uni-marburg.de)

http://zoobank.org/D02CA195-3A71-4F11-9246-AB5990FB41F4

Citation: Reiss M, Chifflard P (2018) Different forest cover and its impact on eco-hydrological traits, invertebrate fauna and biodiversity of spring habitats. Nature Conservation 27: 85–99. https://doi.org/10.3897/natureconservation.27.26024

Abstract

Headwater springs in the German Low Mountain Ranges are local ecotone habitats and biogeographical islands embedded in and interlinked with their adjacent landscape. The structure of forests reflects the eco-hydrological conditions in substrate type occurrence, microhabitat richness and biodiversity in forest springs. This study considers effects from different forest land cover by comparing spring habitats in deciduous beech forests and coniferous spruce forests on eco-hydrological structures and biodiversity. Study areas include six different forest landscapes in the Low Mountain Ranges in Central Germany in Hesse and Thuringia. Hydro-morphological structure mapping and invertebrate sampling was executed within a multi-habitat sampling regime, which involves sampling plots being allocated according to the cover ratio of the occurring substrata. Aquatic and terrestrial spring zones are considered with respect to an ecotone approach. Some *in situ* measurements were implemented, such as pH values, to assess the acidity of the spring water. Results show obvious differences in acidity, substrate type cover ratios and biodiversity in deciduous and coniferous forest springs. Conifer forest springs were found tending to acidification while deciduous forest springs were slightly alkaline. Deciduous forest springs had higher cover ratios of organic microhabitats as well as a higher biodiversity in species richness and total number of individuals. Although it was not possible to clearly distinguish one direct key factor of fauna assemblages, negative effects from forest management practices (e.g. monoculture plantations of conifer forest) on spring habitats can be concluded.

Keywords

springhead, headwater, microhabitats, substrate, habitat assessment, crenobionts

Introduction

Mountainous headwater springs are mostly small water bodies where dominant groundwater occurs at the surface to form an intermittent or permanent discharge influenced by subsurface interflow and overland flow (Frisbee et al. 2013). Spring habitats are ecotones in two spatial dimensions. Firstly, springs are vertical aquatic below-above-ground-interfaces between groundwater and surface water. Secondly, springs are above-ground-horizontal terrestrial-aquatic interfaces between different adjacent areas within a continuum of aquatic, semi-aquatic or amphibian to hygrophilous terrestrial spring areas similar to the riparian zone ecotone (Gibert et al. 1990). Therefore, springs are normally nearly constantly cold and wet island habitats without clarifying benchmarks on the delimitation of hot springs (e.g. Pentecost et al. 2003). Glazier (2009) introduces the term "ambient springs" to characterise the water temperature regime with low minimum and maximum amplitudes and an annual mean related to the local yearly mean air temperature (Thienemann 1924). The relative constantly cold water temperature conditions or isothermy (Odum 1971, Teal 1957) lead to specific fauna adaptation of cold-stenothermic specialists and glacial relicts (Nielsen 1950, Ward and Stanford 1982, Fischer 1996). However, fauna composition is much more heterogeneous in springheads and is often explained as a consequence of a high degree of substratum heterogeneity, which can result in high species richness (Bonettini and Cantonati 1996, Lindegaard et al. 1998, Williams and Williams 1999, Staudacher and Füreder 2007, Koperski et al. 2011). The springhead can be built of different substrate types that build heterogeneous mosaic-like structures called patches, hence, springs which can also be termed patchy ecosystems on the micro scale (Pickett and White 1985). The first quantitative approach for analysing the microhabitat-fauna-relationship in springheads and for calculating ratios of invertebrate substrate preferences regarding the ecotone characteristics was completed by Reiss (2011) and considered mountainous headwater springs in Central Germany.

The objective of this study is to determine effects from different forest cover by comparing spring habitats in deciduous and coniferous forests on eco-hydrological structures and biodiversity. This research focuses on impacts from forest types as a determinant of the occurrence of corresponding microhabitat types, its substrate type composition and diversity, as well as its specific colonisation by invertebrates. Here, acidity is also considered, because conifer forests could be contributing to acidification of surface waters (Nisbet 1990, Cannell 1999).

Methods

Study area

Study sites are located in 6 different forested parts of the Low Mountain Ranges in Central Germany (Fig. 1, Tables 1, 2). They were originally chosen to guarantee a wide range of hydro-morphological structures within diverse substrate types as microhabitats for inver-

Figure 1. Study sites in Central Germany.

Table 1. Overview of the study sites within deciduous forest land cover.

Deciduous Forest	Hainich	Krofdorf	Keller-wald	Vogels-berg	Rhön	Burg-wald	Total
Geology	Limestone	Greywacke, Clay Shale		Volcanic rocks		Sandstone	
No. of objects	4	16	27	9	5	0	61
%	7%	26%	44%	15%	8%	–	100%

Table 2. Overview of the study sites within deciduous forest land cover.

