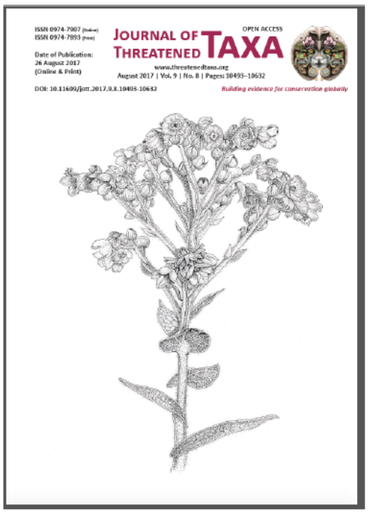

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Short Communication

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Adventitious rooting of mature *Cycas micronesica* K.D. Hill (Cycadales: Cycadaceae) tree stems reveals moderate success for salvage of an endangered cycad

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Abstract: Mature *Cycas micronesica* trees were among the forest trees that were destined to be removed from a 2012 construction site in northern Guam. Forty-nine of these trees were cut at the base and transported to nursery conditions to determine if adventitious root development could be stimulated as a means of saving the trees. Fungicide and auxin were applied to the base of each stem, and perlite was used as the container medium. A nursery maintained by an experienced cycad horticulturist yielded 41% survival, but half of the rooted plants exhibited severe stem dieback. A nursery maintained by general plant nursery specialists without cycad experience resulted in 100% mortality. If numerous caveats are acknowledged, whole-tree stem cuttings may yield moderate success for saving *C. micronesica* trees from construction sites when adequate resources are appropriated to support the nursery phase with experienced cycad biologists.

Keywords: Adaptive management, *Aulacaspis yasumatsui*, conservation genetics, cycad, *Rhyzobius lophanthae*.

Cycas micronesica was the most abundant arborescent species in Guam's forests in 2002 (Donnegan et al. 2004). At that time, no invasive herbivore species had been identified (Image 1A). The species range spans three island groups and four political entities, and is locally known as 'fadang' (United States Territory of Guam and United States Commonwealth of the Northern Mariana Islands), 'faltir' (Federated States of Micronesia), or 'remiang' (Republic of Palau). This species is one of almost 350 described extant cycad species (Calonje et al. 2017). The cycad-specific armored scale *Aulacaspis yasumatsui* Takagi invaded Guam in 2003 (Marler 2012), and infestations of the insect have combined with other threats to cause widespread death of the plant population (Marler & Lawrence 2012). *Cycas micronesica* was Red-Listed as Endangered in 2006 by the International Union for Conservation of Nature (IUCN) due to the acute, rapid decline in population (Marler et al. 2010).

The preparation of a northern Guam construction site in 2012 included the removal of numerous large *C. micronesica* trees as a component of ongoing environmental resource mitigation measures. The protocols available to remove and transplant the trees were constrained by two issues. First, due to the dangers from the unexploded ordnance that has persisted on Guam since World War II, any activity that breaks the surface of the soil on lands that are under federal jurisdiction requires costly clearance by ordnance specialists. Second, the severely limited budget was insufficient to cover the costs of the ordnance clearance or the labor and equipment required to excavate the existing root systems for traditional tree transplanting protocols. Therefore, an adaptive management project was enacted to determine if adventitious root

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development could occur on whole-tree cuttings such that the approach could be used to rescue *C. micronesica* trees from future construction sites.

Large, healthy cycad plants respond well to traditional transplanting procedures where extraction of the existing zygotic roots is possible (Haynes 2012). Additionally, *Cycas* L. species produce adventitious lateral stems (Stopes 1910; Norstog & Nicholls 1997), and adventitious roots are often fostered on these "pups" in successful asexual propagation protocols. However, we are unaware of a case where whole-tree cuttings were used as cycad propagules for a large-scale asexual propagation project. Moreover, general health of the population in 2012 was severely compromised due to years of invasive species infestations (Image 1B). Propagation successes based on healthy trees may not be possible where the propagules are derived from unhealthy trees. Our objective was to assess the feasibility of using whole-tree cuttings to develop adventitious roots in attempts to rescue unhealthy mature *C. micronesica* trees from future construction sites.

