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Diet of a threatened pond frog differs over a small spatial scale

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ABSTRACT: Suitability of habitat patches affects demographic processes and consequently influences the viability of populations. In order for managers to consider the potential of resources on a scale appropriate to their use, it is important to understand the processes that influence the ecology of threatened species. Differential growth rates of *Litoria aurea* (green and golden bell frog) at Sydney Olympic Park, Australia, may be explained by prey availability and diet. We tested: (1) whether food availability differed among precincts at Sydney Olympic Park and (2) whether the diet of *L. aurea* was influenced by availability of invertebrate prey. Diets were distinct among precincts and reflected the variation in biomass and richness of invertebrate assemblages. Precincts with greater biomass corresponded to areas with faster individual growth rates and greater habitat structure. The differences in diet and individual growth rates of *L. aurea* among precincts at Sydney Olympic Park demonstrate how caution must be applied to generalising population function, even within a small area.

KEY WORDS: Patchy habitat · Prey · Amphibian · Food · *Litoria aurea*

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INTRODUCTION

Consideration of scale is crucial when managing threatened species because population function is affected by habitat patch suitability. While some patches may sustain healthy populations, other areas, with lower densities of individuals, are at an increased risk of extinction or are unviable (Hanski 1994). Therefore, for managers to accurately gauge the potential of resources on a scale appropriate to their use, it is important to understand the processes that influence the ecology of threatened species. For example, the distribution of mallee emu wrens at Murray-Sunset reserve, South Australia, is restricted to discrete habitat patches that have been unburnt for 15 yr (Brown et al. 2009). In this situation, managers must allocate species-specific actions, such as disturbance reduction within the isolated occupied patches, while operating large-scale land management, such as wildfire control, over the entire 633 000 ha parkland. This multifaceted approach is necessary for the persistence of many threatened species that occupy patches of habitat.

Urban areas often provide heterogeneous habitats in close proximity to each other because development and restoration occur at different temporal and spatial scales (Cadenasso et al. 2007). In many urban areas, managers regulate ecological practices and, therefore, it is important to determine the most beneficial management regimes for threatened species. Green and golden bell frogs *Litoria aurea* are a threatened species that persist primarily in isolated, urban areas on the south-east coast of Australia (Mahony 1996). They have disappeared throughout 90% of their former range in the last 40 yr, following rapid, widespread declines that are usually attributed to the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, and now persist in iso-

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lated populations in a wide variety of habitats (Mahony 1996). Despite their decline in Australia, *L. aurea* are invasive in New Zealand, Vanuatu and New Caledonia (Bishop 2008). The species' ability to persist in such a diversity of habitats suggests that it is a generalist with few specific requirements. One managed population occurs at Sydney Olympic Park (SOP), Sydney, New South Wales, Australia, a small (320 ha) region with heterogeneous habitat types. The area has been surveyed and managed since 1996 to directly ensure the population's persistence (Darcovich & O'Meara 2008).

The population size of *Litoria aurea* at SOP is small, and is therefore inherently exposed to extinction processes. The population experiences high annual mortality (Pickett et al. in press), probably in part due to *Batrachochytrium dendrobatidis* (Penman et al. 2008, Stockwell et al. 2008, 2010). The compensatory high fecundity and growth rate of *L. aurea* allows the population to persist with high turnover and size variability (Hamer & Mahony 2007, Pickett et al. in press). However, individual growth rates differ among 3 spatially distinct sites: individuals in Narawang Wetland and Kronos Hill grow faster than those in the Brickpit (Pickett et al. in press). Determining factors that influence growth may be important to demography. For example, fast-growing individuals may mature faster and contribute to reproduction earlier, which would in turn increase population growth and viability (Pickett et al. in press).

The difference in growth rates of *Litoria aurea* at SOP could be explained by a number of factors. One hypothesis is that frogs may have differential access to resources due to variability in prey items. We tested: (1) whether prey resource availability differed among precincts at SOP and (2) whether the diet of *L. aurea* was influenced by availability of invertebrate prey.

