Fish species diversity and assemblage structure in seasonal wetlands in the dry dipterocarp forests of Kulen Promtep Wildlife Sanctuary, Cambodia

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មូលន័យសង្ខេប

ព្រៃរបោះ (Dipterocarp forest) ជាព្រៃដែលគេប្រទះឃើញមានច្រើននៅទំនាបភាគខាងជើងនៃប្រទេសកម្ពុជា ប៉ុន្តែមានការ យល់ដឹងតិចតួចនៅឡើយអំពីមច្ឆាជាតិដែលរស់នៅតាមរដូវ នៅតាមដីសើមនៃព្រៃប្រភេទនេះ។ ជាមួយនឹងសម្ពាធកើនឡើងទៅលើ ធនធានតំបន់ដីសើមនៅក្នុងប្រទេសកម្ពុជា ព័ត៌មានស្ដីពីតម្លៃនៃតំបន់ដីសើមទំហំតូចគឺជាភាពចាំបាច់។ យើងបានពិពណ៌នាពី សមាសភាពត្រីនៅតំបន់ដីសើមក្នុងដែនជម្រកសត្វព្រៃកូលែនព្រហ្មទេព ដោយផ្អែកលើការប្រមូលទិន្នន័យនៅដើមរដូវវស្សា ក្នុងរដូវ វស្សា និង រដូវប្រាំងនាអំឡុងឆ្នាំ២០១៥ ដល់ ២០១៦។ ចំនួនត្រីសរុប១៨៩៥ក្បាល ត្រូវជា៥៣ប្រភេទ និង ១៧អំបូរត្រូវបានចាប់។ សមាសភាពរបស់ត្រីមានភាពសម្បូរបំផុតនៅអំឡុងរដូវវស្សា ផ្អែកលើការវិភាគទិន្នន័យតាមម្បូវិធី Shannon-Weiner index និង Species richness។ យើងមិនអាចធ្វើការញែកអំពីសមាសភាពត្រីក្នុងរដូវផ្សេងៗបានទេ តាមកម្មវិធីវិភាគទិន្នន័យ non-metric multi-dimensional scaling ដោយសារភាពឧស្សាហ៍ក្នុងដំណើរការប្រមូលទិន្នន័យរវាងរដូវប្រាំង និង រដូវវស្សា។ គ្មានភាពទំនាក់ ទំនង(correlation)គ្នាគួរឲ្យកត់សម្គាល់ទេ រវាងតំបន់ដីសើម(log-transformed wetland areas) និង នានាភាពដែលបាន វាស់វែង(diversity measure) ទោះបីជាជម្រៅទឹកអតិបរមា និង នានាភាពមានទំនាក់ទំនងគ្នាគួរឲ្យកត់សម្គាល់ និង ជាវិជ្ជមានក៏ ដោយៗ ម៉ូដែលBinomial generalized linear models ត្រូវបានប្រើដើម្បីកំណត់ឲ្យដឹងថា តើរដូវ ភាពភ្ជាប់ជាមួយនឹងប្រភាព ទឹកជាអថេរព្រៃ ឬ ជម្រៅទឹកអតិមាមានភាពជាប់ទាក់ទងទៅនឹងត្រីទាំងប្រាំប្រភេទដែលសម្បូរជាងគេឬទេ។ គ្មានម៉ូដែលណាមួយ បានបង្ហាញពីភាពមានទំនាក់ទំនងគ្នាគួរឲ្យកត់សម្គល់នោះទេ បើទោះជាជម្រៅទឹកគឺជាម៉ូដែលដែលបង្ហាញពីភាពមានទំនាក់ទំនង គ្នាសម្រាប់ប្រភេទត្រីនីមួយៗក៏ដោយៗ លទ្ធផលនៃការសិក្សានេះបង្ហាញថា ជម្រៅទឹកអាចជាកត្តាមួយយ៉ាងសំខាន់ដែលមាន ឥទ្ធិពលទៅដល់ភាពសម្បូរបែបនៃសមាសភាពត្រី និង វត្តមានរបស់ប្រភេទដែលសម្បូរទាំងឡាយនៅក្នុងតំបន់ដីសើមតាមរដូវៗ វិធី សាស្ត្រប្រមូលទិន្នន័យសាកល្បងរបស់យើង ក៏អាចបង្ហាញពីវិធីសម្រាប់ធ្វើការវាយតម្លៃតំបន់ដីសើមតូចៗនៅក្នុងតំបន់ ហើយយើង ផ្ដល់នូវអនុសាសន៍សម្រាប់ធ្វើការកែលម្អវិធីសាស្ត្រទាំងនេះឲ្យកាន់តែល្អប្រសើរៗ

Abstract

Dipterocarp forests are common in the northern plains of Cambodia, but little is known about the fish that occupy seasonal wetlands in these. With pressures increasing on Cambodian wetland resources, more information is needed on the value of small wetlands. We describe fish assemblages in seasonal wetlands in Kulen Promtep Wildlife Sanctuary based on sampling completed during the early-wet, wet and dry seasons in 2015–2016. A total of 1,895 fish were captured, representing 53 species in 17 families. Fish assemblages were most diverse during the wet season according to the Shannon-Weiner index and species richness. We were not able to identify distinct seasonal assemblages using non-

metric multi-dimensional scaling, which may have been due to differences in sampling effort between seasons. There was no significant correlation between log-transformed wetland area and diversity measures, although correlations between maximum water depth and diversity were significant and positive. Binomial generalized linear models were used to examine whether season, connectivity to permanent water bodies (categorical) and maximum water depth were related to presence of the five most common species. None of the models revealed significant relationships, although depth was in the best fit model for each species. These results indicate that water depth may be important in influencing the diversity of fish assemblages and presence of common species in seasonal wetlands. Our pilot of rapid sampling methods can inform protocols for assessing small wetlands in the region and we provide recommendations for improving these methods.

Keywords Forest, intermittent wetlands, Shannon–Weiner diversity index, species richness.

Introduction

Over 30% of Cambodia is covered by wetlands (Kosal, 2004) which are important habitats for humans and wildlife. Cambodian wetlands provide numerous ecosystem services including food, medicines, firewood, irrigation water, aquaculture, tourism, transportation, flood protection and habitat for endangered species (Kol, 2003; Loeung *et al.*, 2015). Freshwater fish and fish products account for a relatively high portion of total protein consumed in Cambodia (Needham & Funge-Smith, 2015) and seasonally-inundated wetlands provide breeding, nursery and feeding habitats for these. Wetland fish also provide ecosystem services beyond food security (Cowx & Portocarrero Aya, 2011), such as supporting piscivorous wildlife. The rapid development and land use changes currently occurring in Cambodia are increasing pressures on wetland resources and highlight the need for more information on their values.

