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Cover image: Bengal slow loris *Nyticebus bengalensis* released in the forests surrounding Phnom Tamao Wildlife Rescue Centre and Zoological Garden, Cambodia (© Jeremy Holden).

Editorial—Only for zoos? The involvement of rescue centres in captive breeding programmes for wildlife conservation in Southeast Asia

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Captive breeding programmes for species reintroductions can maintain animal populations whose wild counterparts are severely threatened once the causes of declines have been mitigated (IUCN SSC, 2013). As early as the 1960s, zoos began implementing captive breeding programs to guard species against extinction in an 'Ark' paradigm (Zimmermann, 2010; Keulartz, 2015). This resulted in the reestablishment of species in habitats where they had become locally extinct, such as the golden lion tamarin *Leontopithecus rosalia* (Kierulff *et al*., 2012), Arabian oryx *Oryx leucoryx* (Spalton *et al*., 1999) and Père David's deer *Elaphurus davidianus* (Cheng *et al*., 2021). By the turn of the century however, the challenges faced by *ex situ* zoos to achieve this with consistent success were becoming apparent. These included limitations in appropriate space, unsuitable climates, shortfalls in funding and securing adequate numbers of founders to ensure genetic variability (Ralls & Ballou, 1992; Zimmerman, 2010).

Further complications include the difficulty in maintaining captive collections of species with strict dietary or habitat requirements outside of their country of origin. Examples include douc langurs *Pygathrix* spp., which require specialized foliage that is difficult and expensive to obtain outside of range countries (Schwitzer *et al*., 2006; Hale *et al*., 2018), and pangolins *Manis* spp., where the difficulty of catering to their insectivorous diets in captivity has often resulted in animals dying within six months (Yang *et al*., 2007). These experiences led to zoos increasingly shifting their breeding and reintroduction programmes to native species (Jakob-Hoff *et al*., 2015; Olive & Jansen, 2017) and employing a more integrated approach towards non-native species. This includes research and training with exposure and providing support to *in situ* projects in natural habitats with local wildlife rescue centres (Cuarón, 2005; Zimmermann, 2010; Conde *et al*., 2011; Keulartz, 2015; Spooner *et al*., 2023).

Wildlife rescue centres situated in countries with rich biodiversity but limited finances are in a different position to *ex situ* zoos. Unlike most zoological gardens, the number of animals in rescue centres in developing countries can differ vastly in a given year depending on the number of rescues or confiscations. Due to poor facilities or a lack of finances, many arrivals may not survive or contribute further to the conservation of their species. However, their potential for conservation should not be discounted. With funding, enhanced capacity, good animal husbandry and protocols for rehabilitating rescued wildlife, these centres can have a positive conservation impact. For instance, while western zoos must ensure they have sufficient space to display a diverse range of charismatic animals so as to attract visitors, wildlife rescue centres can specialize to a greater extent or dedicate their resources to larger numbers of a single species (Gilbert *et al*., 2017; Hosey *et al*., 2020). Further, they are better placed to promote public understanding of the importance of conserving rare, threatened and often understudied species in their own countries.

To help rescue centres determine the best outcomes for animal arrivals, the *IUCN Guidelines for the Placement of Confiscated Animals* details the decision-making process on whether to release, euthanize or permanently house displaced wildlife (IUCN, 2002). Rehabilitation and reintroduction of animals rescued from the illegal wildlife

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Table 1 Mammal species rehabilitated or captive born at Phnom Tamao Wildlife Rescue Center and later released into forests surrounding Phnom Tamao, Takeo, Angkor Archaeological Park, Siem Reap, or Wildlife Release Station (WRS), Koh Kong.

Common Name	Scientific Name	IUCN Listing	Release Site
Sunda pangolin	*Manis javanica*	Critically Endangered	Phnom Tamao, WRS
Germain's silvered langur	*Trachypithecus germaini*	Endangered	Angkor
Long-tailed macaque	*Macaca fascicularis*	Endangered	Phnom Tamao
Pileated gibbon	*Hylobates pileatus*	Endangered	Angkor
Siamese Eld's deer	*Rucervus eldii siamensis*	Endangered	Phnom Tamao
Bengal slow loris[1]	*Nycticebus bengalensis*	Vulnerable	Phnom Tamao, WRS
Binturong	*Arctictis binturong*	Vulnerable	WRS
Sambar deer	*Rusa unicolor*	Vulnerable	Phnom Tamao
Smooth-coated otter	*Lutrogale perspicillata*	Vulnerable	Angkor
Common palm civet	*Paradoxurus hermaphroditus*	Least Concern	Phnom Tamao
Golden jackal	*Canis aureus*	Least Concern	Phnom Tamao
Leopard cat	*Prionailurus bengalensis*	Least Concern	Phnom Tamao, Angkor, WRS
Lesser mouse deer[1]	*Tragulus kanchil*	Least Concern	Phnom Tamao
Malaysian porcupine	*Hystrix brachyura*	Least Concern	Phnom Tamao
Muntjac	*Muntiacus vaginalis*	Least Concern	Phnom Tamao, Angkor, WRS
Small Indian civet[1]	*Viverricula indica*	Least Concern	Phnom Tamao, Angkor
Wild pig	*Sus scrofa*	Least Concern	Phnom Tamao

[1] Only rescued and rehabilitated individuals of this species have been released.

trade has become an accepted component of conservation plans for many species (Cheyne, 2009; Saran *et al*., 2011; Molinari-Jobin *et al*., 2024). Apart from the ethical and in some countries legal concerns with euthanasia, this can lead to the loss of individuals of species that may be perceived as common when their wild populations are actually declining significantly. This was the case for long-tailed macaques *Macaca fascicularis* whose arrivals at rescue centres overwhelmed some to the extent that they could no longer accept new animals during the same 14-year period that the species moved from being regarded as Least Concern to Endangered (Hansen *et al*., 2022). There is also potential for rescued animals that cannot be released due to injury or familiarisation with humans to take part in captive breeding programs while being retained in permanent housing. This is especially appropriate for species that are difficult to maintain outside of their native habitats.

Many wildlife rescue centres in Southeast Asia have been at the forefront of research, rehabilitation and release of rare species targeted by the illegal wildlife trade. These facilities are often run by non-government organizations that collaborate with international experts (including accredited zoos) and local governments and target specific threatened species. As these centres became more established, many have begun captive breeding programmes for target species that enter their facilities. Examples include Save Vietnam's Wildlife, which focuses on the rehabilitation and release of trafficked Sunda pangolins *M. javanica* and Chinese pangolins *M. pentadactyla* and has begun captive breeding of the latter (Challender *et al*., 2011; Gray *et al*., 2023); the Endangered Primate Rescue Center (Vietnam), which has undertaken extensive research on the husbandry of douc langurs in captivity and the wild, as well as captive breeding and release programmes for Delacour's langurs *Trachypithecus delacouri* (Nadler, 2012, 2013, 2023); and the Angkor Center for Conservation of Biodiversity (Cambodia), which rehabilitates and captive breeds endangered bird and reptile species, including the white-shouldered ibis *Pseudibis davisoni* (Woesner *et al*., 2021; CIWG, 2023). Because many of these programmes are still in their infancy, it is difficult to define their success in terms of large-scale reintroduction efforts. Unfortunately, this is not an isolated issue as the fate of most rehabilitated animals released back into the wild remains unknown (Quaglia, 2024). However, as wild populations continue to decline these relatively small-scale programmes will undoubtedly become an increasingly

Fig. 1 The first gibbon born in Angkor (*Ping-peeung*) with her first-born (*K'mum*), Angkor Archeological Park, Siem Reap, April 2024 (© Jeremy Holden).

important component of future captive breeding efforts and conservation plans.

One such example is the Phnom Tamao Wildlife Rescue Centre and Zoological Garden (Phnom Tamao), which accepts all rescued wildlife and is managed by the Cambodian Forestry Administration (FA). The decision in 1995 by the Ministry of Agriculture, Forests and Fisheries (MAFF) to create Cambodia's first national zoo and wildlife rescue centre in a regenerating area of forest south of Phnom Penh was inspired. The centre is open to visitors, although not all the animals are on display (a non-visitor or 'off show' area exists for rehabilitation and release) and almost all have come from the illegal wildlife trade. In the early days, a lack of amenities including electricity and a reliable water source meant that improving conditions for some of the animals took time. However, collaboration between FA/MAFF and several non-governmental organisations (Wildlife Alliance, Free the Bears and Fauna & Flora) has ensured better care for the animals and enabled successful breeding programmes for many species. This has culminated in the release of appropriate animals via responsible protocols into protected natural habitats, often forests where they previously occurred. This includes countless numbers of birds and reptiles and several noteworthy mammal species (Table 1). In future, similar efforts should enable the repopulation of areas where species have been extirpated, provided appropriate management of Phnom Tamao and adequate protection of the forests can be assured.

The benefits that wildlife rescue centres such as Phnom Tamao can provide for wildlife conservation are demonstrated well by a male pileated gibbon *Hylobates pileatus* which was rescued in 2006. Because the individual (named *Pompoi*) had been hand-raised, he was accustomed to people and so not appropriate for release. Following a badly-broken arm post-rescue and setbacks including repeat fractures, the pin of the repaired radius bone in his left arm was removed in 2009 and he was paired with a female of a similar age. Subsequent cooperation between FA, APSARA (which manages the Angkor Archaeological Park in Siem Reap Province) and Wildlife Alliance led to the release of suitable wildlife species into the forests of Angkor in June 2013. This began with the acclimatization of the first pair of pileated gibbons (*Baray* and *Saranick*, which were captive born at Phnom Tamao) and was followed by their release six months later (Leroux *et al*., 2019).

Two years later, *Pompoi* and his mate produced a baby at Phnom Tamao and although they were not considered suitable for release, their mother-raised daughter exhibited appropriate behaviour including a wariness of humans. Consequently, she was paired with a male in a remote area of Phnom Tamao for one year, after which both gibbons were moved to an acclimatization enclosure in Angkor in November 2018. In July 2020, they became the third pair of gibbons from Phnom Tamao to be released in the forests of Angkor. At the time of writing, they have produced two offspring (*Mey-ambough* in September 2021 and *K'touy* in May 2024) and there are four pairs of gibbons living free in Angkor, all of whom can trace their origins back to Phnom Tamao. These have borne 11 gibbons to date. The first of these, a female named *Ping-peeung*, was paired with a young male from Phnom Tamao and their first infant (*K'mum*) was born in January 2024 (Fig. 1). *A-ping* and *Chung-ruth* represent the fourth and final pair of gibbons and were born in Angkor to different parents. Their first child (*Omal*) was born in April 2024.

This summary of rescued gibbons from Phnom Tamao that went on to play a role in restoring the species to an area where it once occurred is an example of what can be achieved when the right balance of circumstances and partners coincide. With wildlife threatened by so many factors, including habitat loss, exploitation and climate change, this demonstrates what rescue centres such as Phnom Tamao can achieve when located as they should be. In natural habitat, not just to give some kind of life to a few less fortunate wild animals, but to enable these animals to play an important role in maintaining and restoring the country's national history and heritage. This is surely something worth hanging on to everywhere, whatever the cost.

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References

Challender, D.W.S., Nguyen V.T., Jones, M. & May, L. (2011) Time budgets and activity patterns of captive Sunda pangolins (*Manis javanica*). *Zoo Biology*, **29**, 1–13.

Cheng Z., Tian X., Zhong Z., Li P., Sun D., Bai J., Meng Y., Zhang S., Zhang Y., Wang, L. & Liu, D. (2021) Reintroduction, distribution, population dynamics and conservation of a species formerly extinct in the wild: A review of thirty-five years of successful Milu (*Elaphurus davidianus*) reintroduction in China. *Global Ecology and Conservation*, **31**, e01860.

Cheyne, S.M. (2009) The role of reintroduction in gibbon conservation: opportunities and challenges. In *The Gibbons* (eds D. Whittaker & S. Lappan), pp. 477–496. Springer, New York, USA.

Conde, D.A., Flesness, N., Colchero, F., Jones, O.R. & Scheuerlein, A. (2011) An emerging role of zoos to conserve biodiversity. *Science*, **331**, 1390–1391.

Cuarón, A.D. (2005) Further role of zoos in conservation: Monitoring wildlife use and the dilemma of receiving donated and confiscated animals. *Zoo Biology*, **24**, 115–124.

[IUCN] International Union for Conservation of Nature (2002) *IUCN Guidelines for the Placement of Confiscated Animals*. IUCN, Gland, Switzerland.

[IUCN SCC] International Union for Conservation of Nature/Species Survival Commission (2013) *Guidelines for Reintroductions and Other Conservation Translocations*. Version 1.0. IUCN SCC, Gland, Switzerland.

Gilbert, T., Gardner, R., Kraaijeveld, A.R. & Riordan, P. (2017) Contributions of zoos and aquariums to reintroductions: historical reintroduction efforts in the context of changing conservation perspectives. *International Zoo Yearbook*, **51**, 15–31.

Gray, R.J., Le D.V., Nguyen H.T.T., Cao L.N., Nguyen T., Pham T., Willcox, D., Chen T. & Nguyen T.V. (2023) Home ranges and activity patterns of Sunda pangolins *Manis javanica* (Pholidota: Manidae) in Vietnam. *Journal of Asia-Pacific Biodiversity*, **16**, 421–431.

[CIWG] Cambodia Ibis Working Group (2023) *CIWG Annual Report 2022*. Https://naturelifecambodia.org/wp-content/uploads/2012/05/CIWG-Annual-Report-2022-Final.pdf [accessed 18 June 2024].

Hansen, M.F., Ang A., Trinh T., Sy E., Paramasiwam, S., Ahmed, T., Dimalibot, J., Jones-Engel, L., Ruppert, N., Griffioen, C., Lwin N., Phiapalath P., Gray, R., Kite, S., Doak, N., Nijman, V., Fuentes, A. & Gumert, M.D. (2022) *Macaca fascicularis. The IUCN Red List of Threatened Species 2022*. Https://dx.doi.org/10.2305/IUCN.UK.2022-1.RLTS.T12551A199563077.en [accessed 25 June 2024].

Hale, V.L., Tan C.L., Niu K., Yang Y., Knight, R., Zhang Q., Cui D. & Amato, K.R. (2018) Diet versus phylogeny: a comparison of gut microbiota in captive colobine monkey species. *Microbial Ecology*. **75**, 515–527.

Hosey, G., Melfi, V. & Ward, S.J. (2020) Problematic animals in the zoo: the issue of charismatic megafauna. In *Problematic Wildlife II* (eds F. Angelici & L. Rossi), pp. 485–508. Springer Cham, Switzerland.

Jakob-Hoff, R., Harley, D., Magrath, M., Lancaster, M. & Kuchling, G. (2015) Advances in the contribution of zoos to reintroduction programs. In *Advances in Reintroduction Biology in Australian and New Zealand Fauna* (eds D.P. Armstrong, M.W. Hayward, D. Moro & P.J. Seddon), pp. 201–215. CSIRO Publishing, Melbourne, Australia.

Kierulff, M.C.M., Ruiz-Miranda, C.R., de Oliveira, P.P., Beck, B.B., Martins, A., Dietz, J.M., Rambaldi, D.M. & Baker, A.J. (2012) The golden lion tamarin *Leontopithecus rosalia*: a conservation success story. *International Zoo Yearbook*, **46**, 36–45.

Keulartz, J. (2015) Captivity for conservation? Zoos at a crossroads. *Journal of Agricultural and Environmental Ethics*, **28**, 335–351.

Leroux, N., Bunthoeun R. & Marx, N. (2019) The reintroduction of captive-born pileated gibbons (*Hylobates pileatus*) into the Angkor Protected Forest, Siem Reap, Cambodia. *Primate Conservation*, **33**, 1–11.

Molinari-Jobin, A., Zimmermann, F., Borel, S., Le Grand, L., Iannino, E., Anders, O., Belotti, E., Bufka, L., Ćirović, D., Drouet-Hoguet, N., Engleder, T., Figura, M., Fuxjäger, C., Gregorova, E., Heurich, M., Idelberger, S., Kubala, J., Kusak, J., Melovski, D., Middelhoff, T.L., Mináriková, T., Molinari, P., Mouzon-Moyne, L., Moyne, G., Mysłajek, R.W., Nowak, S., Ozolins, J., Ryser, A., Sanaja, B., Shkvyria, M., Sin, T., Sindičić, M., Slijepčević, V., Stauffer, C., Tám, B., Trajce, A., Volfová, J., Wölfl, S., Zlatanova, D. & Vogt, K. (2024) Rehabilitation and release of orphaned Eurasian lynx (*Lynx lynx*) in Europe: implications for management and conservation. *Plos One*, **19**, e0297789.

Nadler, T. (2012) Reintroduction of the 'Critically Endangered' Delacour's langur (*Trachypithecus delacouri*)—a preliminary report. *Vietnamese Journal of Primatology*, **2**, 67–72.

Nadler, T. (2013) Twenty years Endangered Primate Rescue Center, Vietnam—Retrospect and Outlook—Report 2012. *Vietnamese Journal of Primatology*, **2**, 1–12.

Nadler, T. (2023) Hand rearing and development of douc langurs (*Pygathrix* sp.) at the Endangered Primate Rescue Center, Vietnam. *Vietnamese Journal of Primatology*, **3**, 51–57.

Olive, A. & Jansen, K. (2017) The contribution of zoos and aquaria to Aichi Biodiversity Target 12: A case study of Canadian zoos. *Global Ecology and Conservation*, **10**, 103–113.

Quaglia, S. (2024) Does wildlife rehabilitation really work? No one knows for sure. *Science*, **385**, 11–12.

Ralls, K. & Ballou, J.D. (1992) Managing genetic diversity in captive breeding and reintroduction programs. In *Transactions of the Fifty-Seventh North American Wildlife and Natural Resources Conference* (ed R.E. McCabe), pp. 263–282. Wildlife Management Institute, Washington DC, USA.

Saran, K.A., Parker, G., Parker, R. & Dickman, C.R. (2011) Rehabilitation as a conservation tool: a case study using the common wombat. *Pacific Conservation Biology*, **17**, 310–319.

Schwitzer, C., Klumpe, K. & Kaumanns, W. (2006) Energy and nutrient intake, feeding behaviour and activity budget of captive Douc langurs (*Pygathrix nemaeus*). In *Zoo Animal Nutrition Vol. 3* (ed A. Fidgett), pp. 109–124. Filander, Verlag, Germany.

Spalton, J.A., Lawrence, M.W. & Brend, S.A. (1999) Arabian oryx reintroduction in Oman: successes and setbacks. *Oryx*, **33**, 168–175.

Spooner, S.L., Walker, S.L., Dowell, S. & Moss, A. (2023) The value of zoos for species and society: the need for a new model. *Biological Conservation*, **279**, 109925.

Woesner, M., Meyerhoff, M. & Wagner, P. (2021) Behavior patterns of the white-shouldered ibis *Pseudibis davisoni* (Hume, 1876) in a captive environment at the Angkor Centre for Conservation of Biodiversity, Cambodia. *Der Zoologische Garten*, **89**, 121–134.

Zimmermann, A. (2010) The role of zoos in contributing to in situ conservation. In Wild Mammals in Captivity: Principles and Techniques for Zoo Management (eds D.G. Kleiman, K.V. Thompson & C.K. Baer), pp. 281–287. University of Chicago Press, Chicago, USA.

Yang C.W., Chen S., Chang C.Y., Lin M.F., Block, E., Lorentsen, R., Chin J.S.C. & Dierenfeld, E.S. (2007) History and dietary husbandry of pangolins in captivity. *Zoo Biology*, **26**, 223–230.

Short Communication

Use of a portable DNA sequencer for invertebrate species identification in Cambodia, a pilot study

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DNA barcoding is a well-established molecular genetics tool that uses a short, standardised reference gene, or genes, to create species-specific DNA sequences known as barcodes. It can be used for a wide range of applications such as creating genetic references for voucher specimens (Ward *et al*., 2009), species identification and taxonomic delimitation (DeSalle *et al*., 2005) and population genetics (Hajibabaei *et al*., 2007b). These applications can be used to aid conservation efforts by identifying illegally traded species (Rehman *et al*., 2015), contributing to population assessments (Wilson *et al*., 2016), identification of invasive species (Armstrong & Ball, 2005) and biodiversity monitoring (Hajibabaei *et al*., 2007a).

The accessibility of barcoding has changed over the years with the advent of portable sequencers increasing accessibility while decreasing costs and turnaround times for data generation. They allow for rapid, in-country identification of samples which is particularly important for conservation efforts as projects can be time sensitive, occur in remote locations, or focus on CITES-protected species where international shipping is restricted (Krehenwinkel *et al*., 2019). As well as providing the ability to sequence samples in situ, portable sequencers also provide opportunities for sequencing in locations where access to traditional sequencing methodologies is limited (Pomerantz *et al*., 2018) or prohibitively expensive (von Rintelen *et al*., 2017). This is pertinent to many conservation priorities as biodiversity hotspots are often located in regions facing these challenges, such as Southeast Asia (Myers *et al*., 2000).

We tested the utility of a portable DNA sequencer, the Oxford Nanopore Technologies (UK) MinION, for identification of invertebrate species in Cambodia during a workshop ran at the Centre for Biodiversity Conservation in February 2023. Briefly, invertebrate species were collected from the Royal University of Phnom Penh campus (Fig. 1) and euthanised before being transferred to a sample tube with absolute ethanol for storage. A basic taxonomic identification was recorded for each sample using simple morphological characteristics but for any future development of voucher specimens we would recommend an expert taxonomic identification and capturing high-resolution images of samples where possible. DNA was extracted from the samples (Fig. 2) using QuickExtract (LGC Biosearch Technologies, Middlesex, UK) following the manufacturers protocol. Approximately 365 bp of the COI barcoding locus were amplified using the mlCOIintF (5′-GGWACWG-

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Fig. 1 Invertebrate sample collection on campus of Royal University of Phnom Penh.

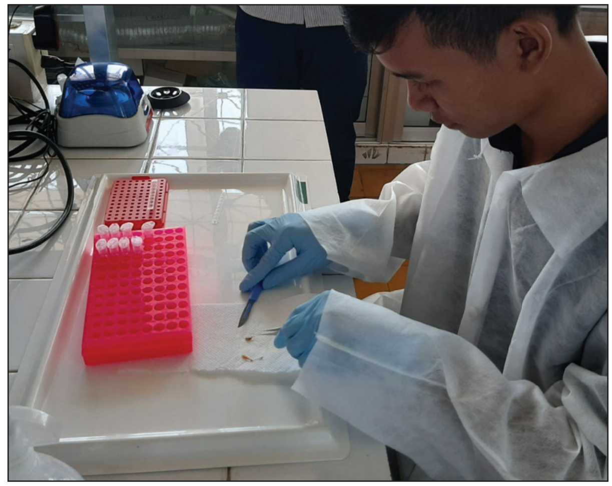

Fig. 2 Preparation of invertebrate samples for DNA extraction in laboratory.

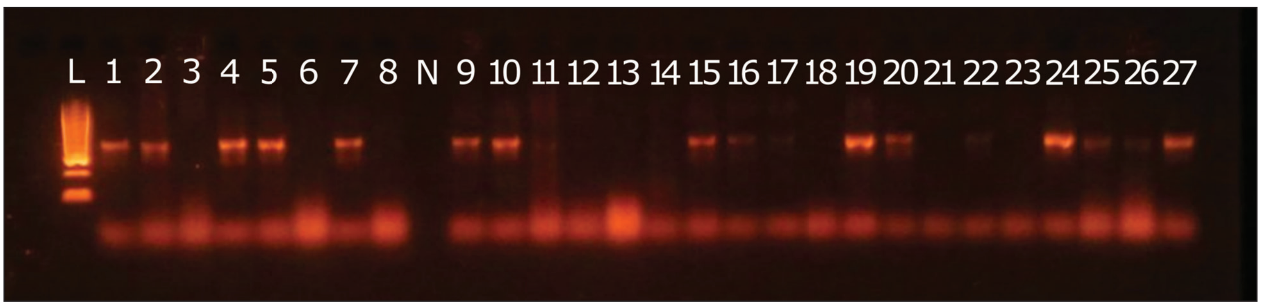

Fig. 3 Gel electrophoresis image visualising which samples were successfully amplified at the COI barcoding loci. The first well (L) contains a DNA ladder (HyperLadder 100 bp) used to determine the length of PCR product amplified. Samples were morphologically identified as: 1) ladybird, 2) fly, 3) treehopper, 4) yellow butterfly, 5) moth, 6) spider, 7) damselfly, 8) butterfly, 9) grasshopper, 10) cockroach, 11) hopper, 12) fly, 13) hopper, 14) beetle, 15) mosquito, 16) butterfly, 17) dragonfly, 18) worm, 19) spider, 20) yellow ant, 21) cockroach, 22) grasshopper, 23) leaf hopper, 24) spider, 25) fly, 26) ant, 27) red ant, and N indicates the negative control. Samples that were successfully amplified are shown by a band over 300 bp (e.g., sample 1) and those that failed have an absence of a band (e.g. sample three).

GWTGAACWGTWTAYCCYCC-3′, Wangensteen *et al*., 2018) and jgHCO2198 (5′-TAIACYTCIGGRTGICCRAARAAYCA-3′, Geller *et al*., 2013) primer set and the following 10 µl PCR master mix: 1.4X DreamTaq Hot Start PCR Master Mix (Thermo Scientific, Massachusetts, US), 1 µM forward primer (mlCOIintF), 1 µM reverse primer (jgHCO2198), 1.5 µl nuclease free water, 0.5 µl bovine serum albumin, and 1 µl DNA template. PCRs were conducted on a MiniPCR (MiniPCR, Massachusetts, US) and conditions were as follows: initial denaturation at 95 °C for five minutes; 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 50 °C for 30 seconds and extension at 72 °C for 30 seconds, followed by final extension at 72 °C for two minutes. PCR products were visualised on a 2% agarose gel (Fig. 3). All 18 samples that successfully amplified at PCR were pooled together to create a sequencing library following the ligation sequencing amplicons protocol (SQK-LSK114, Oxford Nanopore Technologies).