Materials and Methods

In situ operations

A ladder was used to access the apical portion of each of 49 trees located in northern Guam. All epiphytes, debris, termite nests, and other non-cycad materials were removed by hand, and all cycad leaves were removed with hand shears. A chainsaw was used to saw the full-tree specimens. As the actual cutting of specimens commenced, internal cavities or decay became evident in a majority of the specimens (Image 1C). For each of those trees, sequential cuts were made toward the stem apex until a contiguous cross section was reached with no internal cavity or discoloration of the pith and cortex tissues (Image 1D). The cut surfaces of each stem were sealed with tree sealant and each specimen was covered with shade cloth to avoid direct sun exposure. Trucks were used to transport the specimens to the University of Guam for nursery preparation.

Nursery operations

All nursery procedures were conducted under 50% shade cloth in open-air nurseries. Each stem was cleaned with pressurized water to reduce the likelihood of persistent pathogens. The entire specimen was sprayed with an insect growth regulator solution (11.23% pyriproxyfen) to control any surface armored scale infestations. The final stem cut in the nursery was made approximately 10cm from the original field cut. This was done to remove the basal portion of each stem with a fresh cut because this portion may have been subjected to inadvertent damage during the transport operations. Most specimens revealed more internal decay of pith or cortex tissue when this cut was made. Therefore, sequential cuts toward the apex were made until contiguous healthy tissue was reached.

The cut stem surface was allowed to air-dry, then it was covered with tree sealant and allowed to dry again. The sealant limited water loss and acted as a prophylactic against possible pathogens in the rooting medium. The 40–45 cm basal portion of each stem was inserted into a 10% bleach solution and held for a minimum of one minute. A slurry of fungicide (82.5% tetrachloroisophthalonitrile) and rooting hormone (0.3% indole-3-butyric acid) was painted on the basal 30-cm of each stem.

A 15-cm layer of porous volcanic rock was placed in the bottom of each container to ensure adequate aeration below the stem (Image 2A). This layer of volcanic rock also disallowed any compression of the

Image 1. (A) High density populations of healthy *Cycas micronesica* trees existed on Guam until the invasion of several invasive herbivores. (B) Tree health has declined following several years of pressure from the invasive species. (C) Large stem cuttings obtained in 2012 revealed widespread internal decay. (D) Sequential cuts were made toward the apex of the stems until healthy stem tissue was exposed. © Thomas Marler.

container medium under the weight of the trees. A double layer of nylon window screen was placed on top of the volcanic rock to pad the stem base and to ensure the perlite-rooting medium could not erode into the rock layer. The prepared stem section was gently placed on top of the nylon screen and held in place while three wires were sleeved around the apical portion of the plant then tethered to anchors at approximately 120 degree intervals such that they served as guy wires to hold the stem in place. A composite of 50% perlite and 50% sandblasting sand was placed around each stem to 8–10 cm depth above the screen. The remainder of the container was filled in with perlite (Image 2A).

One nursery contained 34 plants and was managed and maintained by experienced cycad specialists (Image 2B). The plants averaged 88cm in height with 17cm basal diameter. A second nursery contained 15 plants and was managed by general forestry nursery specialists. The plants averaged 107cm in height with 16cm basal diameter.

