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Supplementary material

Determining optimal sampling strategies for monitoring threatened endemic macro-invertebrates in Australia's artesian springs

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Table S1. Recommendations for optimal methods of sampling the abundance of each taxa encountered in the present study

Explanations of each column can be found in the 'comment' embedded on the column. 'AREA VARIANCE' denotes the importance of sampling across areas: HIGH, strong differences across 'pool'/'tail'; MOD, indiscernible difference across areas or conflicting patterns across springs; LOW, no evidence for differences in abundance across areas. Recommended method is expressed as the recommended method type and the recommended sampling area/s (as defined in the Method section, Study Site) separated by a hyphen (-). Method type, either that which samples highest abundance with lowest variance, or highest abundance and is most time efficient. Sampling area, if HIGH or MOD 'Area variance', it is recommended that sampling be restricted to the area that is inhabited. The variance in abundance estimates found using the recommended method within a spring: HIGH, standard deviation greater than or equal to mean; MOD, standard deviation between 99 and 25% of the mean; LOW, standard deviation less than 25% of the mean. The importance of sampling across numerous springs because a taxon may be found only in a limited number of springs: HIGH, taxon was found only in one of the three sampled springs, suggesting its distribution is limited across springs and numerous springs should be sampled; MOD, taxon was found in two of the three springs, suggesting there may be some limitations on distribution; LOW, taxon was found in all springs and it is assumed, therefore, to be common across most springs, the importance of sampling numerous springs is therefore reduced. The average number of individuals of that taxon found in 10 cm^2 of the recommended method samples. The maximum and minimum numbers are also given as an indication of the variance across all springs sampled

Taxon	Highest abundance	Lowest variance	Area variance	Recommended method	Proportion of samples present	Within spring variance	Between spring variance	Average number of individuals per 100-cm^2 sample (minimum, maximum)	Observations	Recommendations
Atyidae	Scoop	Scoop	HIGH	Scoop-pool – >	0.67	HIGH	LOW	7 (0, 28)		Due to high within spring variance and rarity across samples it is advised to sample >5 times. No need to sample tail areas. Large and obvious when samples are emptied on to sampling tray so would be efficiently sampled alive then returned to the spring. Some variance in average abundance across springs so estimates will be more accurate if numerous pools are sampled.
Chiltonidae	Scoop	Core	MOD	Scoop-both – >	0.87	MOD	LOW	11 (0, 79)		Common across samples but due to high variance between samples sampling >10 times is advised. Sampling across 'areas' (pools and tails) is recommended as abundance is generally homogenous within the spring. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled.

Taxon	Highest abundance	Lowest variance	Area variance	Recommended method	Proportion of samples present	Within spring variance	Between spring variance	Average number of individuals per 100-cm^2 sample (minimum, maximum)	Observations	Recommendations
Cypridoidea	Scoop	Core	LOW	Scoop-both – >	0.93	MOD	LOW	36 (0, 243)	Scoop obtain slightly lower abundance estimates in area 2 consistently across springs	Common across samples but due to high variance between samples sampling >10 times is advised. Sampling across all 'areas' (pools and tails) is recommended as abundance is generally homogenous within the spring. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled. Sampling for this group may benefit from using a smaller mesh size.
Leptoceridae	Scoop	Scoop	LOW	Scoop-both – >	0.80	MOD	LOW	2 (0, 8)		Common across samples and moderate variance between samples so 10 samples across all 'areas' (pools and tails) should suffice as abundance is generally homogenous within the spring. Large and obvious when samples are emptied on to sampling tray so would be efficiently sampled alive then returned to the spring. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled.
Chironomidae	Scoop	Scoop	MOD	Scoop-both – >	0.93	HIGH	LOW	10 (0.58)	Higher abundance in tails but high variance obscures the overall comparison	Common across samples but due to high variance between samples sampling >10 times is advised. Sampling across 'areas' (pools and tails) is recommended as abundance is generally homogenous within the spring. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled.