MATERIALS AND METHODS

Study site

SOP is a restored industrial wasteland in the western suburbs of Sydney, Australia. *Litoria aurea* was discovered in the grounds of the park prior to developing the site for the 2000 Sydney Olympic Games (Darcovich & O'Meara 2008). A large restoration strategy was implemented to offset habitat loss and increase the distribution of *L. aurea* in an attempt to ensure the persistence of the population (Pickett et al. 2013). Currently, the park contains 150 ponds of varying size and hydrological regimes, providing a selection of habitat for aquatic fauna. Within the park, *L. aurea* was studied in 3 distinct precincts (33° 51′ S, 151° 04′ E; Fig. 1). The Brickpit precinct is a disused quarry where works finished in 1998; it is 24 ha in size and contains 45 ponds of varying size. Transects were established around the central and largest ponds. The Kronos Hill/Wentworth Common area, located north of the Brickpit, was completed between 1998 and 2000 (Darcovich & O'Meara 2008), is 40 ha in size and contains 37 habitat ponds. However, owing to a skewed distribution of frogs at this site, our study focused on the western ponds of Kronos Hill. In 1999, additional habitat was created on remediated land north of Kronos Hill/Wentworth Common in the area now known as Narawang Wetland. Narawang Wetland consists of 20 ha containing 22 habitat ponds. Transects were set around the most easterly part of the wetlands (Fig. 1) and were selected to represent the area where *L. aurea* were most heavily sampled for stomach flushing. Long-term demographic studies have detected little movement between precincts (Pickett et al. in press).

Surveys and invertebrate sampling

Surveys for *Litoria aurea* were conducted in 14 ponds within the 3 precincts between 2 and 22 February 2012 at SOP. Sampling of each pond occurred between 20:00 and 04:00 h. Individual *L. aurea* were captured inside a single-use disposable plastic bag to prevent potential pathogen transmission. Individuals were only stomach flushed once. Individuals greater than 35 mm snout–vent length (SVL) were identified by a passive integrated transponder tag (Christy 1996, Christy et al. 2007), whereas juveniles were identified by skin biopsy taken from toe webbing for genetic purposes. Stomach flushing was completed within 30 min of capture (Solé et al. 2005), and frogs were then released at the site of their capture. Dietary items were stored in 80% ethanol, identified to order (Zborowski & Storey 2010), dried and weighed to the nearest 0.0001 g using a Sartorious BP 210S electronic microbalance. Unidentifiable prey items were rare and excluded from count data, which was quantified by the number of whole animals or, where the organism was in many pieces, e.g. arachnids, by the number of abdomens.

Invertebrate sampling in the environment was completed between 19 and 23 February 2012. Between 5 and 7 belt transects, 20 m by 1 m, were posi-

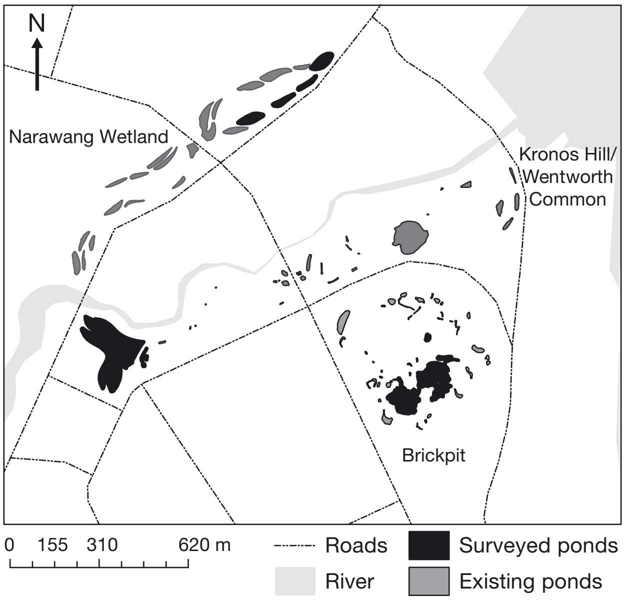

Fig. 1. Sydney Olympic Park, Sydney, NSW, Australia, showing the 3 precincts where invertebrate sampling and stomach flushing of *Litoria aurea* occurred in 2012

