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Wildlife Research

Supplementary Material

Predator-free short-hydroperiod wetlands enhance metamorph output in a threatened amphibian: insights into frog breeding behaviour evolution and conservation management

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Appendix S1. Methods and results of the detection probability modelling of metamorphs

To determine the detection probability of each habitat type, a binomial distribution was used and the data was presented with the R function cbind(seen, notseen). Seen = the amount of model frogs seen during a survey and notseen = the number of model frogs missed during a survey. A quasibinomial function was tested to determine if there was overdispersion. There was overdispersion found which led to the implementation of a general linear mixed effects model with a betabinomial distribution, with the formula cbind(seen, notseen)~habitat_type + (1|pond), where pond was used as a random effects. This was conducted in the glmmTMB package in R statistics. This resulted in an acceptable level of overdispersion and improved model fit.

With the acceptance of this model, there was a statistically significance different found ($\chi^2[1]=15.0$, $P = 0.020$) and an alpha = 0.05. Differences in the detection probability of each were examined with Tukey honestly significance difference (HSD) post-hoc tests. It was found that all the habitat types were relatively similar in detection probability except for one, which was significantly different compared to all other habitat types (see Figure S1 and Table S1).

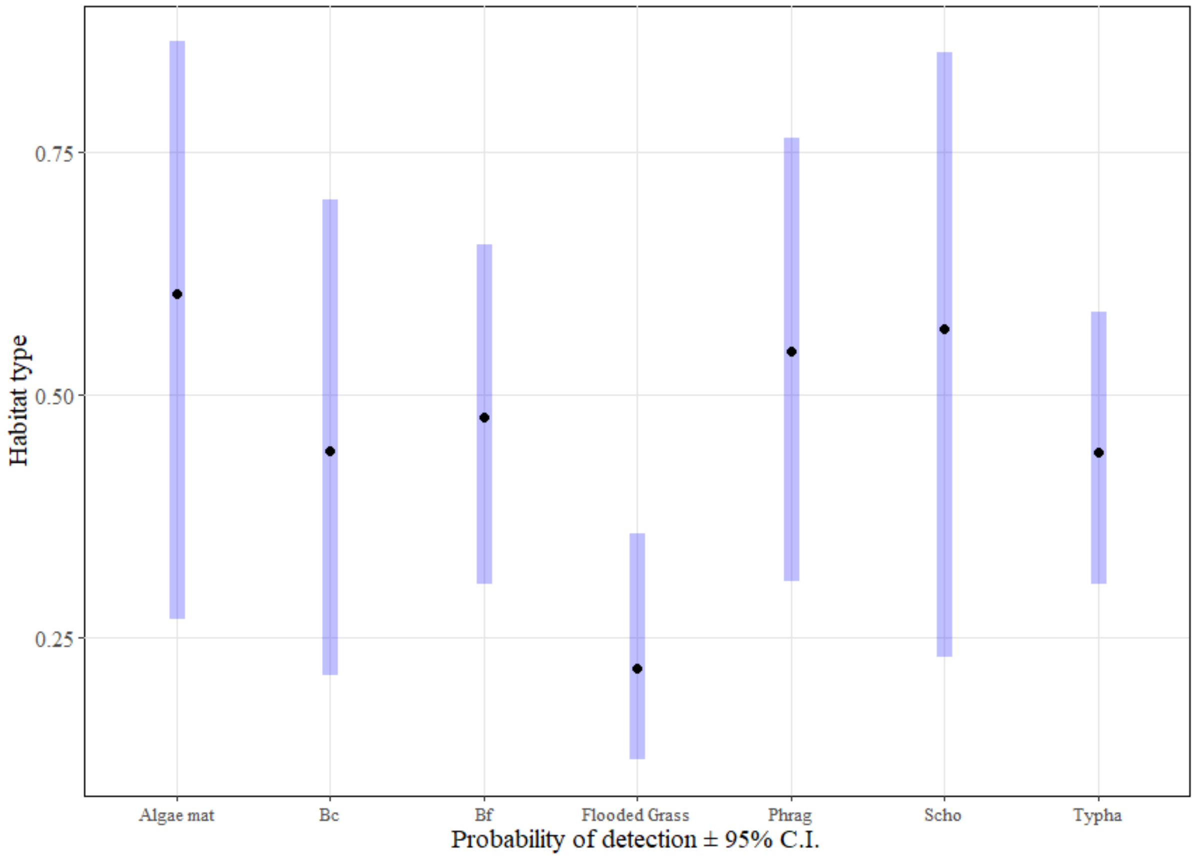

Figure S1. Comparison of detection probability among habitat types. Algae matt; *n* = 3 surveys in one wetland. Flooded grass; *n* = 15 survey among five wetlands. Typha; *n* = 18 surveys among six wetlands. Bc = *Bolbochoenus caldwelli*; *n* = 6 surveys among two wetlands. Bf = *Bolboschoenus fluviatalis*; *n* = 12 surveys among four wetlands. Phrag = *Phragmites australis*; *n* = 6 surveys among two wetlands. Scho = *Schoenoplectus validus*; *n* = 3 surveys in one wetland.

Table S1. Detection probability significance comparisons with Tukey HSD tests among habitat types

Shading indicated significant different at an alpha level = 0.05

Variable 1	Variable 2	Estimate	SE	df	T ratio	P value
Algae mat	Bc	0.653	0.73	54	0.892	0.376
Algae mat	Bf	0.511	0.78	54	0.655	0.515
Algae mat	Flooded grass	1.694	0.70	54	2.417	0.019