Coniferous Forest	Hainich	Krofdorf	Keller-wald	Vogels-berg	Rhön	Burg-wald	Total
Geology	Limestone	Greywacke, Clay Shale		Volcanic rocks		Sandstone	
No. of objects	2	3	3	0	0	17	25
%	8%	12%	12%	–	–	68%	100%

tebrates. In total, 86 springheads were analysed, split amongst 61 springs in deciduous forest land cover dominated by *Fagus sylvatica* (Beech) and 25 springs in coniferous forest land cover dominated by *Picea abies* (Spruce).

Data analysis and field methods

Data analysis was based on a data set from a study on the microhabitat-fauna-relationship and the importance of invertebrate substrate preferences (Reiss 2011). Here,
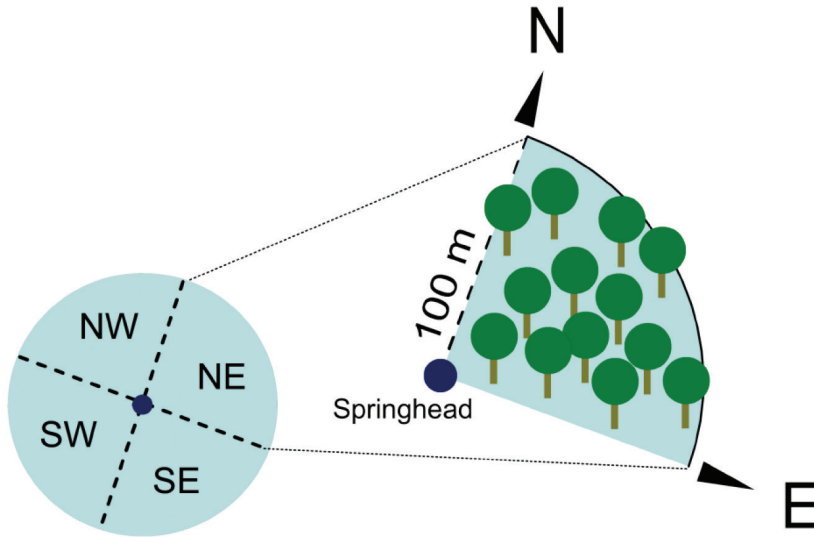
Figure 2. Four-segment approach of adjacent biotope types mapping for springs.

the evaluation of possible effects from different forest cover on abiotic parameters and biodiversity was not an objective of the previous research. However, data regarding adjacent biotope types were mapped. The determination of new taxa was also added.

Adjacent biotope field mapping was done by observing a length of 100 metres from the springhead and by considering four separated quarters, orientated by compass directions (Fig. 2) (Zollhöfer 1997).

In situ measurements of physical-chemical parameters, such as water temperature (°C), pH, electrical conductivity (μS^*cm^{-1}), oxygen concentration (mg/l) and oxygen saturation (%), were taken with hand held sensors.

Hydro-morphological structure mapping and invertebrate sampling was conducted using a novel integrated technique for multi-habitat sampling (Reiss 2011, Reiss and Chifflard 2015). The technique was based on a modified approach and substrate type terminology was used similar to the AQEM/STAR procedure (Hering et al. 2003). Here, the substantial difference is the subdivision of mineral and organic substrate types within the determination of the coverage ratios of microhabitats. Mineral and organic layers were considered individually in a 2-layer approach by taking the area of the whole springhead habitat as a reference surface (5-10 m^2). Distribution of sub-sample replicates in each layer should be done according to the estimated share of microhabitats (100 percent coverage means 20 samples in total per layer: 1 sample taken for 5 percent coverage). For example, if the sampling layer of mineral microhabitats consists of 50% psammopelal (mud), 25% microlithal (coarse gravel), 25% macrolithal (coarse blocks and head-sized cobbles) then 10 replicates of benthic macroinvertebrate samples should be taken in the psammopelal, 5 replicates in the microlithal, 5 replicates in the macrolithal. Fauna sampling corresponds with each 5 percent coverage fraction by sampling invertebrates for 2 minutes over a 10 cm x 10

cm reference area for each fraction. Invertebrates were preserved in ethanol alcohol (90%) for transport and subsequent identification in the laboratory.

Data preparation was carried out by filtering and sorting all data related to 100% of the deciduous and coniferous forest cover within the four-segment approach of the adjacent biotope type field mapping procedure. Multiple methods were used to characterise diversity indices: Margalef richness (d) after Margalef (1958), Shannon index (H') after Shannon and Weaver (1949) and Pielou's evenness (J') after Pielou (1966), using the statistical analysis tool PRIMER-E (Clarke and Gorley 2006). Box-Whisker-Plots were generated with BoxPlotR using the online version (shiny.chemgrid.org/boxplotr) (Spitzer et al. 2014).