tioned perpendicular to the water's edge. The proportion of vegetation cover in each 2 m^2 section of transect was summed and the total proportion of coverage was calculated. Vegetation was grouped into one of 5 main categories that reflected its structure: grass, reeds, bare ground, woodland or leaf litter. Invertebrate sampling was completed between 09:00 and 11:00 h over 5 d. Each metre of transect was sampled by 2 min of aerial sweep netting (net 152 mm diameter, 0.9 × 0.3 m mesh, 18 cm long, Australian Entomological Supplies Pty Ltd) and 2 min of active searching. Invertebrates were stored in 80% ethanol and identified to order (Zborowski & Storey 2010). The cumulated sample from each transect was weighed to the nearest 0.0001 g using a Sartorious BP 210S electronic microbalance. Our research was conducted in accordance with institutional, national and international guidelines concerning the use of animals in research and the sampling of endangered species.

Statistical analysis

Primer 6 (version 6.1.15) was used to analyse all multi-dimensional scaling (MDS) and diversity indices. Euclidean distance was used to investigate the composition of prey taxa among sites by using the presence/absence of prey taxa detected in *L. aurea* stomachs in each pond; the precinct was used as a grouping variable. Bray-Curtis similarity was used to investigate the similarity of invertebrate composition in the sites by using the abundance of invertebrate taxa in each transect as the site, and the habitat precinct as a grouping factor. A one-way ANOVA was used to test for differences in diversity, transformed richness (natural log), evenness, abundance and biomass of invertebrates sampled in transects among precincts. The proportion of vegetation in each transect was transformed (arc sine) to meet assumptions of normality before a one-way ANOVA was employed to compare variance among precincts. Linear mixed-effects models (assuming a normal distribution) were used to explore variability in the mass of frog gut contents. Precinct was included as a fixed effect; individual frogs were included as random effects. Pond and SVL of frogs were included in the initial model, and then removed in the final model owing to non-significance. Univariate models were completed in SPSS version 9.

RESULTS

The proportion of vegetation cover in each transect differed among precincts ($F_{8,75} = 5.97$, $p < 0.001$; Fig. 2). The Brickpit habitat primarily comprised bare ground (56%) and grass (44%), with few trees. Narawang Wetland was dominated by grass (44%), woodlands (33%) and leaf litter (23%). Kronos Hill was predominantly grassy (83%) with small areas of woodland (12%).

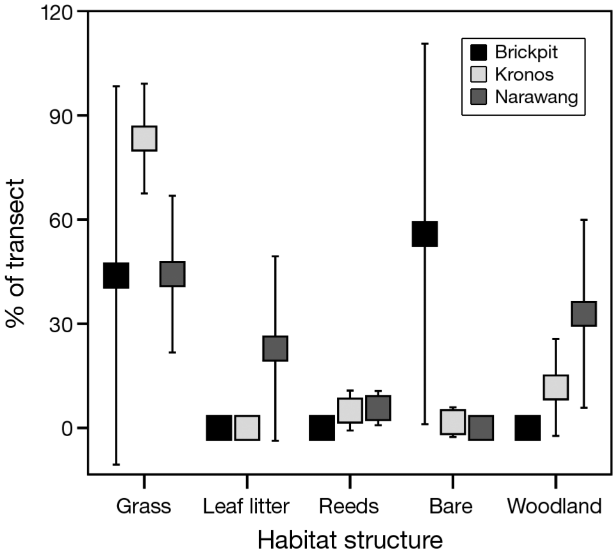

Fig. 2. Mean percentage of habitat type in 3 precincts at Sydney Olympic Park in 2012. Error bars are 95% confidence intervals (CI)