Algae mat	Phrag	0.240	0.82	54	0.292	0.771
Algae mat	Scho	0.150	0.98	54	0.153	0.879
Algae mat	Typha	0.659	0.75	54	0.874	0.386
Bc	Bf	-0.142	0.63	54	-0.226	0.822
Bc	Flooded grass	1.042	0.54	54	1.919	0.060
Bc	Phrag	-0.413	0.70	54	-0.591	0.557
Bc	Scho	-0.503	0.89	54	-0.567	0.573
Bc	Typha	0.006	0.57	54	0.011	0.992
Bf	Flooded grass	1.184	0.45	54	2.610	0.012
Bf	Phrag	-0.271	0.51	54	-0.535	0.595
Bf	Scho	-0.361	0.74	54	-0.491	0.625
Bf	Typha	0.148	0.38	54	0.387	0.701
Flooded grass	Phrag	-1.454	0.57	54	-2.562	0.013
Flooded grass	Scho	-1.544	0.79	54	-1.949	0.057
Flooded grass	Typha	-1.036	0.36	54	-2.885	0.006
Phrag	Scho	-0.090	0.76	54	-0.119	0.906
Phrag	Typha	0.419	0.53	54	0.796	0.430
Scho	Typha	0.509	0.78	54	0.656	0.514

Since there two major groups of detection probability estimates in the results (group 1 = algae mat, *Bolboschoenus caldwelli*, *Bolboschoenus fluviatalis*, *Phragmites australis*, *Schoenoplectus validus* and *Typha* sp., and group 2 = flooded grass), the habitat types of group 1 were combined. The model was rerun with this new combination ($\chi^2[1]=13.2$, P = 0.000), which lead to the following detection probability estimates; group 1 detection probability = 0.47 ± 0.34-0.61 95% CI and group 2 detection probability = 0.22 ± 0.12-0.37 95% CI.

Appendix S2. Summary and justification of predator species considered in each covariate

There were several species identified as potential tadpole predators, including reptiles, birds and freshwater macroinvertebrates, and the counts were included as covariates in the model. There was three species of terrestrial reptile predators that have been previously identified as predators of *Litoria aurea* (Pyke and White 2001), including the eastern marsh snake (*Hemiaspis signata*), the eastern water skink (*Eulamprus quoyii*) and the red-bellied black snake (*Pseudechis porphyriacus*), and observations of these predators were combined into a presence/absence categorical variable. There was one aquatic reptile, the eastern long-necked turtle (*Chelodina longicollis*), which was also represented as a categorical variable. Bird predators consisted of the Australian little bittern (*Ixobrychus dubius*), Australian white ibis (*Threskiornis molucca*), eastern great egret (*Ardea alba modesta*), pacific heron (*Ardea pacifica*), royal spoonbill (*Platalea regia*), and white-faced heron (*Egretta novaehollandiae*). Their combined presence/absence was used as a categorical variable.