The library ran for two hours and generated a total of 2.99 million reads. Reads were basecalled with Guppy v6.4.6 (Oxford Nanopore Technologies) using default parameters where a total of 2.19 million reads passed quality control. These sequences were compared to a local database containing COI reference sequences taken from NCBI GenBank (<www.ncbi.nlm.nih.gov/>) and BOLD (<www.boldsystems.org/>) repositories (Meglécz, 2023; database available from <https://zenodo.org/

Table 1 Details of 12 specimens identified using the sequences generated during the workshop. Each specimen was given a morphological ID and the consensus sequences generated were compared to NCBI GenBank and BOLD repositories where the top match was recorded alongside the query coverage and identity match. Supercript figures indictate the taxonomic level each specimen was identified to: [1] Species, [2] Genus, [3] Family, [4] Order.

Morphological ID	NCBI GenBank				BOLD			
	DNA Barcoding ID	Top Match Accession	Query Cover	Identity Match	DNA Barcoding ID	Top Match BIN	Query Cover	Identity Match
Spider	*Tetragnatha mandibulata* [1]	MK057477	100%	99.37%	*Tetragnatha mandibulata* [1]	AAK2567	100%	97.59%
Yellow butterfly	*Eurema hecabe* [1]	MN609532	100%	100%	*Eurema hecabe* [1]	AAA6082	100%	100%
Ladybird	*Illeis bistigmosa* [1]	MZ325765	100%	100%	*Illeis* sp. [2]	-	100%	97.78%
Fly sp.1	Dolichopodidae sp. [3]	KX052896	98%	90.42%	Dolichopodidae [3]	ACV0962	-	99.04%
Cockroach	*Pycnoscelus surinamensis* [1]	MW535117	100%	99.69%	*Pycnoscelus surinamensis* [1]	AAG9904	100%	99.68%
Dragonfly	*Brachythemis contaminata* [1]	MG885560	98%	100%	*Brachythemis contaminata* [1]	ADC3495	100%	98.41%
Grasshopper	*Pseudoxya diminuta* [1]	KC139999	100%	100%	*Pseudoxya diminuta* [1]	ACD4638	100%	100%
Fly sp.2	Diptera sp. [4]	GU675516	100%	97.17%	*Calliphora dispar* [1]	AAH7137	100%	97.17%
Yellow ant	*Anoplolepis gracilipes* [1]	MK482686	100%	99.69%	*Anoplolepis gracilipes* [1]	AAA9474	100%	100%
Red ant	*Oecophylla smaragdina* [1]	AB185478	100%	99.69%	*Oecophylla smaragdina* [1]	AAA5846	100%	99.68%
Moth	*Hypena* sp. [2]	KX860485	100%	99.69%	*Hypena simplicalis* [1]	AAG5843	100%	99.68%
Damselfly	*Agriocnemis pygmaea* [1]	MK506257	100%	100%	*Agriocnemis pygmaea* [1]	ABW0502	100%	100%

record/6555985#.ZHb953bMKUk>). Positive matches to the database were made for 1.78 million reads (81%). Matches were grouped by taxonomic ID and ranked by number of positive matches. All taxonomic ID groups that had less than 10,000 hits were discarded, and the remaining sequences were removed if they were less than 100 bp in length or had a BLAST identity match below 90%.

A total of 12 taxonomic ID groups passed quality control measures. Each group was mapped to a taxonomically appropriate reference sequence (i.e. the top GenBank or BOLD hit for each group) in Geneious Prime (v2021.1.1: Biomatters, Auckland, New Zealand) using the Geneious mapper algorithm and default parameters. Consensus sequences 318 bp in length were generated for each of the 12 taxonomic ID groups. Consensus nucleotide sequences were translated into protein sequences to check for the presence of NUMTs (nuclear mitochondrial DNA segments) and none were identified. Finally, consensus sequences were again compared to the NCBI GenBank and BOLD repositories for classification. We successfully classified 12 samples from the generated sequencing data (Table 1).

We were able to identify a total of 12 different specimens from the 18 samples that successfully amplified at PCR. The disparity between the number of samples amplified and the number of specimens identified may be due to several factors. Firstly, the level of PCR amplification was not consistent across all samples so weaker samples may not have sequenced at a sufficient depth to pass quality control measures. Secondly, as samples were given a broad taxonomic classification based on basic morphology some samples may have been duplicates of the same species (e.g., two samples sequenced were identified as grasshoppers but only one species of grasshopper was identified). Lastly, our reference database may not have had sufficient taxonomic breadth to identify every sample, although it is unlikely that samples would not have been identified at a higher taxonomic level (i.e. Order).

Basecalled sequences used to generate the consensus sequence for each of the 12 taxonomic groups are available via Figshare (<https://figshare.com/>), as follows, Taxonomic ID Groups 1–12 (respectively): 10.6084/m9.figshare.23300720, –.23301173, –.23301197, –.23301203, –.23301212, –.23301224, –.23301230, –.23301239, –.23301242, –.23301251, –.23301263 and –.23301269. Consensus sequences are also available from Figshare (10.6084/m9.figshare.24525697).

We showed the utility of a portable DNA sequencer for invertebrate species identification in Cambodia. We were able to identify samples with a high degree of certainty but not all specimens could be identified to species level; this highlights the paucity of barcoding voucher sequences for species in Cambodia. Most samples identified to species level were taxa with widespread distributions that had been catalogued and barcoded from elsewhere in their ranges. Developing a barcoding database from morphologically-identified museum voucher specimens for species in Cambodia would be of vast benefit to conservation efforts in the region (Francis *et al*., 2010). As well as providing valuable information about the biology and ecology of native species, it would also provide the baseline data required for species identification and biodiversity monitoring (Krishnamurthy & Francis, 2012).

Portable DNA sequencers provide an important opportunity to increase genetics capacity for conservation in Cambodia where access to sequencing facilities has often been limited, prohibitively expensive, or where transportation of samples has been challenging. Specifically, the Oxford Nanopore Technologies portable sequencer, MinION, offers a competitively priced sequencing solution (Watsa *et al*., 2020) and the newly established distribution facility in Singapore will increase the accessibility of materials across the Asia-Pacific region (Oxford Nanopore Technologies, 2023). Given its portable nature, MinION also presents the opportunity for use in remote locations as well as acting as a teaching tool (Watsa et al., 2020) and while it is still often purported to produce data with a high error rate, recent developments in pore technology and chemistry have greatly improved sequencing accuracy (van Djik *et al*., 2023).

As with any new technology careful consideration does need to be made for the use of portable DNA sequencers in Cambodia. In particular, flow cells for MinION have a limited shelf life and the longevity of flow cells and other required reagents is affected by temperature storage. Preserving these items could be difficult as access to stable temperature storage is challenging in this region. It is also important that appropriate training is acquired on the use of flow cells, preparation of input materials and the bioinformatic pipelines required for analysis. Because our protocol was tested within a laboratory setting, additional development would be required to ensure all components operate effectively in field conditions before deployment in remote work locations. Overall, portable DNA sequencers provide an opportunity to increase capacity domestically and a means to develop genetic resources in country (Pomerantz *et al*., 2018; Krehenwinkel *et al*., 2019; Watsa *et al*., 2020).

Demonstrating the use of a portable sequencer in Cambodia is a first step to developing its potential for conservation in the region. Our pilot study used established protocols and acts as a proof of concept for the utility of portable sequencers in Cambodia. Its success has shown that with further development there is potential for in-country generation of barcoding data for invertebrate voucher specimens where protocols can be adapted to a range of taxa, as well as other applications such as identification of wildlife products that are traded illegally. While the protocols and techniques demonstrated here may not be novel to the wider scientific community, they represent the potential for a shift in the landscape of conservation genetics in Cambodia.

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References

Armstrong, K.F. & Ball, S.L. (2005) DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society B*, **360**, 1813–1823.

DeSalle, R., Egan, M.G. & Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B*, **360**, 1905–1916.

Francis, C.M., Borisenko, A.V., Ivanova, N.V., Eger, J.L., Lim, B.L., Guillén-Servent, A., Kruskop, S.K., Mackie, I. & Hebert, P.D.N. (2010) The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. *PLoS ONE*, **5**, e12575.

Geller, J., Meyer, C., Parker, M. & Hawk, H. (2013) Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, **13**, 851–861.

Hajibabaei, M., Singer, G.A.C., Clare, E.L. & Hebert, P.D.N., (2007a) Design and applicability of DNA assays and DNA barcodes in biodiversity monitoring. *BMC Biology*, **5**, 24.

Hajibabaei, M., Singer, G.A.C., Hebert, P.D.N. & Hickey, D.A. (2007b) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics*, **23**, 167–172.

Krehenwinkel, H., Pomerantz, A. & Prost, S. (2019) Genetic biomonitoring and biodiversity assessment using portable sequencing technologies: current uses and future directions. *Genes*, **10**, 858.

Krishnamurthy, K.P. & Francis, R.A. (2012) A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodiversity and Conservation*, **21**, 1901–1919.

Meglécz, E. (2023) COInr and mkCOInr: building and customizing a nonredundant barcoding reference database from BOLD and NCBI using a semi-automated pipeline. *Molecular Ecology Resources*, **23**, 933–945.

Myers, N., Mittermeier, R., Mittermeier, C. da Fonseca, G.A.B & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Oxford Nanopore Technologies (2023) *Oxford Nanopore Technologies Teams up with UPS Healthcare to Accelerate Delivery of New-Generation DNA Sequencing Technology to More Customers in Locations across Asia Pacific*. Https://nanoporetech.com/about-us/news/oxford-nanopore-technologies-teams-ups-healthcare-accelerate-delivery-new-generation [accessed 21 May 2023].

Pomerantz, A., Peñafiel, N., Arteaga, A., Bustamante, L., Pichardo, F., Coloma, L.A., Barrio-Amorós, C.L., Salazar-Valenzuela, D. & Prost, S. (2018) Real-time DNA barcoding in a rainforest using nanopore sequencing: opportunities for rapid biodiversity assessments and local capacity building. *GigaScience*, **7**, giy033.

Rehman, A., Jafar, S., Raja, N. A. & Mahar, J. (2015) Use of DNA barcoding to control the illegal wildlife trade: a CITES case report from Pakistan. *Journal of Bioresource Management*, **2**, 3.

van Dijk, E. L., Naquin, D., Gorrichon, K., Jaszczyszyn, Y., Ouazahrou, R., Thermes, C. & Hernandez, C. (2023) Genomics in the long-read sequencing era. *Trends in Genetics*, **39**, 649–671.

von Rintelen, K., Arida, E. & Häuser, C. (2017) A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. *Research Ideas and Outcomes*, **3**, e20860.

Wangensteen, O.S., Palacín, C., Guardiola, M. & Turon, X. (2018) DNA metabarcoding of littoral hard-bottom communities: high diversity and database gaps revealed by two molecular markers. *PeerJ*, **6**, e4705.

Ward, R.D., Hanner, R. & Herbert, P.D.N. (2009) The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology*, **74**, 329–356.

Watsa, M., Erkenswick, G.A., Pomerantz, A. & Prost, S. (2020) Portable sequencing as a teaching tool in conservation and biodiversity research. *PLoS Biol*, **18**, e3000667.

Wilson, J.J., Sing K.W., Lee P.S. & Wee A.K.S. (2016) Applications of DNA barcodes in wildlife conservation in tropical East Asia. *Conservation Biology*, **30**, 982–989.

Short Communication

First record of the Mekong arrowhead puffer *Pao suvattii* (Sontirat & Soonthornsatit, 1985) (Tetraodontiformes: Tetraodontidae) from Cambodia

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Tetraodontid fishes (Tetraodontidae), commonly known as puffers, have a soft, inflatable body, numerous minute spines (or entirely naked in some), four fused teeth, no pelvic fins or fin spine, and a truncate to rounded caudal fin (Nelson, 2006; Nelson *et al*., 2016; Taki *et al*., 2021). Many pufferfishes are confined to marine waters, but some enter into or spend their entire life in brackish and/or freshwater areas throughout tropical and subtropical, Atlantic, Indian and Pacific waters (Nelson, 2006; Nelson *et al*., 2016; Taki *et al*., 2021). Many also contain a strong poison (tetrodotoxin) in their bodies (Zhu *et al*., 2020; Taki *et al*., 2021). The Tetraodontidae family is one of the most speciose families of fish, comprising 196 species in 26 genera (Nelson *et al*., 2016). In Southeast Asia, the family includes an estimated 40 species in 12 genera (Kottelat, 2013), whereas 15 species in five genera (*Carinotetraodon*, *Langocephalus*, *Auriglobus*, *Arothron* and *Pao*) have been recorded in the Mekong River (Taki *et al*., 2021). Within the *Pao* genus, at least six species have been reported from the Indochinese portion of the Mekong River (Taki *et al*., 2021) and five species (*P. abei*, *P. baileyi*, *P. cambodgjiensis*, *P. turgidus* and *P. fangi*) from the Cambodian portion of the system (Rainboth, 1996; So *et al*., 2018).

Taxonomic confusion concerning freshwater pufferfishes in Asia has been reported since Dekkers's review of the *Tetraodon* genus in 1975 (Matsuura, 2015) and revisions of pufferfish genera have continued to the present (Kottelat, 2013; Matsuura, 2015; Froese & Pauly, 2023; Fricke *et al*., 2024). Kottelat (2013) discussed taxonomic issues related to *Tetraodon*, *Tetrodon* and *Monotrete* and created a new genus, *Pao*, for the taxa found in the Southeast Asia. We follow Kottelat (2013) in adopting *Pao* in relation to the Mekong River and in considering *Tetraodon* and *Monotrete* as synonyms of *Pao* within the region (Froese & Pauly, 2023; Fricke *et al*., 2024). Currently, the *Tetraodon* genus is used for only six freshwater pufferfishes in Africa (Matsuura, 2015).

The Mekong arrowhead puffer *Pao suvattii* (Sontirat & Soonthornsatit, 1985) is endemic to the Lower Mekong

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Basin (Vidthayanon, 2012) and has hitherto been reported from Thailand and Laos PDR only (Roberts, 1998; Kottelat, 2001; Vidthayanon, 2012; Taki *et al*., 2021; Froese & Pauly, 2023). The species was not included for Cambodia by Rainboth (1996), Rainboth *et al*. (2012), So *et al*. (2018) or Taki *et al*. (2021) and no photographs or voucher specimens have been reported from the country.

In February 2022, we caught one tetraodontid fish resembling *Pao* sp. in the Ou Nampha stream, which drains into the Sekong River of the Mekong system (Fig. 1). The capture site is situated within Siem Pang Wildlife Sanctuary in northeast Cambodia. Through a careful check of keys in field guides (Kottelat, 2001; Taki *et al*., 2021), the individual was subsequently identified as *Pao suvattii* (Sontirat & Soonthornsatit, 1985). We document this record as the first for the species from the Cambodian Mekong drainage.

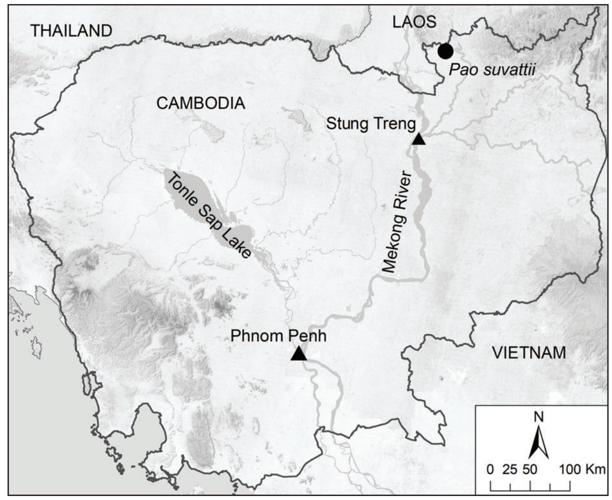

Fig. 1 Location of first record of *Pao suvattii* (Sontirat & Soonthornsatit, 1985) (black circle) in Cambodia.

Fig. 2 Field photographs of *Pao suvattii*, Stung Treng Province, February 2022.

Pao suvattii (Sontirat & Soonthornsatit, 1985)

Diagnosis: *Pao suvattii* is characterized by an upturned mouth, a long and tapering snout with curved lips, a depressed head and body, slightly elongated body covered with spinules, an arrowhead-shaped dusky marking behind the interorbital area and before the origin of dorsal fin, and a series of oblique stripes on the cheeks and lower anterior part of the body. Live specimens have a brown to dark body covered with small dark and white spots. The ventral surface of body is white (Fig. 2). Because the individual we encountered was not kept as a voucher specimen, our identification was based on careful examination and photographs taken in the field, both of which agreed with the identification keys and descriptions for *Pao* species (Sontirat & Soonthornsatit, 1985; Roberts, 1998; Kottelat, 2001; Taki *et al*., 2021).

Distribution: Based on existing records, *P. suvattii* is restricted to the Lower Mekong Basin in Thailand and Laos, extending into northern Cambodia (Roberts, 1998; Vidthayanon, 2012; Taki *et al*., 2021; this study).

Habitat: We captured *P. suvattii* in a stationary gillnet set in a small deep pool (20 m wide x 30 m length x 3 m deep) in the slightly-flowing, dark and clear water habitat surrounded by a flooded forest with a rocky substrate in the Ou Nampha stream (Fig. 3). The stream drains into the Sekong River of the Mekong system. The capture site is located within the area encompassed by Khamphok Village, Siem Pang District (and Siem Pang Wildlife Sanctuary), Stung Treng Province, northeast Cambodia (Fig. 1).

Although evidenced only by photographs (taken with an iPhone11 Promax camera), our record nonetheless provides new information on the distribution of *P. suvattii* which can be included for Cambodia in FishBase (<www.fishbase.se>), Fishes of Mainland Southeast Asia (<http://ffish.asia>; Kano *et al*., 2013) and future field guides for the region. Notwithstanding this, further studies should consider collection of voucher specimens for biometric and molecular analyses to confirm identifications of future records of the species from the Mekong River basin.

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Fig. 3 Stretch of the Ou Nampha stream where *Pao suvatti* was recorded, Stung Treng Province, February 2022.

References

Fricke, R., Eschmeyer, W.N. & van der Laan, R. (2023) *Eschmeyer's Catalog of Fishes*. Http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [accessed 4 January 2024].

Froese, R. & Pauly, D. (2023) *FishBase*. Https://www.fishbase.us/ [accessed 10 September 2023].

Kano Y., Adnan, M.S., Grudpan, C., Grudpan, J., Magtoon, W., Musikasinthorn, P., Natori Y., Ottomanski, S., Praxaysonbath, B., Phongsa, K., Rangsiruji, A., Shibukawa K., Shimatani Y., So N., Suvarnaraksha A., Thach P., Nguyen T.P., Tran D.D., Utsugi K. & Yamashita T. (2013) An online database on freshwater fish diversity and distribution in mainland Southeast Asia. *Ichthyological Research*, **60**, 293–295.

Kottelat, M. (2001) *Fishes of Laos*. WHT Publications Ltd., Colombo, Sri Lanka.

Kottelat, M. (2013) The fishes of the inland waters of Southeast Asia: a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. *Raffles Bulletin of Zoology*, **27**, 1–663.

Matsuura K. (2015) Taxonomy and systematics of tetraodontiform fishes: a review focusing primarily on progress in the period from 1980 to 2014. *Ichthyological Research*, **62**, 72–113.

Nelson, J.S. (2006) *Fishes of the World*. John Wiley & Sons, New Jersey, US.

Nelson, J.S., Grande, T.C. & Wilson, M.V.H. (2016) *Fishes of the World*. John Wiley & Sons, New Jersey, US.

Rainboth, W.J. (1996) *FAO Species Identification Field Guide for Fishery Purposes: Fishes of the Cambodian Mekong*. Food and Agriculture Organization of the United Nations, Rome, Italy.

Rainboth, W.J., Vidthayanon C. & Mai D.Y. (2012) *Fishes of the Greater Mekong Ecosystem with Species List and Photographic Atlas*. Museum of Zoology, University of Michigan, US.

Roberts, T.R. (1998) Freshwater fugu or pufferfishes of the genus *Tetraodon* from the Mekong basin, with descriptions of two new species. *Ichthyological Research*, **45**, 225–234.

So N., Utsugi K., Shibukawa K., Thach P., Chhuoy S., Kim S., Chin D., Nen P. & Chheng P. (2018) *Fishes of Cambodian Freshwater Bodies*. Inland Fisheries Research and Development Institute, Fisheries Administration, Phnom Penh, Cambodia.

Sontirat, S. & Soonthornsatit, S. (1985) A new puffer species of Thailand: *Tetraodon suvattii* n. sp. In *The 23rd Academic Conference on Fisheries at Kasetsart University, 5–7 February 1985*, pp. 49–53). Kasetsart University, Bangkok.

Taki Y., Ohtsuka R., Komoda M., Natori Y., Utsugi K., Shibukawa K., Oizumi T., Ottomanski S., Praxaysombath, B., Phongsa, K., Magtoon, W., Musikasinthorn, P., Grudpan, C., Grudpan, J., Suvarnaraksha, A., So N., Thach P., Nguyen P.T., Tran D.D. & Tran L.X. (2021) *Fishes of the Indochinese Mekong*. Nagao Natural Environment Foundation, Tokyo, Japan.

Vidthayanon C. (2012) *Monotrete suvattii. The IUCN Red List of Threatened Species*. Http://dx.doi.org/10.2305/IUCN.UK.2012-1.RLTS.T187933A1836631.en [accessed 1 October 2023].

Zhu H., Yamada A., Goto Y., Horn L., Ngy L., Wada M., Doi H., Lee J.S., Takatani T. & Arakawa O. (2020) Phylogeny and toxin profile of freshwater pufferfish (genus *Pao*) collected from two different regions in Cambodia. *Toxins*, **12**, 689.

Short Communication

Confiscation and rehabilitation of native and alien turtle species (*Malayemys* spp.) intended for prayer animal release in the Angkor Landscape

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Cambodia is both a transit and a source country for illegally trafficked wildlife, with many species being trafficked to neighbouring countries or sold domestically (Gray *et al*., 2017; Heinrich *et al*., 2019). Chelonians are among the most consistently targeted species, often for consumption, medical use, and pet markets (Stanford, *et al*., 2020; Cox *et al*., 2022; Nguyen, 2023). An alternative incentive for chelonian trafficking, less represented within the scientific literature, is their use in prayer-animal release events. These events are primarily carried out by Buddhists, the dominant religious group in Cambodia, where approximately 95% of the population identifies as Buddhist (Manira *et al*., 2019).

In Cambodia, prayer-animal release is viewed by many as a demonstration of kindness and benevolence towards the animals, with the belief that participants will gain some form of merit or good karma for releasing the animal into the wild (Magellan, 2019). Buddhism teaches compassion for all living organisms, and the act of prayer-animal release is meant to be a physical display of this compassion. However, if not performed under scientific guidance, introducing new animals through prayer-animal release can have detrimental ecological impacts (Liu *et al*., 2013). Non-native species can introduce foreign diseases and parasites, to which native populations have no resistance. The presence of these non-native species can disrupt the local ecosystems, leading to altered food webs and the decline of native biodiversity. These changes can have cascading effects, resulting in long-term environmental harm, an outcome well-documented in species such as the pond slider *Trachemys scripta*, which has become the most abundant and invasive terrapin worldwide (Fanaru *et al*., 2024). Prayer-animal release with native species also demands careful consideration. Releasing native species can pose similar risks, including transmission of diseases or possible genetic interference, resulting in the loss of biodiversity (Awoyemi *et al*., 2016). Most animals used for prayer-animal release in Cambodia are native; wildlife trafficking records have shown over 95% of all confiscations in Cambodia between 2001–2018 were native species (Heinrich *et al*., 2020).

On 28 August 2023, the Siem Reap Provincial Department of Environment (PDoE) confiscated 11 snail-eating turtles (*Malayemys* spp.) and one red-eared slider *T. scripta elegans* from an illegal trader at Angkor Wat. We suspected that these turtles were intended for prayer-animal release because they were being sold next to the temple inside Angkor Wat, a popular prayer-animal release site. While we could not confirm the intentions of those involved in their trade, the purchase of turtles at this location is highly unlikely to be for any other

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purpose in our experience. The turtles were transported to the PDoE Angkor Landscape office where they were temporarily housed in tubs with shallow water to allow them to hydrate while avoiding possible drowning of any weaker animals. The following day, the 12 turtles were transported to the Angkor Centre for Conservation of Biodiversity (ACCB) in transport crates lined with wet towels to avoid potential further dehydration. Unfortunately, one of the snail-eating turtles succumbed to its injuries before arriving at ACCB, whereas the remainder arrived in poor condition.

Morphological characteristics enabled ACCB staff to correctly identify two distinct species from the eleven snail-eating turtles rescued. Six were Mekong snail-eating turtles *M. subtrijuga* (Fig. 1a), a species with two distinct allopatric populations: one isolated population restricted to the island of Java in Indonesia, likely due to anthropogenic introduction or a naturally occurring relict population, and another with an extensive distribution across the lower Mekong River system of the Indochinese Peninsula, spanning Cambodia, Laos, Vietnam and northeastern Thailand (Dawson *et al*., 2020). Five were Malayan snail-eating turtles *M. macrocephala* (Fig. 1b), a species native to northern and central Thailand, south through peninsular Thailand to northwestern peninsular Malaysia (Ihlow *et al*., 2015, 2016; Dawson *et al*., 2018). Trade records confirm the presence of *M. macrocephala* in food markets in Vientiane and Champassak provinces in Laos (Auer, 2011; Suzuki *et al*., 2015). *Malayemys macrocephala* is not known to naturally occur in Cambodia, but its distribution approaches extreme western Cambodia and could thus marginally extend into a small area of the country, though this has yet to be confirmed (Dawson *et al*., 2018). The phenotypical differences used by ACCB staff to distinguish these species included the number of nasal stripes, because *M. macrocephala* generally has four or fewer nasal stripes, whereas *M. subtrijuga* typically has six or more. *Malayemys macrocephala* also has a much wider infraorbital stripe that seldom extends above the loreal seam in front of the eye, compared to *M. subtrijuga* which has a narrower infraorbital stripe that usually connects to the supraorbital stripe on the snout (Fig. 1) (Brophy, 2004).