The lower Mekong River Basin has a monsoonal climate with a dynamic annual flood pulse and the productivity of wetlands in the region depends on the substantial differences between the wet and dry seasons (Kosal, 2004). During the wet season, flooding occurs in forested areas of Cambodia and receding waters are retained in seasonal wetlands that persist into the dry season (Kol, 2003). Deciduous dipterocarp forests in Cambodia are generally understudied and under-protected (Wohlfart *et al.*, 2014), but are common across the Northern Plains where an estimated 12,000 wetlands exist (Barzen, 2004). While descriptions of fish assemblages exist for the floodplains of the Tonle Sap Lake (e.g., Campbell *et al.*, 2006) and flooded forest habitats adjacent to the Mekong River (e.g., Baird, 2007), little information is available for the many seasonal wetlands scattered across the Northern Plains.

Understanding what influences the structure of wetland fish assemblages is valuable because they may not form a single management unit and a large variety of wetlands may need to be conserved to adequately represent fish species diversity within a region (Pazin *et al.*, 2006). Seasonal wetlands can become harsh environments for fish if they are disconnected during the dry season (e.g., low oxygen, high water temperatures, exposure to predation, complete loss of water) and these stresses can structure aquatic communities. Studies of habitat relationships with fish assemblages in seasonal wetlands have had mixed results. For instance, Fernandes *et al.* (2010) examined the influence of depth, vegetation biomass and distance from permanent water bodies on fish in temporary wetlands in Pantanal, Brazil and found a positive relationship between water depth and species richness, but no relationship with the linear distance from the nearest permanent water body. Another study of seasonal wetlands in Florida revealed that connectivity with permanent water bodies was the dominant influence on fish assemblages, but that correlated variables such as depth and hydro-period were also important (Baber *et al.*, 2002). In artificial and natural depressions adjacent to the Oueme River in Africa, fish communities were dominated by piscivores tolerant of hypoxia during low water periods, indicating that these communities were likely influenced by dissolved oxygen and predation or both. Consequently, understanding the relationships between wetland characteristics and fish assemblages can help to determine how human activities that influence these characteristics may also affect fish and their ecosystem services.

This paper was prepared as part of a multi-disciplinary project that sought to advance understanding of the value of wetland ecological functions and ecosystem services through a rapid assessment of seasonal wetlands in the dry dipterocarp forests of Cambodia and Vietnam. The purpose of project in Cambodia was to provide managers at the Ministry of the Environment and the Kulen Promtep Wildlife Sanctuary with baseline infor-

mation to inform effective management and establish a basis for more extensive studies in the future.

Our study had three objectives: 1) to describe the fish diversity and assemblages of wetlands sampled, 2) to explore how variations in wetland size (area and maximum water depth) and connectivity (isolated or connected) influence fish diversity and the presence of the most common fish species, and 3) to pilot rapid sampling methods that could be used in protocols for sampling small, seasonal wetlands throughout the Mekong River Basin. We are unaware of any previous systematic surveys of fish in the seasonal dry forest wetlands in Cambodia, and so our overall aim was to improve understanding of the value of this ecosystem and inform its management.

Methods

Study Area

Our study was undertaken in Kulen Promtep Wildlife Sanctuary (KPWS), Cambodia's largest protected area, which is located in the country's Northern Plains (Edwards, 2012; Fig. 1). The sanctuary covers 4,099 km^2 and is managed by the Ministry of Environment with assistance from the Wildlife Conservation Society, which has supported ecotourism and efforts to improve livelihoods of communities inside the sanctuary through conservation-friendly rice cultivation (Souter *et al.*, 2016). The wildlife sanctuary is situated in the upper Stung Sen River catchment, a tributary of the Tonle Sap Lake.

We used rapid sampling techniques to gather data on wetlands in KPWS. Fish sampling was conducted near four communities (Tmart Boey, Rum Check, Sambour and Prey Veng) within the sanctuary in Preah Vihear Province. Landcover in this part of Preah Vihear mainly comprises open deciduous dipterocarp forests, grassland savannah and seasonal wetlands. Wetlands for sampling were selected to cover a range of habitat types based on interviews with village leaders.

Sampling was undertaken in June 2015 (early-wet season), October 2015 (wet season) and January 2016 (dry season). The wet season was considered to occur from June through October and the dry season from November through May. Sampling in June 2015 and January 2016 included our entire team, whereas October 2015 comprised sampling for fish diversity only. Consequently, some our analyses are limited to the early-wet and dry seasons when broader datasets were gathered.

Fish collection

Wetlands are dynamic environments that can be difficult to sample for fish (Kaller *et al.*, 2013). Those with underwater vegetation are especially difficult to sample because the vegetation can get in the way and capture
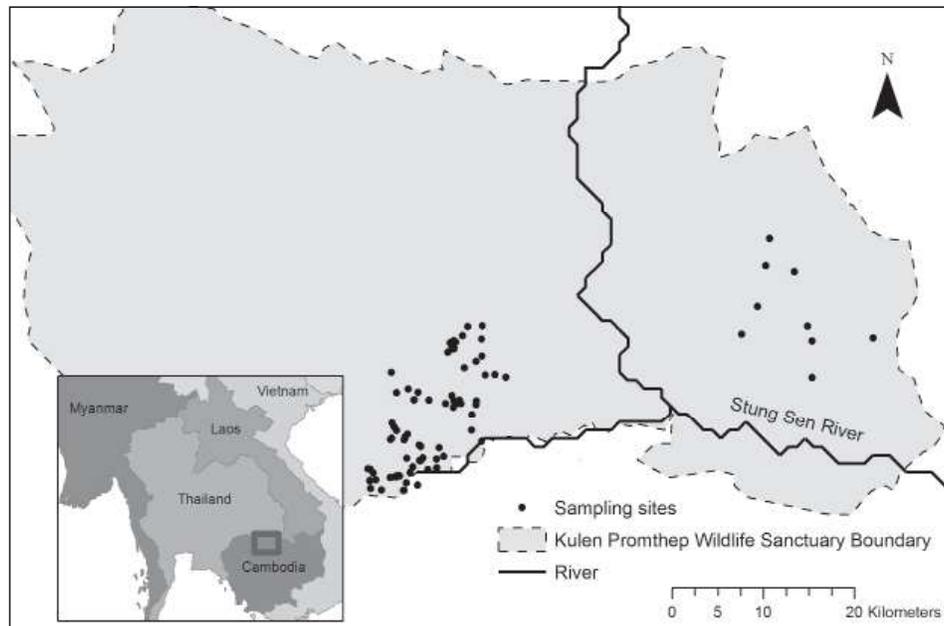
Fig. 1 Sampling sites at Kulen Promtep Wildlife Sanctuary for which GPS data were available.

efficiency can vary by gear type (Knight & Bain, 1996). We used a combination of gear types to address these challenges and employed a rapid survey approach with active sampling methods because each wetland was typically visited for one hour or less due to time constraints. Consequently, active gear methods were used at all wetlands where water was found and passive sampling gear was used opportunistically where time permitted.