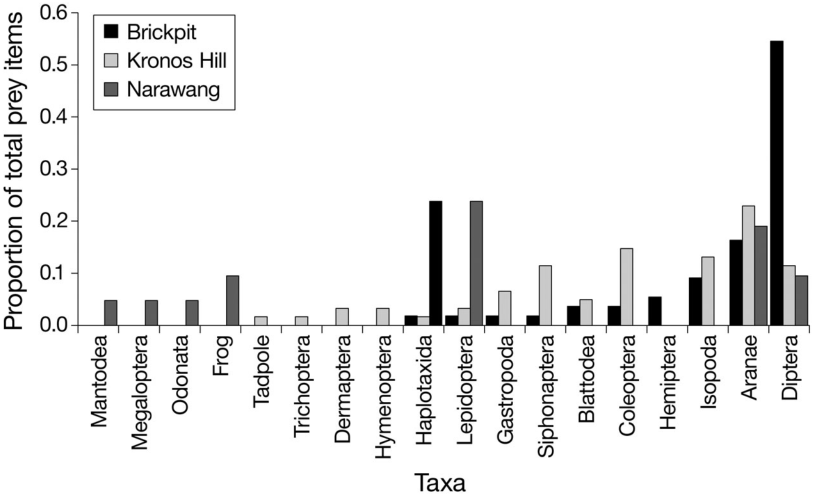

Fig. 3. Proportion of *Litoria aurea* gut contents sampled from each taxa at 3 precincts at Sydney Olympic Park in 2012. Frogs and tadpoles are presented separately to demonstrate the occurrence of aquatic foraging

Invertebrate surveys sampled 38 taxa. Mean biomass of dried invertebrates in transects at Narawang Wetland (1.5 ± 0.4 g) was higher than in the Brickpit (0.3 ± 0.5 g), while Kronos Hill was intermediate (1.0 ± 0.3 g; $F_{2,15}$ = 7.62, $p < 0.005$). Taxa richness was higher in transects in Narawang Wetland (17.6 ± 0.78) and Kronos Hill (17.3 ± 1.9) than in the Brickpit (13 ± 0.6; $F_{2,15}$ = 7.02, $p < 0.01$). However, the mean abundance (605 ± 67, $F_{2,15}$ = 1.244, p = 0.32), evenness (0.62 ± 0.62, $F_{2,15}$ = 0.60, p = 0.56) and diversity (0.7 ± 0.2, $F_{2,15}$ = 2.49, p = 0.12) of invertebrates in transects did not differ significantly among precincts.

Sixty *Litoria aurea* gut samples contained 137 individual prey items, with 1 to 16 items per gut. Sixteen invertebrate taxa were identified from gut contents, all of which were sampled during invertebrate surveys (Fig. 3). Gut contents included both aquatic and terrestrial invertebrates, but also tadpoles (unknown sp.) and 2 other frog species (*Litoria fallax* and *Limnodynastes peronii*). Stomach flushing of 13 *L. aurea* did not return any prey items.

The similarity of prey contents from *Litoria aurea* guts differed according to precinct in an ordination (MDS) (Fig. 4). Diptera (n = 33) comprised 54% of food items in *L. aurea* in the Brickpit, but only represented a small proportion of the diet in Kronos Hill and Narawang Wetland. Individual prey items in guts of *L. aurea* from Narawang Wetland had significantly greater mean biomass than those from Kronos Hill and the Brickpit ($F_{2,23}$ = 5.06, $p < 0.02$; Fig. 5). However, differences in prey biomass among precincts were not determined by frog size; there was no significant interaction between precinct and SVL ($F_{2,13}$ = 0.34, p = 0.721).