Taxon	Highest abundance	Lowest variance	Area variance	Recommended method	Proportion of samples present	Within spring variance	Between spring variance	Average number of individuals per 100-cm^2 sample (minimum, maximum)	Observations	Recommendations
Ceratopogonidae	Scoop	Scoop	MOD	Scoop-both – >	0.87	HIGH	LOW	14 (0, 96)	Higher abundance in tails but high variance obscures the overall comparison	Common across samples but due to high variance between samples sampling >10 times is advised. Sampling across 'areas' (pools/tails) is recommended as abundance is generally homogenous within the spring. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled.
Hydryphantidae	Core	Core	MOD	Large core-tail – >	0.17	HIGH	LOW	< 1 (0, 4)	Higher abundance in tails but high variance obscures the overall comparison, cores were often only method with group present	Rare across space and few captured in each sample so a large sample size is recommended. Large core method recommended however, if sampling for other Acari a combined effort of numerous scoops within tail areas only may be more advisable. Found in most springs so numerous springs may not need be sampled.
Arrenuridae	Scoop	Scoop	HIGH	Scoop-tail – >	0.40	MOD	HIGH	< 1 (0, 1)	Rare	Rare across space and few captured in each sample so a large sample size is recommended. Large core method recommended however, if sampling for other Acari a combined effort of numerous scoops within tail areas only may be more advisable. Found in most springs so numerous springs may not need be sampled.

Taxon	Highest abundance	Lowest variance	Area variance	Recommended method	Proportion of samples present	Within spring variance	Between spring variance	Average number of individuals per 100-cm^2 sample (minimum, maximum)	Observations	Recommendations
Hydrophilidae	Core	Core	HIGH	Scoop-tail – >	0.93	MOD	LOW	12 (0, 38)	Includes larvae though adults were the majority of individuals	Common across samples with low variance so 5 samples within tails should suffice. Large core method recommended however, if sampling for other aquatic beetles (i.e. in combination with Dytiscidae) a combined effort of numerous scoops within tail areas may be more advisable. Large and obvious when samples are emptied on to sampling tray so would be efficiently sampled alive then returned to the spring. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled.
Dytiscidae	Scoop	Scoop	HIGH	Scoop-tail – >	0.60	HIGH	LOW	4 (0, 21)	Includes larvae though adults were the majority of individuals	Common across samples with low variance so 5 samples within tails should suffice. Large core method recommended however, if sampling for other aquatic beetles (i.e. in combination with Hydrophilidae a combined effort of numerous scoops within tail areas may be more advisable. Large and obvious when samples are emptied on to sampling tray so would be efficiently sampled alive then returned to the spring. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled.
Notonectidae	Scoop	Scoop	HIGH	Scoop-tail – >	0.20	HIGH	HIGH	< 1 (0, 1)	Rare	Rare across space and few captured in each sample so a large sample size is recommended. Only need sample in 'tail' as they are rare in pools. This group were only found in one spring suggesting patchy occupancy across springs – numerous springs may need to be sampled to ensure populations are found.

Taxon	Highest abundance	Lowest variance	Area variance	Recommended method	Proportion of samples present	Within spring variance	Between spring variance	Average number of individuals per 100-cm^2 sample (minimum, maximum)	Observations	Recommendations
Stratiomyidae	Scoop	Scoop	HIGH	Scoop-tail – >	0.27	HIGH	LOW	< 1 (0, 5)		Rare across space, few captured in each sample and highly variable across samples so a large sample size is recommended. Only need sample in 'tail' as they are rare in pools. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled.
Odonata larvae (OR)	Scoop	Core	MOD	Scoop-both – >	0.33	MOD	LOW	< 1 (0, 3)		Rare across space, few captured in each sample and moderate variance across samples so a large sample size is recommended. Large and obvious when samples are emptied on to sampling tray so would be efficiently sampled alive then returned to the spring. Those sampling this group would benefit from refining methods for sampling adults as well as larvae.
Tubifinidae	Core	Core	MOD	Large core-both – >	0.50	MOD	LOW	2 (0, 10)		Common across samples and moderate variance between samples so 10 samples across all 'areas' (pools and tails) should suffice as abundance is generally homogenous within the spring. Found in most springs and abundance across springs generally similar so numerous springs may not need be sampled.
Dugesidae	Scoop	Core	MOD	Scoop-both – >	0.15	HIGH	MOD	< 1 (0, 2)	Rare in samples but common when counts are made of visible individuals before physical sampling.	Other methods, possibly those that do not destructively sample, may need to be developed for this group as their abundance does not seem to be effectively captured by the methods tested here. Observed in most areas of the spring but not often.