In active sampling, we first used backpack electro-fishing for up to 10 minutes in each wetland, depending on the size of the water body. As some were very small (i.e., less than 2 m^2 in area), less than ten minutes was considered sufficient for sampling. In shallow wetlands where researchers could wade, electrofishing was undertaken in zig-zag transect lines across the centre of the wetland from one end to the other. For deeper wetlands where researchers could not wade, electrofishing was confined to areas along the shoreline. Start and end times were recorded to calculate the associated effort. Second, we also used a 1.2 x 3.7 m polyethylene fibre seine net with 0.64 cm mesh (Frabill, Plano, USA) to sweep-sample each wetland. To this end, two people completed a five-metre pass adjacent to the shoreline which was undertaken three times at each site, for a total seine distance of 15 m, covering different areas each time where possible. While some of the sampling gear we employed is prohibited for fishing in Cambodia, its use was permitted in our study by the Inland Fisheries Research and Development Institute (IFReDI) and KPWS and staff from both organisations participated in the sampling.

Our survey was intended to be rapid, but where time allowed, passive sampling gear (i.e., gill nets and minnow traps) were deployed at sites with a water depth of >30 cm to obtain further data on species presence. As these gears were not consistently used, the data are not included in analysis apart from summary information on overall species richness and total abundance of each species. Gill nets (mesh size 1.5 cm and 1.25 cm) were set across the deepest parts of wetlands if these were small, or in waist-deep water near the shore if they were large. The gill nets were left in the water for at least 30 minutes. Four minnow traps (Primer TR-501; 25.4 x 25.4 x 45.7 cm; Gardena, USA) were set in wetlands sufficiently deep to submerge the trap opening. Each trap had two entrances (6.35 cm in width) and a piece of bread or ball of sticky rice was placed into the bait pocket inside. Where possible, wetlands sampled with the traps were divided into four approximately equal quadrants with one minnow trap placed in the centre of each. If the wetland was prohibitively large, individual traps were deployed at least 5 m apart along the shore line, close to where active sampling took place. Traps were left to soak for at least 30 minutes. The start and end times of gill net and minnow trap deployments were recorded.

Sample processing

Fish were collected alive and processed separately by sampling gear at each site. Each fish was identified to species based on Rainboth (1996), Vidthayanon (2008), and an unpublished IFReDI fish identification guide. Where species identification was uncertain, the individual was preserved in ethanol for later identification at the IFReDI laboratory. All other fish were released alive into the wetlands following processing, which included photographs of individuals of most species. Fish names in this study follow valid species names in Eschmeyer *et al.* (2016).

Habitat characteristics

Data on wetland size and connectivity were recorded at each wetland during the June 2015 (early wet season) and January 2016 (dry season) sampling.

Wetland length and width were measured in metres in the field (longest diameter either way) for smaller wetlands. These measurements were multiplied to give an estimate for wetland area. When too large to measure this way, the surface area of wetlands was derived from GPS tracks of the boundary or from satellite images. Water depth was measured in centimetres at one metre intervals along a profile transect from the edge to the centre of each wetland. The deepest point along the transect was taken as the maximum depth for a wetland. Wetlands deeper than approximately 1.5 m were not measured further for depth. Wetlands were recognized as either 'connected' (via channel or sheet flow from a nearby river) or 'isolated'. In the early wet season, connectivity was determined through site-based observations. As this was not always clear during the dry season however, connectivity with a permanent water body during this period was sometimes assigned using information obtained from local villagers or field guides familiar with the site.

Analysis

The extent of fish occupation in each season was determined by comparing the percentage of inhabited wetlands between the three sampling periods. The relative abundance of each wetland was calculated as the combined total of fish recorded from the two active sampling methods (electrofishing and seine nets) undertaken at every site.

We calculated species diversity metrics for all three sampling periods, although analyses of relationships between diversity and wetland characteristics were confined to the early-wet and dry season sampling events (when these data were collected). Species diversity was examined using two metrics based on data from electrofishing and seine netting: species richness (total number of species per wetland) and the Shannon–Weiner diversity index (H'). The Shannon-Weiner index was calculated for all wetland sites where fish were collected using the Vegan package in the R statistical programme (R Core Team, Austria). Pearson's correlations were used to explore relationships between wetland size and diversity metrics.

Use of a linear mixed-effect regression model was initially considered in analysis, but plots of relationships between species diversity, richness and wetland size and depth did not indicate any patterns for isolated and connected wetlands that warranted such a model. Binomial generalized linear models were consequently employed to examine whether sampling season, wetland connectivity and depth were related to the presence and absence of the five most common fish species. These species were selected because they represented the vast majority of the catch and were the only species that individually comprised >5% of the total catch. Sample sizes for other species were too small to justify such analysis.

Prior to model development, correlation analysis was undertaken to test for multi-collinearity between wetland size and depth. A positive correlation was found between maximum depth and the log-transformed wetland size (Pearson's correlation: $df=15$, $r=0.66$, $p=0.004$). Wetland area was not necessarily a good indicator of water volume because different approaches were used to generate these data and water depth measurements might better reflect the water volume of a wetland during sampling. Because the two variables were correlated, depth was employed in candidate models. Because depth data were not collected from a few sites where fish were sampled, our dry season

analysis was confined to 17 sites where both types of data were available (Table 1). Eight candidate models were tested for each species: 1) null, 2) season, 3) connectivity, 4) depth, 5) connectivity + depth, 6) season + depth, 7) season + connectivity, and 8) season + connectivity + depth. Model selection was performed using Akaike's Information Criterion (AIC) to determine the best model or set of models for each species. The AIC scores were used to quantitatively rank each model and the model with the lowest AIC value (AICmin) was considered the best. Differences in using Akaike's Information Criterion (AIC) from the lowest value were calculated as $\Delta i=AICi-AICmin$.

Non-metric Multidimensional Scaling (NMDS) was applied using the 'metaMDS' function in the Vegan package of R software to visualize differences in species assemblages between sampling sites based on Bray-Curtis dissimilarity values. This analysis was confined to data from active sampling methods and only included species found in more than two sites. Clusters of similar assemblages were defined in the visual analysis to distinguish the separate seasons. Three dimensions produced adequate configuration between observed dissimilarity and ordination stress.

Cluster analysis was used to examine whether certain species assemblages occurred due the connectivity categories of the wetland (connected vs. isolated). Bray-Curtis dissimilarities were calculated using data from the January 2016 dry season using the 'vegdist' function in the Vegan package of R software. An agglomerative hierarchical clustering analysis using the 'hclust' function in R with a complete-linkage algorithm was used to characterize fish assemblages in the dry season alone based on log-transformed abundance data from active sampling ($n=20$ sites). This was done because our early-wet season sample size was too small to analyze this way and because our wet season dataset lacked connectivity data. Complete-linkage looks at similarity between a sample and the farthest member of its cluster, which

Table 1 Summary characteristics of wetlands sampled at Kulen Promtep Wildlife Sanctuary. Area and depth data were not collected at all sites (sample sizes are given in parentheses). [1] Three sites had water deeper than 100 cm.