Results

Hydro-physical-chemical parameters

The results from hydro-physical-chemical *in situ* measurements in springs (Table 3) indicated that there is not much difference between deciduous forest and coniferous forest land cover for water temperature (Wtemp) and electrical conductivity (EC). In contrast, differences are obvious for pH, oxygen concentration (O_2 Conc.) and oxygen saturation (O_2 Satur.).

The acidification of spring water seems to be one major effect in conifer forest springheads, showing that the pH measurements giving a much lower median value in coniferous forest with pH 5.4, than in deciduous forest with pH 7.7 (Fig. 3).

Microhabitats

Microhabitats, which cover ratios for deciduous and coniferous forest springs (Fig. 4), highlight differences within the two dissimilar forest types. Generally, 13 substrate types are equally present in deciduous forest and coniferous forest springs. However, cover ratios of substrate types in coniferous forest springs are obviously rarer, so that most of the microhabitats, especially organic substrate types are under-represented. Otherwise, certain microhabitats, such as coniferous litter and moss cushions, show slightly higher cover ratios in coniferous forest than in deciduous forest springs. Emergent macrophytes in coniferous forest springs are considerably under-represented in comparison to springheads in deciduous forests.

Fauna and Biodiversity

Overall, 52 taxa and 2617 individuals in springs of deciduous forest land cover and 33 taxa and 326 individuals in springs of coniferous forest land cover were found. Tables 4 and 5 show taxa lists of found invertebrates including individuals per taxon,

Table 3. Hydro-physical-chemical measurements of deciduous forest and coniferous forest land cover.

Parameter	Wtemp		pH		EC		O₂ Conc.		O₂ Satur.	
	°C				µS*cm⁻¹		mg/l		%	
Max	15.9	13.4	8.5	8.3	960	790	13.0	14.8	134%	142%
75-Q	12.4	11.5	7.9	7.4	320	230	9.7	10.7	107%	102%
Median	10.2	10.3	7.7	5.4	200	180	8.9	7.0	92%	75%
25-Q	8.1	8.1	7.5	4.9	130	140	7.1	4.3	79%	43%
Min	3.1	3.0	6.0	4.0	70	110	3.6	2.4	38%	25%

Figure 3. pH of springs of coniferous (left boxplot) and deciduous (right boxplot) forest land cover.
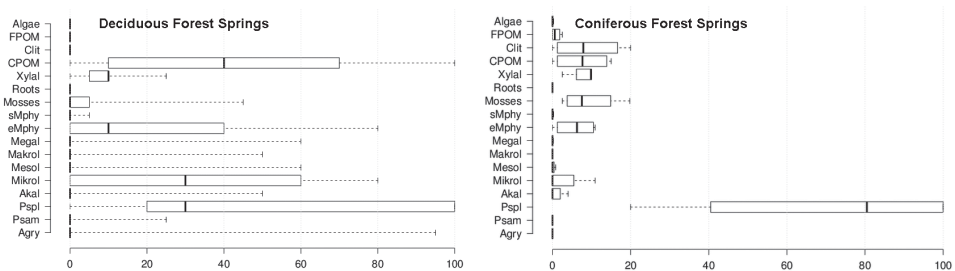
Figure 4. Cover ratio of microhabitats in deciduous (left) and coniferous (right) forest headwater springs. Substrate terminology: Argyllal – Agry; Psammal – Psam; Psammopelal – Pspl; Akal – Akal; Microlithal – Mikrol; Mesolithal – Mesol; Macrolithal – Makrol; Megalithal – Megal; Emergent macrophytes – eMphy; Submerged macrophytes – sMphy; Moss cushions – Mosses; Fine roots – Roots; Xylal (Dead Wood) – Xylal; Coarse particular organic material – CPOM; Coniferous litter – Clit; Fine particular organic material – FPOM; Algae – Algae.

Table 4. Taxa list of springs within deciduous forest land cover. x – good substrate type preference identification; xx – very good substrate type preference identification; m – mosses (spring dwelling taxa are in bold).