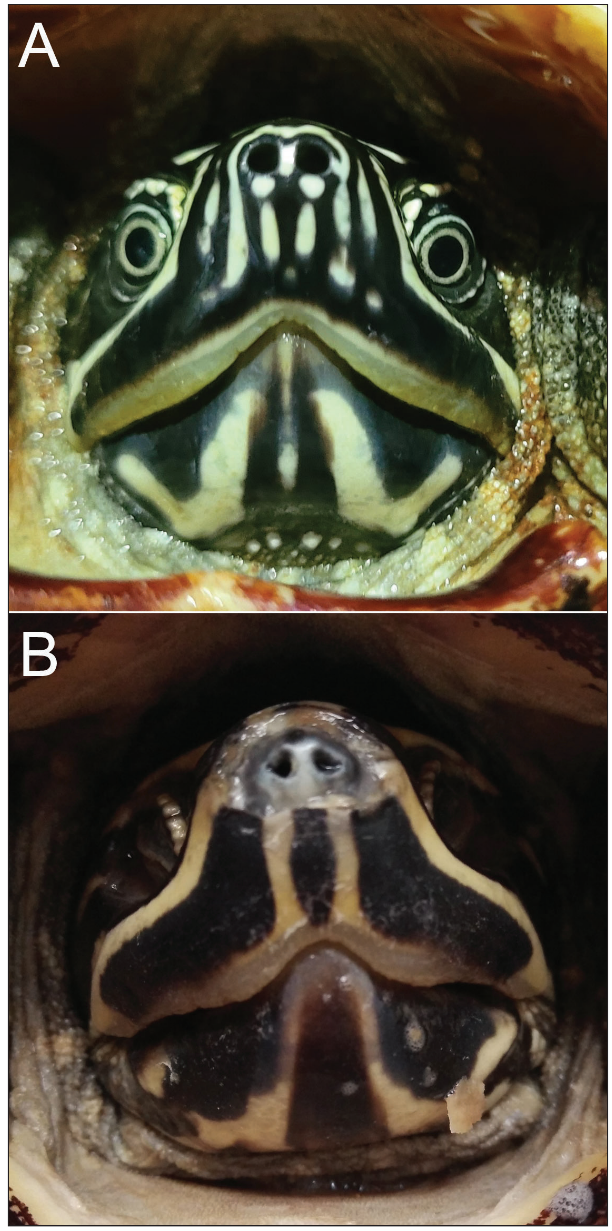

Fig. 1 Variations in nasal markings between A) *Malayemys subtrijuga* (© ACCB / Maria Blümm) and B) *M. macrocephala* (© ACCB / Jason Miller). *Malayemys subtrijuga* has six or more nasal stripes, whereas *M. macrocephala* has four or fewer nasal stripes.

Preliminary examination of the turtles upon arrival at ACCB showed that all were severely dehydrated (Fig. 2a) and presented with a lesser or greater degree of septicaemic cutaneous ulcerative disease (SCUD, also known as shell rot) on the plastron and/or carapace (Fig. 3). Complications with shell health are common in rescued turtles (Cortés *et al*., 2022) and in our experience, confiscated snail-eating turtles almost always display signs of SCUD. This suggests that the physiology of these two species is severely prone to environmental stressors, which is also consistent with other reports of *Malayemys* in human care (Dawson *et al*., 2018).

Three of the turtles also presented bilateral keratitis, a simultaneous infection of the cornea in both eyes, resulting in their eyes displaying a white colouration (Fig. 2b). We also noted that these three turtles displayed

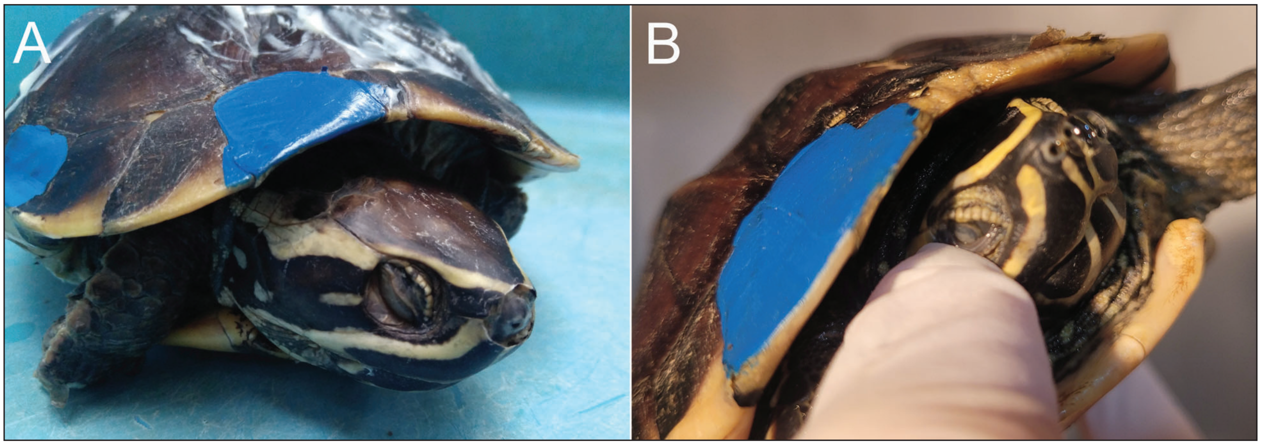

Fig. 2 Two confiscated snail-eating turtles before treatment began at ACCB. These showed signs of weakness such as closed eyes and relaxed limbs, whereas the sunken eyes (A) indicated a high degree of dehydration. The white corneal colouration of the eye (B) is a symptom of keratitis. All of the turtles showed similar signs of weakness and dehydration. Topical ointment (A) can also be seen on the shell as part of treatment and blue nail varnish was painted on differing scutes to identify individuals (© ACCB / Maria Blümm).

Fig. 3 Three confiscated snail-eating turtles after arrival at ACCB, all with plastron damage caused by septicaemic cutaneous ulcerative disease. Lesions are indicated by white arrows (© ACCB / Maria Blümm).

sunken eyes and produced a severe odour from their oral cavities. Although showing no obvious signs of disease aside from severe dehydration and SCUD, a fourth turtle suddenly became very weak 24 hrs after its arrival at ACCB. Despite intensive treatment and husbandry care from the ACCB veterinary team, all four turtles regrettably died less than 48 hours after their arrival on site. At the time of writing, all of the remaining turtles from the confiscation are receiving intensive treatment, antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), and fluid therapy, in addition to being placed on a treatment schedule for shell care (Doneley *et al*., 2017).

On 15 September 2023, Siem Reap PDoE confiscated a further five *M. macrocephala* from traders at Angkor Wat and we suspect again these were intended for prayer-animal release. These turtles were also transported to ACCB for rehabilitation and they too showed signs of severe dehydration and SCUD. One had a hole penetrating its carapace (Fig. 4). The extent of this wound was found to have completely perforated the shell and damaged a lung and the turtle unfortunately died several days later as a consequence. Another turtle had buoyancy issues, being unable to submerge itself fully. The prognosis for this turtle at the time of writing was poor, because the buoyancy concerns most likely stemmed from internal complications requiring an extended period of intensive care.

Our experience with rescued *M. subtrijuga* turtles is that the species generally does not adjust well to an ex-situ environment. The poor state of health of turtles on arrival at ACCB and the intensive treatment required often result in major problems during their acclimation. Despite the high standards of husbandry ensured by ACCB veterinary staff and keepers, these turtles often continue to develop minor SCUD. As a result, ACCB strives to release *M. subtrijuga* individuals in suitable, secure habitats in collaboration with government authorities, following scientific guidance from experienced staff and conservation allies. To this end, staff ensure that their medical condition has improved and that no further medication is required. The turtles must also exhibit clear signs they are healthy and active, to maximise the prospects for survival on release back into the wild.

Fig. 4 A snail-eating turtle with a small, but deep hole in the carapace which perforated the shell and damaged a lung (© ACCB / Maria Blümm).

Confiscation of *M. macrocephala* turtles also pose concerns. Although most animals used in prayer-animal release in Cambodia are native species, release of *M. macrocephala* appears to be increasing. *Malayemys macrocephala* is readily available in Thailand, where the species is frequently caught and sold for prayer-animal release (Dawson *et al*., 2018). The presence of *M. macrocephala* in confiscations by Siem Reap PDoE suggests continued illegal wildlife trade between the native range of this species (Thailand, Malaysia, Myanmar; see above) and Cambodia. It also suggests that introductions of non-native species are frequently occurring to the Angkor Landscape due to prayer-animal release events (Heinrich *et al*., 2020), although this needs to be verified by a larger field study in the area.

ACCB focuses primarily on Endangered and Critically Endangered turtles and birds native to Cambodia. *Malayemys macrocephala* is currently classified by the IUCN Red List as Least Concern, whereas *M. subtrijuga* is classified as Near Threatened (Cota, 2021; Horne *et al*., 2021). Neither is a priority species for ACCB and resource constraints prevent long-term care for either species. However, addressing the ecological challenges unintentionally posed by prayer-animal releases involving both species is essential. Investigating the frequency and scale of *M. macrocephala* releases in Cambodia, and understanding the origins and pathways is crucial for developing targeted conservation strategies. If a native *M. macrocephala* population exists in western Cambodia, then release plans could be considered. Until confirmed, no confiscated *M. macrocephala* can be released in Cambodia because they are currently considered a non-native species.

Education could play a key role in influencing prayer-animal release practices. Religion has been of interest to many biologists worldwide who believe that there is potential for this to have a positive impact on conservation (Liu *et al*., 2013). Practices such as prayer-animal releases can adapt to current ecological realities without losing their core values of compassion for wildlife (Wasserman *et al*., 2019). If performed under the guidance of ecological experts and with native species, these could have a positive impact. This large undertaking begins with educating those who have the most influence: monks. Since 2021, ACCB's outreach and education programme has focused on capacity building of Buddhist monks and laypeople at six pagodas in four provinces. This effort is specifically focused on addressing conservation issues, including a sustainable approach to prayer-animal release. Further educational programmes such as this likely offer a way forward in bridging the gap between science, religion and tradition. For example, Liu *et al*. (2013) found that organisers of prayer-animal release events with ecological knowledge were less likely to release invasive species. While releasing native species can still have a negative impact if not guided by science, this demonstrates the influence of education on traditional and religious practices, as well as the open-mindedness of those organising these events.

The continued release of non-native species such as *M. macrocephala* through prayer-animal release could be a major driver of ecological change. The typical survival rate of a species released into a novel habitat has been found to be extremely low and relies on what is known as a propagule, the minimum number of organisms required to reproduce and survive under favourable conditions (Liu *et al*., 2013; Awoyemi *et al*., 2016). Dawson *et al*. (2018) suggested that religious practices such as prayer-animal release could introduce *M. macrocephala* into areas outside of the species distribution. With large or frequent release events, *M. macrocephala* would be increasingly likely to establish itself in areas such as the Angkor Landscape, if this has not already occurred. *Malayemys subtrijuga* and *M. macrocephala* are closely related, and Ihow *et al*. (2016) documented past introgression and relatively low differentiation in the mitochondrial DNA of the two species. The establishment of *M. macrocephala* in the Angkor Landscape could threaten the genetic integrity of native *M. subtrijuga* in this area through interbreeding. Such genetic pollution of native chelonian species by anthropogenically introduced species is well documented in the genus *Trachemys* and has been shown to have negative implications for conservation (Parham *et al*., 2013). We consequently recommend surveys to establish whether *M. macrocephala* currently resides at the known prayer-animal release locations within the Angkor Landscape. Collaborations are also essential between conservationists and religious leaders to ensure that prayer-animal releases are conducted in an ecologically responsible manner and thereby mitigate the associated risks.

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References

Auer, M. (2011) Beobachtungen zu Vorkommen und Handel von Schildkröten in Nordlaos. *Sauria*, **33**, 3–11.

Awoyemi, S.M., Kraus, F., Li Y., Magellan, K. & Schaefer, J. (2016) *Prayer Animal Release can Embody Conservation Principles: a Call to Action*. Http://st.scb.org/images/content_groups/Religion/Policy_Brief_for_Prayer_Animal_Release__RCRC__FINAL_SCB_Letterhead.pdf [accessed 18 January 2024].

Brophy, T.R. (2004) Geographic variation and systematics in the South-east Asian turtles of the genus *Malayemys* (Testudines: Bataguridae). *Hamadryad*, **29**, 63–79.

Cortés, A.A.C., Brieva, C. & Witte, C. (2022) Implications of wildlife trafficking on the health and conservation of an endangered turtle species in Colombia. *Conservation Science and Practice*, **4**, e595.

Cota, M. (2021) *Malayemys macrocephala. The IUCN Red List of Threatened Species 2021*. DOI 10.2305/IUCN.UK.2021-1.RLTS.T123770233A123770237.en [accessed 30 January 2024].

Cox, N., Young, B.E., Bowles, P., Fernandez, M., Marin, J., Rapacciuolo, G., Böhm, M., Brooks, T.M., Hedges, S.B., Hilton-Taylor, C., Hoffmann, M., Jenkins, R.K.B., Tognelli, M. F., Alexander, G.J., Allison, A., Ananjeva, N.B., Auliya, M., Avila, L.J., Chapple, D.G., Cisneros-Heredia, D.F., Cogger, H.G., Colli, G.R., De Silva, A., Eisemberg, C.C., Els, J., Fong G.A., Grant, T.D., Hitchmough, R.A., Iskandar, D.T., Kidera, N., Martins, M., Meiri, S., Mitchell, N.J., Molur, S., Nogueira, C.D.C., Ortiz, J.C., Penner, J., Rhodin, A.G.J., Riva, G.A., Rödel, M. O., Roll, U., Sanders, K. L., Santos-Barrera, G., Shea, G.M., Spawls, S., Stuart, B.L., Tolley, K.A., Trape, J.F., Vidal, M.A., Wagner, P., Wallace, B.P. & Xie Y. (2022) A global reptile assessment highlights shared conservation needs of tetrapods. *Nature*, **605**, 285–290.

Dawson, J.E., Ihow, F., Ettmar, S., van Dijk, P.P. & Thirakhupt, K. (2018) *Malayemys macrocephala* (Gray 1859) - Malayan snail-eating turtle, rice-field terrapin. In *Conservation Biology of Freshwater Turtles and Tortoises* (eds A.G.J. Rhodin, J.B. Iverson, P.P. van Dijk, C.B. Stanford, E.V. Goode, K.A. Buhlmann & R.A. Mittermeier), pp. 108.1–108.16. Chelonian Research Foundation and Turtle Conservancy, Arlington, USA.

Dawson, J.E., Ihlow, F., & Platt, S.G. (2020) Malayemys subtrijuga (Schlegel and Müller 1845) - Mekong Snail-Eating Turtle. In *Conservation Biology of Freshwater Turtles and Tortoises* (eds A.G.J. Rhodin, J.B. Iverson, P.P. van Dijk, C.B. Stanford, E.V. Goode, K.A. Buhlmann & R.A. Mittermeier), pp. 111.1–111.24.

Chelonian Research Foundation and Turtle Conservancy, Arlington, USA.

Doneley, B., Monks, D., Johnson, R. & Carmel, B. (2018) *Reptile Medicine and Surgery in Clinical Practice*. Wiley-Blackwell, New Jersey, USA.

Fănaru, G., Petrovan, S., Băncilă, R.I., Vizireanu, M.G., Drăgan, O., Vlad, S.E., Rozylowicz, L. & Cogălniceanu, D. (2024) Nesting ecology and confirmed breeding of the invasive pond slider *Trachemys scripta* in an urban environment, Romania. *European Journal of Wildlife Research*, **70**, 1–10.

Gray, T.N.E., Marx, N., Khem V., Lague, D., Nijman, V. & Gauntlett, S. (2017) Holistic management of live animals confiscated from illegal wildlife trade. *Applied Ecology*, 54, 726–730.

Heinrich, S., Ross, J.V., Gray, T.N.E., Delean, S., Marx, N. & Cassey, P. (2020) Plight of the commons: 17 years of wildlife trafficking in Cambodia. *Biological Conservation*, **241**, 108379.

Horne, B.D., McCormack, T. & Timmins, R.J. (2021) *Malayemys subtrijuga. The IUCN Red List of Threatened Species 2021*. DOI 10.2305/IUCN.UK.2021-1.RLTS.T123770834A2929454.en. [accessed 30 January 2024].

Ihlow, F., Flecks, M., Hartman, T., Cota, M., Makchai, S., Meewattana, P., Dawson, J.E., Kheng L., Rauh, A., Rödder, D. & Fritz, U. (2015) Diversity in Southeast Asian snail-eating turtles (Geoemydidae: Malayemys): implications for phylogeography and taxonomy. 18th European Congress of Herpetology, Wroclaw, Poland. DOI 10.13140/RG.2.1.4762.4086

Ihlow, F., Vamberger, M., Flecks, M., Hartman, T., Cota, M., Makchai, S., Meewattana, P., Dawson, J.E., Kheng, L., Rauh, A., Rödder, D. & Fritz, U. (2016) Integrative taxonomy of Southeast Asian snail-eating turtles (Geoemydidae: Malayemys) reveals a new species and mitochondrial introgression. *PLOS One*, **11**, e0153108.

Liu X., McGarrity, M.E., Bai C., Ke Z. & Li Y. (2013) Ecological knowledge reduces religious release of invasive species. *Ecosphere*, **4**, 1–12.

Magellan, K. (2019) Prayer animal release: an understudied pathway for introduction of invasive aquatic species. *Aquatic Ecosystem Health and Management*, **22**, 452–461,

Manira, L., Utari, P. & Sri, H. (2019) Cultural identification and adaptation of muslim minority: evidence from Cambodia. *International Journal of Multicultural and Multireligious Understanding*, **6**, 709–719.

Nguyen T.H. (2023) Factors influencing the pattern of wildlife product consumption in Indochina: case study of Cambodia. *E3S Web of Conferences*. DOI 10.1051/e3sconf/202342004021

Parham, J.F., Papenfuss, T.J., van Dijk, P.P., Wilson, B.S., Marte, C., Schettino, L.R & Simison, W.B. (2013) Genetic introgression and hybridization in Antillean freshwater turtles (Trachemys) revealed by coalescent analyses of mitochondrial and cloned nuclear markers. *Molecular Phylogenetics and Evolution*, **67**, 176–187.

Stanford, C.B., Iverson, J.B., Rhodin, A.G.J., van Dijk, P.P., Mittermeier, R.A., Kuchling, G., Berry, K.H., Bertolero, A., Bjorndal, K.A., Blanck, T.E.G., Buhlmann, K.A., Cayot, L.J., Ceballos, C.P., Das, I., Frazier, J., Fretey, J., Girondot, M., Guillon, J.-M., Hofmeyr, M.D., Jackson, D.R., Janzen, F.J., Georges, A., Kaska, Y., Kemenes, I., Kiester, A.R., Lau, M., Laurenti, A., Lawson, D.P., Le, M., Limpus, C., Lovich, J.E., Luiselli, L., McCormack, T., Meyer, G.A., Páez, V.P., Platt, K., Pritchard, P.C.H., Quinn, H.R., Roosenburg, W.M., Seminoff, J.A., Shaffer, H.B., Sharma, D.S.K., Spencer, R., van de Merwe, J., Vogt, R.C. & Walde, A.D. (2020) Turtles and tortoises are in trouble. *Current Biology*, **30**, R721–R735.

Suzuki D., Fuse, K., Aizu M., Yoshizawa S., Tanaka W., Araya, K. & Praxaysombath, B. (2015) Reptile diversity in food markets in Laos. *Current Herpetology*, **34**, 112–119.

Wasserman, R.J., Dick, J.T.A., Welch, R.J., Dalu, T. & Magellan, K. (2019) Site and species selection for religious release of non-native fauna. *Conservation Biology*, **33**, 969–971.

Use of trapeangs by Eld’s deer *Rucervus eldii siamensis* in Siem Pang Wildlife Sanctuary, Cambodia

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មូលន័យសង្ខេប

ត្រពាំងមានសារសំខាន់សម្រាប់សត្វជាច្រើនប្រភេទដែលរស់នៅព្រៃរបោះនៅភាគខាងជើងនិងភាគខាងកើតនៃប្រទេសកម្ពុជា។ ជាពិសេសត្រពាំងផ្តល់ប្រភពទឹកដ៏សំខាន់សម្រាប់សត្វទំពារអៀង និងប្រភេទសត្វដទៃទៀត ដោយសារត្រពាំងកាន់តែខ្វះខាតទឹកខ្លាំងនៅរដូវប្រាំង។ យើងបានវាយតម្លៃវត្តមានរបស់សត្វក្តាន់ *Rucervus eldii siamensis* ដែលជាសត្វជិតផុតពូជជាសាកលនៅត្រពាំងក្នុងដែនជម្រកសត្វព្រៃសៀមប៉ាងនៅភាគខាងជើងប្រទេសកម្ពុជា។ គោលបំណងរបស់យើងគឺមើលកំណើនវត្តមានសត្វក្តាន់នារដូវប្រាំងដោយការដាក់ទឹកបន្ថែមក្នុងត្រពាំង ហើយយើងបានពិនិត្យមើលអន្ទាក់កាមេរ៉ានៅឆ្នាំ២០២១ និងឆ្នាំ២០២២ ដែលដាក់ពង្រាយចំនួន ២៤ នៅ ១២ ត្រពាំង (អន្ទាក់កាមេរ៉ាពីរក្នុងមួយត្រពាំង) នៅព្រៃរបោះក្នុងទីជម្រកសត្វព្រៃសៀមប៉ាង។ កម្រិតទឹកត្រូវបានរក្សានៅត្រពាំងចំនួន០៦នៃការសិក្សា ដោយម៉ាស៊ីនបូមទឹកប្រើថាមពលពន្លឺព្រះអាទិត្យដើម្បីប្រៀបធៀបជាមួយនឹងត្រពាំងចំនួន០៦ទៀតដែលមិនបានបន្ថែមទឹក។ យើងមិនបានរកឃើញភាពខុសគ្នាជាតំលៃស្ថិតិនូវចំនួនវត្តមានសត្វក្តាន់រវាងត្រពាំងបន្ថែមទឹក និងត្រពាំងមិនបន្ថែមទឹកទេ ទោះបីការប្រើប្រាស់ត្រពាំងបន្ថែមកាន់តែច្រើនដោយសត្វក្តាន់ត្រូវបានកត់ត្រានៅឆ្នាំទី២ក៏ដោយ។ លទ្ធផលរបស់យើងផ្តល់នូវព័ត៌មានអេកូឡូស៊ីអំពីសកម្មភាពសត្វក្តាន់នៅត្រពាំង និងជួយក្នុងការសម្រេចចិត្តសម្រាប់ការគ្រប់គ្រងសត្វក្តាន់នាពេលអនាគត។

Abstract

Waterholes (*trapeang* in Khmer) are important for a wide range of species inhabiting deciduous dipterocarp forests in northern and eastern Cambodia. In particular, they provide a critical source of water for ungulates and other species as this becomes increasingly scarce during the dry season. We evaluated visits by the globally Endangered Eld’s deer *Rucervus eldii siamensis* to trapeangs in Siem Pang Wildlife Sanctuary, northern Cambodia. Our aim was to test if supplementary water provisioning would increase visitation rates of the deer during the dry season and we conducted camera trap surveys in 2021 and in 2022, deploying 24 camera traps at six pairs of trapeangs (two cameras per trapeang) in deciduous dipterocarp forests within the sanctuary. Water levels were maintained at six of our study trapeangs with solar water pumps to allow comparisons with our six control trapeangs. We did not find a statistically significant difference in the number of deer visits between the two groups of trapeangs, although greater use of supplemented trapeangs by the deer was recorded during the second year. Our results provide ecological information on the activity of Eld’s deer at trapeangs and will aid decision-making for their future management.

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Keywords Camera trap, camtrapR, deciduous dipterocarp forest, Eld's deer, seasonal pools, Wildlife Insights.

Introduction

Deciduous dipterocarp forests (DDF) are a unique ecosystem and species assembly found across the Mekong Basin in Southeast Asia, where high temperatures and pronounced seasonal precipitation patterns predominate (Pennington *et al*., 2009). These are characterized by a savanna landscape comprising a mix of deciduous trees (predominantly Dipterocarpaceae) and grasslands (Ratnam *et al*., 2011; Wohlfart *et al*., 2014). In northern and eastern Cambodia, DDF supports a wide range of important and endangered species, including Asian elephant *Elephas maximus*, banteng *Bos javanicus* and Eld's deer *Rucervus eldii*. This ecosystem is functionally distinct and evolved in response to abiotic factors (soil characteristics, seasonality and climate) and disturbances caused by herbivores or the frequent fires that occur during the dry season (Pletcher *et al*., 2022).