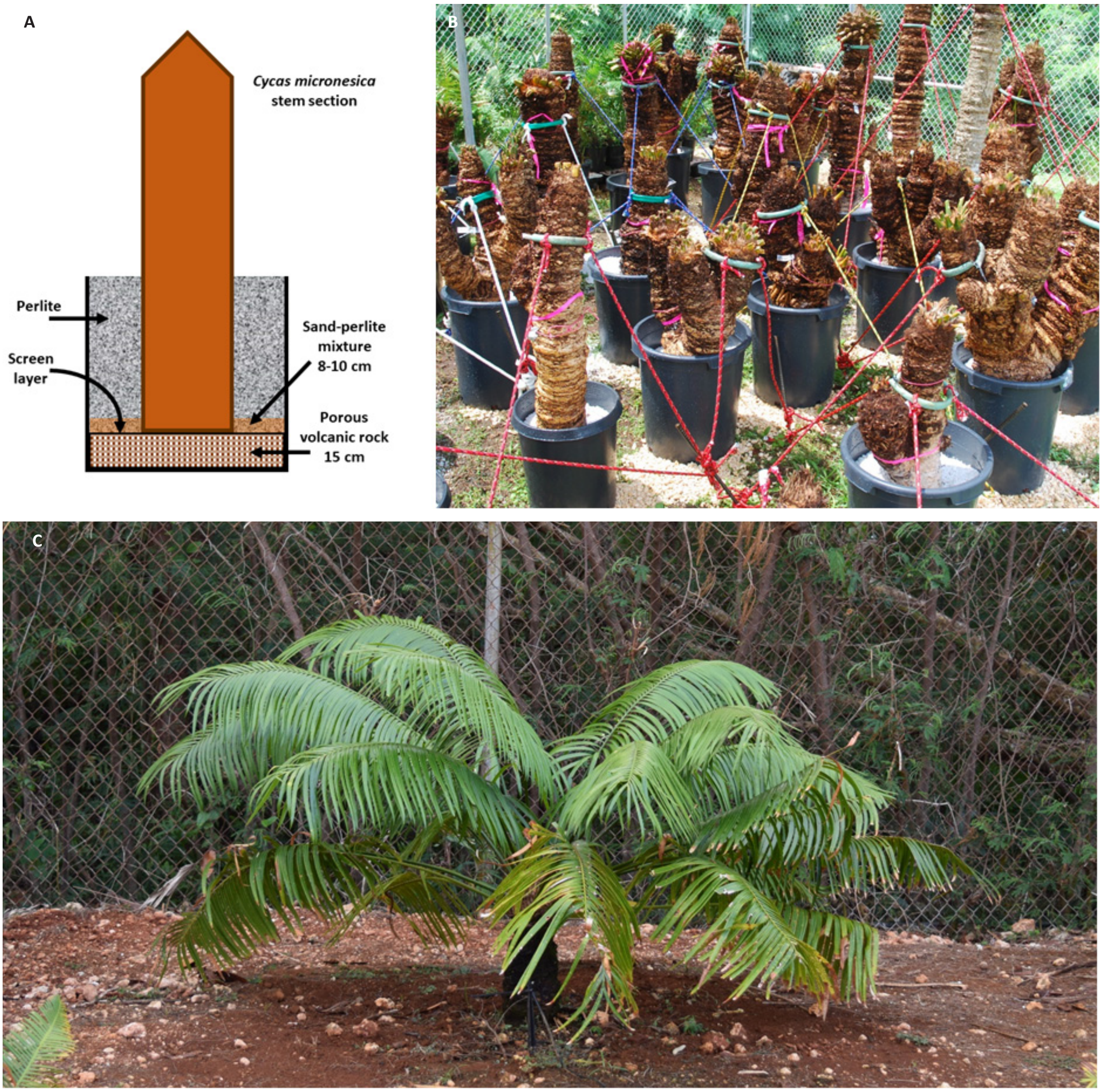

Image 2. (A) Large *Cycas micronesica* stems were rooted in large containers, which ensured adequate aeration surrounding the stem. (B) Appearance of a nursery in which large stems were treated and managed to form adventitious roots, 12 June 2012. (C) Successfully propagated mature stem planted in the University of Guam campus being supported exclusively by an adventitious root system, 06 February 2017. © Thomas Marler.