Invertebrates – taxa and abundances			Invertebrates – substrate preference					
Taxa	Individuals (Ind.)	Springs	Ind./springs	Pspl	Microl	CPOM	Xylal	eMphy	Others
Bythinella dunkeri	577	26	22.2			x			
Bythinella compressa	198	11	18.0	x					
Gammarus fossarum	402	26	15.5			x			
Gammarus pulex	110	10	11.0	x		x			
Crenobia alpina	112	15	7.5			x			
Niphargus schellenbergi	50	7	7.1			x			
Sericostoma sp.	188	34	5.5		x				
Crunoecia irrorata	177	38	4.7			x			
Pisidium cf. *personatum*	165	36	4.6	xx					
Oniscus asellus	17	4	4.3				xx		
Nemoura sp.	86	29	3.0			x			
Carychium tridentatum	20	7	2.9	x			x		
Niphargus aquilex	16	6	2.7			xx			
Leuctra sp.	71	28	2.5	x		x			
Trichoniscus sp.	58	23	2.5			x	x		
Niphargus sp.	19	8	2.4						
Oligolophus tridens	7	3	2.3					xx	
Monachoides incarnatus	30	13	2.3					xx	
Hydroporinae sp.	17	8	2.1						
Dixa sp.	57	27	2.1					x	
Clinocera sp.	2	1	2.0						
Euconulus fulvus	8	4	2.0			x			
Helophorus sp.	2	1	2.0			x	x		
Anacaena sp.	72	37	1.9			x			m
Galba truncatula	27	15	1.8			x			
Ligidium hypnorum	7	4	1.8						m
Protonemura sp.	7	4	1.8				x		
Eiseniella tetraedra	30	18	1.7			x	x		
Discus rotundatus	13	8	1.6				x		
Velia sp.	8	5	1.6		x	x			
Carychium sp.	17	11	1.5						
Pedicia rivosa	4	3	1.3					xx	
Bezzia sp.	11	10	1.1	xx					
Agabus sp.	4	4	1.0			x			
Amphinemura sp.	1	1	1.0						
Arianta arbustorum	1	1	1.0						
Cicadella viridis	3	3	1.0					xx	
Cochlicopa sp.	1	1	1.0						
Cordulegaster bidentatus	2	2	1.0	x	xx	x			

Glomeris marginata	1	1	1.0						
Habroleptoides confusa	3	3	1.0			xx			
Ixodes sp.	1	1	1.0						
Leiobunum blackwalli	1	1	1.0					xx	
Lithobius sp.	4	4	1.0					x	
Mitopus morio	1	1	1.0						
Nemastoma lugubre	1	1	1.0						
Neobisium sp.	2	2	1.0				x		m
Platybuninae sp.	1	1	1.0						
Polydesmus sp.	1	1	1.0			x	xx		
Radix sp.	1	1	1.0						
Trichia sp.	2	2	1.0						
Trombidium holosericeum	1	1	1.0						
Averages	Ø 50	Ø 10	Ø 3						
Identification of substrate type preferences				7	3	20	9	7	3
Identification of substrate type preferences / total no. of taxa				0.14	0.06	0.38	0.17	0.14	0.06
Identification of substrate type preferences in total				49					

occurrence of a taxon (no. of springs), the ratio of individuals and springs (occurrence of a taxon) and the classification of the substrate preference. Typical spring dwelling taxa are in bold letters. In deciduous forest, the ratio of the total number of individuals in a taxon and their level of occurrence (no. of springs) is 43 individuals per spring and, in coniferous forest, 13 individuals per spring. The average number of springs, colonised by individuals of a taxon, is much higher for deciduous forest springs (Ø 10 no. of springs) than it is for coniferous springs (Ø 3 no. of springs). The identification of substrate preferences is also higher for springs in deciduous forest (49 identified with 12 very good substrate type preference identification), than in springs in coniferous forest (40 identified with 7 very good substrate type preference identification). Taxa, with an organic substrate type preference, are much more dominant in both forest types than taxa with a mineral substrate type preference. A clear decline of cold stenothermal species, such as the two groundwater amphipods *Niphargus schellenbergi*, *Niphargus aquilex* and the spring-dwelling flatworm *Crenobia alpina*, is particularly apparent in coniferous forest springs regarding the individuals per spring ratios. The number of spring dwelling taxa – as a group of well-known crenobionts and crenophilous taxa – is almost equal in both forest types. In deciduous forest springs, 11 spring dwelling taxa and, in conifer forest springs, 10 spring dwelling taxa occur. Certainly, the median of the individuals per spring ratio is different; while in deciduous forest springs, it is 4.7 individuals per spring and in conifer forest springs, it is 2.8 individuals per spring. Overall, the amount of spring dwelling taxa is higher in proportion to the total number of taxa in conifer forest springs than in deciduous forest springs. Characterising biodiversity, using biodiversity indices like Margalef richness (d) and Shannon index (H') with deciduous and coniferous forest cover, shows only marginal differences (Table 6). Overall, biodiversity is slightly

Table 5. Taxa list of springs within coniferous forest land cover. x: good substrate type preference identification; xx: very good substrate type preference identification; m – mosses; Ml – Megalith (spring dwelling taxa are in bold).