Season	No. of sample sites	No. of sites with fish	Connected / isolated	Size (m²)	Maximum depth (cm)
Early-wet (June 2015)	5	4	2 / 3	896–12,821 (4)	28–63 (3)
Wet (October 2015)	13	12	n/a	n/a	n/a
Dry (January 2016)	23	20	12 / 11	305–425,258 (17)	10–124 (17[1])
Total	41	36			

tends to produce tight, compact clusters (Krebs, 1999). Isolated sites were labelled separately and the data were visually examined using a dendrogram to identify clear groupings of assemblages that corresponded to our connectivity categories.

Results

Species composition and relative abundance

A total of 41 sites were sampled in KPWS for fishes during the early-wet (*n*=5), wet (*n*=13) and dry (*n*=23) seasons (Table 1). We were not able to sample all sites visited, because although 56 sites were visited during the early-wet season, most did not contain water yet. Fish were present in 80%, 92%, and 87% of wetlands sampled in the early-wet, wet and dry seasons, respectively. The mean relative abundance of fish (combined data for all species from active sampling methods) was highest in the early-wet season with 56 fish per wetland (SD=56.53; Table 2), which was highly influenced by a single wetland which included 120 individuals of *Trichopodus trichopterus*. In general, there was high variability in fish abundance between wetlands in all seasons.

Combining data from all sampling methods (passive and active), a total of 53 species arranged in 32 genera and 17 families were represented among the 1,895 fish captured (Table 3). All species recorded were native to Cambodia and included one Near Threatened (*Clarias macrocephalus*) and one Vulnerable (*Oxygaster pointoni*) taxon according to the IUCN Red List, in addition to one species considered rare in Cambodia (*Puntigrus partipentazona*). *Trichopodus trichopterus* was found in the greatest number of wetlands (85% of sites sampled) and was captured in all seasons. This was also the most common species encountered, comprising 22.3% of all fish, particularly during the early-wet season. *Trichopsis vittata* was also common in the wet (October) and dry (January) seasons and occasionally in the early-wet season. This occurred at 83% of sites and comprised 11.8% of all individuals. *Esomus metallicus* (present at 66% of sites) and *Rasbora paviana* (68%) were similarly common, comprising 13.7% and 13.4% of all individuals respectively, although neither was captured in the early-wet season. Following these, *Rasbora borapetensis* comprised 7.4% of all individuals (present 34% of sites) and *Anabas testudineus* was frequently encountered in all seasons (at 66% of sites), although at lower abundances (3.6% of all individuals).

Ordination results (NMDS stress=0.123; Fig. 2) based on the standardized abundance of fish species recorded in active sampling did not exhibit distinct seasonal clusters and differences were mostly driven by sites dominated by a single species. The early-wet season contained the most distinctive group, but this was driven mainly by one site (KP28) where a single *Monopterus albus* was captured. The early-wet season also had a very low sample size (four sites where fish were captured) compared to other seasons. One dry season site (KP103) only had abundant *A. testudineus*, while another (KP92) contained many *T. vittata*. In the wet season, three sites (KP122, KP123, KP124) each contained *E. metallicus* and different *Trichopsis taxa*, although *E. metallicus* was found in both the wet and dry seasons. Our analysis included species captured at three or more sites, but when all species were included the resulting clusters were even less distinct by season. Excluding sites with a single species (*n*=3), ordination results (NMDS stress = 0.133; Figs 3–4) appeared as concentric polygons, the largest of which was the dry season which had the greatest variation in species. However, this was also the season with the greatest number of samples, whereas the season with the least variability also had the lowest number of samples (early-wet season).

Fish richness and diversity

Based on active sampling methods, the maximum species richness per wetland was 12, 15 and 15 species in the early-wet, wet and dry seasons respectively. Individual

Table 2 Fish abundance, species richness and diversity (Shannon-Weiner *H′*) of wetlands in Kulen Promtep Wildlife Sanctuary by season. Figures given in parenthesis represent standard deviation.

Season	Abundance		Diversity		Species richness	
	Mean	Range	Mean	Range	Mean	Range
Early-wet (June 2015)	56.00 (56.53)	1–135	1.01 (0.93)	0.00–2.07	6.25 (4.50)	1–12
Wet (October 2015)	30.25 (20.69)	2–80	1.39 (0.72)	0.16–2.43	7.50 (4.81)	2–15
Dry (January 2016)	45.70 (77.77)	2–362	1.10 (0.61)	0.00–2.36	5.40 (3.66)	1–15

Table 3 Fish species richness and abundance by season in Kulen Promtep Wildlife Sanctuary based on active and passive sampling methods. Status: LC=Least Concern, DD=Data Deficient, NA=Not Assessed, NT=Near Threatened, VU=Vulnerable.

No.	Family, Species	Status	Season			Total
			Early-Wet	Wet	Dry	
	Ambassidae					
1	*Parambassis apogonoides*	LC			1	1
	Anabantidae					
2	*Anabas testudineus*	DD	7	15	47	69
	Bagridae					
3	*Mystus atrifasciatus*	LC		1		1
4	*Mystus multiradiatus*	LC			9	9
5	*Mystus mysticetus*	LC		7	5	12
	Balitoridae					
6	*Nemacheilus pallidus*	LC			1	1
	Belonidae					
7	*Xenentodon* sp.	N/A		1	1	2
	Channidae					
8	*Channa gachua*	LC	1	2		3
9	*Channa striata*	LC	6	4	5	15
	Clariidae					
10	*Clarias batrachus*	LC			2	2
11	*Clarias macrocephalus*	NT			1	1
	Cobitidae					
12	*Lepidocephalichthys hasselti*	LC	3	3	26	32
	Cyprinidae					
13	*Amblypharyngodon chulabhornae*	LC		1	3	4
14	*Barbodes aurotaeniatus*	LC	4	19	67	90
15	*Cyclocheilichthys apogon*	LC		7	4	11
16	*Cyclocheilichthys armatus*	LC		6	6	12
17	*Cyclocheilichthys lagleri*	LC			6	6
18	*Esomus longimanus*	DD		2		2
19	*Esomus metallicus*	LC		48	212	260
20	*Henicorhynchus lobatus*	LC			2	2
21	*Henicorhynchus siamensis*	N/A	1		8	9
22	*Labiobarbus leptocheilus*	N/A	3	2		5
23	*Labiobarbus siamensis*	LC			1	1
24	*Laubuka caeruleostigmata*	N/A		1		1
25	*Laubuka lankensis*	N/A		10	1	11
26	*Osteochilus lini*	LC			7	7
27	*Osteochilus vittatus*	LC	10	2	2	14
28	*Oxygaster anomalura*	LC		8		8
29	*Oxygaster pointoni*	VU		6	4	10
30	*Parachela maculicauda*	LC			1	1
31	*Parachela oxygastroides*	LC		6		6

Table 3 Continued.