DISCUSSION

Litoria aurea consumed a wide variety of prey types, but diet was distinct among precincts in close proximity to one another at SOP. *L. aurea* in the Brickpit ate lighter prey items than at Narawang Wetland, potentially because of differences in the availability of prey and taxa among sites. Invertebrate sampling demonstrated that a much lower biomass occurred in the Brickpit, where *L. aurea* diet had a larger representation of dipterans and fewer taxa overall. In Narawang Wetland, the invertebrate assemblage was higher in both biomass and richness relative to the other sites, providing the frogs with greater choice and biomass of food items. Kronos Hill was less distinct from the other sites and had invertebrate assemblages resembling both the Brickpit and Narawang Wetland.

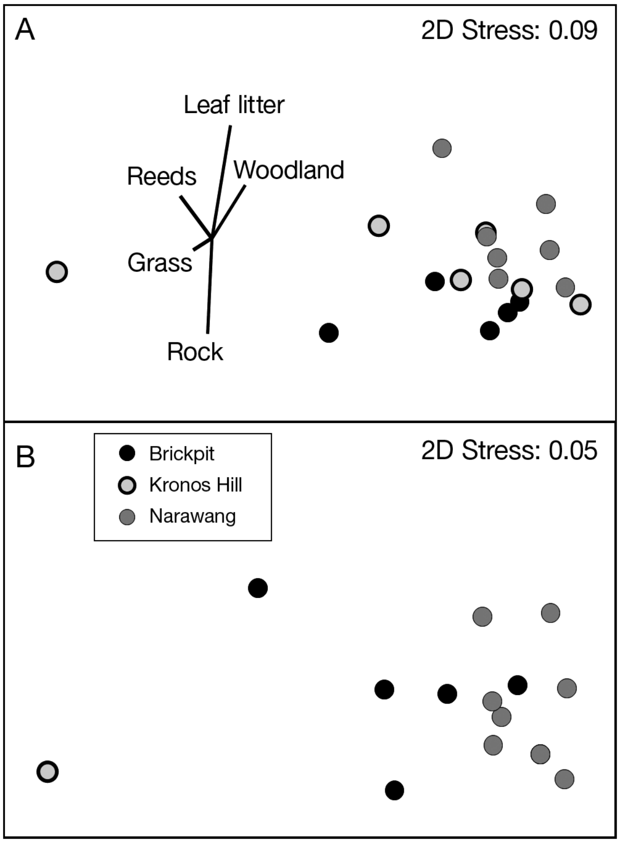

Fig. 4. Ordination of (A) invertebrates sampled on transects and (B) invertebrates sampled from gut contents of *Litoria aurea* at Sydney Olympic Park in 2012, grouped by habitat precinct