Invertebrates – taxa and abundances				Invertebrates – substrate preference					
Taxa	Individuals (Ind.)	Springs	Ind./springs	Pspl	Microl	CPOM	Xylal	eMphy	Others
Bythinella dunkeri	77	2	38.5			x			
Leuctra sp.	14	1	14.0	x		x			
Gammarus fossarum	34	3	11.3			x			
Gammarus pulex	5	1	5.0	x		x			
Pisidium cf. *personatum*	24	5	4.8	xx					
Nemoura sp.	32	7	4.6			x			
Crunoecia irrorata	16	4	4.0			x			
Cordulegaster bidentatus	3	1	3.0	x	xx	x			
Sericostoma sp.	11	4	2.8		x				
Anacaena sp.	44	18	2.4			x			
Trichoniscus sp.	18	8	2.3			x	x		
Dixa sp.	6	3	2.0					x	
Euconulus sp.	4	2	2.0			x			
Habroleptoides confusa	4	2	2.0			xx			
Niphargus schellenbergi	2	1	2.0			x			
Polycelis felina	2	1	2.0			x			
Leiobuninae sp.	3	2	1.5						
Agabus sp.	3	3	1.0			x			
Bezzia sp.	3	3	1.0	xx					
Carychium sp.	2	2	1.0	x		x			
Clinocera sp.	1	1	1.0						
Crenobia alpina	1	1	1.0			x			
Eiseniella tetraedra	3	3	1.0			x	x		
Galba truncatula	1	1	1.0			x			
Helophorus sp.	1	1	1.0			x	x		m
Lacinius ephippiatus	1	1	1.0						
Ligidium hypnorum	2	2	1.0						m
Metellina merianae	1	1	1.0						
Neobisium sp.	2	2	1.0				x		m
Niphargus aquilex	1	1	1.0			xx			
Paranemastoma quadripunctatum	3	3	1.0				xx		Ml
Polydesmus sp.	1	1	1.0			x	xx		
Rilaena triangularis	1	1	1.0						
Averages	Ø 10	Ø 3	Ø 4						
Identification of substrate type preferences				6	2	21	6	1	4
Identification of substrate type preferences / total no. of taxa				0.18	0.06	0.64	0.24	0.03	0.12
Identification of substrate type preferences in total				40					

Table 6. Diversity indices in deciduous and coniferous forest headwater springs.

Forest land cover	Margalef richness (d)	Shannon index (H')	Pilou's evenness (J')
Deciduous Forest	6.48	2.78	0.70
Coniferous Forest	5.53	2.65	0.76

higher in deciduous forest springs. The distribution of individuals within occurring invertebrate taxa is more homogenous in coniferous forest springs regarding Pielou's evenness (J').

Discussion

Results show three main forest land cover impacts on eco-hydrological structures and biodiversity in mountainous headwater springs concerning deciduous and coniferous forest: 1) Acidification in conifer forest springs; 2) Higher cover ratios of organic substrate type based microhabitats in deciduous forest springs; 3) Higher biodiversity in species richness and total number of individuals as well as abundances in deciduous forest springs.

Acidification of springs in coniferous forests of the German Low Mountain Ranges is a well-known problem, especially in siliceous springs with poor acid buffer capacities and can causes a decrease in the total number of species and individuals (Klös 1984, Lükewille et al. 1984, Meijering 1984, Mauden 1994, Orendt and Reinhart 1997, Audorff and Beierkuhnlein 1999, Hahn 2000). Therefore, it can be expected that the lower values of the total number of species and individuals in conifer forest springs is mainly a consequence of acidification. This interpretation is congruent with findings from a multivariate statistical analysis to characterise the main components of environmental factors influencing the absent and present data on spring fauna using a principal component analysis in the entire data set (Reiss 2011). It is important to note that only in-situ measurements (on-site pH values) were taken into account and no further investigations could be executed to analyse the dynamics or additional reasons for low pH values in order to deduce more accurate and detailed interpretations about acidification.

Higher cover ratios of organic substrate type based microhabitats in deciduous forest springs compared with springs in coniferous forest can be seen as a higher hydromorphological representation of specific microhabitats (e.g. emergent macrophytes or CPOM). However, conifer forest springs have the same quantitative share of occurring substrate types. CPOM (Coarse particular organic material), mainly composed by leaf litter from deciduous trees, is more important as allochthonous organic material as the most important food source for spring fauna communities with a high proportion of primary consumers in particular like herbivorous shredders (e.g. *Gammarus fossarum*, *Gammarus pulex*) or grazer and scrapers (e.g. *Bythinella dunkeri*, *Bythinella compressa*) (Teal 1957, Mninshall 1967, Whiles and Wallace 1997, Kemp and Boynton 2004). There is no microhabitat preference in coniferous litter, because it is commonly known