No.	Family, Species	Status	Early-Wet	Wet	Dry	Total
			Season			
32	*Parachela siamensis*	LC			4	4
33	*Puntius brevis*	LC		8	37	45
34	*Puntigrus partipentazona*	LC		1		1
35	*Rasbora aurotaenia*	LC			10	10
36	*Rasbora borapetensis*	LC	10	3	127	140
37	*Rasbora paviana*	LC		121	133	254
38	*Rasbora trilineata*	LC		16		16
39	*Rasbosoma spilocerca*	N/A	2			2
40	*Systomus orphoides*	N/A		10	1	11
41	*Thynnichthys thynnoides*	LC			25	25
	Eleotridae					
42	*Oxyeleotris marmorata*	LC			1	1
	Hemiramphidae					
43	*Dermogenys siamensis*	LC	4	16	6	26
	Mastacembelidae					
44	*Macrognathus siamensis*	LC			1	1
	Nandidae					
45	*Pristolepis fasciata*	LC		4		4
	Notopteridae					
46	*Notopterus notopterus*	LC			1	1
	Osphronemidae					
47	*Trichopodus microlepis*	LC	5	2	37	44
48	*Trichopodus trichopterus*	LC	123	59	240	422
49	*Trichopsis pumila*	LC	12	4	21	37
50	*Trichopsis vittata*	LC	31	32	161	224
	Siluridae					
51	*Ompok siluroides*	N/A		8	5	13
52	*Ompok eugeneiatus*	N/A			3	3
	Synbranchidae					
53	*Monopterus albus*	LC	2	1		3
	Abundance		1235	224	436	1895
	Species richness		16	34	41	53

wetlands in the wet season had higher species richness and were most diverse (Table 2; Fig. 5), although overall species richness for all sites combined was highest in the dry season (Table 3). There was a significant correlation between log-transformed total abundance and log-transformed species richness (Pearson's correlation: df=38, r=0.80, p<0.001; Fig. 6) for all seasons combined, indicating that more species were generally found in wetlands with a greater abundance of fish.

### Wetlands characteristics and fish diversity

Sites surveyed for wetland characteristics were well balanced between the connected and isolated categories (Table 1). Individual wetlands ranged in size from 305 m²
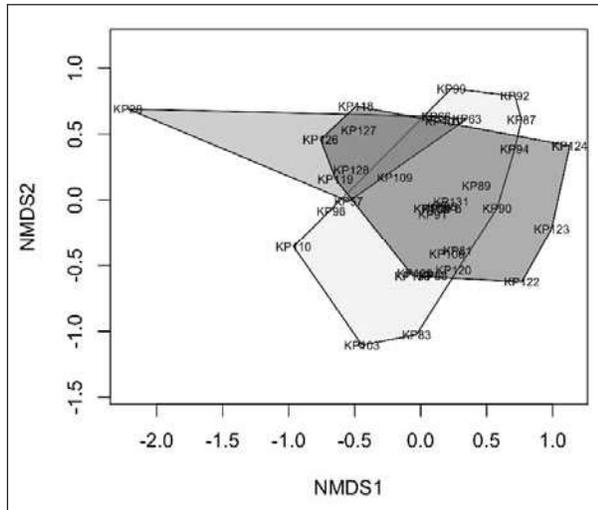
Fig. 2 Non-metric multidimensional scaling ordination plot of sampling sites based on standardized fish species abundance in the early-wet (mid-grey), wet (dark grey) and dry (light grey) seasons for taxa at ≥3 sites in Kulen Promtep Wildlife Sanctuary.
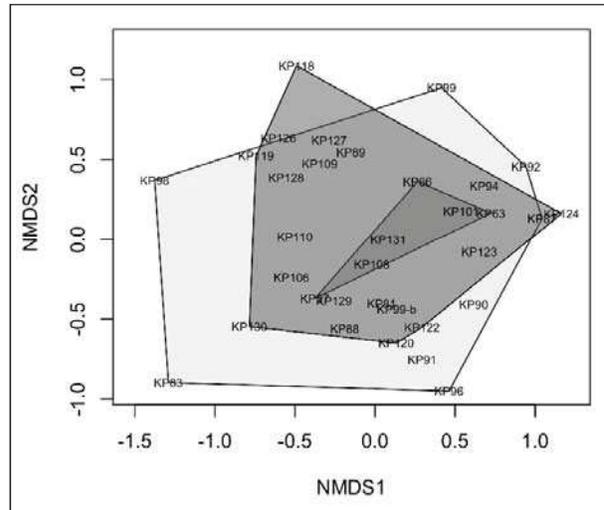
Fig. 3 Non-metric multidimensional scaling ordination plot of sampling sites based on standardized fish species abundance in the early-wet (mid-grey), wet (dark grey) and dry (light grey) seasons for taxa at ≥3 sites and sites with >1 taxon in Kulen Promtep Wildlife Sanctuary.
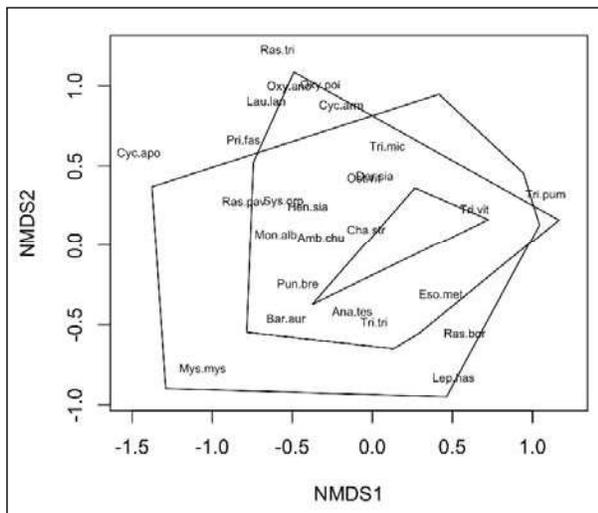
Fig. 4 Non-metric multidimensional scaling (NMDS) ordination plot of sampling sites based on standardized fish species (labeled) abundance in the early-wet, wet and dry seasons for taxa at ≥3 sites and sites with >1 taxon in Kulen Promtep Wildlife Sanctuary. The polygons define the same seasons depicted in Fig. 3.
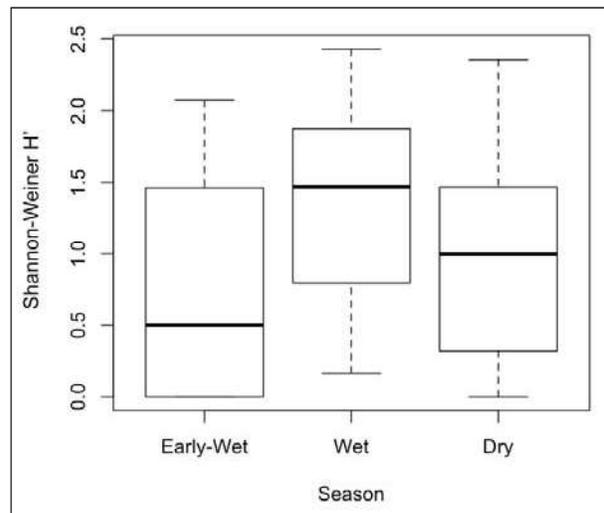
Fig. 5 Box plot of species diversity by season based on active sampling of sites in early-wet (n=4), wet (n=12) and dry (n=20) season in Kulen Promtep Wildlife Sanctuary.