Results

Several plants developed new leaves shortly after being added to the nursery. These leaves were pruned from the plants to ensure stem water loss via transpiration did not increase prior to root formation. All of the plants in the nursery managed by general forestry specialists were unsuccessful in developing adventitious roots and died. Within six months, 41% of the plants in the nursery managed by cycad specialists had successfully rooted and resumed stem growth. Half of these plants exhibited severe stem dieback, then developed adventitious buds to resume stem growth. As sustained stem growth occurred, the surface of the container medium was top-dressed with screened field soil such that the large pores of the perlite were filled in with the field soil. The mature stem sections supported by adventitious root systems (Image 2C) exhibited the appearance of several-year-old juvenile seedling plants supported by zygotic root systems.

Discussion

Two ongoing developments illuminate the timeliness of this adaptive management research for Guam conservation efforts. First, the 2012 project was conducted when *C. micronesica* was not protected by the United States Endangered Species Act. But the species was listed as Threatened in November 2015 (United States Fish & Wildlife Service 2015). All future plant rescue operations will be constrained by federal research and collection rules and regulations. Second, a large-scale military buildup has been in the planning stages for years (Marler & Moore 2011). As the construction activities to support this military buildup increase, more greenspace will be destroyed and the most appropriate protocols for rescuing *C. micronesica* trees are urgently needed.

The study also highlights an important international conservation agenda. Cycads are the most threatened group of plants, with more than 63% of described taxa listed as threatened (Brummitt et al. 2015; Fragniere et al. 2015). Published research on cycad horticulture and physiology has been inadequate (Cascasan & Marler 2016) even though past reports have illuminated that efforts to cultivate cycads to advance conservation efforts are hindered by the lack of research (Norstog & Nicholls 1997; Donaldson 2003). Our study adds to this inadequately established literature.

Conservation applications

a. Rescuing native threatened trees from construction sites brings to light a need to understand genetic structuring of in situ forests. Indeed, genetic considerations are required for adequately managing native forest resources (Bozzano et al. 2014). Guam's cycad population is highly structured genetically with historical gene flow not occurring well among habitats (Cibrian-Jaramillo et al. 2010). The benefits of conserving the genetics of the donor Guam sub-population by rescuing trees prior to destruction of greenspace is difficult to justify if the conservation project adulterates the genetics of a second recipient sub-population. Conservation funding agencies would benefit by retaining the services of a qualified ecologist to oversee large-scale transplant operations of threatened tree species to ensure genetic threats to the recipient sub-populations are not perpetrated by the conservation project.

b. Perennial forest species in the Mariana Islands are equipped with traits that enable resilience to chronic pressures of tropical cyclones (Marler 2001). Experimental approaches were used to predict an increase in major structural damage of *C. micronesica* trees by tropical cyclones that would occur following the invasions of the exotic insect herbivores (Marler 2013). A tropical cyclone occurred in 2015, which tested this prediction, and confirmed an increase in major stem snapping or toppling occurred in exposed sites (Marler et al. 2016). Rescuing a portion of these snapped or toppled trees following each tropical cyclone is possible using the protocols described in this case study.

c. Our collective experiences indicate that an increase in the percentage of successful adventitious rooting may occur in the nursery if smaller stem sections are harvested in the field. While the ultimate transplants will be smaller in stature than transplants using the protocols described herein, the approach would save a more diverse segment of the gene pool from the donor sub-population. This approach would also reduce harvesting costs, transportation costs, and minimize the need to cut off the internal stem decay sections at the nursery site.

d. Another approach to reduce costs of the rescue attempts would be to bypass the nursery expenses and space requirements by transferring stem sections from the donor sub-population directly to the recipient sub-population. Research to pursue this approach may best focus on hygiene. Use of sterilized soil at the bottom of the planting hole and perlite to surround the stem may mitigate pathogen pressures that tend to reduce asexual propagation success.

e. No empirical methods have been employed to determine the means by which *A. yasumatsui* infestations reduce health and ultimately kill a *Cycas* tree. A decline in non-structural carbohydrates is likely one of the mechanisms. Our overall success when both nursery sites are considered was adventitious root formation on only 28% of the large stems. The reduced internal carbohydrate pool may be responsible for the lack of success for a majority of the stems. Excavating fewer trees with intact zygotic roots may be more successful in rescuing trees from construction sites than cutting off a larger number of stems then hoping to achieve successful adventitious root initiation. This approach may also lead to greater long-term success considering Guam's frequency of tropical cyclone damage. For a range of species, trees with adventitious root systems suffer greater damage during tropical cyclones than trees with zygotic root systems.