that the chemical composition of needles tend to retard biological activity in decomposition (Olson 1963). Here, detritus processing takes a much longer time (Sedell et al. 1974, Whiles and Wallace 1997) and can be characterised as a low quality detritivore food resource (Taylor et al. 1989, Klemmedson 1992, Friberg and Jacobsen 1994). Furthermore, comparing deciduous and coniferous spring microhabitats, one main conclusion is that substrate types are more important than substrate heterogeneity in contrast to a more general finding by Kubíková et al. (2012). However, in this study, it is not possible to distinguish between acidification and substrate types for describing a prevailing key factor causing taxa and individuals' richness in forest spring habitats.

Higher biodiversity in species richness, total number of individuals as well as abundances in deciduous forest springs in contrast to conifer forest springs, are the most obvious impacts caused by different forest stands. The result of a higher amount of spring dwelling taxa in conifer forest springs than in deciduous forest springs, in proportion to the total number of all found taxa, is consistent with findings by Hahn (2000). Hahn (2000) concluded that spring dwelling species are more tolerant to low pH-values than rhithrobiontic ones due to their adaptation to special crenal living conditions. However, conifer forest springs in our investigation, with their tendency to acidification, are characterised by considerably less individuals per spring ratios and a total number of individuals considering only spring dwelling taxa. Higher cover ratios of organic substrate type based microhabitats in deciduous forest springs compared with springs in coniferous forest seem to be less crucial for biodiversity. Nevertheless, although organic substrate type based microhabitats are of importance, they are more decisive on taxa composition. Some taxa which are under-represented in deciduous forest springs become more quantitatively relevant in conifer forest springs. The abundance of the shredding plecopteran *Leuctra* spp. is much higher in conifer forest springs, which is in line with the findings in a study about leaf litter decomposition in macroinvertebrate communities in pine and hardwood headwater catchments of the southern Appalachian Mountains in North Carolina, USA (Whiles and Wallace 1997). *Leuctra* spp. is known as an acid-tolerant aquatic invertebrate (Dangles and Guérold 2000), which is an indicator for acidification as an important factor on taxa composition. Otherwise, acid-sensitive amphipod *Gammarus fossarum* is similarly abundant, which means no acidification shift in taxa composition is noticeable. It can be inferred that both factors – acidification and microhabitat – govern fauna assemblages or biodiversity and no strong key variable can be characterised. However, this study indicates impacts from different forest cover when comparing spring habitats in deciduous and coniferous forest on eco-hydrological structures and biodiversity.

Conclusion

Different forest land cover causes considerable contrasts in microhabitat structures; obvious organic substrate type composition and cover ratios; as well as differences in species richness and invertebrate abundance of spring habitats in deciduous and coniferous

forest. This means, land cover as an ecological mesoscale, properly determined by different forest types, has an impact on eco-hydrological structures and biodiversity on the micro scale. It implies an essential consideration for adjacent biotope type mapping and is an important integrative parameter for spring habitat assessment approaches. Furthermore, the recognition of substrate preferences of invertebrates, within an ecotone based assessment approach, characterises microhabitats explicitly for all parts of a springhead, regarding aquatic and terrestrial spring habitat zones. Here, the importance of forest land cover and the substrate type diversity relationship is taken into account within an ecological spring habitat assessment methodology and characterises its consequences on invertebrate biodiversity. Therefore, negative effects from forest management practices (e.g. forest conversion) within a nature conservation perspective can be included in decision-making and action plans to realise national or regional strategies on biodiversity.

Acknowledgements

References

Audorff V, Beierkuhnlein C (1999) Versauerung und Stoffausträge aus Quelleinzugsgebieten. In: Beierkuhnlein C, Gollan T (Eds) Ökologie silikatischer Waldquellen in Mitteleuropa. Bayreuther Institut für Terrestrische Ökosystemforschung. Bayreuth, 103–117.

Bonettini AM, Cantonati M (1996) Macroinvertebrate assemblages of springs of the River Sarca catchment (Adamello-Brenta Regional Park, Trentino, Italy). Crunoecia 5: 71–78.

Cannell MG (1999) Environmental impacts of forest monocultures: Water use, acidification, wildlife conservation, and carbon storage. New Forests 17(1/3): 239–262. https://doi.org/10.1023/A:1006551018221

Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial. Plymouth Marine Laboratory, Plymouth, 190 pp.

Dangles O, Guérold F (2000) Structural and functional responses of benthic macroinvertebrates to acid precipitation in two forested headwater streams (Vosges Mountains, northeastern France). Hydrobiologia 418(1): 25–31. https://doi.org/10.1023/A:1003805902634

Fischer J (1996) Kaltstenothermie – einziger Schlüssel zum Verständnis der Krenobionten. Crunoecia 5: 91–96.