to 425,258 m^2 (mean=28,637 m^2) and in depth from 10 cm to >100 cm (mean=46 cm for precisely measured depths). There was no significant correlation between log-transformed wetland area and log-transformed species rich-

ness (Pearson's correlation: df=20, r=0.12, p=0.589; Fig. 7) or between log-transformed wetland area and H' (Pearson's correlation: df=20, r=0.23, p=0.298; Fig. 8). The same was true for log-transformed maximum depth and log-transformed species richness (Pearson's correlation: df=18, r=0.40, p=0.080) and for log-transformed depth and H' (Pearson's correlation: df=18, r=0.38, p=0.101).
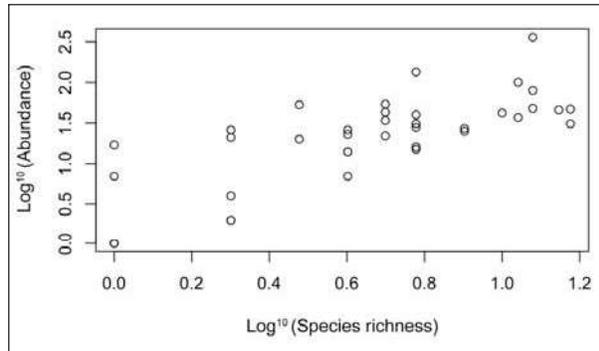
Fig. 6 Relationship between log-transformed abundance and species richness for all seasons (active sampling only) in Kulen Promtep Wildlife Sanctuary.

However, maximum depth and species richness (all connectivity levels and seasons combined) were significantly correlated, although this was heavily influenced by a single outlier (Pearson's correlation: df=18, r=0.48, p=0.032; Fig. 9). There was also significant correlation between maximum depth and H' (Pearson's correlation: df=18, r=0.53, p=0.015; Fig. 9).

No patterns related to the wetland connectivity were apparent in the dendrogram characterizing fish assemblages actively sampled in the dry season. However, the dendrogram indicated that geographically closer sites and those sampled closer in time were more similar, suggesting potential issues of spatial or temporal autocorrelation.

Species-habitat associations

None of the eight regression models we fitted for each of the five most common species (*T. trichopterus*, *E. metallicus*, *T. vittata*, *A. testudineus* & *R. paviana*) appeared to have strong support. However, the models based on depth alone were most supported according to AIC fit (except for *E. metallicus*, for which the best model was depth + season), although coefficient estimates indicated the depth variable was never significant (Appendix 1).

Discussion

Fish assemblages in seasonal wetlands of KPWS

Fish assemblages in the seasonal wetlands of KPWS were dominated by common species native to Cambodia. For example, *T. trichopterus* and *T. vittata* are known to seasonally occupy shallow, sluggish or standing water habitats and *E. metallicus* moves into seasonally flooded habitats like rice paddies, canals and ditches (Rainboth,
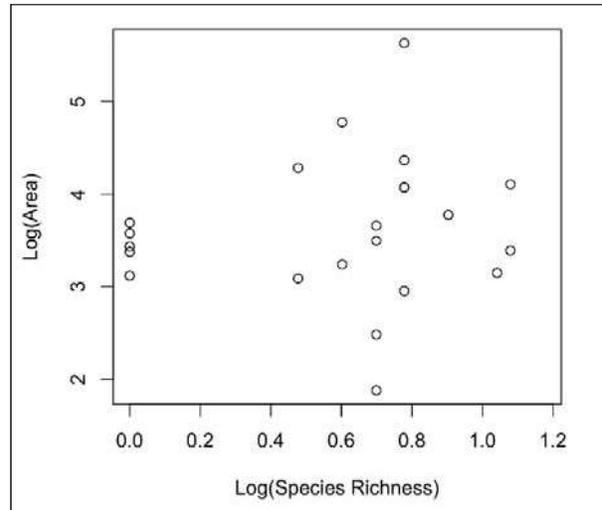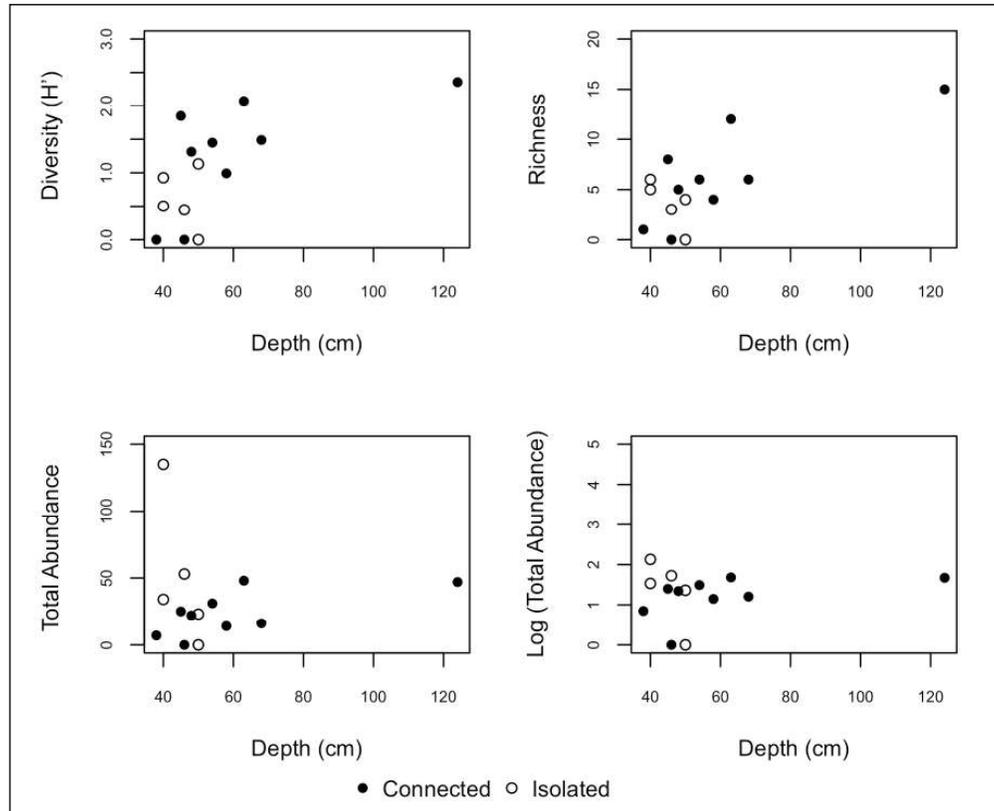