Taxon	Highest abundance	Lowest variance	Area variance	Recommended method	Proportion of samples present	Within spring variance	Between spring variance	Average number of individuals per 100-cm^2 sample (minimum, maximum)	Observations	Recommendations
Planorbidae										
Gyraleus edgbastonensis	Small core	Small core	HIGH	Small core-pool – >	0.67	MOD	MOD	2 (0, 3)		Common across samples when taken within the pool of springs where they exist, but numerically rare and moderately variable so >5 samples within pools recommended. A large number of springs should be sampled as this species is not found in the pools of all springs. Often nestles amongst and upon vegetation so likely to suffer vegetation-based sampling biases if cores are not used.
Glyptophysa n. sp.	Large core	Large core	HIGH	Scoop-pool – >	0.60	HIGH	HIGH	< 1(0, 4)		Common across samples when taken within the pool of springs where they exist, but numerically rare and moderately variable so >5 samples within pools recommended. A large number of springs should be sampled as this species is not found in the pools of all springs. Large, low numbers per sample and obvious when samples are emptied on to sampling tray so would be efficiently sampled alive then returned to the spring.
Bithyniidae										
Gabbia fontana	Small core	Large core	MOD	Small core-both – >	0.83	MOD	LOW	6 (0, 13)	Highly variable patterns across tail and pool depending on the spring sampled (i.e. more abundant in tail of some springs, pool of others)	Common throughout the spring with moderate within spring variance meaning >10 samples across all areas of the spring recommended. Occupies different areas of different springs so it is recommended that a large number of springs be sampled.
Hydrobiidae										

Taxon	Highest abundance	Lowest variance	Area variance	Recommended method	Proportion of samples present	Within spring variance	Between spring variance	Average number of individuals per 100-cm^2 sample (minimum, maximum)	Observations	Recommendations
Jardinella acuminata	Small core	Small core	HIGH	Small core-both – >	1.00	LOW	MOD	5 (0, 6)	High potential for spatial variance across seasons within a spring as more prevalent in tails and up to four times more numerous overall in July	Common across samples in the springs in which the species is found and low within spring variance so 5 samples across areas may suffice. Numerous springs should be sampled as they are not found in all springs and average abundance varied considerably across springs (~100 per sample – ~10). Seems to expand distribution into tail areas in cooler months meaning abundance estimates may vary considerably across seasons – any enquiry into this species should consider incorporating a seasonal component.
Jardinella jesswiseae	Large core	Small core	HIGH	Small core-pool – >	0.33	HIGH	LOW	1 (0, 2)	High potential for spatial variance across seasons within a spring as more prevalent in tails and up to four times more numerous overall in July	Rare across samples, when only taken within pools, and high within spring variance so > 5 samples recommended. Numerous springs should be sampled despite the fact they were found in all springs as average abundance varied considerably across springs (~200 per sample – ~10). Seems to expand distribution into tail areas in cooler months meaning abundance estimates may vary considerably across seasons – any enquiry into this species should consider incorporating a seasonal component.

Taxon	Highest abundance	Lowest variance	Area variance	Recommended method	Proportion of samples present	Within spring variance	Between spring variance	Average number of individuals per 100-cm^2 sample (minimum, maximum)	Observations	Recommendations
Jardinella pallida	Small core	Large core	HIGH	Small core-pool – >	1.00	HIGH	LOW	9 (0, 20)	High potential for spatial variance across seasons within a spring as more prevalent in tails and up to four times more numerous overall in July	Rare across samples, when only taken within pools, and high within spring variance so > 5 samples recommended. Numerous springs should be sampled despite the fact they were found in all springs as average abundance varied considerably across springs (~200 per sample – ~10). Seems to expand distribution into tail areas in cooler months meaning abundance estimates may vary considerably across seasons – any enquiry into this species should consider incorporating a seasonal component.
Jardinella corrugata	Small core	Large core	LOW	Small core-both – >	0.33	HIGH	LOW	1 (0, 2)		Common across samples but high variance between samples so >5 samples across all 'areas' (pools and tails) should suffice. Found in most springs but abundance across springs ranges considerably so numerous springs need be sampled. Differentiating juvenile individuals of this species from closely related *J. edgbastonensis* is difficult and may affect estimates.