Waterholes, known as *trapeang* in Khmer, are an integral feature of DDF in Cambodia. These wetlands fill with water during the rainy season and typically dry out during the subsequent dry season months, creating patches of muddy substrates that provide ideal living or foraging conditions for a range of species, including critically endangered giant ibises *Thaumatibis gigantea* and white-shouldered ibises *Pseudibis davisoni* (Wright *et al*., 2010; Eang *et al*., 2021). They likely also provide a critical source of drinking water for ungulates such as Eld's deer during the peak dry season (Pin *et al*., 2018). Eld's deer is a large tropical cervid that historically occurred across DDF in Southeast Asia. The species has suffered a significant decline, with just two numerically significant units of wild animals now remaining, *R. e. thamin* in Myanmar and *R. e. siamensis* in Cambodia (Ladd *et al*., 2022a). In Cambodia, populations of the species have declined by over 90% since 2000 and while the most recent *IUCN Red List Assessment* in 2015 estimated 700 individuals remained in functionally isolated subpopulations (Gray *et al*., 2015a), the current population is likely less than 400 individuals (Ladd *et al*., 2022a).

Despite their importance, the ecological roles performed by trapeangs in DDF are increasingly disturbed by human activities such as land conversion to agriculture (Gray *et al*., 2015b) and ecological succession associated with the disappearance of the native wallowing megafauna (Eames *et al*., 2018). Climate change also represents a major threat, as the expected changes in rainfall patterns and increased temperatures associated with global warming will significantly impact water resources and water availability in Cambodia and this in turn impact the biodiversity and ecological balance of wetlands, as well as the livelihoods of communities that depend on them (Oeurng *et al*., 2019). Further, Cambodia's climate is affected by the El Niño–Southern Oscillation (ENSO) (Thirumalai *et al*., 2017), which usually returns every two to seven years (Climate Prediction Center, 2023). The warming phase El Niño causes hotter and dryer conditions than usual during the dry season months from November to April, and the frequency of extreme El Niño events is expected to increase due to climate change (Nicholls *et al*., 2005; Wang *et al*., 2019). For instance, the 2014–2016 episode culminated in a severe drought in Cambodia that affected both agriculture and wildlife, with a significant toll on animal life (Crothers, 2016).

Ecological management of trapeangs and ensuring their resilience in the face of climate change is of paramount importance given the number of species that depend on them (Timmins, 2012). Ungulates vary in their requirements for water, but as most species show some dependency towards specific drinking locations, water availability in seasonal ecosystems with temporal and/or spatial water scarcity is important for maintaining ungulate populations (Western, 1975; Hayward & Hayward, 2012; Montalvo *et al*., 2019). As such, creation of artificial waterholes or modifications to existing waterholes are integral to wildlife (particularly ungulate) conservation strategies in tropical savannah and dry forest ecosystems (Chamaillé-Jammes *et al*., 2007; Smit *et al*., 2007; Dar *et al*., 2012; Weeber *et al*., 2020). To this end, several pilot projects have been undertaken in Cambodia in recent years to determine whether such efforts are beneficial to local wildlife and how they might impact the use of trapeangs by globally threatened large ungulates and waterbirds. Trial modifications include the deepening of existing trapeangs on the assumption that they would hold water for longer periods during the dry season (Gray *et al*., 2015b), or by artificially creating new waterholes. These suggest that artificial deepening of natural trapeangs is effective in increasing water availability during the dry season, although the effects of this on local wildlife have yet to be investigated.

Pin *et al*. (2018) found that size and depth characteristics of trapeangs in eastern Cambodia was positively correlated with visitation by threatened waterbird species. Notwithstanding this, the influence of waterholes characteristics on their use by other wildlife species requires further investigation as trapeangs in DDF are likely of particular importance for wildlife in these ecosystems. In Siem Pang Wildlife Sanctuary in northern Cambodia, 66 trapeangs have been modified by manual or mechanical excavation to date and six trapeangs have been equipped with solar pumps that draw water up from the water table, including four which have been deepened (Rising Phoenix, unpubl. data).

SPWS likely hosts the largest remaining population of *R. e. siamensis* (Ladd, 2022; Ladd *et al*., 2022b), although this is particularly vulnerable to illegal hunting. During the 2016 drought, almost all of the trapeangs known in Siem Pang Wildlife Sanctuary dried out whereas the sole waterhole that retained water (trapeang Chambork), was increasingly visited by Eld's deer due to the scarcity of water elsewhere (as indicated by deer trails). Local hunters erected *machans*—wooden platforms in trees—at this trapeang from which to shoot the animals. To reduce the risk of hunting in future, we selected six waterholes throughout the DDF in SPWS for a pilot project aimed at providing permanent water sources. To this end, we created bore-wells at the six waterholes and equipped these with solar pumps in January 2021. The pumps were tasked with maintaining water in the trapeangs during the dry season (and were turned off during the wet season), drawing water from the water table 30–50 m below ground level. Consequently, the purpose of our study was to test if artificial provisioning of water at the six trapeangs during the dry season increased their visitation and use by Eld's deer relative to unmodified trapeangs.

Methods

Study area

Siem Pang Wildlife Sanctuary (SPWS) is a forest landscape mosaic with wetland elements and covers 130,000 ha in northern Cambodia. Deciduous dipterocarp forests account for 50% of the sanctuary, whereas semi-evergreen forests account for another 40%, with the remainder comprising degraded forests or grasslands (8%) and riverine habitats (2%) (BirdLife International Cambodia Programme, 2012). In Cambodia, DDF is characterized by a pronounced seasonal monsoon cycle, with alternating dry and wet seasons (Fan & Luo, 2019). Mean annual precipitation in SPWS is around 1,300 mm according to models, but rainfall is highly seasonal with most occurring during the wet season (mean 1,200 mm) from May to October and less than 100 mm during the dry season from November to April (Global Modelling & Assimilation Office, 2015). Over 200 trapeangs have been documented in SPWS (Rising Phoenix, unpubl. data). Most dry out during the dry season whereas others usually maintain water throughout the year. Domestic cattle *B. taurus* and water buffalo *Bubalus bubalus* from nearby villages roam freely in DDF within the wildlife sanctuary. The largest wild mammals occurring in these are Eld's deer, wild pig *Sus scrofa* and northern red muntjac *Muntiacus vaginalis*, with other large ungulates such as gaur *B. gaurus*, banteng *B. javanicus* and sambar deer *Rusa unicolor* now largely restricted to the semi-evergreen forests. Asian elephants occur east of the Sekong River and wild water buffaloes *B. arnee*, if formerly present, are now extirpated (Loveridge *et al*., 2018). Because waterholes provide a source of water for domestic ungulates and food (fish and frogs) for local communities, humans and their domestic dogs *Canis familiaris* are also frequent visitors (Ladd *et al*., 2023).

Study sites

Our study was conducted in trapeangs situated within DDF inside the wildlife sanctuary. The six trapeangs with pumps installed were paired with six control trapeangs without pumps (Fig. 1). Where possible, the paired trapeangs were selected with similar characteristics to the trapeangs with pumps. This included consideration of the surrounding vegetation and trapeang size. Paired trapeangs were at least 400 m and a maximum of 1,300 m apart (Fig. 2, Table 1). Water was maintained in the trapeangs with pumps throughout the dry season, whereas the other trapeangs were allowed to dry out naturally.

Camera trapping

We deployed camera traps (BTC-6PXD Dark Ops, Browning Trail Cameras, New South Wales, Australia) at 12 trapeangs in 2021 (six supplemented & six controls) and 11 trapeangs in 2022 (six supplemented & five

Table 1 Distance between paired trapeangs in Siem Pang Wildlife Sanctuary.

Supplemented	Control	Distance (km)
Angkrong	Thmor	0.9
Kdoeung	Umpiel	0.4
Khmun	Ktum	1.2
Lumporn	Trakoun	0.6
Lumtier	Thmat Kon	1.3
Thmea	Kontout	0.8

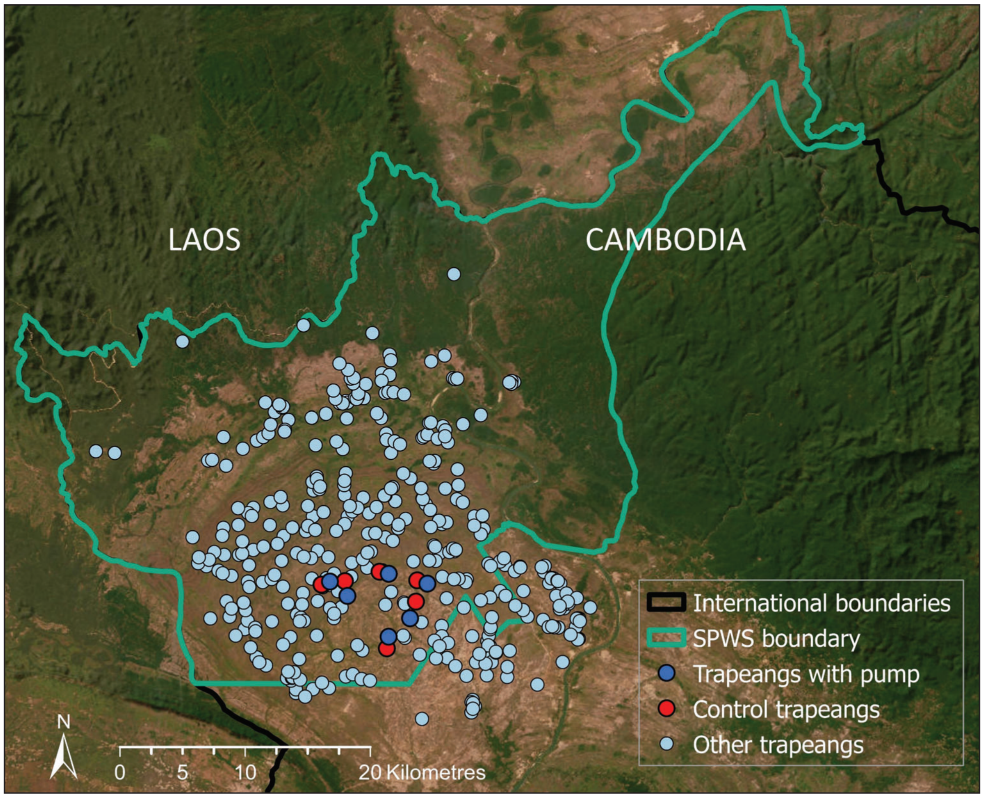

Fig. 1 Distribution of known trapeangs and trapeangs with camera traps deployed in deciduous dipterocarp forest in Siem Pang Wildlife Sanctuary. Two camera traps were deployed at each trapeang.

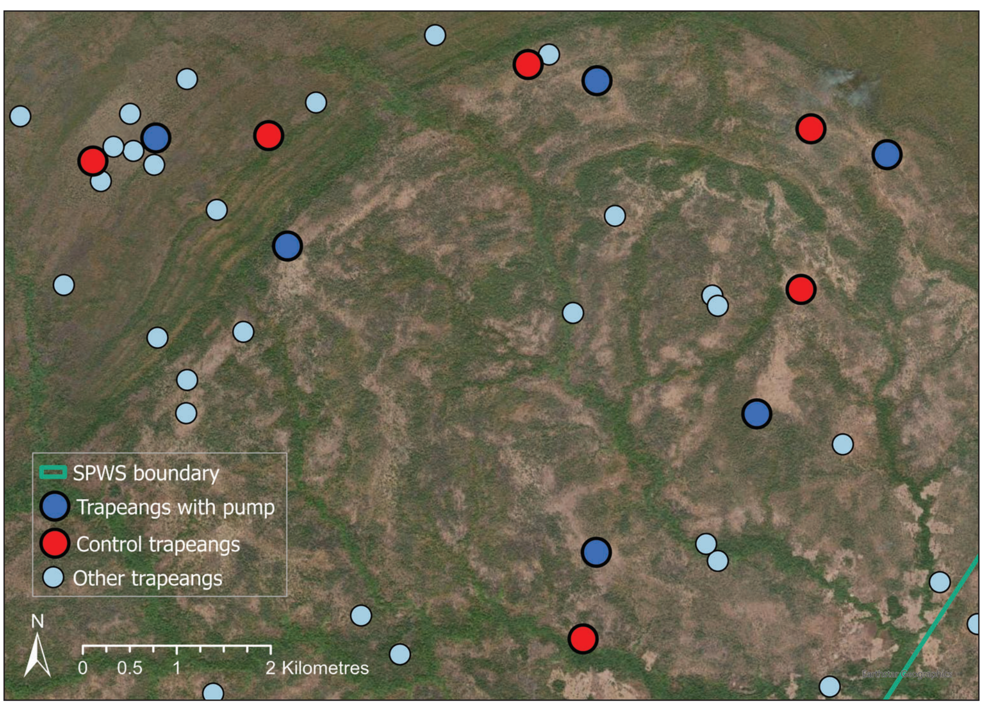

Fig. 2 Indepth view of study trapeangs and other trapeangs not monitored. Darker green shading represents semi-evergreen forest.

controls). Two cameras were deployed at each trapeangs and were placed to maximise coverage of the area and increase detection of visiting wildlife, providing a total of 24 cameras deployed in 2021 and 22 in 2022. Each trapeang was considered as a single station in our study design, although camera images were treated separately in analysis. Camera placement at each site was determined by signs of wildlife activity such as tracks around the waterhole, optimal placement for passive infrared sensor detection, or based on expert opinion and advice from field staff as to the best location. The proximity of dirt roads, direction of the sun, location of an appropriate tree to mount the camera and nearby vegetation were also considered in placement. As Eld's deer were the study target, cameras were attached ca. 0.8m above ground level to appropriately positioned trees close to the edges of trapeangs. Vegetation that might obstruct the field of view was removed. Cameras were set to take photographs at set time intervals and when motion was detected. A time-lapse plus mode was employed to take a picture every 60 minutes. Rapid-fire mode was enabled, resulting in eight photos per detection with a one second delay between triggers. Sensitivity was set to high and flash to long range (detection & flash range up to 25 m).

Cameras were deployed in the field from 9 December 2020 to 10 June 2021 and from 1 February to 15 May 2022. This corresponded to 366 camera-trap-nights per station in 2020–2021 and 206 camera-trap-nights per station in 2022, both coinciding with the dry season.

Image analysis

A total of 67,346 images were obtained for 2020–2021, and 36,871 for 2022 (totalling 104,217 images). Images were uploaded on Wildlife Insights (<www.wildlifeinsights.org/>) for species identification. Wildlife Insights is an online platform that provides tools for users to upload, manage, and analyse camera trap data, as well as share it with other researchers and conservationists. The platform uses artificial intelligence and machine learning algorithms to identify species in camera trap images, helping researchers to analyse their data. Images were automatically grouped and treated in sequences of 60 seconds. Images taken less than 60 seconds apart were considered as belonging to the same sequence, resulting in the segregation of 14,592 different sequences. All sequences were visually checked by one reviewer and tagged as blank or identified to species level where possible.

Data analysis

Data exported from Wildlife Insights were analysed using R 4.2.2 (R core team, 2022) with the camtrapR package (Niedballa *et al*., 2016). In line with previous research on Eld's deer in SPWS, we defined independent events as sequences of the same species at the same station separated by six minutes or more (Ladd, 2022). Patterns of activity (i.e., how animals distribute their activity throughout the 24 h day) were determined by plotting a kernel density estimation of activity based on timestamps for each independent event in 2020–2021 and 2022. To compare the number of sequences recorded for each of the two groups of trapeangs, we used Mann-Whitney tests to compare numbers of sequences recorded for each of the two groups of trapeangs each year as well as the entire study period. We also compared data for the two groups from March and April in 2021 and 2022, as water is minimal during these months and so differences in visitation between the two groups could be greatest until the first rains arrive (usually at the end of April).

Rainfall data

Empirical data on rainfall are not recorded using rain gauges or other devices in the Siem Pang area and the closest meteorological station is located some 80 km away in Stung Treng Province (Smith, 2023). However, retrospective datasets are available through NASA's global modelling and assimilation office tool MERRA-2 (Modern-Era Retrospective analysis for Research and Applications, Vers. 2). MERRA-2 provides a long-term global reanalysis of the atmosphere (from 1980 onwards), incorporating space-based observations into its atmospheric general circulation model to generate estimates of rainfall on a particular land surface, with a monthly resolution.

Results

Our six treatment trapeangs were served with pumps that maintained water levels throughout the dry season, whereas our six control trapeangs were left to dry out naturally. Only one of the latter retained some water throughout the dry season in 2021, whereas all but one retained some water through the 2022 dry season (when rain occurred). Our total effective sampling effort was 3,690 camera-trap-nights in 2020–2021 (representing 84% of the total potential effort) and 1,731 camera-trap-nights in 2022 (76.4% of total potential effort). The difference is attributable to vandalism (i.e., memory cards stolen or cameras turned off) or cameras running out of battery power during the sampling period.

A total of 14,592 60-second data sequences were recorded and tagged in Wildlife Insights, of which 7,580 were blank (51.9%), 1,385 recorded Burmese hare *Lepus*

Table 2 Eld's deer detections at study trapeangs using a six-minute independence threshold. Only one camera trap was active at trapeang Thmor in 2021 and no camera was set there in 2022.

Supplemented Trapeang (2021)	#	**Control Trapeang (2021)**	#	**Supplemented Trapeang (2022)**	#	**Control Trapeang (2022)**	#
Angkrong	0	Thmor	0	Angkrong	4	Thmor	N/A
Kdoeung	92	Umpiel	132	Kdoeung	192	Umpiel	5
Khmun	36	Khtum	41	Khmun	3	Khtum	7
Lumporn	7	Trakoun	13	Lumporn	20	Trakoun	9
Lumtier	8	Thmat Kon	17	Lumtier	5	Thmat Kon	1
Thmea	50	Kontout	24	Thmea	93	Kontout	14
	193		**227**		**317**		**36**

peguensis (9.5%), 1,208 recorded domestic water buffalo (8.3%), 932 recorded Eld's deer (6.4%), 858 recorded humans (staff & villagers) (5.9%), 800 recorded northern red muntjac (5.5%), 448 recorded domestic cattle (3.1%), and 445 recorded wild pig (3.1%).

When the data were considered in terms of independent events, Eld's deer were detected in 773 out of a total of 5,939 independent events for all species (compared to 661 events provided using a 30-minute threshold). Eld's deer were detected at ten of 12 stations in 2020–2021, for a total of 420 events, with between 0 and 132 events per station ($\bar{x}$=35, SD=40.2 events per station). In 2022, the species was detected at all 11 stations, for a total of 353 events, with between 1 and 192 events per station ($\bar{x}$=32.1, SD=59.1 events per station) (Table 2).

Of the 773 independent events obtained for Eld's deer, we counted 1,387 Eld's deer, with a mean group size of 1.8 (SD=1.2, with 1–10 animals simultaneously counted). Eld's deer occurred in groups of two or more in 326 events (42.2%), with a mean group size of 2.9 (SD=1.2). Males were evident in 310 events (40.1%) and were recorded alone in 239 of these (77.1%). Detections of males increased over the course of the dry season and peaked in March. They were present in groups in 9% of images in January (2/22), but in more than a quarter of images for February (15/63), March (39/165) and April (15/57) (Fig. 3).

Combining both study years, 510 sequences of Eld's deer were recorded at supplemented trapeangs (with pumps) whereas 263 sequences were recorded at control trapeangs. However, no significant difference was found between the two groups (number of sequences at supplemented vs. control trapeangs) using a Mann-Whitney test (U=59.5, p=0.36). The same was found when detections were segregated by year (2021: U= 16.5, p=0.44; 2022: U= 11.5, p=0.29) and month (number of independent events recorded in March or April of each year) (March 2021: U=17.0, p=0.47; April 2021: U=10.5, p=0.13; March 2022: U=9.5, p=0.10; April 2022: U=10.5, p=0.13). Given the small size of our two groups however, no definitive conclusion can be drawn from the absence of statistically significant differences.

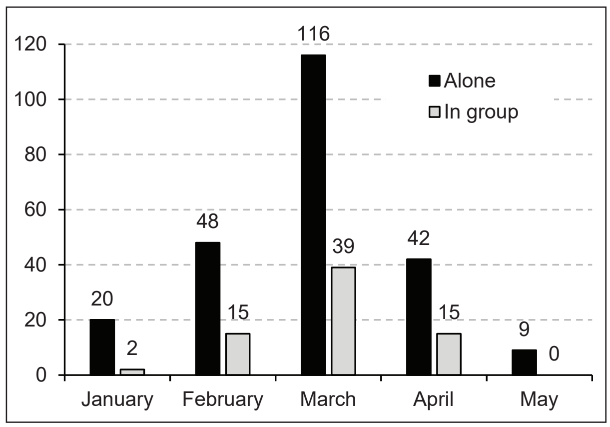

Fig. 3 Detection of male Eld's deer as solitary animals and in groups. Detections increased over the dry season and peaked in March.

The mean duration of the 773 independent events was 34.1 seconds from the first to the last image (range 1–464, SD=54.5 seconds). When adult males were alone in sequences (n=239), their mean duration was 15.9 seconds (range 1–275, SD=26.4 seconds).

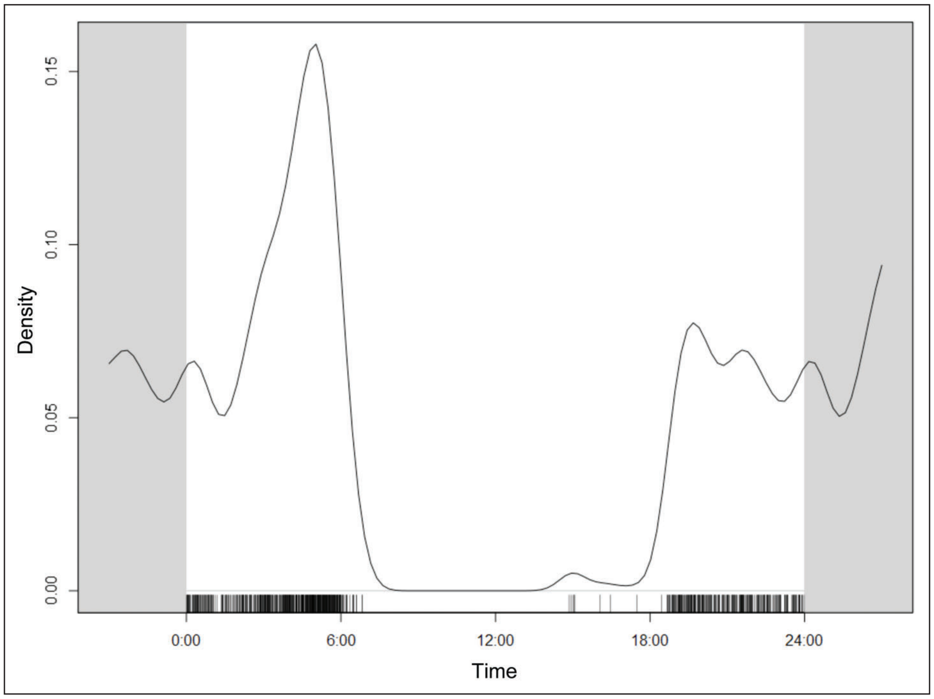

Fig. 4 Kernel density estimates of activity of Eld's deer, based on camera trap records (*n*=773) at 12 study trapeangs in Siem Pang Wildlife Sanctuary, 2021 and 2022.

Fig. 5 Two male Eld's deer bathing in late afternoon at a trapeang in Siem Pang Wildlife Sanctuary.

Activity patterns

Eld's deer visits to trapeangs were mainly nocturnal, with most activity occurring between 1900 and 0600 hrs and a peak of activity between 0400 and 0600 hrs (Fig. 4). It was not possible to identify the behaviour of animals in most images, aside from walking/standing still and head up/down, although some animals were infrequently seen bathing, fighting or drinking (Fig. 5).

Discussion

Numbers of detections of Eld's deer varied greatly between trapeangs and years. For example, in the supplemented trapeang group, 192 events were recorded at Kdoeung station but only three at Khmun during the same 2022 survey. Likewise, at Umpiel station (control), 132 events were recorded in 2021 but only five in 2022. Additionally, large differences were observed between the first and second survey years, beyond those of individual trapeangs. In the second survey year, four of the five trapeang pairs showed higher use of supplemented trapeangs, versus only one out of six in the first year. Two trapeang pairs especially displayed large differences (Kdoeung/Umpiel & Thmea/Kontout). Factors underlying these differences are likely diverse and probably include variations in meteorological conditions as well as ecological factors.

The 2020–2023 period was a rare triple-dip La Niña event (NASA Earth Observatory, 2022) and regular rainfall occurred throughout the dry season in Cambodia. According to the MERRA-2 model, an unusual amount of rain occurred in February 2021 and January–March 2022 period (Table 3). This above average rainfall may have resulted in Eld's deer visiting these trapeangs less than compared to more typical, drier years, as water would have been more available throughout the DDF. This variability in rainfall, combined with the lack of baseline data on trapeang use before our study, makes it difficult to discern possible associations between water manipulation and trapeang use by Eld's deer. A major caveat of our study is that we were not able to reliably record changes in water availability at trapeangs or local rainfall during the study period. As a result, we cannot look for correlations between these variables and monthly detection rates of Eld's deer. Marker pegs were deployed to monitor changes in water levels during the first study year, but these were found to be ineffective due to wallowing buffalo and subsequently removed. Variability between years at the same trapeang could also be due to other factors such as increased disturbance or illegal hunting pressure, whereas variability between stations could be due to shifts in the occupancy and density of Eld's deer within the sanctuary, though no data are available to confirm this theory. Many factors could be at play, such as differences in the habitats surrounding trapeangs (e.g., open grassland vs. trees at a higher density), plant species richness, water quality or occupation of the trapeangs by water buffalo.