Friberg N, Jacobsen D (1994) Feeding plasticity of two detritivore-shredders. Freshwater Biology 32(1): 133–142. https://doi.org/10.1111/j.1365-2427.1994.tb00873.x

Frisbee MD, Phillips FM, White AF, Campbell AR, Liu F (2013) Effect of source integration on the geochemical fluxes from springs. Applied Geochemistry 28: 32–54. https://doi.org/10.1016/j.apgeochem.2012.08.028

Gibert J, Dole-Oliver M-J, Marmonier P, Vervier P (1990) Surface Water-Groundwater Ecotones. In: Naiman RJ, Déchamps H (Eds) The ecology and management of aquatic-terrestrical ecotones. UNESCO, Paris, 199–225. https://doi.org/10.1017/CBO9780511753381

Glazier DS (2009) Springs. In: Likens GE (Ed.) Encyclopedia of inland waters, Vol 1. Acadenic Press (San Diego): 734–755. https://doi.org/10.1016/B978-012370626-3.00259-3

Hahn HJ (2000) Studies on Classifying of Undisturbed Springs in Southwestern Germany by Macrobenthic Communities. Limnologica 30(3): 247–259. https://doi.org/10.1016/S0075-9511(00)80055-9

Hering D, Buffagni A, Moog O, Sandin L, Sommerhäuser M, Stubauer I, Feld C, Johnson R, Pinto P, Skoulikidis N, Verdonschot P, Zahrádková S (2003) The development of a system to assess the ecological quality of streams based on macroinvertebrates – design of the sampling program within the AQEM project. International Review of Hydrobiology 88(34): 345–361. https://doi.org/10.1002/iroh.200390030

Kemp WM, Boynton WR (2004) Productivity, trophic structure, and energy flow in the steady-state ecosystems of Silver Springs, Florida. Ecological Modelling 178(1/2): 43–49. https://doi.org/10.1016/j.ecolmodel.2003.12.020

Klemmedson JO (1992) Decomposition and nutrient release from mixtures of Gambel Oak and Pondersoa Pine leaf litter. Forest Ecology and Management 47(1–4): 349–361. https://doi.org/10.1016/0378-1127(92)90284-G

Klös W (1984) Der Einfluss von Umweltfaktoren auf die Gewässergüte in den Quellbereichen hessischer Gewässer. In: Wieting J, Lenhart B, Steinberg C (Eds) Gewässerversauerung in der Bundesrepublik Deutschland: Ergebnisse und Wertung eines Statusseminars des Umweltbundesamtes in Zusammenarbeit mit dem. Bayerischen Landesamt für Wasserwirtschaft vom 23–24 Februar 1984 in München. Erich Schmidt Verlag, Berlin, 93–95.

Koperski P, Dumnicka E, Galas J (2011) Abiotic parameters determining fauna composition in karstic springs. Polish Journal of Ecology 59(1): 153–163.

Kubíková L, Simon OP, Tichá K, Maciak M, Douda K, Bílý M (2012) The influence of mesoscale habitat conditions on the macroinvertebrate composition of springs in a geologically homogeneous area. Freshwater Science 31(2): 668–679. https://doi.org/10.1899/11-174.1

Lindegaard C, Brodersen KP, Wiberg-Larsen P, Skriver J (1998) Multivariate analyses of macrofaunal communities in Danish springs and springbrooks. In: Botosaneanu L (Ed.) Studies in Crenobiology. The biology of springs and springbrooks. Backhuys Publishers, Leiden, 201–219.

Lükewille A, Späh H, Thesing U (1984) Aluminiumhydroxidausfällungen in Quellbächen der Senne (Teutoburger Wald) als Folge saurer Niederschläge. In: Wieting J, Lenhart B, Steinberg B (Eds) Gewässerversauerung in der Bundesrepublik Deutschland: Ergebnisse und Wertung eines Statusseminars des Umweltbundesamtes in Zusammenarbeit mit dem. Bayerischen Landesamt für Wasserwirtschaft vom 23–24 Februar 1984 in München. – Erich Schmidt Verlag, Berlin, 106–120.

Margalef R (1958) Information theory in ecology. International Journal of General Systems 3: 36–71.

Mauden R (1994) Der Einfluss der Gewässerversauerung auf die Quellbachzoozönose. LÖBF-Mitteilungen 1/1994: 24–28.

Meijering MPD (1984) Die Verbreitung von Indikatorarten der Gattung Gammarus im Schlitzerland (Osthessen) in 1968 und 1982. In: Wieting J, Lenhart B, Steinberg C (Eds) Gewässerversauerung in der Bundesrepublik Deutschland: Ergebnisse und Wertung eines Statusseminars des Umweltbundesamtes in Zusammenarbeit mit dem. Bayerischen Landesamt für Wasserwirtschaft vom 23–24 Februar 1984 in München. Erich Schmidt Verlag, Berlin, 96–105.