Fig. 7 Relationship between log-transformed wetland area and species richness for all seasons (active sampling only) in Kulen Promtep Wildlife Sanctuary.
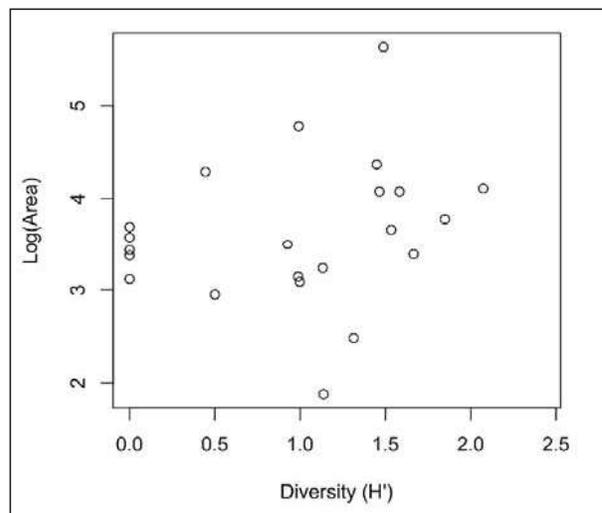
Fig. 8 Relationship between log-transformed wetland area and species diversity for all seasons (active sampling only) in Kulen Promtep Wildlife Sanctuary.

1996). In all seasons, most wetlands sampled (>80%) were occupied by fish and some contained at least 15 species, indicating that these sites can provide valuable fish-related ecosystem services such as food for piscivorous wildlife (e.g., endangered water birds) and humans, even in the dry season and early-wet season. It was not possible in our visual analysis of ordination results to describe separate fish assemblages or define groups of indicator species based on season. Differences in sample

Fig. 9 Relationships between maximum depth (cm) and fish diversity (top left), species richness (top right), total abundance (bottom left) and log-transformed total abundance (bottom right) for isolated (empty symbols) and connected (solid symbols) wetlands at Kulen Promtep Wildlife Sanctuary.

size between season may have confounded interpretation. While a few species were only collected in the dry season (e.g., *Parachela maculicauda, Oxyeleotris marmorata* & *Parambassis apogonoides*), these were only encountered at one or two sites and this may reflect greater sampling effort.

We found several species that were well adapted to drying or low oxygen conditions, such as *Clarias batrachus*, which can survive in poorer quality water. Similarly, *A. testudineus*, which was captured in all seasons, is commonly found in ponds, swamps and wetlands throughout Southeast Asia and tolerates stagnant water conditions (Rainboth, 1996). This species can hibernate in mud, has special organs that allow it to breathe air and can walk on land using spines on its gill plates. It is also easier to transport to markets from remote areas because the species can stay alive for days in water containers (Valbo-Jørgensen *et al.,* 2009). Needham & Funge-Smith (2015) reported that air-breathing "black fish" species including *A. testudineus* are common in the diet of Cambodians, comprising 30% of total consump-

tion. Jackson *et al.* (2013) found that artificial and natural floodplain depressions adjacent to an African river had a higher percentage of fishes that could tolerate dissolved oxygen than nearby river channels, indicating that the water quality in these isolated pools likely influences assemblage structure. Jackson *et al.* (2013) also found that piscivores dominated isolated, seasonal ponds during low water periods in the Oueme River in Africa. We similarly found that where only one species was encountered in a wetland, the species was almost always *A. testudineus*, a known piscivore.

Relationships between wetland characteristics and fish

We examined whether wetland size (area and maximum water depth) and connectivity (isolated or connected) influenced fish diversity, but did not find a significant correlation between area and species richness or diversity (*H'*). This accords with the findings of similar studies. Snodgrass *et al.* (1996), although not studying a tropical system, found no correlation between wetland size and species richness. Similarly, Tondato *et al.* (2013) found

that while depth was one of the most important variables influencing species occurrence and that wetland area had no effect. In contrast, Pazin *et al.* (2006) found that species richness was positively related to area, canopy cover, hydro-period and conductivity, but not to depth. However, this might be because the temporary wetlands studied by Pazin *et al.* (2006) were much smaller (mean area=2.42 m²) and shallower (mean depth=8.1 cm) compared to our study (mean area=28,637 m²) and Tondato *et al.* (2013) (mean area=1,591 m²). Furthermore, our measurements of wetland size might not have been a good indicator of water volume at the time of sampling. Due to the large variation in the size of the wetlands sampled, their area was either estimated from length and width measurements in the field or calculated from GPS tracks or satellite images of the wetland boundary. As a consequence, depth may have been a better indicator of total water volume at the wetland during sampling.

Although none of our models found a significant relationship between presence of a given fish species and depth, depth was in the best model for each of the five most common species. Likewise, maximum depth was also correlated with overall species richness and diversity. This suggests that water depth may be an important factor influencing the diversity of fish assemblages and presence of common species in KPWS. It has also been found to be important in determining fish communities in many small, seasonal wetlands (Escalera-Vazquez & Zambrano, 2010; Tondato *et al.*, 2013; Fernandes *et al.*, 2015). For instance, Fernandes *et al.* (2015) found that fish abundance and species richness were generally higher in deeper and more connected wetland patches. Our findings collectively suggest that a more comprehensive study of the influence of wetland inundation patterns and depth on fish diversity could provide useful information for conservation efforts, as described below.

One strategy that has been proposed for wildlife conservation in the dry forest habitats of Cambodia is to physically deepen wetlands so that they maintain water year-round, thereby converting seasonal wetlands to permanent wetlands (Gray *et al.*, 2015). While we found a correlation between maximum depth and fish diversity, we caution that limited conclusions can be drawn from our rapid survey. Escalera-Vazquez and Zambrano (2010) suggest that different communities in temporary and permanent wetlands may help to maintain diversity at a landscape level. Deepening wetlands to create permanently inundated habitats may increase species richness in those wetlands, but could also lead to homogeneity in species assemblages among modified wetlands. Conversely, maintaining a variety of depths may support greater diversity overall. We therefore

recommend further examination of the effects of such wetland modifications on fish assemblages to determine potential benefits or negative impacts on fish resources and diversity.

We also found indications that wetlands closer to each other may have more similar fish assemblages. To protect greater fish diversity, it could therefore prove valuable to select scattered rather than clustered wetlands for conservation purposes. Further research to improve understanding of the spatial distribution of fish assemblages would help site managers direct resources towards areas with higher biodiversity and ecosystem services.