Several other factors linked to the study design may also have influenced the detectability of Eld's deer. For instance, we selected camera traps with infrared flash over white flash due to time and budget constraints. This makes individual identification of large animals less efficient and can impact the ability of a reviewer to identify species, in our case to differentiate between Eld's deer and northern red muntjac (Meek *et al*., 2014; Ladd *et al*., 2022c). The images produced by the cameras were also substandard in daylight hours and blurring made identification difficult at times. Despite having two cameras at each station, the field of view and detection zone did not always fully cover all edges of trapeangs and so could have under-detected visits by Eld's deer. Another caveat lies in the difficulty of finding statistical differences between the treatment and groups. Only six trapeangs were deepened and equipped with solar pumps, a rather low sample size that would necessitate a high number of Eld's deer detections to provide clear results. We did however observe an increase in visitation at supplemented trapeangs.

Table 3 MERRA-2 precipitation corrected figures (mm) for the study area (14.1457°N, 106.2477°E).

Year	Jan	Feb	Mar	Apr	May
2020	0.0	0.0	15.8	52.7	0
2021	0.0	15.8	0.0	183.8	8.1
2022	20.7	16.3	105.5	80.9	N/A
Mean 1981-2020	2.9	5.1	17.5	48.3	6.2

More Eld's deer events were recorded in March (*n*=332, almost 43% of those recorded) than February (*n*=235) or April (*n*=104). Our survey in 2022 was shorter than in 2021 which precluded comparisons with January and May. Although the breeding cycle of *R. e. siamensis* is unclear, it likely follows the pattern observed for *R. e. thamin* in Myanmar, with mating occurring in March or April and fawns born in November–December (Aung *et al*., 2001). Because Eld's deer increase their activity during the rut period, this could lead to an increase in detections and indeed our detections were significantly higher in February, March and April. Changes in detection likely also reflect season and water availability, with animals concentrating at fewer trapeangs as these dry out and wider water availability reduces within the sanctuary. In 2021, the first rains in SPWS occurred at the end of April when most of the control trapeangs had dried out, whereas scattered rain occurred during the dry season in 2022 and most trapeangs retained some water. Additionally, rains are not evenly distributed across the landscape and some trapeangs may benefit from earlier rain than others.

While this could explain our increased detections in March, our results differ from Ladd (2022) who reported higher detections in SPWS in May (which corresponds to the early rainy season) and lower detections in March during the rut and dry season when the landscape has dried out (Ladd, 2022). However, it is important to note that we deployed camera traps at trapeangs whereas Ladd (2022) deployed camera trap arrays throughout the dry forest. It is likely that our higher detections in March are related to the extreme scarcity of water in the DDF.

Previous studies have described the social organization and group size of *R. e. thamin* in Myanmar during the hot dry season. Our mean group size of 1.8±1.2 found in our study is much smaller than that found in Shwesettaw Wildlife Sanctuary in Myanmar where mean group size was 7.6±0.9 (Thu *et al*., 2019) and in Chatthin Wildlife Sanctuary where this peaked in April at 5.9±8.3 individuals (Aung *et al*., 2001). The maximum number of individuals we recorded simultaneously was ten, which is consistent with group sizes usually spotted in SPWS, although groups of up to 29 individuals have also been recorded simultaneously (Rising Phoenix, unpubl. data). Groups of up to 28 and more than 70 individuals were described by Thu *et al*. (2019) and Aung *et al*. (2001) respectively. These differences could be due to a lower population density in SPWS, as a positive relationship has been identified between group size and population density has been documented in other cervids, or by the fact that according to Thu *et al*. (2019), large groups of Eld's deer in Shwesettaw Wildlife Sanctuary avoid areas near water sources as predation and hunting pressure are higher. Eld's deer visitations at trapeangs were mainly nocturnal in our study, which may be a predator avoidance behaviour triggered by human disturbance, as observed in Hainan (Pan *et al*., 2011).

In camera traps studies, time-to-independence intervals of 30 to 60 minutes are frequently used, whereby all images of the same species are filtered and discarded within this interval for each camera (Peral *et al*., 2022). However, the interval setting is largely arbitrary and is probably species dependent. For example, Ladd (2022) used the lorelogram technique of Iannarilli *et al.* (2019) on a set of data for Eld's deer to empirically determined an independence interval of six minutes for the species. We adopted this treatment in our study. The definition of Ladd (2022) for independent events was based on an analysis of detections from 40 camera traps deployed in the dry forest which provided 368 detections of Eld's deer over 4,026 camera trap nights between December 2018 and May 2019. In addition to being arbitrary, choosing a longer interval usually results in loss of data, which is problematic for studying species that occur at low density and are difficult to detect. In our case, a 30-minute threshold would have reduced the number of independent events by roughly 15% but would not have changed our data analysis and interpretation unduly.

Finally, reviewing and tagging camera trap images is a time-consuming process. Use of the Wildlife Insights platform proved to be effective in removing barriers related to image cataloguing and data storage that are often associated with large datasets such as the >100,000 images in our study (Glover-Kapfer *et al*., 2019; Ahumada *et al*., 2020). Though artificial intelligence was ineffective most of the time in recognizing animals to the species level (and the class and family level to some extent), it was helpful in grouping the images by sequences for review, identifying blank images with adequate accuracy and common species such as dogs or cattle. Recognition of humans or vehicles in the images was also very good.

Conclusions

Siem Pang Wildlife Sanctuary is one of the last strongholds for Eld's deer in Cambodia, but the population is threatened by human activities and climate change which risks changes to rainfall patterns and longer and harsher droughts and ecosystem modifications. To mitigate these threats, we modified several trapeangs by deepening these and installing solar-powered water pumps. We designed our camera-trap study to compare visits of Eld's deer between supplemented and control trapeangs over the course of the dry season. However, we did not find a significant difference due to a small sample size and high variability between years and within groups. This was likely due to factors including variations in rainfall locally and between years, as well as ecological factors and Eld's deer behaviour, but given the design of our survey, it was not possible to resolve the multiple hypotheses. Future studies of trapeang use need to occur over a longer temporal scale to account for variation in rainfall and subsequent trapeang use by wildlife. Our study could be improved on by expanding sample size, monitoring local rainfall using rain gauges and employing water gauges to objectively record water levels at trapeangs. Nevertheless, our results provide a baseline for future studies of trapeang use by Eld's deer trapeang in SPWS and will aid future decision-making in management of trapeangs.

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References

Ahumada, J.A., Fegraus, E., Birch, T., Flores, N., Kays, R., O'Brien, T.G., Palmer, J., Schuttler, S.G., Zhao, J.Y., Jetz, W., Kinnaird, M.F., Kulkarni, S., Lyet, A., Thau, D., Duong, M., Oliver, R.Y. & Dancer, A. (2020) Wildlife insights: a platform to maximize the potential of camera trap and other passive sensor wildlife data for the planet. *Environmental Conservation*, **47**, 1–6.

Aung, M., Mcshea, W.J., Htung, S., Than, A., Soe, T.M., Monfort, S. & Wemmer, C. (2001) Ecology and social organization of a tropical deer (*Cervus eldi thamin*). *Journal of Mammalogy*, **82**, 836–847.

BirdLife International Cambodia Programme (2012) *The biodiversity of the proposed Western Siem Pang protected forest Stung Treng Province, Cambodia*. BirdLife International Cambodia Programme, Phnom Penh, Cambodia.

Chamaillé-Jammes, S., Valeix, M. & Fritz, H. (2007) Managing heterogeneity in elephant distribution: interactions between elephant population density and surface-water availability: Surface water and elephant distribution. *Journal of Applied Ecology*, **44**, 625–633.

Climate Prediction Center (2023) *Historical El Nino / La Nina episodes (1950-present)*. Https://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php [accessed 8 August 2023].

Crothers, L. (2016) *Animals die as Cambodia is gripped by worst drought in decades*. *The Guardian,* 5 May 2016. Https://www.theguardian.com/global-development/2016/may/05/animals-die-cambodia-worst-drought-decades [accessed 08 August 2023].

Dar, T.A., Habib, B. & Khan, J.A. (2012) Group size, habitat use and overlap analysis of four sympatric ungulate species in Shivalik Ecosystem, Uttarakhand, India. *Mammalia*, **76**, 31–41.

Eames, J., Eang S., Loveridge, R. & Gray, T.N.E. (2018) Impact of experimental domestic water buffalo *Bubalus bubalis* grazing on waterhole dynamics in north-eastern Cambodia. *Cambodian Journal of Natural History*, **2018**, 101–109.

Eang S., Vann V. & Eames, J.C. (2021) A second population assessment of the Critically Endangered giant ibis Thaumatibis gigantea in Siem Pang Wildlife Sanctuary, Cambodia. *Cambodian Journal of Natural History*, **2021**, 12–20.

Fan X. & Luo X. (2019) Precipitation and flow variations in the Lancang–Mekong river basin and the implications of monsoon fluctuation and regional topography. *Water*, **11**, 2086.

Global Modelling And Assimilation Office (2015) *MERRA-2 tavgM_2d_lfo_Nx: 2d, monthly mean, time-averaged, single-level, assimilation, land surface forcings V5.12.4*. Https://doi.org/10.5067/5V7K6LJD44SY [accessed 30 August 2023].

Glover-Kapfer, P., Soto-Navarro, C.A. & Wearn, O.R. (2019) Camera-trapping version 3.0: current constraints and future priorities for development. *Remote Sensing in Ecology and Conservation*, **5**, 209–223.

Gray, T., Brook, S.M., McShea, W.J., Mahood, S.P., Ranjitsingh, M.K., Miyunt, A., Hussain, S.A., Timmins, R. (2015a) *Rucervus eldii. The IUCN Red List of Threatened Species*. Https://doi.org/10.2305/IUCN.UK.2015-2.RLTS.T4265A22166803.en [accessed 11 September 2023].

Gray, T., McShea, W., Koehncke, A., Sovanna P. & Wright, M. (2015b) Artificial deepening of seasonal waterholes in eastern Cambodia: impact on water retention and use by large ungulates and waterbirds. *Journal of Threatened Taxa*, **7**, 7189-7195.

Hayward, M.W. & Hayward, M.D. (2012) Waterhole use by African fauna. *South African Journal of Wildlife Research*, **42**, 117–127.

Iannarilli, F., Arnold, T.W., Erb, J.D. & Fieberg, J.R. (2019) Using lorelograms to measure and model correlation in binary data: Applications to ecological studies. *Methods in Ecology and Evolution*, **10**, 2153–2162.

Ladd, R. (2022) *Deer, dogs, demographics and detection: conservation management of Eld's deer (Rucervus eldii) in Cambodia*. PhD Thesis, University of Queensland, Australia.

Ladd, R., Crouthers, R., Brook, S. & Eames, J.C. (2022a) Reviewing the status and demise of the Endangered Eld's deer and identifying priority sites and conservation actions in Cambodia. *Mammalia*, **86**, 407–421.

Ladd, R., Meek, P., Eames, J.C. & Leung L.K.-P. (2023) Activity range and patterns of free-roaming village dogs in a rural Cambodian village. *Wildlife Research*. DOI 10.1071/WR23024

Ladd, R., Meek, P., Eames, J.C. & Leung L.K.-P. (2022b) *Deriving a population estimate for Eld's deer in Siem Pang Wildlife Sanctuary, Cambodia*. Unpublished manuscript.

Ladd, R., Meek, P. & Leung L.K.-P. (2022c) The influence of camera-trap flash type on the behavioural response, detection rate and individual recognition of Eld's deer. *Wildlife Research*, **50**, 475–483.

Loveridge, R., Cusack, J., Eames, J., Eang S. & Willcox, D. (2018) Mammal records and conservation threats in Siem Pang Wildlife Sanctuary and Siem Pang Khang Lech Wildlife Sanctuary. *Cambodian Journal of Natural History*, **2018**, 76–89.

Meek, P.D., Ballard, G., Claridge, A., Kays, R., Moseby, K., O'Brien, T., O'Connell, A., Sanderson, J., Swann, D.E., Tobler, M. & Townsend, S. (2014) Recommended guiding principles for reporting on camera trapping research. *Biodiversity Conservation*, **23**, 2321–2343.

Montalvo, V.H., Sáenz-Bolaños, C., Alfaro, L.D., Cruz, J.C., Guimarães-Rodrigues, F.H., Carrillo, E., Sutherland, C. & Fuller, T.K. (2019) Seasonal use of waterholes and pathways by macrofauna in the dry forest of Costa Rica. *Journal of Tropical Ecology*, **35**, 68–73.

NASA Earth Observatory (2022) *La Niña Times Three*. Https://earthobservatory.nasa.gov/images/150691/la-nina-times-three [accessed 30 August 2023].

Nicholls, N., Baek, H.-J., Gosai, A., Chambers, L.E., Choi Y., Collins, D., Della-Marta, P.M., Griffiths, G.M., Haylock, M.R., Iga, N., Lata, R., Maitrepierre, L., Manton, M.J., Nakamigawa, H., Ouprasitwong, N., Solofa, D., Tahani L., Thuy D.T., Tibig, L., Trewin, B., Vediapan, K. & Zhai P. (2005) The El Niño–Southern Oscillation and daily temperature extremes in east Asia and the west Pacific. *Geophysical Research Letters*, **32**, L16714.

Niedballa, J., Sollmann, R., Courtiol, A. & Wilting, A. (2016) CamtrapR: an R package for efficient camera trap data management. *Methods in Ecology and Evolution*, **7**, 1457–1462.

Oeurng, C., Cochrane, T., Chung, S., Kondolf, M. & Piman, T., Arias, M. (2019) Assessing climate change impacts on river flows in the Tonle Sap Lake Basin, Cambodia. *Water*, **11**, 618.

Pan D., Teng L., Cui F., Zeng Z., Bravery, B.D., Zhang Q. & Song Y. (2011) Eld's deer translocated to human-inhabited areas become nocturnal. *AMBIO*, **40**, 60–67.

Pennington, R.T., Lavin, M. & Oliveira-Filho, A. (2009) Woody plant diversity, evolution, and ecology in the tropics: perspectives from seasonally dry tropical forests. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 437–457.

Peral, C., Landman, M. & Kerley, G.I.H. (2022) The inappropriate use of time-to-independence biases estimates of activity patterns of free-ranging mammals derived from camera traps. *Ecology and Evolution*, **12**, e9408.

Pin C., Ngoprasert, D., Gray, T.N.E., Savini, T., Crouthers, R. & Gale, G.A. (2018) Utilization of waterholes by globally threatened species in deciduous dipterocarp forest of the Eastern Plains Landscape of Cambodia. *Oryx*, **54**, 572–582.

Pletcher, E., Staver, C. & Schwartz, N.B. (2022) The environmental drivers of tree cover and forest–savanna mosaics in Southeast Asia. *Ecography*, **2022**, e06280.

R Core Team (2022) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ratnam, J., Bond, W.J., Fensham, R.J., Hoffmann, W.A., Archibald, S., Lehmann, C.E.R., Anderson, M.T., Higgins, S.I. & Sankaran, M. (2011) When is a 'forest' a savanna, and why does it matter? *Global Ecology and Biogeography*, **20**, 653–660.

Smit, I.P.J., Grant, C.C. & Devereux, B.J. (2007) Do artificial waterholes influence the way herbivores use the landscape? Herbivore distribution patterns around rivers and artificial surface water sources in a large African savanna park. *Biological Conservation*, **136**, 85–99.

Smith, M. (2023) *CLIMWAT for CROPWAT: A Climatic Database for Irrigation Planning and Management*. Https://www.fao.org/land-water/databases-and-software/climwat-for-cropwat/en/ [accessed 14 September 2023].

Thirumalai, K., DiNezio, P.N., Okumura Y. & Deser, C. (2017) Extreme temperatures in Southeast Asia caused by El Niño and worsened by global warming. *Nature Communication*, **8**, 15531.

Thu A.M., Li G.-G., Zhang M., Thang T.H., Soe, A.M., Naing W. & Quan R.-C. (2019) Group size and social organization of the endangered Eld's deer (*Rucervus eldii thamin*): Results from a long-term study in Myanmar. *Global Ecology and Conservation*, **18**, e00618.

Timmins, R.J. (2012) *An Assessment of the "Vulnerability" of the Proposed Western Siem Pang Protected Forest to Climate Change, with Recommendations for Adaptation and Monitoring*. BirdLife International Cambodia Programme, Phnom Penh, Cambodia.

Wang B., Luo X., Yang Y.-M., Sun W., Cane, M.A., Cai W., Yeh S.-W. & Liu J. (2019) Historical change of El Niño properties sheds light on future changes of extreme El Niño. *Proceedings of the National Academy of Sciences*, **116**, 22512–22517.

Weeber, J., Hempson, G.P. & February, E.C. (2020) Large herbivore conservation in a changing world: surface water provision and adaptability allow wildebeest to persist after collapse of long-range movements. *Global Change Biology*, **26**, 2841–2853.

Western, D. (1975) Water availability and its influence on the structure and dynamics of a savannah large mammal community. *African Journal of Ecology*, **13**, 265–286.

Wohlfart, C., Wegmann, M. & Leimgruber, P. (2014) Mapping threatened dry deciduous dipterocarp forest in South-east Asia for conservation management. *Tropical Conservation Science*, **7**, 597–613.

Wright, H.L., Buckingham, D.L. & Dolman, P.M. (2010) Dry season habitat use by critically endangered white-shouldered ibis in northern Cambodia. *Animal Conservation*, 13, 71–79.

Lessons from the first successful ex situ conservation breeding of the Critically Endangered white-shouldered ibis [Threskiornithidae: *Pseudibis davisoni* (Hume, 1875)] in Cambodia

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មូលន័យសង្ខេប

ការបង្កាត់ពូជអភិរក្សក្រៅតំបន់ធម្មជាតិ (ex situ) ត្រូវបានប្រើប្រាស់កាន់តែច្រើនឡើងដើម្បីការពារការផុតពូជនៃប្រភេទបក្សីដែលកំពុងរងការគំរាមកំហែង។ ប្រទេសកម្ពុជា ជាជម្រករបស់សត្វត្រយ៉ងចង្កំកស *Pseudibis davisoni* ដែលកំពុងរងការគំរាមកំហែង។ ដោយមានការគាំទ្រពីអាជ្ញាធររដ្ឋាភិបាល មជ្ឈមណ្ឌលអង្គរសម្រាប់ការអភិរក្សជីវចម្រុះបានបង្កើតប៉ូពុយឡាស្យុង ex situនៃប្រភេទសត្វដោយផ្អែកលើឯកត្តៈដែលបានជួយសង្គ្រោះ និងរឹបអូស ដោយមានគោលដៅបង្កើតឡើងនូវប៉ូពុយឡាស្យុងដែលធានានិរន្តរភាពសេនេទិច។ ក្នុងរដូវបង្កាត់ឆ្នាំ២០២២ - ២០២៣ យើងបង្កាត់បានកូនបក្សីពីរក្បាលដោយជោគជ័យ ដែលតំណាងឲ្យការបង្កាត់ក្រៅតំបន់ធម្មជាតិ (ex situ) ប្រកបដោយភាពជោគជ័យជាលើកដំបូងនៅលើពិភពលោក។ បទពិសោធន៍នេះបានផ្តល់ឲ្យយើងនូវឱកាសដើម្បីពិចារណាពីមេរៀនដែលយើងទទួលបាន និងជាព័ត៌មាននាពេលអនាគត។ សកម្មភាពប្រចាំថ្ងៃរបស់យើងមានដូចជាការផ្គត់ផ្គង់វត្ថុធាតុដើមសម្រាប់ធ្វើសំបុក ការផ្លាស់ប្តូរបបអាហារតាមរដូវកាលដើម្បីធ្វើត្រាប់តាមលក្ខខណ្ឌធម្មជាតិ និងការផ្តល់ឱកាសជ្រើសរើសដៃគូបន្តពូជដោយសេរី ដែលមានសារៈសំខាន់នៅក្នុងការជម្រុញការបង្កាត់ពូជ។ ត្រូវមានការប្រុងប្រយ័ត្នដើម្បីការពារការខូចទ្រង់ទ្រាយរូបសាស្ត្រដែលអាចបង្កឡើងដោយវិធីសាស្ត្រនៃការចិញ្ចឹមសត្វ។ ការកែប្រែកន្លែងបង្កាត់ពូជចាំបាច់ត្រូវធ្វើឱ្យប្រសើរឡើងនូវការដាក់ដោយឡែកនៃគូបង្កាត់ពូជ ដើម្បីកាត់បន្ថយអកប្បកិរិយាខឹងច្រឡោតរវាងឯកត្តៈនីមួយៗ។

Abstract

Ex situ conservation breeding is increasingly used to prevent the extinction of threatened bird species. Cambodia is home to the highly threatened white-shouldered ibis *Pseudibis davisoni*. With support from government authorities, the Angkor Centre for Conservation of Biodiversity has developed an ex situ population of the species based on rescued and confiscated individuals with the goal of establishing a genetically sustainable assurance population. During the 2022–2023 breeding season, we successfully bred two chicks, which represents the first successful ex situ breeding effort worldwide. This experience provided us an opportunity to consider lessons learned and inform future efforts. Our daily provision of nesting material, seasonal change of diet to mimic natural conditions and allowance of free mate choice may have been important in stimulating breeding. Caution must be taken to prevent morphological deformities

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that could be caused by husbandry procedures. Modifications to our breeding complex are needed to improve separation of breeding pairs so as to minimize aggressive behaviour between individuals.

Keywords Captive breeding, hatching success, mate choice, nestling success, reproductive success, seasonal diet.

Introduction

Ex situ conservation breeding is increasingly used to prevent the extinction of threatened bird species and populations (Seddon *et al*., 2007; Redford *et al*., 2011; Biega *et al*., 2019; Bussolini *et al*., 2023). Successful conservation breeding programmes include the California condor *Gymnogyps californianus* in North America (Snyder & Snyder, 2000), the crested ibis *Nipponia nippon* in Asia (Xi *et al*., 2002) and most recently, the Spix's macaw *Cyanopsitta spixii* in Brazil (Vercillo *et al*., 2023). While captive breeding programmes have suceeded for some bird species (Butchart *et al*., 2006), they can be time-consuming, resource-intensive and difficult to implement (D'Elia 2010; Hoffmann *et al*., 2010; Collar & Butchart 2014; Bussolini *et al*., 2023). Success is by no means guaranteed, because many programmes are limited by decreased reproductive success in captive environments.

Decreased reproductive success in captive environments is not the only reason for the failure of ex situ conservation projects. Other challenges such as lack of suitable habitat for reintroduction and difficulties in monitoring and managing populations post-release also contribute to the failure of these programmes (Lees & Wilcken, 2009; Mason, 2010; Leus, 2011; Farquharson *et al*., 2018; Bubac *et al*., 2019). As such, a thorough understanding of potential challenges is crucial to planning and successfully implementing ex situ conservation projects.

Removing individuals from the wild to create founder populations for ex situ breeding programmes can increase species extinction risk (Snyder *et al*., 1996). On the other hand, ex situ conservation efforts can buy time for the causes of population declines in the wild to be addressed through actions such as eliminating introduced predators, restoring habitats or enacting legislative changes that create conditions for species to survive (Andrew *et al*., 2016; Mahood *et al*., 2021). If individuals need to be taken from the wild, ex situ breeding programmes should only be undertaken when sufficient numbers remain in situ. Determining the necessity and feasibility of establishing a founder generation from the wild should be based on a One Plan Approach (Byers *et al*., 2013).

Cambodia supports the white-shouldered ibis *Pseudibis davisoni*, which is regarded as Critically Endangered (BirdLife International, 2018). The species was once widely distributed in Southeast Asia but has declined substantially in the last 50 years (Thewlis & Timmins, 1996; BirdLife International, 2018). Currently, it is almost entirely confined to northern and eastern parts of Cambodia (ca. 87–95 % of the global population) with a fragmented population estimated at 670 mature individuals globally (BirdLife International, 2018). Conversion of dry forests for infrastructure development, settlement and agriculture have been projected to cause further, severe declines in the species (Wright *et al*., 2010b, 2012b).

During the dry season in Cambodia (November–May), white-shouldered ibises nest in dipterocarp trees in dry forests close to seasonally abandoned wet-season rice-fields, large contiguous areas of dry forest and in flooded forest within the Mekong channel (Wright *et al*., 2013b; BirdLife International, 2018). During this season, they forage at waterholes (known as *trapeangs* in Khmer) (Wright *et al*., 2010a; BirdLife International, 2018) and the species appears to benefit from the presence of large wild herbivores, which are believed to have historically played a role in maintaining trapeangs in dry forest landscapes. As such, the decline of large herbivore species in the region may have contributed to the decline of the ibis, with only a few suitable areas now remaining for the species in Cambodia. In the absence of large herbivores, the wallowing and grazing behaviour of domestic water buffaloes may serve a similar function (Wright *et al*., 2010a; Eames *et al*., 2018).

Actions have been undertaken to conserve populations of white-shouldered ibises in Cambodia over the last two decades. For example, its population has been monitored annually through a coordinated national census and its nests are monitored and protected in priority areas for the species through schemes which offer incentives to community members to locate and report nest locations (Clements *et al*., 2010; Wright *et al*., 2010b, 2012b, 2013a; Cambodia Ibis Working Group [CIWG], 2023). A community-managed ecotourism initiative has been initiated at Tmatboey and Siem Pang incorporating nest-finding and monitoring (BirdLife International, 2018). Communities have also been engaged in low-intensity, sustainable and wildlife-friendly agriculture through the promotion of an Ibis Rice scheme, which protects foraging areas for the species and encourages coexistence between the farmers and ibises (Ibis Rice, 2023; Sansom Mlup Prey, 2023). Finally, the CIWG was established by a national government decree in 2023 and facilitates cooperation between different stakeholders striving to protect the species.

While a One Plan Approach (Byers *et al*., 2013) incorporating in situ and ex situ conservation efforts does not exist for white-shouldered ibises in Cambodia, the need for the latter has been acknowledged (CIWG, 2023). To this end, an ex situ population of white-shouldered ibises has been established at the Angkor Centre for Conservation of Biodiversity (ACCB) using rescued and confiscated individuals with the goal of establishing a genetically viable assurance population (Woesner *et al*., 2021). Situated in Siem Reap Province (13°40'41''N, 104°01'32''E), the ACCB is a species conservation centre of Allwetterzoo Münster (Germany) which focuses on establishing assurance colonies of threatened chelonians and bird species. This paper describes the first successful ex situ breeding of white-shouldered ibises at ACCB and lessons learned during the 2022–2023 breeding season (November 2022 to July 2023).