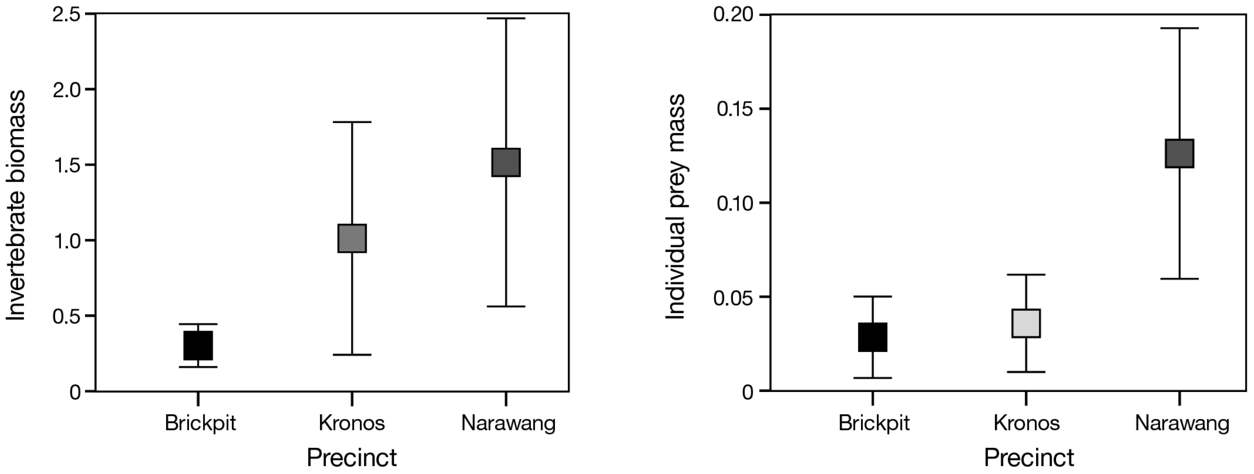

Fig. 5. (A) Mean biomass of invertebrates collected from transects in each precinct at Sydney Olympic Park in 2012. (B) Mean mass of prey items from *Litoria aurea* guts in each precinct Error bars are 95 % CI

Differences in invertebrate assemblages among precincts may be explained by disparity in habitat structure at SOP. A higher proportion of grass and trees, and a strong ground layer of leaf litter, dominated Narawang Wetland, with no areas of bare ground. Invertebrate assemblages are responsive to small-scale changes in microhabitat (Kremen et al. 1993), suggesting that differences in habitat among precincts likely account for the greater biomass and richness of invertebrates in Narawang Wetland compared with the Brickpit. Narawang Wetland had habitat with multiple strata which provide a variety of fossorial and arboreal shelter sites, and consequently had the highest biomass and richness of invertebrates. In stark contrast, the lack of soil and large areas of bare ground and rock that accounted for over half the transect area at the Brickpit provided little habitat diversity or refuge for invertebrates. The mown grass at Kronos Hill lacked the woodland and leaf litter of Narawang Wetland, but had more structure than the bare rock in the Brickpit. The intermediate structure in Kronos Hill was reflected in the invertebrate assemblages, which resembled those in both of the other precincts.

Differences in prey availability among precincts reflect the differences in growth rates of frogs among precincts in SOP. *Litoria aurea* in Narawang Wetland and Kronos Hill reach a length indicative of maturity within a single season of growth, whereas females from the Brickpit take an extra 50 d on average (Pickett et al. in press). Differences in growth rate reflect the comparatively high biomass of invertebrates eaten by *L. aurea* in Narawang Wetland compared with the Brickpit. However, in Kronos Hill the growth rates and prey availability were greater than those in the Brickpit, while the mass of prey was more similar to that in the Brickpit, suggesting that the diet biomass may not have been reflected by the stomach flushing. This may be explained by differences in the decomposition rate of prey, which can confound dietary studies by over representing slow-digesting prey items (Hyslop 1980). In addition, while the difference in resource availability reflected variation in growth rates of *L. aurea* in the present study, other alternative factors need to be considered as potential contributors to differential growth rates, such as differences in microclimate, including temperature (Browne & Edwards 2003), which affects growth rate of ectothermic animals.

Areas of highly productive habitat, with a high richness and abundance of invertebrate taxa supporting faster-growing *Litoria aurea*, may be crucial components for an optimal design of constructed *L. aurea* habitat. *L. aurea* is often the subject of translocations to newly constructed habitat relating to development applications, because populations often reside on industrial and urban land (White & Pyke 2008). Designing habitat that will maximise the growth rate of *L. aurea* could be particularly beneficial, because susceptibility to *Batrachochytrium dendrobatidis* is implicated in failure of reintroductions (Stockwell et al. 2008). For species that suffer high mortality, the difference between reaching maturity in the first or second breeding season could alter the viability of the population. The differences in diet and demographic rates of *L. aurea* among precincts at SOP demonstrate how caution must be applied to generalising population function, even within a small area. Ecological studies aimed at measuring baseline resource requirements required to optimise demographics of managed populations should take heterogeneity at the small to micro-landscape scales, as well as larger landscape scales, into account.

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