Rapid sampling methods

Further data on wetland distribution, biodiversity and ecosystem services is needed to demonstrate the importance of wetland conservation to decision makers (Kingsford *et al.*, 2016). The swift pace of development in Cambodia emphasizes the need to gather this information rapidly to support conservation and we sought to test a rapid method for collecting basic data on fish in small wetlands. The methods we used were effective as a rapid sampling technique and could be incorporated into future protocols for wetland assessments. In this context, we describe challenges and lessons learnt regarding our approach.

To balance the need for rapid sampling and adequate levels of effort, our methods could be improved by repeatedly sampling the same wetlands and developing species accumulation curves to determine the level of effort (electrofishing time or number of seine passes) required to accurately estimate species richness. In our study, we were limited to using a similar level of effort at each wetland, irrespective of their size.

While we did not examine the influence of aquatic vegetation, this may also play a role in structuring fish assemblages in seasonal wetlands. For example, Tondato *et al.* (2013) found that macrophyte richness and cover were important in influencing fish species occurrence. Escalera-Vazquez & Zambrano (2010) also found that community structure was related to macrophyte cover, in addition to water temperature, depth and pH. Jackson *et al.* (2013) found greater macrophyte coverage in artificial depressions compared to natural depressions, which led to differences in dissolved oxygen and consequently also in fish assemblages. We therefore recommend measurement of aquatic plant cover in future wetland studies, alongside instantaneous water quality characteristics such as temperature, pH, dissolved oxygen and conductivity. Given the presence of fish that are known to tolerate hypoxia, dissolved oxygen likely plays a role

in structuring species assemblages in the dry season in smaller wetlands (e.g., Jackson *et al.* 2013).

We also did not explicitly address spatial autocorrelation, although our cluster analysis suggested that wetlands closer together may be more similar in fish composition than distant wetlands. Although few researchers quantify and adjust for spatial autocorrelation (Tondato *et al.,* 2013), we recommend its consideration in future wetland studies. This could be achieved by including a measure of the degree of spatial correlation in analysis based on the coordinates for each wetland.

We recognize that our methods are biased towards species associated with shallower waters because we did not sample the deeper waters of larger wetlands. Future studies would benefit from access to deeper wetland locations to deploy traps and nets and accurately measure all depths (e.g., using a small lightweight boat and a weighted rope). In addition, an electronic range finder could be used to measure the length and width of smaller wetlands. Our study would also have been strengthened by quantitative data on connectivity, rather than a simple qualitative (i.e., isolated or connected) category based on direct observation and local knowledge.

Our results can be used to develop specific research questions about the environmental characteristics of wetlands that influence the structure of the fish communities. To expand on our work, research on the effects of changes in connectivity would shed light on the potential influence of changes to hydrology due to development or climate change. In this context, wetlands could be selected for a year-long study where these are resampled monthly for water depth and connectivity to determine the influence of specific inundation and duration patterns (e.g., Baber *et al.,* 2002) on fish assemblages. This would improve understanding of how fish assemblages form and change during the dry season when the wetlands begin to dry up. Source river populations could also be sampled to learn how the species assemblages in wetlands compare to those of their source rivers (e.g., Jackson *et al.,* 2013). The results of such studies would help site managers understand the importance of connectivity and flood-timing on fish diversity and resources in small wetlands, because these may be altered by water management and land use changes in a watershed.

Acknowledgements

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Appendix 1 Model relationships between sampling season, wetland connectivity, water depth and the presence and absence of the five most common fish species at Kulen Promtep Wildlife Sanctuary

Species	Model	Residual deviance	Residual degrees of freedom	AIC	Δ AIC	*p*
Trichopodus trichopterus	1) Null	54.548	39	56.55	26.3	
	2) Season	52.067	37	58.07	27.8	
	3) Connectivity	36.498	26	40.50	10.3	
	4) Max. Depth	26.226	18	30.23	0.0	Intercept 0.260, depth 0.308
	5) Max. Depth + Connectivity	24.334	17	30.33	0.1	

Appendix 1 Continued

Species	Model	Residual deviance	Residual degrees of freedom	AIC	Δ AIC	p
	6) Max. Depth + Season	26.057	17	32.06	1.8	
	7) Season + Connectivity	36.361	25	42.36	12.1	
	8) Season + Connectivity + Max. Depth	24.245	16	32.25	2.0	
Esomus metallicus	1) Null	55.352	39	57.35	29.8	
	2) Season	48.142	37	54.14	26.6	
	3) Connectivity	38.243	26	42.24	14.7	
	4) Max. Depth	26.551	18	30.55	3.0	
	5) Max. Depth + Connectivity	26.49	17	32.49	4.9	
	6) Max. Depth + Season	21.568	17	27.57	0.0	Intercept 0.207, Depth 0.292, Season 0.996
	7) Season + Connectivity	31.794	25	37.79	10.2	
	8) Season + Connectivity + Max. Depth	21.566	16	29.57	2.0	
Trichopsis vittata	1) Null	54.548	39	56.55	24.8	
	2) Season	54.523	37	60.52	28.8	
	3) Connectivity	37.657	26	41.66	9.9	
	4) Max. Depth	27.726	18	31.73	0.0	Intercept 0.993, Depth 0.992
	5) Max. Depth + Connectivity	27.413	17	33.41	1.7	
	6) Max. Depth + Season	27.326	17	33.33	1.6	
	7) Season + Connectivity	37.652	25	43.65	11.9	
	8) Season + Connectivity + Max. Depth	27.068	16	35.07	3.3	
Anabas testudineus	1) Null	53.841	39	55.84	24.8	
	2) Season	53.82	37	59.82	28.7	
	3) Connectivity	36.16	26	40.16	9.1	
	4) Max. Depth	27.072	18	31.07	0.0	Intercept 0.436, Depth 0.515
	5) Max. Depth + Connectivity	26.739	17	32.74	1.7	
	6) Max. Depth + Season	26.896	17	32.90	1.8	
	7) Season + Connectivity	36.154	25	42.15	11.1	
	8) Season + Connectivity + Max. Depth	26.600	16	34.60	3.5	
Rasbora paviana	1) Null	51.796	39	53.80	32.2	
	2) Season	41.679	37	47.68	26.1	
	3) Connectivity	25.454	26	29.45	7.8	
	4) Max. Depth	17.614	18	21.61	0.0	Intercept 0.0324*, Depth 0.1727
	5) Max. Depth + Connectivity	17.224	17	23.22	1.6	
	6) Max. Depth + Season	16.283	17	22.28	0.7	
	7) Season + Connectivity	23.003	25	29.00	7.4	
	8) Season + Connectivity + Max. Depth	15.907	16	23.90	2.3	

Significance values are provided for best fit model only (* indicates significant value).