Methods

Captive breeding site

Cambodia's climate is tropical, with relatively high temperatures and two distinct seasons: a monsoon-driven wet season (May–October) with south-westerly winds ushering in clouds and moisture that accounts for between 80–90% of the country's annual rainfall and a dry season (November–April), with cooler temperatures between November and January (World Bank, 2021). The area around ACCB experiences a mean minimum temperature of 20.5 °C and a mean maximum temperature of 34.6 °C (World Bank, 2021), although temperatures can be more extreme with lowest and highest records of 15.4 °C and 40.0°C (WorldData, 2024). During the 2022–2023 breeding season, minimum and maximum temperatures recorded were 20 °C and 40 °C respectively (AccuWeather, 2023). The mean maximum relative humidity in Cambodia is around 82% (WorldData, 2024).

Our white-shouldered ibises are kept in an off-show complex consisting of four aviaries which are interconnected through sliding doors at ground level. This includes one large aviary (W20) which measures 18x18 m (Fig. 1) and three smaller breeding aviaries (W20.1, W20.2 & W20.3) each measuring 12x6 m. All four aviaries range in height between 4–5 m (Fig. 2), are planted with

Fig. 1 White-shouldered ibis (upper centre) inside the largest aviary (W20) at the ACCB (© ACCB / K. Groot).

Fig. 2 One of three smaller breeding aviaries (W20.2) at the ACCB. The platform pair 1 used to build their nest and raise their chick is located in the far right corner of the enclosure (© ACCB / K. Groot).

trees, bushes and grasses and are equipped with a small pond and perching options at differing heights. Nest sites are also installed in all four. The largest aviary contains multiple woven bamboo baskets, whereas the three smaller aviaries contain a high nest platform (ca. 50x50 cm) placed ca. 4 m above ground level and a smaller round nest basket on a pole.

White-shouldered ibises at ACCB

At the start of the 2022–2023 breeding season, the ex situ population of white-shouldered ibis at ACCB comprised nine individuals (three males, three females and three birds whose sex was undetermined), including some birds of unknown age. Some of these individuals were rescued from the illicit pet trade whereas others were recovered from snares or rescued as nestlings or fledglings that had fallen from nests during bad weather. Through engagement with partner organizations and local communities monitoring nests, injured ibises are promptly identified, enabling swift interventions. Individuals rescued as chicks or juveniles could have been imprinted on humans prior to their arrival at ACCB. The individuals that bred or are suspected to have bred in the 2022–2023 breeding season are shown in Table 1. All of the birds were kept together and had access to all four aviaries at the start of the breeding season.

Diet

The natural diet of white-shouldered ibises comprises small invertebrates such as mole-crickets, insect larvae, amphibians (*Fejervarya limnocharis* and *Microhyla* spp.) and occasionally eels, snakes and leeches. Amphibians appear to form the bulk of the diet, especially during the dry season when these almost excluisvely occur at trapeangs (Wright *et al*., 2012a, 2013b; BirdLife International, 2018).

White-shouldered ibises at ACCB are fed twice a day. The non-breeding diet consists of 75 g of freshwater fish (a mixture of *Barbonymus* spp. and *Cirrhinus* spp.) and 75 g of frogs (East Asian bullfrog *Hoplobatrachus chinensis* bred nearby) per bird feeding, both of which are cut up into small strips or pieces. While none of these species have been recorded in the diet of wild white-shouldered ibises, amphibians form a key part of their diet during the dry season (Wright *et al*., 2010a, 2012a, 2013b). The breeding season diet comprises different quantities of the same components, namely 50 g of freshwater fish and 100 g of frogs per bird twice a day. 'Superworms' (larvae of the tenebrionid beetle *Zophobas morio*) and the supple-

Table 1 Summary information on individuals that potentially bred out of a total of three males, three females and three unsexed birds. The male involved in pair 1 is unknown, hence both males are considered as potential partners in this context. The individual marked * died during the 2022–2023 breeding season, potentially after a mating event.

Pair	ACCB ID	Sex	Arrival date	Estimated birth date	Acquired from
Pair 1	0180006	Male*	18 March 2017	15 February 2017	Ratanakiri
Pair 1	0180007	Male	18 March 2017	15 February 2017	Ratanakiri
Pair 1	0180009	Female	16 January 2018	30 December 2017	Kratie
Pair 2	0180012	Male	7 November 2018	1 January 2015	Stung Treng
Pair 2	0180010	Female	15 March 2018	8 February 2018	Kratie

ment Korvimin ZVT + Reptil (Tierarzt24, Germany) are provided daily during the breeding season.

Nesting materials

Prior to the present study, nesting material was only provided every second day if the birds interacted with it. From early November 2022 onwards, nesting material was provided every two days in the largest aviary (W20) and daily from mid-December onwards. Following incubation, nest material was provided less frequently. The material consisted of a mixture of small branches with green leaves and small bare branches, with a circumference of 14–27 mm, diameter of 4–6 mm and length of 20–50 mm. The ibises also foraged for grass in the enclosure and employed this for nest building.

Data collection

Data were opportunistically collected by ACCB keepers and curators via behavioural observations made with binoculars during the study period. These data included intraspecies interactions (positive and negative), nest building, breeding events and other noteworthy observations and events. All data collected were maintained using the Zoological Management Information System (https://zims.species360.org/).

Results

Nest building

Pair 1 started building a nest on the high breeding platform in the middle aviary (W20.2). However, staff were unable to identify the sire in pair 1 during the 2022–2023 breeding season (Table 1). Nest material was first observed on the platform on 8 February 2023. On 8 March, pair 2 were found to have a nest of their own in one of the trees in the large aviary (W20). It was unknown when this pair began building the nest because the nest area was not accessible.

Incubation

Due to the height and angle of the nest locations, it was not feasible to access and monitor these without disturbing the breeding pairs. The dates that eggs were laid are consequently unknown. There was no indication that a second egg was laid by either pair, although this cannot be confirmed. However, pair 1 began incubating on 13 February, ceased on 16 March, and the chick (ACCB ID 0180022, hereafter chick 1) was first heard (but not seen) on 18 March. We therefore assume that the incubation period of this pair was 31–33 days. However, the pair could have been brooding a very young (and undetected) chick for a few days before incubation ceased. This introduces some uncertainty in estimating the duration of the incubation period. In terms of pair 2, a bird was observed sitting on the nest for the first time on 16 March. However, it is not known for certain whether the pair started incubating earlier. Chick 2 (ACCB ID 0180023) was first heard on 15 April, which would indicate an incubation period of at least 30 days.

Chick development

Chick 1 was not seen until 27 March, nine days after it was first heard. Following this, chick 1 could be monitored daily from outside the aviary (Fig. 3). On 25 April, a deformity in its wings was observed, whereby its primary feathers were rotated outwards on both wings. This could indicate a bilateral valgus deformity known as ‘angel wings’ (Fig. 4). As the chick was 40 days old and approaching fledging by this time, it was decided to bring it to the on-site veterinary clinic for examination and treatment. Because the superworms were suspected as the cause of the deformity due to their high levels of proteins, these were removed from all diets for the remainder of the breeding season.

The chick was treated by placing a figure of eight bandage on each wing to keep these in the correct position and holding these together in place with a body wrap. Selenium-Vitamin E was also administered (dosage 0.1 mg Se/kg IM) to prevent neuromuscular diseases such as capture myopathy. Because voluntary feeding was not observed, the chick had to be force-fed for the first three days of treatment. Bandages were changed every two days during the first week of treatment. Light-coloured bandages with a camouflage pattern were used, but the bird managed to remove these every two to three days, likely due to the high colour contrast of the bandage against the feathers. Bandages were then changed to a dark-green colour, which proved more effective as these lasted for a week. During each bandage replacement, passive movements of the wings were undertaken to avoid contractures and reduced joint motion due to prolonged bandaging. Despite this, we noticed on 8 May that the wings were starting to become slightly stiff, impairing their complete or normal extension. Because the deformities on both wings were corrected at this point, it was decided to cease bandaging and limit the treatment to physiotherapy, so as to increase wing mobilisation and correct motion. By 15 May, chick 1 had a fully recovered wing motion and was able to fly normally.

Chick 2 was first observed perching next to the nest on 8 June and seen walking on the ground on 9 June, 55 days after hatching. At this point, the decision was made to remove the chick from its parents to prevent predation by Javan mongooses *Urva javanica*, which are capable of occasionally entering the aviary. Following a medical check-up, chick 2 was found to be healthy and did not develop any valgus deformity.

Mate choice and aggressive behaviour

During the preceding breeding season (2021–2022), video camera-traps were employed to assess pair bonding among adult birds. Although bonded pairs were subsequently identified and separated into the smaller breeding aviaries (W20.1–W20.3) just before the breeding season, this did not lead to successful breeding as only initial nest building was observed. As a consequence, it was decided to keep all of the birds together during the 2022–2023 breeding season, allowing these to access all four aviaries of the complex and free mate choice. While this yielded positive results, there were also losses in the population and aggressive behaviour was observed between the birds. On 13 March, a male (thought to be a potential mate in pair 1) was found dead in one of the breeding aviaries and necropsy concluded

Fig. 3 Chick 1 on the nest at approximately 18 days old (© ACCB / K. Groot).

Fig. 4 Valgus deformity before treatment on chick 1, noticeable on both right and left wing where the primary feathers protrude outwards from the body (© ACCB / M. Blümm).

this was due to a fight with a conspecific. Subsequently, another male (ACCB ID 0180007, Table 1) raised chick 1 together with the pair 1 female. Six days later, a second male (not thought to be a breeding bird) was found dead under similar circumstances. Further, an unsexed bird was attacked by a pair 2 individual through the netting separating the aviaries when it approached this too closely. Following these incidents, it was decided to separate all individuals except the breeding pairs for the remainder of the breeding season, although they still had visual contact. Following separation, two adults were observed pecking each other through the netting and another unsexed bird was attacked by pair 2 while in the vicinity of the barrier. A visual barrier was therefore erected between the adjacent aviaries to reduce the risk of aggressive behaviour and associated stress.

Discussion

The fledging of two white-shouldered ibis chicks during the 2022–2023 breeding season at ACCB was the first breeding documented for the species in captivity. Although firm conclusions cannot be drawn on our techniques based on two breeding pairs in just one season, the experience gained and lessons learned nonetheless represent valuable information which warrant consideration in future ex situ conservation efforts.

While the age that white-shouldered ibises reach sexual maturity is unknown, northern bald ibis *Geronticus eremita* generally attain this within three years (Sorato & Kotrschal, 2006). Clutch sizes for crested ibises laid by 2–3 years-old and ≥10 years-old birds are significantly smaller than those of 4–10 years-old birds (Yu *et al*., 2014). As 83 % (seven of nine birds) of our suspected breeding individuals of white-shouldered ibis are 3–4 years old, it is possible that these reached reproductive maturity several years before the 2022–2023 breeding season (assuming homogeneous reproductive physiology among members of the Threskiornithidae).

Both of our pairs raised one chick during in the 2022–2023 breeding season, which is less than the average number of fledged chicks per nest in the wild (mean ± SD: 1.8 ± 0.6) (Wright, 2012). However, due to the elevated nest platforms at ACCB, it is unknown whether a second egg or chick existed in an early stage of incubation. Our data indicate the incubation period for chick 1 lasted 31–33 days and at least 30 days for chick 2. The mean incubation period observed in the wild is 30.4 ± 2.7 days (*n*=17), although challenges in determining laying and hatching dates from ground-based observations create some uncertainty in this figure (Wright, 2012). Nonetheless, the incubation duration for both of the chicks at ACCB appears to fall within the range documented in the wild. This contrasts with the overall nesting period (including incubation). Whereas chick 2 left the nest naturally after 55 days, the average nesting period in the wild has been documented at 67.6 ± 5.9 days (*n*=20) (Wright, 2012), indicating that chick 2 left the nest late compared to wild fledglings.

We regularly offered nest material from the start of November (the start of the breeding season in Cambodia: Wright *et al*. 2013b; BirdLife International, 2018; CIWG, 2023) until incubation began in mid-February. This differed from previous breeding seasons, where nest material was offered sporadically and only resulted in initial nest building. As such, our daily provision of material could have encouraged nest building in our successful pairs, as suggested by Böhm (2006) and Huyghe *et al*. (2023) for other species. Additionally, the aviary complex required maintenance during the breeding season in previous years. This meant the birds had to be separated into temporary housing which could have fostered a sense of insecurity and instability, contributing to the lack of breeding success. This was not the case during the 2022–2023 season, as there was no need to relocate or disturb the birds for maintenance purposes.

The 2022–2023 breeding season was the first time a seasonal diet (based on breeding) was provided to the white-shouldered ibis colony. This resembled expected changes to the diet of wild white-shouldered ibises during the breeding season, when they forage most extensively on amphibians in the desiccating mud of trapeangs (Wright *et al*., 2010a, 2012a, 2013b). As seasonal changes in diet may encourage breeding in captivity (Greggor *et al*., 2018; Rose, 2021; SNZ & CBI, 2023), this action could have conceivably encouraged the pairs at ACCB to breed.

While the diet of our birds resembled the diets of other captive ibises (Böhm, 2006; Bracko & King, 2019), one of our chicks developed ''angel wings'', a well-known deformity in captive waterfowl which can occur due to a high protein diet (Kear, 1973; Smith, 1997). The diets of captive ibises often include superworms and mealworms (larvae of a tenebrionid beetle, *Tenebrio molitor*) (Böhm, 2006; Bracko & King, 2019) which are provided during the rearing period (Xi *et al*., 2001; Böhm, 2006). These normally possess high levels of protein (39.5 % crude protein) (Dragojlović *et al*., 2022). Following identification of the angel wing deformity in chick 1, superworms were eliminated from all diets. This naturally resulted in a decrease in protein intake of the chick 2, which was ten days old at the time and developed normally thereafter. Nonetheless, other factors associated with angel wings, such as genetics (Sun *et al*., 2023) or excessive heat in the early stages of growth (Wade, 2022), among others, could also have caused the valgus deformity in chick 1.

We allowed free mate choice for the first time during the 2022–2023 breeding season. This may enhance breeding success in conservation breeding programmes (Asa *et al*., 2010; Greggor *et al*., 2018; Martin-Wintle *et al*., 2018; Rose, 2021) and studies on zebra finches *Taeniopygia guttata* (Ihle *et al*., 2015) and mallards *Anas platyrhynchos* (Bluhm & Gowaty, 2004) found that birds given free mate choice had a greater chance of producing a clutch than those with assigned partners. However, caution must be exercised due to the potential for aggressive behaviour, especially in solitary breeders. Unlike many other ibis species commonly kept ex situ (Olmos & Silva, 2001; Tomlinson, 2007; Boucheker *et al*., 2009; Martinez *et al*., 2020), white-shouldered ibises are territorial, display solitary nesting behaviour (Wright *et al*., 2013b) and may be more aggressive when confined together in the breeding season. Nevertheless, the loss of two birds due to aggressive interactions in a short period was unforeseen and is not often seen in other captive ibis species (McCreesh *et al*., 2023). Although most studies to date have focused on more solitary breeding species, aggressive behaviour such as pecking between individuals was only sporadically observed in a study conducted at ACCB (Woesner *et al*., 2021). However, Rutkowski & Gerdsen (2011) found that a hadada ibis *Bostrychia hagedash* (a solitary breeder) showed aggression towards a sacred ibis *Threskiornis aethiopicus* housed in the same enclosure. The timing of the aggressive behaviour in our study was also unexpected, because it occurred later in the breeding season after pairs had already begun incubation approximately one month before, rather than during the initial stages of pair bonding, nest construction and incubation. As a consequence, allowing free mate choice in captive white-shouldered ibises presents challenges. Monitoring of individuals that have formed pair bonds prior to the breeding season and separating pairs once bonded is necessary. Development of additional aviaries to separate bonded pairs within the complex at ACCB will also be essential.

While the 2022–2023 breeding season resulted in successful fledging of two white-shouldered ibises, our experience highlights the need for caution in embarking on ex situ conservation actions for this and other Critically Endangered species, as any fatalities could be detrimental to their overall recovery. We therefore recommend risk assessment of the white-shouldered ibis breeding programme according to IUCN guidelines (IUCN SSC, 2014). This could adopt an approach similar to Mahood *et al*. (2021), which evaluated the benefits and risks of establishing an ex situ captive management program for the Critically Endangered Bengal florican *Houbaropsis bengalensis blandini* in Cambodia.

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References

AccuWeather (2023) *Phnom Penh, Cambodia Monthly Weather*. Https://www.accuweather.com/en/kh/phnom-penh/49785/may-weather/49785?year=2023 [accessed 19 October 2023].

Andrew, P., Cogger, H., Driscoll, D.A., Flakus, S., Harlow, P.S., Maple, D., Misso, M., Pink, C., Retallick, K., Rose, K., Tiernan, B., West, J. & Woinarski, J.C.Z. (2016) Somewhat saved: a captive breeding programme for two endemic Christmas Island lizard species, now extinct in the wild. *Oryx*, **52**, 171–174.

Asa, C.S., Traylor-Holzer, K. & Lacy, R.C. (2010) Can conservation-breeding programmes be improved by incorporating mate choice? *International Zoo Yearbook*, **45**, 203–212.

Biega, A.M., Lamont, M., Mooers, A.Ø., Bowkett, A.E. & Martin, T.E. (2019) Guiding the prioritization of the most endangered and evolutionary distinct birds for new zoo conservation programs. *Zoo Biology*, **38**, 305–315.

BirdLife International (2018) *IUCN Red List of Threatened Species: Pseudibis davisoni*. Https://www.iucnredlist.org/fr/species/22697531/134189710 [accessed 12 December 2023].

Bluhm, C.K. & Gowaty, P.A. (2004) Social constraints on female mate preferences in mallards, *Anas platyrhynchos*, decrease offspring viability and mother productivity. *Animal Behaviour*, **68**, 977–983.

Böhm, C. (2006) *Husbandry Guidelines for the Northern Bald Ibis (Geronticus eremita)*. Unpublished report. Https://www.researchgate.net/publication/311563050_Husbandry_guidelines_for_the_Northern_Bald_Ibis [accessed 18 Retrieved 2023].

Boucheker, A., Nedjah, R., Samraoui, F., Menaï, R. & Samraoui, B. (2009) Aspects of the breeding ecology and conservation of the glossy ibis in Algeria. *Waterbirds*, **32**, 345–351.

Bracko, A. & King, C.E. (2019) The captive glossy ibis *Plegadis falcinellus* population and ex situ conservation opportunities. *SIS Conservation*, **1**, 72–77.

Bubac, C.M., Johnson, A.C., Fox, J.A. & Cullingham, C.I. (2019) Conservation translocations and post-release monitoring: identifying trends in failures, biases, and challenges from around the world. *Biological Conservation*, **238**, 108239.

Bussolini, L.T., Crates, R., Herrod, A., Magrath, M.J.L., Troy, S., & Stojanovic, D. (2023) Carry-over effects of nestling physical condition predict first-year survival of a critically endangered migratory parrot. *Animal Conservation*, **27**, 78–85.

Butchart, S.H.M., Stattersfield, A.J. & Collar, N.J. (2006) How many bird extinctions have we prevented? *Oryx*, **40**, 266–278.

Byers, O., Lees, C., Wilcken, J. & Schwitzer, J. (2013) The one plan approach: the philosophy and implementation of CBSG's approach to integrated species conservation planning. *WAZA-Magazine*, 14, 2–5.

[CIWG] Cambodia Ibis Working Group (2023) *CIWG Annual Report 2022*. Https://naturelifecambodia.org/wp-content/uploads/2012/05/CIWG-Annual-Report-2022-Final.pdf [accessed 22 February 2024].

Clements, T., John, A., Nielsen, K., An D., Tan S. & Milner-Gulland, E. (2010) Payments for biodiversity conservation in the context of weak institutions: Comparison of three programs from Cambodia. *Ecological Economics*, **69**, 1283–1291.

Collar, N. & Butchart, S.H.M. (2014) Conservation breeding and avian diversity: chances and challenges. *International Zoo Yearbook*, 48, 7–28.

D'Elia, J.G. (2010) Evolution of avian conservation breeding with insights for addressing the current extinction crisis. *Journal of Fish and Wildlife Management*, **1**, 189–210.

Dragojlović, D., Đuragic, O., Pezo, L., Popović, L., Rakita, S., Tomičić, Z. & Spasevski, N. (2022) Comparison of nutritional profiles of super worm (*Zophobas morio*) and yellow mealworm (*Tenebrio molitor*) as alternative feeds used in animal husbandry: is super worm superior? *Animals*, **12**, 1–16.

Eames, J.C., Eang S., Loveridge, R. & Gray, T.N.E. (2018) Impact of experimental domestic water buffalo *Bubalus bubalis* grazing on waterhole dynamics in north-eastern Cambodia. *Cambodian Journal of Natural History*, **2018**, 101–109.

Farquharson, K.A., Hogg, C.J. & Grueber, C.E. (2018) A meta-analysis of birth-origin effects on reproduction in diverse captive environments. *Nature Communications*, **9**, 1–10.

Greggor, A.L., Vicino, G.A., Swaisgood, R.R., Fidgett, A., Brenner, D.J., Kinney, M.E., Farabaugh, S.M., Masuda, B.M., & Lamberski, N. (2018) Animal welfare in conservation breeding: applications and challenges. *Frontiers in Veterinary Science*, **5**, 1–6.

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T.M., Butchart, S.H.M., Carpenter, K.E., Chanson, J., Collen, B., Cox, N.A., Darwall, W., Dulvy, N.K., Harrison, L.R., Katariya, V., Pollock, C., Quader, S., Richman, N.I., Rodrigues, A.S.L., Tognelli, M.F., Molur, S. (2010) The impact of conservation on the status of the world's vertebrates. *Science*, **330**, 1503–1509.

Huyghe, M., Izquierdo, P., Bureau, E. & Llopis, A. (2023) *EAZA Best Practice Guidelines, Aegypius monachus - Cinereous Vulture*. European Association of Zoos and Aquaria, Amsterdam, The Netherlands.

IBIS Rice (2023) *Our Story - Rooted in Conservation*. Https://ibisrice.com/our-story/ [accessed 1 November 2023].

Ihle, M., Kempenaers, B. & Forstmeier, W. (2015) Fitness benefits of mate choice for compatibility in a socially monogamous species. *PLOS Biology*, **13**, 1–21.

IUCN SSC (2014) *Guidelines on the Use of Ex Situ Management for Species Conservation*. Version 2. IUCN Species Survival Commission, Gland, Switzerland.

Kear, J. (1973) Notes on the nutrition of young waterfowl, with special reference to slipped-wing. *International Zoo Yearbook*, **13**, 97–100.

Lees, C. & Wilcken, J. (2009) Sustaining the ark: the challenges faced by zoos in maintaining viable populations. *International Zoo Yearbook*, **43**, 6–18.

Leus, K. (2011) Captive breeding and conservation. *Zoology in the Middle East*, **54**, 151–158.

Mahood, S.P., Hong C., Meyerhoff, M., Ferrando, P.P., Sum P., Son V., Ouch P. & Garnett, S.T. (2021) IUCN captive management guidelines support ex situ conservation of the Bengal florican *Houbaropsis bengalensis blandini*. *Oryx*, **55**, 903–915.

Martinez, C.B., Miranda, A.C. & Ruiz, X. (2020) Breeding biology and brood reduction of herons and ibis in a northern Brazilian mangrove swamp: eggs do not starve. *Waterbirds*, **43**, 55–64.

Martin-Wintle, M.S., Wintle, N.J.P., Díez-León, M., Swaisgood, R.R. & Cheryl, S. (2018) Improving the sustainability of ex situ populations with mate choice. *Zoo Biology*, **38**, 119–132.

Mason, G.J. (2010) Species differences in responses to captivity: stress, welfare and the comparative method. *Trends in Ecology and Evolution*, **25**, 713–721.

McCreesh, K., Yaffy, D., Spiro, S., Patterson, S.D. & Guthrie, A. (2023) A retrospective analysis of the morbidity and mortality of captive northern bald ibis (*Geronticus eremita*), African sacred ibis (*Threskiornis aethiopicus*), and scarlet ibis (*Eudocimus ruber*) housed at the London Zoo from 2000 to 2020. *Journal of Zoo and Wildlife Medicine*, **54**, 94–101.

Olmos, F. & Silva, R.S.E. (2001) Breeding biology and nest site characteristics of the scarlet ibis in southeastern Brazil. *Waterbirds*, **24**, 58–67.

Redford, K.H., Amato, G., Baillie, J., Beldomenico, P.M., Bennett, E.L., Clum, N.J., Cook, R.L., Fonseca, G., Hedges, S.B., Launay, F., Lieberman, S., Mace, G.M., Murayama, A., Putnam, A.S., Robinson, J.G., Rosenbaum, H.C., Sanderson, E.W., Stuart, S.N., Thomas, P. & Thorbjarnarson, J.B. (2011). What does it mean to successfully conserve a (vertebrate) species? *BioScience*, **61**, 39–48.

Rose, P. (2021) Evidence for aviculture: identifying research needs to advance the role of ex situ bird populations in conservation initiatives and collection planning. *Birds*, **2**, 77–95.

Rutkowski, C. & Gerdsen, G. (2011) Hadada ibis at the Oregon Zoo. *Watchbird*, **XXXVIII**, 18–26.

Sansom Mlup Prey (2023) *About - Sansom Mlup Prey*. Https://sansommluppreykh.org/about/ [accessed 24 May 2024].