f. The time that has elapsed since an invasion may influence many population traits of an invasive species (Yokomizo et al. 2017). For restoration and conservation projects, adaptive management strategies should be formulated with caution when available data were obtained in the past. The *A. yasumatsui* population immigrated into the study site in 2005 (personal observations), and our *C. micronesica* stem adventitious rooting data were obtained seven years later. Interpreting our results through the lens of context dependency mandates that these phenomena be considered. For example, propagation of whole-tree *C. micronesica* stems from healthy trees exhibiting no signs of herbivory should be more efficacious than our 28% success, because time since immigration would be zero. Alternatively, propagation of stems from the same Guam sub-population in 2017 should be much less successful than the 28% from 2012, because time since immigration would be 12 years.

g. Numerous biological control efforts have been implemented (Marler & Terry 2013; Moore et al. 2005). Annual mortality in Guam's *C. micronesica* habitats has not declined despite establishment of the predator *Rhyzobius lophanthae* Blaisdell and an unidentified *Arrhenophagus* Aurivillius parasitoid as biological control agents of *A. yasumatsui*. Continuing the pursuit of an effective biological control program for *A. yasumatsui* is arguably the most important endeavor for conserving *C. micronesica*. Indeed, a majority or all of the trees rescued from construction sites then transplanted to other sites will be killed by *A. yasumatsui* damage during the years after successful transplanting. The funds that conservation agencies make available for tree rescue may be more successfully invested into expanding the biological program to manage the insular *A. yasumatsui* population.

In summary, success of adventitious root formation on large *C. micronesica* stems may be moderately successful, provided an experienced cycad horticulturist is involved in the nursery phase. Large-scale transplanting of rescued *C. micronesica* trees from one sub-population may generate a conservation dilemma if contamination of the genetic integrity of the recipient sub-population is not considered. The established biotic threats to the populations of this tree species persist throughout Guam, so most or all of the rescued *C. micronesica* trees from a construction site will die during the years following successful transplantation. Investing all available conservation funds into mitigating the persistent, ubiquitous threats would help conserve the species with more success than investing those funds into tree rescue projects.

References

Bozzano, M., R. Jalonen, E. Thomas, D. Boshier, L. Gallo, S. Cavers, S. Bordács, P. Smith & J. Loo (eds.) (2014). *Genetic Considerations in Ecosystem Restoration Using Native Tree Species*. State of the World's Forest Genetic Resources - Thematic Study. Rome, FAO and Bioversity International, 281pp.

Brummitt, N.A., S.P. Bachman, J. Griffiths-Lee, M. Lutz, J.F. Moat, A. Farjon, J.S. Donaldson, C. Hilton-Taylor, T.R. Meagher, S. Albuquerque, E. Aletrari, A.K. Andrews, G. Atchison, E. Baloch, B. Barlozzini, A. Brunazzi, J. Carretero, M. Celesti, H. Chadburn, E. Cianfoni, C. Cockel, V. Coldwell, B. Concetti, S. Contu, V. Crook, P. Dyson, L. Gardiner, N. Ghanim, H. Greene, A. Groom, R. Harker, D. Hopkins, S. Khela, P. Lakeman-Fraser, H. Lindon, H. Lockwood, C. Loftus, D. Lombrici, L. Lopez-Poveda, L. Lyon, K. Malcolm-Tompkins, L. Moreno, L. Murray, K. Nazar, E. Power, M.Q. Tuijelaars, R. Salter, R. Segrott, H. Thacker, L.J. Thomas, S. Tingvoll, G. Watkinson, K. Wojtaszekova & E.M.N. Lughadha (2015). Green plants in the red: A baseline global assessment for the IUCN sampled Red List Index for plants. *PloS one* 10(8): e0135152.