Potential freshwater macroinvertebrate tadpole predators included the Australian emperor dragonfly larvae (*Anax papuensis*), the large Dytisicid beetle *Cybister tripunctatus*, damselfly larvae (taxon: Zygoptera), the water spider *Dolomedes facetus*, the Hydrophilid beetle *Hydrophilus* sp., dragonfly larvae in the Libullidae family, and backswimmers (family: Notonectidae). Additionally, medium and small sized Dytiscid beetles which were not identified below family level were grouped into one catch per unit effort covariate. There was only one instance where *Gambusia holbrooki* co-occurred with *L. aurea* tadpoles, and hence *G. holbrooki* was not used as a predator covariate against metamorph recruitment.

A potential tadpole competitor, tadpoles of the striped marsh frog (*Limnodynastes peronii*) were tested against metamorph counts of *L. aurea*. Additionally, Litoria *aurea* tadpole catch per unit effort was also tested against *L. aurea* metamorph count as a control to test the assumption that more tadpoles caught in Fyke nets predicted more metamorphs observed. If this assumption was satisfied then more inference can be made of the fact that less tadpoles observed meant less metamorphs would be observed.

Reference

Pyke, G.H. and White, A.W. (2001) A review of the biology of the Green and Golden Bell Frog *Litoria aurea*. *Australian Zoologist* **31**, 563–598.

Appendix S3. Observations of predation among aquatic fauna

There were several tadpole predator observations made during the course of this study. There were two instances of the freshwater turtle *C. longicollis* predating on large (~25 mm SVL) *L. aurea* tadpoles. In both instances the turtle was in ambush position submerged approximately 40 cm on the bank (the first observed at 20:03 24/01/2019 in permanent wetland 14B and the second at 18:30 24/01/2019 in permanent wetland 3A) of the wetland and would strike when a tadpole passed its position. In the first instance, five tadpoles were consumed and in the second instance four tadpoles were consumed, both being monitored for a 30-40 minute period. In both instances the turtles regurgitated the skin and tail of the tadpoles. There was also one instance of the dragonfly larvae of *A. papuensis* feeding on a *Limnodynastes peronii* tadpole (size: ~15 mm SVL) on 16:15 14/12/2018. This was observed from individuals caught within a Fyke net in permanent wetland 14B.

There was an instance of a predation interaction between two species of freshwater macroinvertebrates. A *Cybister tripunctatus* was observed feeding on a metamorphasising *A. papuensis*, which occurred at 21:49 12/02/2019 in permanent wetland 3A.

Predators of the eggs of *L. aurea* were made from opportunistic observations of one egg clutch for ~10 minutes from 20:25 8/12/2016 within wetland 2C. There were several freshwater macroinvertebrate predators including small Dytiscid beetles (carapace length = ~5 mm, $n = 2$) and Notonectids ($n = 1$) were observed consuming ova. Large *L. aurea* tadpoles were also observed canabilising the eggs (size = ~30 mm SVL, $n = 1$).

Appendix S4. List of Earthwatch volunteers

Disappearing Frogs Expedition January 2018 – Earthwatch representative: Andrea Haas, attendees: Chayton Barber, Stella Cross, Aidan Fong, Matthew Harris, Cassandra Ho, Rebecca Jenkins, Elijah Kinnane, Mila Norquay-Whitford, Niza Salarda and Glen Sands

Disappearing Frogs Expedition December 2018 – Earthwatch representative: Andrea Haas, attendees: Emily Bridges, Eirene Carajias, Zara Edmond, Abaigh Gleeson, Abby Howes, Siobhan Kirk, Andrew Lim, Kirralee Seaman, Michela Skipp, Chenxin Tu and Riley Warwick

Disappearing Frogs Expedition January 2019 – Earthwatch representative: Maria Garcia-Rojas, attendees: Rosemary Bergin, Lucy Capurso, Isaac Cheng, Renee Kennedy, Claire Larkin, Victoria Mok, Emma Peterson, Emily Saddington, Anna Tran and Adrian Yeung