Seddon, P.J., Armstrong, D.P. & Maloney, R.F. (2007) Developing the science of reintroduction biology. *Conservation Biology*, **21**, 303–312.

Smith, K. (1997) Angel wing in captive-reared waterfowl. *Journal of Wildife Rehabillitation*, **20**, 3–5.

[SNZ & CBI] Smithsonian's National Zoo & Conservation Biology Institute (2023) *Guam Kingfisher (Sihek)*. Https://nationalzoo.si.edu/animals/guam-kingfisher-sihek [accessed 9 September 2023].

Snyder, N.F.R., Derrickson, S.R., Beissinger, S.R., Wiley, J.W., Smith, T.B., Toone, W.D. & Miller, B.J. (1996) Limitations of captive breeding in endangered species recovery. *Conservation Biology*, **10**, 338–348.

Snyder, N.F.R. & Snyder, H. (2000) *The California Condor: A Saga of Natural History and Conservation*. Princeton University Press, New Jersey, USA.

Sorato, E., & Kotrschal, K. (2006) Hormonal and behavioural symmetries between the sexes in the northern bald ibis. *General and Comparative Endocrinology*, **146**, 265–274.

Sun Y., Sang Q., Yin Z., Zhang F., Zhu F. & Hou Z. (2023) Genome-wide association study identified the candidate genes associated with angel wing trait in Pekin duck. *Animal Genetics*, **54**, 211–215.

Thewlis, R. M., & Timmins, R. J. (1996) The rediscovery of giant ibis *Pseudibis gigantea* with a review of previous records. *Bird Conservation International*, **6**, 317–324.

Tomlinson, C. (2007) A review of red-cheeked ibis or waldrapp *Geronticus eremita*: conservation. *International Zoo Yearbook*, **33**, 67–73.

Vercillo, U.E., Oliveira-Santos, L.G.R., Novaes, M.C., Purchase, C., Purchase, C., Lugarini, C., Ferreira, A.L., De Marco Júnior, N.P., Marcuk, V. & De Andrade Franco, J.L. (2023) Spix's Macaw *Cyanopsitta spixii* (Wagler, 1832) population viability analysis. *Bird Conservation International*, **33**, e67.

Wade, L. (2022) Treating angel wing deformity: a sling for the wing. *Today's Veterinary Practice*, **68**, 69–74.

Woesner, M., Meyerhoff, M. & Wagner, P. (2021) Behavior patterns of the white-shouldered ibis *Pseudibis davisoni* (Hume, 1875) in a captive environment at the Angkor Centre for Conservation of Biodiversity, Cambodia. *Der Zoologische Garten*, **89**, 121–134.

World Bank (2021) *Climate Change Knowledge Portal: Cambodia*. Https://climateknowledgeportal.worldbank.org/country/cambodia [accessed 19 August 2023].

WorldData (2024) *Cambodia*. Https://www.worlddata.info/asia/cambodia/index.php#:~:text=Climate%20in%20Cambodia,attractive%20high%20season%20in%20December [accessed 19 August 2023].

Wright, H.L. (2012) *Synanthropic survival: low-impact agriculture and white-shouldered ibis conservation ecology*. PhD thesis, University of East Anglia, UK.

Wright, H.L., Buckingham, D.L. & Dolman, P.M. (2010a) Dry season habitat use by critically endangered white-shouldered ibis in northern Cambodia. *Animal Conservation*, **13**, 71–79.

Wright, H.L., Collar, N., Lake, I.R. & Dolman, P.M. (2013b) Amphibian concentrations in desiccating mud may determine the breeding season of the white-shouldered ibis (*Pseudibis davisoni*). *The Auk*, 130, 774–783.

Wright, H.L., Collar, N.J., Lake, I.R., Norin N., Vann R., Ko S., Phearun S. & Dolman, P.M. (2013a) Experimental test of a conservation intervention for a highly threatened waterbird. *The Journal of Wildlife Management*, **77**, 1610–1617.

Wright, H.L., Collar, N., Lake, I.R., Norin N., Vann R., Ko S., Phearun S. & Dolman, P.M. (2012b) First census of the white-shouldered ibis *Pseudibis davisoni* reveals roost-site mismatch with Cambodia's protected areas. *Oryx*, **46**, 236–239.

Wright, H.L., Collar, N.J., Lake, I.R., Vorsak B. & Dolman, P.M. (2012a) Foraging ecology of sympatric white-shouldered ibis *Pseudibis davisoni* and giant ibis *Thaumatibis gigantea* in northern Cambodia. *Forktail*, **28**, 93–100.

Wright, H.L., Vorsak B., Collar, N., Gray, T.N.E., Lake, I.R., Phearun S., Rainey, H., Vann R., Ko S. & Dolman, P.M. (2010b) Establishing a national monitoring programme for white-shouldered ibis in Cambodia. *Ibis*, **152**, 206–208.

Xi Y., Lu B. & Fujihara N. (2001) Captive rearing and breeding of the crested ibis, *Nipponia nippon*. *Journal of Poultry Science*, **38**, 213–224.

Xi Y., Lu B., Zhang Y.M. & Fujihara N. (2002) Restoration of the crested ibis, *Nipponia nippon*. *Journal of Applied Animal Research*, **22**, 193–200.

Yu X., Li X. & Huo Z. (2014) Breeding ecology and success of a reintroduced population of the endangered crested ibis *Nipponia nippon*. *Bird Conservation International*, **25**, 207–219.

Evidence for a major decline of the Endangered large-spotted civet in a former stronghold in eastern Cambodia

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តំបន់ការពារទេសភាពភាគឦសាន្តប្រទេសកម្ពុជាសម្បូរទៅដោយជីវចម្រុះ។ ទោះបីជាការកើនឡើងនៃសកម្មភាពខុសច្បាប់បានជះឥទ្ធិពលអវិជ្ជមានដល់ថនិកសត្វធំៗ ប៉ុន្តែផលប៉ះពាល់លើថនិកសត្វតូចៗមិនទាន់ត្រូវបានសិក្សានៅឡើយ។ នៅឆ្នាំ២០១៩ យើងបានធ្វើការស្រាវជ្រាវដោយប្រើម៉ាស៊ីនថតស្វ័យប្រវត្តិក្នុងដែនជម្រកសត្វព្រៃស្រែពក (SWS) ដើម្បីកំណត់វត្តមាន (occupancy) សត្វសំពោចបីប្រភេទ៖ សត្វខ្វីន *Viverra zibetha* សត្វសំពោចវល្លិ៍ *Viverricula indica* និង សត្វសំពោចធំ *V. megaspila*។ យើងក៏បានធ្វើការប្រៀបធៀបផងដែរនូវសកម្មភាព (temporal patterns) និងអត្រាកត់ត្រា (encounter rates) សត្វសំពោចទាំងបីប្រភេទនេះនៅឆ្នាំ២០០៩-២០១០។ នៅឆ្នាំ២០១៩ សត្វខ្វីនមានអត្រាកត់ត្រាខ្ពស់ជាងគេ (encounter rate = 3.28) តាមដោយសត្វសំពោចវល្លិ៍ (2.85) និងសត្វសំពោចធំ (0.73)។ ផ្ទុយទៅវិញ សត្វសំពោចធំមានអត្រាកត់ត្រាខ្ពស់នៅឆ្នាំ២០០៩-២០១០ ដែលអត្រាកត់ត្រានេះមានអត្រាកត់ត្រាទាបជាងសត្វសំពោចប្រភេទផ្សេងទៀត។ ការសិក្សានេះ ដូចទៅនឹងការរកឃើញពីឆ្នាំ២០០៩-២០១០ ដែលសត្វសំពោចទាំងបីប្រភេទនេះធ្វើសកម្មភាពនៅពេលយប់ ហើយបានបង្ហាញពីភាពត្រួតស៊ីគ្នាខ្ពស់នៅឆ្នាំ២០១៩។ តំបន់ទំនាបព្រៃល្បោះស្ងួតដែលគ្រប់ដណ្តប់នៅ SWS មានទំនាក់ទំនងវិជ្ជមានជាមួយ occupancy សត្វខ្វីន និងសត្វសំពោចវល្លិ៍ ប៉ុន្តែគ្មានទំនាក់ទំនងវិជ្ជមានជាមួយ occupancy សត្វសំពោចធំទេ។ លទ្ធផលនៃការសិក្សារបស់យើងបង្ហាញពីលទ្ធភាពបម្រែបម្រួលគួរឱ្យកត់សម្គាល់នៃពពួកសត្វសំពោចក្នុងរយៈពេលមួយទសវត្សរ៍ចុងក្រោយនេះនៅ SWS និងការថយចុះយ៉ាងគំហុកនៃសត្វសំពោចធំដែលជាប្រភេទសត្វរងគ្រោះ។ សកម្មភាពល្មើស ជាពិសេសការប្រមាញ់ខុសច្បាប់បានកើនឡើងយ៉ាងខ្លាំងនៅកំឡុងពេលចុះសិក្សាដាក់ម៉ាស៊ីនថតស្វ័យប្រវត្តិនៅដែនជម្រកសត្វព្រៃស្រែពក ដែលប្រហែលជាមូលហេតុចម្បងនៃការថយចុះនេះ។

Abstract

The eastern plains landscape of Cambodia is rich in biodiversity, although increases in illegal activities have negatively impacted large mammals whereas the impacts on smaller mammals are unknown. We conducted a camera-trap survey in Srepok Wildlife Sanctuary (SWS) in 2019 to determine the occupancy of three ground-dwelling civets: large Indian civets *Viverra zibetha*, small Indian civets *Viverricula indica* and large-spotted civets *V. megaspila*. We also compared the

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temporal patterns and encounter rates of these species to those recorded during a survey in 2009–2010. In 2019, large Indian civets were recorded most (encounter rate = 3.28), followed by small Indian civets (2.85) and large-spotted civets (0.73). In contrast, large-spotted civets had the highest encounter rate in 2009–2010, whereas the rates were much lower for the other civet species. In agreement with findings from 2009–2010, all civet species were primarily nocturnal and showed high temporal overlap in 2019. The lowland deciduous dipterocarp forests that dominate SWS had a positive relationship with the occupancy of large and small Indian civets, but not large-spotted civets. Our results demonstrate the possibility of radical changes in the terrestrial civet community over the last decade in SWS, with a strong possibility of a major decline in the Endangered large-spotted civets. Illegal human activities in SWS, notably poaching, increased dramatically between the surveys and are probably at the root of this apparent decline.

Keywords Cambodia, camera traps, deciduous dipterocarp forest, occupancy modeling, small carnivore, Srepok Wildlife Sanctuary.

Introduction

The eastern plains landscape (EPL) of Cambodia is one of the largest extant deciduous dipterocarp forests (DDF) in Southeast Asia (Tordoff *et al*., 2005). The landscape has supported a wide range of globally threatened species and subspecies, including mammals such as Asian elephant *Elephas maximus*, banteng *Bos javanicus*, gaur *B. gaurus*, Eld's deer *Rucervus eldii*, dhole *Cuon alpinus*, Indochinese leopard *Panthera pardus delacouri*, and large birds such as giant ibis *Thaumatibis gigantea*, white-shouldered ibis *Pseudibis davisoni*, lesser adjutant *Leptoptilos javanicus*, green peafowl *Pavo muticus* and several vulture species (Pin *et al*., 2018; Rostro-García *et al*., 2018; Groenenberg *et al*., 2020; Kamler *et al*., 2020).

Deciduous dipterocarp forests are characterized by an open canopy and grassy understory and currently cover about 15–20% of Southeast Asia (Tordoff *et al*., 2005; Wohlfart *et al*., 2014). However, DDF has become the most threatened of all forest types in the region due to illegal logging and habitat transformation (Wohlfart *et al*., 2014; Pin *et al*., 2018; Rostro-García *et al*., 2021). Additionally, a snaring crisis is devastating wildlife populations in Southeast Asia, especially in Cambodia, Laos and Vietnam (Gray *et al*., 2018). These twin factors of habitat loss and indiscriminate snaring are taking their toll on larger mammal species of Southeast Asia (Gray *et al*., 2021; Groenenberg *et al*., 2023), with the result that most are experiencing rapid declines in numbers and local extirpations. In a microcosm of what is happening at a larger scale throughout Southeast Asia, the EPL has experienced an exponential increase in snaring and other forms of poaching along with widespread illegal logging and habitat transformation during the last decade (Groenenberg *et al*., 2020). Consequently, ungulate populations have decreased dramatically in the EPL (Groenenberg *et al*., 2020, 2023; Nuttall *et al*., 2022), whereas tigers have been extirpated (O'Kelly *et al*., 2012) and Indochinese leopards have become functionally extinct (Rostro-García *et al*., 2023).

While the recent increase in poaching and other illegal human activities in EPL have had severe negative impacts on all large mammal populations, it is not known if small carnivore populations have also been impacted. If small carnivores are not targeted in snaring and other forms of poaching, their populations may not be impacted to the same degree as large mammals (Gray *et al*., 2021; Groenenberg *et al*., 2023). Recent research has shown that the abundance and densities of leopard cats *Prionailurus bengalensis*, a small habitat generalist, are relatively high in EPL (Rostro-García *et al*., 2021; Pin *et al*., 2022). This indicates that they probably have not been severely impacted by recent increases in illegal human activities. In contrast, the abundance of jungle cats *Felis chaus*, a DDF-dependent species in this region, was extremely low (Duckworth *et al*., 2005; Rostro-García *et al*., 2021). This might indicate that species restricted to DDF might be more negatively impacted by illegal human activities in EPL compared to habitat generalists. However, more research is needed on the subject.

Of all small carnivore groups in the EPL, civets are particularly diverse. These comprise at least four species including the semi-arboreal common palm civet *Paradoxurus hermaphroditus* and three ground-dwelling civets: the large-spotted civet *Viverra megaspila*, the large Indian civet *V. zibetha* and the small Indian civet *Viverricula indica*. Large-spotted civets are classified as Endangered by IUCN due to population declines resulting from habitat loss and hunting (Timmins *et al*., 2016b). In contrast, none of the other three civet species have experienced major population declines or have small populations or small geographic ranges. As such, they are classified as Least Concern by IUCN (Choudhury *et al*., 2015; Timmins *et al*., 2016a). Large-spotted civets are associated with forests at lower altitudes (Gray *et al*., 2010; Jennings & Veron, 2011; Hamirul *et al*., 2015) and the EPL, with its vast tracts of lowland deciduous dipterocarp forests, is considered a global stronghold for the species (Gray *et al*., 2010; Timmins *et al*., 2016b). In contrast, all of the other civet species in this area of Cambodia are not restricted to lowland areas and consequently occur across different forest types (Francis, 2008; Jennings & Veron, 2022).

The current status and habitat requirements of civet species in the EPL are unknown. However, camera-trapping surveys conducted for large carnivores in the EPL (Rostro-García *et al*., 2023) have provided important data on the three ground-dwelling civet species. For example, camera-trap surveys conducted in 2009–2010 in Srepok Wildlife Sanctuary, the largest protected area within the EPL, showed that the large-spotted civet had the highest encounter rate and naïve occupancy of these species (Gray *et al*., 2010). This study indicated that large-spotted civets appear to be DDF-dependent in EPL, likely due to its confinement to lowland areas dominated by DDF. In contrast, the other civet species were found across a wider range of altitudes and terrain encompassing various forest types. To determine the current status and habitat use of ground-dwelling civets, we conducted a camera-strap survey in Srepok Wildlife Sanctuary in 2019. We focused on the three ground-dwelling civet species, namely the large-spotted civet, the large Indian civet and the small Indian civet (Fig. 1). We used occupancy analysis to determine the habitat use of these and investigated their activity patterns. We calculated camera-trap encounter rates and naïve occupancy for each species and compared our results to those from 10 years earlier. If camera-trap methodology and other possible confounding variables were comparable between the two surveys, this comparison would allow us to determine any changes in the civet community over the last decade in the EPL.

Fig. 1 From top to bottom, a large-spotted civet *Viverra megaspila*, large Indian civet *V. zibetha* and a small Indian civet *Viverricula indica* in Srepok Wildlife Sanctuary, 2019.

Methods

Study site

Our camera-trap study was conducted in the core zone of Srepok Wildlife Sanctuary (3,729 km^2; 12°50′N, 107°50′E; Fig. 2) within the EPL. This is under the management of the Cambodian Ministry of Environment. Srepok Wildlife Sanctuary (SWS) is categorized into four distinct zones: 1) core zones covering 1,876 km^2, 2) conservation zones (756 km^2) with severely restricted human access by law, 3) sustainable use zones (657 km^2) and 4) community zones (439 km^2) where local communities engage in grazing cattle and subsistence hunting using traditional methods, and collection of non-forest timber products. The sanctuary is part of the Lower Mekong Dry Forest Eco-region within Southeast Asia and borders several other protected areas in Cambodia and Vietnam (Fig. 2). It is predominantly covered by DDF which accounts for over 70% (1,050 km^2) of the area, with smaller patches of mixed-deciduous forest and semi-evergreen forest (SEF) on hilltops and along rivers (530 km^2) (Pin *et al*., 2013; Rostro-García *et al*., 2021). During the dry season, the DDF experiences high-frequency forest fires, which result in extensive open understory vegetation and sparse canopy cover (Pin *et al*., 2013; Kamler *et al*., 2021). There is a distinct dry season from about November to April, with average monthly rainfall ranging from 3 to 121 mm. The rainy season typically spans from about May to October, with monthly rainfall ranging from 248 to 370 mm (rainfall data from nearby Sen Monorom, Cambodia, 1982–2012; climate-data.org; accessed 10 July 2019).

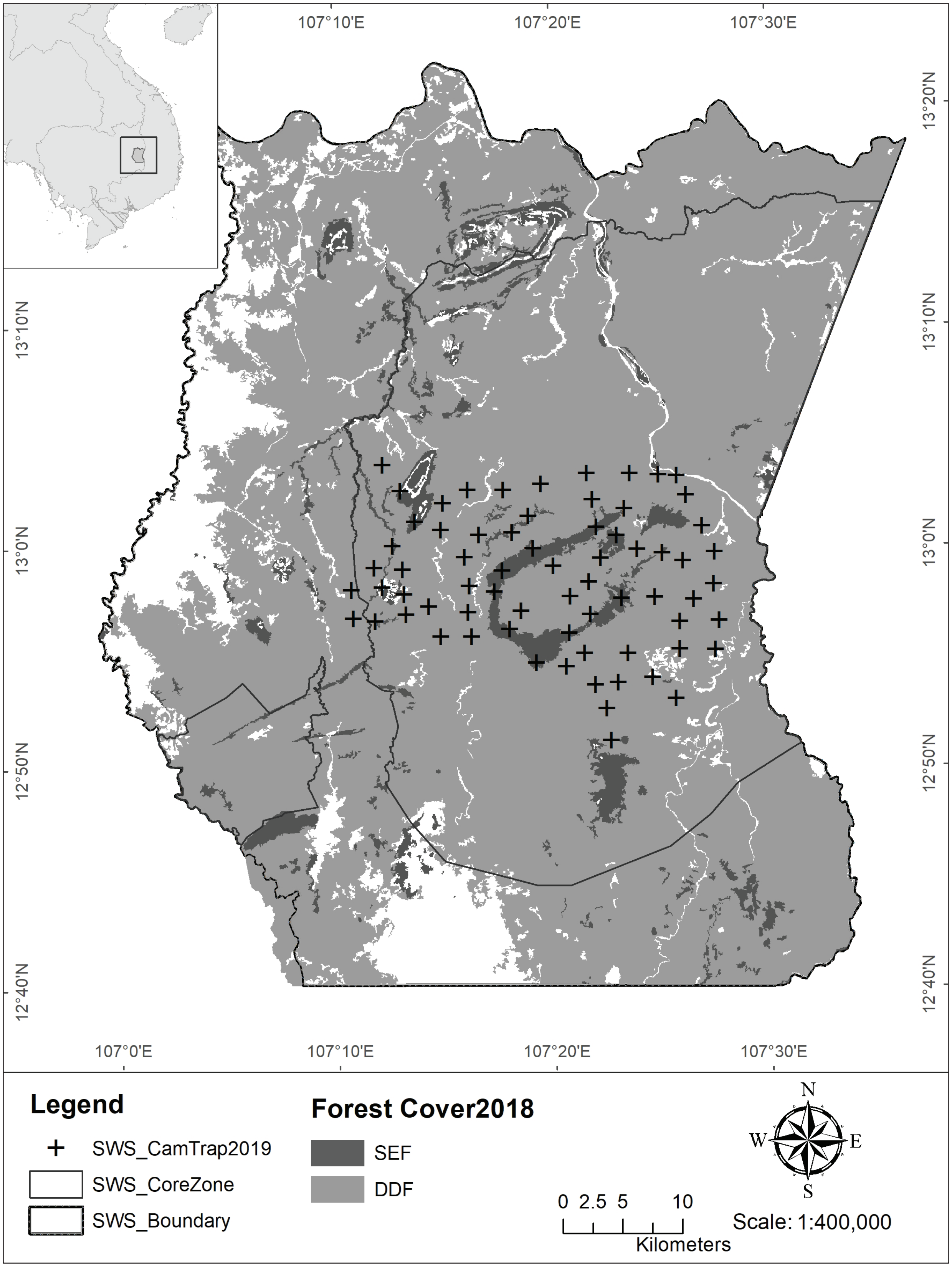

Fig. 2 Distribution of forest types and 69 camera traps used in the 2019 survey in Srepok Wildlife Sanctuary (SWS). The inset map indicates the location of the sanctuary within the broader region.

The wildlife sanctuary supports a globally important banteng population (Gray *et al*., 2012) and several large and medium-sized carnivores, including Indochinese leopards, dholes, sun bears *Helarctos malayanus*, golden jackals *Canis aureus* and jungle cats (Rostro-García *et al*., 2018, 2021; Kamler *et al*., 2020, 2021). It also supports several small carnivores, including leopard cats, yellow-throated martens *Martes flavigula*, small Asian mongooses *Herpestes javanicus*, crab-eating mongooses *H. urva*, and ferret-badgers (*Melogale* sp.) (Rostro-García *et al*., 2021; Pin *et al*., 2022).

Camera-trapping survey

We used records of civets from a camera-trap survey conducted for leopards in the core zone of SWS from 8 February to 8 May 2019 (a total of 4,402 trap nights). The survey employed 69 camera trap stations using three different models of cameras, namely Panthera V6, Reconyx PC900 and Bushnell Trophy. Camera stations were spaced 2–3 km apart along dirt tracks and trails, and were checked once each month until they were collected. Each camera station comprised paired cameras set on opposite sides of the trail. The cameras were attached to trees, positioned 40–50 cm above the ground, at distances of 2–3 m from the centre of the trail. Unbaited cameras were set up in all habitat types present in the core zone, covering an area of approximately 766 km² (Fig. 2).

We compared our results with Gray *et al*. (2010) who conducted camera-trapping during 2009–2010 (December 2009 to June 2010) in the same area of the SWS core zone, also using 69 camera stations (a total of 4,264 trap nights). In that survey, Reconyx RapidFire Professional PC90 cameras were used, but the sampling protocols were otherwise the same e.g., camera stations were set between 2–3 km apart along dirt roads and trails. The camera grid in 2019 covered almost the same area as that used in 2009–10 and both were primarily conducted during the dry season. There was no significant change in vegetation conversion between 2009 and 2019, although the area experienced significant increases in poaching, illegal logging and other human activities (Groenenberg *et al*., 2020; Nuttall *et al*., 2022; Rostro-García *et al*., 2023).

Data analysis

Management of camera-trap photos from 2019 was undertaken with the DigiKam program (vers. 6.0) and detection histories were created using R (R Core Team, 2020) and the 'camtrap R' package (Niedballa *et al*., 2016). Nominally independent encounters of each species were defined as successive photographs >30 minutes apart, or non-consecutive photographs of the same species at the same station (MacKenzie & Royle, 2005; Chutipong *et al*., 2014; Pin *et al*., 2018). We used 24 hours (starting at 00:00:01 and ending at 23:59:59) as an occasion (one day), yielding 88 occasions in total.

We did not re-analyse the survey data collected in 2009, but instead compared our results to Gray *et al*. (2010). We calculated the camera-trap encounter rate for each species based on their number of independent events, divided by total trap nights and multiplied by 100. Although analysis of simulated data has suggested that encounter rate has limitations when compared to actual densities (Sollmann *et al*., 2013), we feel that this method was adequate for our purpose of comparing the encounter rate of species over time at the same site (Clements *et al*., 2021). We calculated naïve occupancy based on the number of camera stations each species was detected, divided by the total number of stations (MacKenzie *et al*., 2017).

Activity patterns were examined for each species using the R package 'overlap' v.0.3.2 (Meredith & Ridout, 2018). We estimated coefficients of overlapping kernel densities based on times of observations between the large Indian civet and large-spotted civet, the large Indian civet and small Indian civet, and the large-spotted civet and small Indian civet. We also compared the overlap values between species in our study and those in Gray *et al*. (2010) to determine if these changed over time among the civet species. For the comparison between study periods, we used Dhat1 for the estimated coefficients because this is recommended for small sample sizes.

We modelled single-season single-species occupancy (MacKenzie *et al*., 2002) in a Bayesian approach for our target species using the 'wiqid' package (Quick and Dirty Estimates for Wildlife Population) (Meredith, 2015). Two environmental covariates (i.e., distance to water and habitat type) were used as site covariates in the model (MacKenzie & Royle, 2005) and we kept the detection probability constant. We used ArcGIS to define forest cover types at each camera trap station (with a 1 km radius around each camera station) and we calculated the distance from each camera station to streams (km). The forest type layer was produced by WWF Cambodia. We ran three models including constant (null) denoted by [psi(.),p(.)], distance to water denoted by [psi(DWater).p(.)], and forest cover type denoted by [psi(habit).p(.)].