Mninshall GW (1967) Role of allochthonous detritus in the trophic structure of a woodland springbrook community. Ecology 48(1): 139–149. https://doi.org/10.2307/1933425

Nielsen A (1950) On the zoogeography of springs. Hydrobiologia 2(4): 313–321. https://doi.org/10.1007/BF00053524

Nisbet T (1990) Forestry and surface water acidification. Forestry Commission (Farnham): 1–16.

Odum EP (1971) Fundamentals of Ecology. W. B. Saunders Company, Philadelphia, 1–546.

Olson JS (1963) Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44(2): 322–331. https://doi.org/10.2307/1932179

Orendt C, Reinhart U (1997) The distribution of *Gammarus pulex* (L.), *Asellus aquaticus* L. and *Pisidium* sp. in an acidified forest brook and some tributary springs in assessing the local state of acidity a small scale level. Limnologica 27: 271–280.

Pentecost A, Jones B, Renaut RW (2003) What is a hot spring? Canadian Journal of Earth Sciences 40(11): 1443–1446. https://doi.org/10.1139/e03-083

Pickett STA, White PS (1985) The Ecology of Natural Disturbance and Patch Dynamics. Academic Press, San Diego, 472 pp.

Pielou EC (1966) The measurement of diversity in different types of biological collections. Journal of Theoretical Biology 13: 131–144. https://doi.org/10.1016/0022-5193(66)90013-0

Reiss M (2011) Substratpräferenz und Mikrohabitat-Fauna-Beziehung im Eukrenal von Quellgewässern. PhD-thesis Philipps-Universität Marburg. University of Marburg (Marburg), 1–245. https://doi.org/10.17192/z2011.0108

Reiss M, Chifflard P (2015) Hydromorphology and Biodiversity in headwaters: An Eco-faunistic substrate preference assessment in forest springs of the German subdued mountains. In: Lo Y-H, Blanco JA, Roy S (Eds) Biodiversity in Ecosystems-Linking Structure and Function. InTech, Rijeka, 223-258. https://doi.org/10.5772/59072

Sedell JR, Triska FJ, Hall D, Anderson NH, Lyford JH (1974) Sources and fates of organic inputs in coniferous forest streams. In: Waring RH, Edmonds RI (Eds) Intergraded Research in the Coniferous Forest Biome. Proceedings AIBS Symposium on Coniferous Forest Ecosystems 5. Coniferous Forest Biome, Ecosystem Analysis Studies, U.S./International Biological Program, Seattle, 57–69.

Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois, Urbana, 1–117. https://doi.org/10.1063/1.3067010

Spitzer M, Wildenhain J, Rappsilber J, Tyers M (2014) BoxPlotR: A web tool for generation of box plots. Nature Methods 11(2): 121–122. https://doi.org/10.1038/nmeth.2811

Staudacher K, Füreder L (2007) Habitat Complexity and Invertebrates in Selected Alpine Springs (Schütt, Carinthia, Austria). International Review of Hydrobiology 92(4-5): 465–479. https://doi.org/10.1002/iroh.200610988

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: A microcosm test. Ecology 70(1): 97–104. https://doi.org/10.2307/1938416

Teal JM (1957) Community metabolism in a temperate cold spring. Ecological Monographs 27(3): 283–302. https://doi.org/10.2307/1942187

Thienemann A (1924) Die Gewässer Mitteleuropas. Eine hydrobiologische Charakteristik ihrer Haupttypen. Schweizerbart, Stuttgart, 144 pp.

Ward JV, Stanford JA (1982) Thermal responses in the evolutionary ecology of aquatic insects. Annual Review of Entomology 27(1): 97–117. https://doi.org/10.1146/annurev.en.27.010182.000525

Whiles MR, Wallace JB (1997) Leaf litter decomposition and macroinvertebrate communities in headwater streams draining pine and hardwood catchments. Hydrobiologia 353(1/3): 107–119. https://doi.org/10.1023/A:1003054827248

Williams DD, Williams NE (1999) Canadian Springs: postglacial development of the invertebrate fauna. In: Batzer DP, Rader RB, Wissinger SA (Eds) Invertebrates in Freshwater Wetlands of North America. Ecology and Management. Wiley, New York, 447–467.

Zollhöfer JM (1997) Quellen – die unbekannten Biotope im Schweizer Jura und Mittelland – erfassen – bewerten – schützen. Bristol-Schriftenreihe 6. Bristol-Stiftung, Zürich, 1–153.