Calonje, M., D.W. Stevenson & L. Stanberg (2017). *The world list of cycads*, online edition. 2013–2017. Available from: http://www.cycadlist.org. Downloaded on 11 July 2017.

Cascasan, A.N. & T.E. Marler (2016). Publishing trends for Cycadales, the most threatened plant group. *Journal of Threatened Taxa* 8(3): 8575–8582; http://doi.org/10.11609/jott.2369.8.3.8575-8582

Cibrian-Jaramillo, A., A.C. Daly, E. Brenner, R. DeSalle & T.E. Marler (2010). When north and south don't mix: genetic connectivity of a recently endangered oceanic cycad, *Cycas micronesica*, in Guam using EST-microsatellites. *Molecular Ecology* 19: 2364–2379.

Donaldson, J.S. (ed.) (2003). *Cycads. Status Survey and Conservation Action Plan*. IUCN/SSC Cycad Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK, ix+86pp.

Donnegan, J.A., S.L. Butler, W. Grabowiecki, B.A. Hiserote & D. Limtiaco (2004). *Guam's Forest Resources, 2002.* Resour. Bull. PNW-RB-243. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, USA, 32pp.

Fragniere, Y., S. Bétrisey, L. Cardinaux, M. Stoffel & G. Kozlowski (2015). Fighting their last stand? A global analysis of the distribution and conservation status of gymnosperms. *Journal of Biogeography* 42(5): 809–820.

Haynes, J. (2012). Transplanting a large cycad. *The Cycad Newsletter* 35(3): 22–23.

Marler, T.E. (2001). Tropical cyclones and perennial species in the

Mariana Islands. *Hortscience* 36: 264–268.

Marler, T.E. (2012). Cycad aulacaspis scale invades the Mariana Islands. *Memoirs New York Botanical Garden* 106: 20–35.

Marler, T.E. (2013). Increased threat of island endemic tree's extirpation via invasion-induced decline of intrinsic resistance to recurring tropical cyclones. *Communicative & Integrative Biology* 6: e223611–e223616.

Marler, T.E., J. Haynes & A. Lindström (2010). *Cycas micronesica*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.2. Available at: http://www.iucnredlist.org. Downloaded on 11 July 2017.

Marler, T.E. & J.H. Lawrence (2012). Demography of *Cycas micronesica* on Guam following introduction of the armoured scale *Aulacaspis yasumatsui***.** *Journal of Tropical Ecology* 28: 233–242; http://doi.org/10.1017/S0266467412000119

Marler, T.E., J.H. Lawrence & G.N. Cruz (2016). Topographic relief, wind direction, and conservation management decisions influence *Cycas micronesica* K.D. Hill population damage during tropical cyclone *Journal of Geography & Natural Disasters* 6: 178; http://doi.org/10.4172/2167-0587.1000178

Marler, T.E. & A. Moore (2011). Military threats to terrestrial resources not restricted to wartime: a case study from Guam. *Journal of Environmental Science & Engineering* 5: 1198–1214.

Marler, T.E. & I. Terry (2013). The continuing demise of *Cycas micronesica*. *The Cycad Newsletter* 36(1): 22–26.

Moore, A., T. Marler, R.H. Miller & R. Muniappan (2005). Biological control of cycad aulacaspis scale on Guam. *The Cycad Newsletter* 28(5): 6–8.

Norstog, K.J. & T.J. Nicholls (1997). *The biology of the cycads*. Cornell University Press, Ithica, New York, xi+363pp.

Stopes, M.C. (1910). Adventitious budding and branching in *Cycas*. *New Phytologist* 9: 235–241.

United States Fish & Wildlife Service (2015). Endangered and threatened wildlife and plants; endangered status for 16 species and threatened status for 7 species in Micronesia. *Federal Register* 80: 59424–59497.

Yokomizo, H., T. Takada, K. Fukaya & J.G. Lambrinos (2017). The influence of time since introduction on the population growth of introduced species and the consequences for management. *Population Ecology* 59: 89–97; http://doi.org/10.1007/s10144-017-0581-6

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