For the Bayesian approach, we ran three chains of Markov Monte Carlo (MCMC) simulations of 100,000 iterations each, discarded 15,000 as initial burn-in, and thinned by one. We compared candidate models using

Watanabe-Akaike information criterion (WAIC) scores (Vehtari *et al*., 2015, 2016; Hollenbach *et al*., 2020). Model convergence was based on the Gelman-Rubin statistic for each parameter, where models successfully converged with a Rhat value <1.1 (Penjor *et al*., 2019; Pin *et al*., 2022). We report posterior means with standard deviations and 95% highest density credible intervals (Penjor *et al*., 2018, 2019; Pin *et al*., 2022). All data analyses were performed in R (R Core Team, 2020).

Results

Our 2019 survey generated 4,402 camera trap days and 129,069 photographs. The encounter rate was lowest for large-spotted civets, whereas it was over four times higher for large Indian civets and almost four times higher for small Indian civets (Table 1). Naïve occupancy was similarly lowest for large-spotted civets, whereas it was over six times higher for large Indian civets and small Indian civets (Table 1). In contrast, during the 2009–2010 survey, the encounter rate and naïve occupancy were higher for large spotted civets than the other two species (Table 1). Between 2009–2010 and 2019, the encounter rate for large-spotted civets decreased by 26% and naïve occupancy decreased by 65% (Table 1). In contrast, the encounter rate and naïve occupancy for large Indian civets increased about threefold from 2009–2010 to 2019 (Table 1). Similarly, the encounter rate for small Indian civets increased twelve-fold and naïve occupancy increased four-fold (Table 1).

For large-spotted civets and large Indian civets, our null model exhibited the lowest WAIC, followed by the model incorporating forest cover type as a site covariate (Table 2). Conversely, the model incorporating distance to water as a site covariate achieved the lowest WAIC score for small Indian civets. For all three civet species, differences in WAIC scores were less than 10, indicating that the null model was preferred (Hollenbach *et al*., 2020). The estimated coefficients of all models for each species are shown in Table 3. For the null model, the estimated detection probabilities for all species were relatively small (less than 0.1) (Fig. 3). The estimated

Table 1 Encounter rate (nominally independent encounters/trap days x 100), number of nominally independent encounters, number of camera locations where each species was recorded, and naïve occupancy (number of camera locations where species was recorded/total number of camera locations) for ground-dwelling civets in Srepok Wildlife Sanctuary, 2019. Camera-trap survey results (Gray *et al*., 2010) from the same site ten years earlier are included for comparative purposes.

Species	Encounter Rate	Nominally Independent Encounters	No. Locations	Naive Occupancy (%)
Present study (*n*=69 camera stations in 2019)				
Large-spotted civet *Viverra megaspila*	0.73	55	07	10.1
Large Indian civet *Viverra zibetha*	3.28	249	44	63.8
Small Indian civet *Viverricula indica*	2.85	216	43	62.3
Gray *et al*. (2010) (*n*=69 camera stations in 2009–2010)				
Large-spotted civet	0.99	48	20	29.0
Large Indian civet	0.93	45	15	21.7
Small Indian civet	0.23	13	11	15.9

Table 2 (Left) Occupancy models for three ground-dwelling civet species, including the number of parameters (df) and the Watanabe-Akaike information criterion (WAIC). DWater = distance to water; habit = forest cover.

Species	Models	df	WAIC
Large-spotted civet *Viverra megaspila*	psi (.), p(.)	2	273.795
	psi (habit), p(.)	3	275.564
	psi (DWater), p(.)	3	275.764
Large Indian civet *Viverra zibetha*	psi (.), p(.)	2	1244.971
	psi (habit), p(.)	3	1245.477
	psi (DWater), p(.)	3	1247.188
Small Indian civet *Viverricula indica*	psi (DWater), p(.)	3	1278.583
	psi (.), p(.)	2	1279.519
	psi (habit), p(.)	3	1281.328

Table 3 Estimated coefficients for occupancy modeling of three ground-dwelling civet species provided using Bayesian inference, including posterior means, standard deviations (SD), 95% highest density credible intervals, Rhat values and Monte Carlo standard errors (MCEpc).

Parameters	Mean	SD	Lower 95	Upper 95	Rhat	MCEpc
			Large-spotted civet *Viverra megaspila*			
psi(.), p(.)						
psi(Intercept)	-1.217	0.202	-1.618	-0.824	1.000	0.742
p(Intercept)	-1.362	0.094	-1.548	-1.177	1.001	0.750
psi(habit), p(.)						
psi(DDF)	-1.151	0.221	-1.585	-0.718	1.000	0.722
psi(SE)	-1.266	0.421	-2.098	-0.457	1.000	0.746
p(Intercept)	-1.364	0.096	-1.551	-1.176	1.000	0.794
psi(Dwater), p(.)						
psi(Intercept)	-1.238	0.206	-1.647	-0.841	1.000	0.711
psi(DWater)	-0.134	0.214	-0.563	0.277	1.000	0.737
p(Intercept)	-1.363	0.096	-1.555	-1.180	1.000	0.754
			Large Indian civet *Viverra zibetha*			
psi(.), p(.)						
psi(Intercept)	0.457	0.170	0.128	0.793	1.000	0.527
p(Intercept)	-1.508	0.042	-1.588	-1.426	1.000	0.829
psi(habit), p(.)						
psi(DDF)	0.575	0.203	0.178	0.974	1.000	0.648
psi(SE)	0.111	0.318	-1.516	0.730	1.000	0.503
p(Intercept)	-1.510	0.042	-1.593	-1.430	1.001	0.849
psi(Dwater), p(.)						
psi(Intercept)	0.462	0.171	0.129	0.798	1.000	0.569
psi(Dwater)	0.023	0.169	-0.352	0.352	1.001	0.530
p(Intercept)	-1.509	0.041	-1.427	-1.427	1.000	0.834
			Small Indian civet *Viverricula indica*			
psi(Dwater), p(.)						
psi(Intercept)	0.510	0.191	0.136	0.887	1.000	0.691
psi(DWater)	-0.320	0.187	-0.687	0.047	1.000	0.667
p(Intercept)	-1.523	0.041	-1.603	-1.442	0.999	0.892
psi(.), p(.)						
psi(Intercept)	0.451	0.173	0.108	0.790	1.000	0.589
p(Intercept)	-1.517	0.041	-1.597	-1.438	1.001	0.811
psi(habit), p(.)						
psi(DDF)	0.394	0.199	0.006	0.788	1.000	0.610
psi(SE)	0.578	0.330	-0.073	1.218	1.000	0.514
p(Intercept)	-1.516	0.041	-1.595	-1.435	1.000	0.856

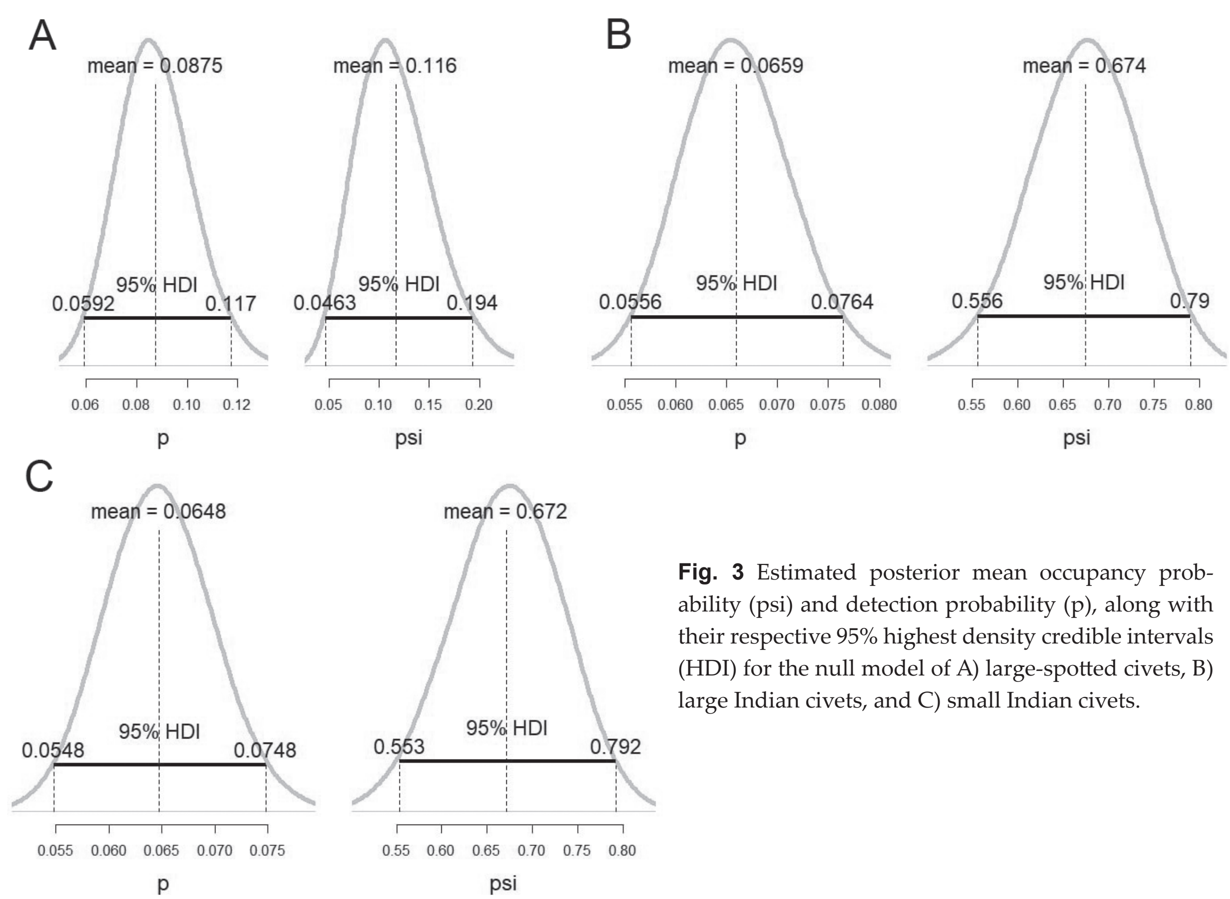

Fig. 3 Estimated posterior mean occupancy probability (psi) and detection probability (p), along with their respective 95% highest density credible intervals (HDI) for the null model of A) large-spotted civets, B) large Indian civets, and C) small Indian civets.

occupancy probabilities for large Indian civets and small Indian civets were similar (both 0.67) and significantly higher than those for large-spotted civets (Fig. 3).

The DDF-dominated lowland areas of SWS had a positive relationship with the occupancy probability of large and small Indian civets and a negative relationship with large-spotted civets (Table 3). The SEF that dominated higher areas of SWS had no effect on the occupancy of large and small Indian civets and a negative relationship with large-spotted civets. Large-spotted civets showed similar occupancy probabilities in DDF (mean = 0.131, CI = 0.047–0.220) and SEF (mean = 0.121, CI = 0.003–0.273). The estimated occupancy probability of large Indian civets was 0.713 (CI = 0.579–0.841) in DDF and 0.542 (CI = 0.309–0.773) in SEF, whereas the small Indian civets had comparable occupancy probabilities in DDF (mean = 0.650, CI = 0.510–0.791) and SEF (mean = 0.708, CI = 0.500–0.909). Distance to water (i.e., rivers) did not significantly affect the occupancy probability of any civet species (Table 3).

All three civet species had similar activity patterns, being almost exclusively nocturnal (Fig. 4). The highest activity peaks for large and small Indian civets occurred just before dawn, whereas large-spotted civets had two peaks in activity, just before dawn and just after sunset (Fig. 4). Estimated coefficients of overlapping kernel densities were 0.752 between large Indian civets and large-spotted civets, 0.898 between large Indian civet and small Indian civets and 0.791 between large-spotted civets and small Indian civets. Overall, activity patterns for each civet species in 2019 were similar to those in 2009–2010 (Gray *et al*., 2010), indicating these had changed little if any between the two periods (Table 4).

Discussion

Our results show that drastic changes have apparently occurred within the terrestrial civet community in SWS from 2009–2010 to 2019. Although encounter rates were highest for large Indian civets and small Indian civets

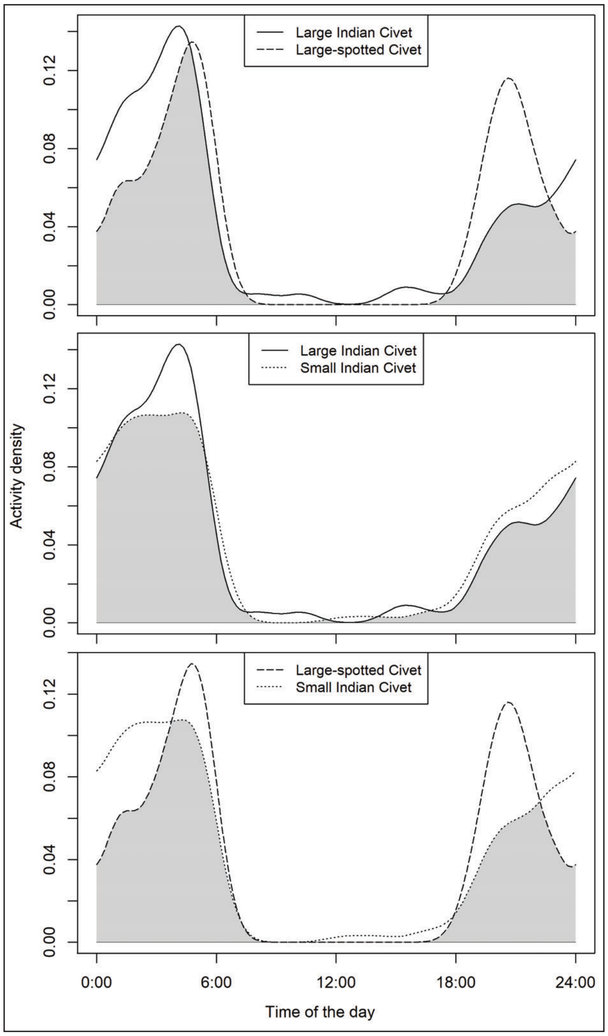

Fig. 4 Comparative activity of large-spotted civets *Viverra megaspila*, large Indian civets *V. zibetha* and small Indian civets *Viverricula indica* in Srepok Wildlife Sanctuary, 2019. Shaded areas represent the coefficient of overlap.

Table 4 Activity overlap based on the estimated coefficients (Dhat1) of overlapping kernel densities between three ground-dwelling civet species in Srepok Wildlife Sanctuary.

Species Overlap	Dhat1	
	2009	2019
Large Indian civet vs. large-spotted civet	0.8157275	0.7529812
Large Indian civet vs. small Indian civet	0.8081864	0.8988771
Large-spotted civet vs. small Indian civet	0.8738956	0.7914684

in 2019, they were highest for large-spotted civets in 2009–2010. Between the two periods, naïve occupancy increased substantially for large Indian civets and small Indian civets, but decreased substantially for large-spotted civets. Following ten years of increased illegal logging when snaring also increased exponentially in SWS (Groenenberg *et al*., 2020), the encounter rate and distribution of large-spotted civets decreased considerably, becoming the least-recorded terrestrial civet in the core zone of SWS based on camera-trap data. Encounter rates and recorded distributions of large Indian civets and small Indian civets increased severalfold in SWS, possibly because these species use a wider altitudinal range and variety of terrain than large-spotted civets. Consequently, the former species could have multiple source populations in the landscape and appear to have flourished in the face of increasing anthropogenic pressures in SWS. We note that encounter rates between studies should be viewed with caution as they might not reflect actual differences in numbers. This is because they could be influenced by other factors such as the use of different camera-trap models, set-up techniques, surveys in different seasons or criteria for station locations. That said, large-spotted and large Indian civets are similar in size and shape, thus differences in camera-trap models should not have affected the detection of these species differently. Additionally, the survey designs were similar between studies and the camera stations in 2019 were actually placed along the same roads and trials as in 2009–2010. Although some camera trapping was conducted during the wet season in the earlier study, most data from that study came from the dry season, so seasonal differences between studies should have been minimal. We therefore conclude that differences in encounter rates between the two studies was influenced most by population changes between the study periods.

Our findings for large and small Indian civets are consistent with studies in other parts of their distributions. Large Indian civets are reported to tolerate considerable habitat modification, and habitat loss and fragmentation are not sufficient to drive population declines (Timmins *et al*., 2016a). The species also appears to be more resilient than large-spotted civets to poaching and indiscriminate snaring (Timmins *et al*., 2016a). In the Nakai-Nam Theun National Protected Area in Laos for example, where intensive market-driven snaring has increased over several decades, large Indian civets were the third most frequently recorded and widespread carnivore (Coudrat *et al*., 2014). In Vietnam, where widespread indiscriminate snaring has led to a decline in most terrestrial mammalian fauna including most small felids, large Indian civets were found in almost half of 13 camera-trapped surveys across the country, albeit in very low numbers (Wilcox *et al*., 2014). This habitat plasticity and relatively greater resilience to poaching therefore seems likely to explain the dramatic increase in encounter rate and recorded distribution for the species in SWS from 2009–2010 to 2019, a time when populations of larger mammal species were devastated in SWS (Groenenberg *et al*., 2020; Rostro-García *et al*., 2023). Additionally, the decrease in large carnivores, some of which consume civets in SWS (Rostro-García *et al*., 2018; Kamler *et al*., 2020), could have also benefited large Indian civets.

Small Indian civets exhibit wide use of and in some cases a preference for degraded and fragmented natural habitats. The species has many healthy populations in agricultural and secondary forest landscapes (Choudhury *et al*., 2015) and can be more common near protected area edges with more disturbed habitats compared to undisturbed forests within protected areas (Johnson *et al*., 2009). It is considered an open forest and edge species and thus has probably benefited from extensive degradation and fragmentation of evergreen forests throughout Southeast Asia (Choudhury *et al*., 2015). The species is also known to persist in the face of heavy hunting that devastates other small terrestrial carnivore populations (Choudhury *et al*., 2015). These attributes probably contributed to the twelve-fold increase in encounter rates for small Indian civets from 2009–2010 to 2019, despite increased poaching and illegal logging in SWS.

Large-spotted civets have much narrower habitat requirements, as the species is typically restricted to elevations <300 m and areas of gentle terrain (Timmins *et al*., 2016b). It also appears to affected more by human hunting than the other two terrestrial civets (Timmins *et al*., 2016b), although it is not clear why indiscriminate snaring (the most common type of poaching in SWS) would negatively affect this species more. It could be the large-spotted civets have a lower intrinsic growth rate (e.g., smaller litters, less frequent births, etc), allowing their populations to be more negatively impacted by mortalities caused by poaching. Regardless of the reasons, our results suggest that a major decline has occurred in large-spotted civet populations in SWS over a ten-year period. This does not bode well for the future of the species in the landscape and these findings have global implications because eastern Cambodia has been considered a stronghold for the species.

Our occupancy modelling showed that large and small Indian civets were positively associated with DDF, whereas the reverse was true for large-spotted civets, suggesting some level of habitat partitioning among the civet species. This is surprising given previous research in SWS showed that large-spotted civets preferred DDF, whereas the large Indian civet was more of a habitat generalist (Gray *et al*., 2010). Our findings are more similar to those made in the northern plains of Cambodia (Suzuki *et al*., 2017) and other parts of their range (Timmins *et al*., 2016b), where large-spotted civets were found in all forest types, albeit at low elevations. Nearly all illegal logging and snaring within SWS is within SEF patches with much less in DDF, so perhaps the small and large Indian civets used more DDF because it was less affected by logging and other human activities. Because long-term data are essential for accurately assessing population density trends, we recommend future camera-trap surveys with similar efforts for civets to monitor their population changes and habitat use in SWS. Future research should also focus on investigating the mechanisms facilitating coexistence within the civet community, particularly exploring the existence of a dominance hierarchy among the species.

Our results suggest the activity patterns of all three civet species are similar and almost completely nocturnal, indicating no temporal partitioning between them. These results are consistent with those in 2009–2010 (Gray *et al*., 2010) and suggest that a decade of increasing human activities within SWS have not affected the activity patterns of the civet community. Because illegal human activity within SWS is primarily diurnal (Rostro-García *et al*., 2023), it presumably did not affect the activities patterns of the nocturnal civet species.

Conservation implications

The Endangered large-spotted civet is experiencing a decrease in encounter rates and recorded distribution in SWS in eastern Cambodia, which is considered a global stronghold for the species (Gray *et al*., 2010). This decrease is plausibly related to the exponential increase in snaring and illegal logging that has occurred in the EPL over the last decade, devastating large mammal populations (Groenenberg *et al*., 2020; Rostro-García *et al*., 2023). In contrast, ground-dwelling small carnivores not confined to level terrain in lowland areas are persisting and even apparently increasing in the face of poaching and habitat-induced changes to the landscape, as evidenced by large and small Indian civets. Unfortunately, indiscriminate snaring is still increasing in SWS and other areas within the EPL (Rostro-García *et al*., 2023) and illegal logging and other forms of habitat loss are expected to continue. Unless a concerted effort is made to reduce poaching and habitat loss in the EPL, which would be a massive task, large-spotted civets may follow tigers, leopards and other larger carnivores in the EPL in becoming extinct in one of its last strongholds in the near future (O'Kelly *et al*., 2012; Rostro-García *et al*., 2023). To protect remaining wildlife in the EPL, especially globally threatened species, immediate actions such as strengthening law enforcement, intensifying anti-poaching efforts and engaging local communities to reduce poaching pressure are crucial. These will require coordinated efforts from government agencies, NGOs, and local communities, which are difficult to implement but necessary for successful wildlife conservation in eastern Cambodia.

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References

Choudhury, A., Duckworth, J., Timmins, R., Chutipong, W., Willcox, D., Rahman, H., Ghimirey, Y. & Mudappa, D. (2015) *Viverricula indica*, small Indian civet. The IUCN Red List of Threatened Species 2015, e.T41710A45220632.

Chutipong, W., Lynam, A.J., Steinmetz, R., Savini, T. & Gale, G. A. (2014) Sampling mammalian carnivores in western Thailand: Issues of rarity and detectability. *Raffles Bulletin of Zoology*, **62**, 521–535.

Clements, G.R., Rostro-García, S., Kamler, J.F., Liang, S.H. & Bin Abu Hashim, A.K. (2021) Conservation status of large mammals in protected and logged forests of the greater Taman Negara Landscape, Peninsular Malaysia. *Biodiversitas*, **22**, 272–277.

Coudrat, C.N.Z., Nanthavong, C., Sayavong, S., Johnson, A., Johnson, L.B. & Robichaud, W.G. (2014) Conservation importance of Nakai-Nam Theun National Protected Area, Laos, for small carnivores based on camera trap data. *Raffles Bulletin of Zoology*, **62**, 31–49.

Duckworth, J., Poole, C., Tizard, R., Walston, J. & Timmins, R. (2005) The jungle cat *Felis chaus* in Indochina: a threatened population of a widespread and adaptable species. *Biodiversity & Conservation*, **14**, 1263–1280.

Francis, C.M. (2008) *A Guide to the Mammals of Southeast Asia*. Princeton University Press, Princeton, New Jersey, USA.

Gray, T.N., Belecky, M., O'Kelly, H.J., Rao, M., Roberts, O., Tilker, A., Signs, M. & Yoganand, K. (2021) Understanding and solving the South-East Asian snaring crisis. *The Ecological Citizen*, **4**, 129–141.

Gray, T.N.E., Hughes, A.C., Laurance, W.F., Long, B., Lynam, A.J., O'Kelly, H., W.J., Seng T., Scotson, L. & Wilkinson, N.M. (2018) The wildlife snaring crisis: an insidious and pervasive threat to biodiversity in Southeast Asia. *Biodiversity and Conservation*, **27**, 1031–1037.

Gray, T. N., Pin C. & Pin C. (2010) Status and ecology of large-spotted civet *Viverra megaspila* in eastern Cambodia. *Small Carnivore Conservation*, **43**, 12–15.

Gray, T.N., Prum S., Pin C. & Phan C. (2012) Distance sampling reveals Cambodia's eastern plains landscape supports the largest global population of Endangered banteng *Bos javanicus*. *Oryx*, **46**, 563–566.

Groenenberg, M., Crouthers, R. & Yoganand, K. (2020) *Population Status of Ungulates in the Eastern Plains Landscape: Srepok Wildlife Sanctuary and Phnom Prich Wildlife Sanctuary, Cambodia*. WWF-Cambodia, Phnom Penh, Cambodia.

Groenenberg, M., Crouthers, R., Yoganand, K., Banet-Eugene, S., Bun S., Muth S., Kim M., Mang T., Panha M., Pheaktra P., Pina T., Sopheak K., Sovanna P., Vibolratanak P., Wyatt, A.G. & Gray, T.N.E. (2023) Snaring devastates terrestrial ungulates whilst sparing arboreal primates in Cambodia's Eastern Plains Landscape. *Biological Conservation*, **284**, 110195.

Hamirul, M., Wong, C.C.T., Mohamed, A., Lau C.F., Mohamad, S.W., Siwan, E.S. & Rayan, D.M. (2015) Recent records of large spotted civet Viverra megaspila from Peninsular Malaysia. *Small Carnivore Conservation*, **52**, 74–83.

Hollenbach, F.M. & Montgomery, J.M. (2020) Bayesian model selection, model comparison, and model averaging. In *The Sage Handbook of Research Methods in Political Science and International Relations* (eds L. Curini & R. Franzese), pp. 937–960. Sage Publications, USA.

Jennings, A.P. & Veron, G. (2011) Predicted distributions and ecological niches of 8 civet and mongoose species in Southeast Asia. *Journal of Mammalogy*, **92**, 316–327.

Jennings, A.P. & Veron, G. (2022) Ecology and conservation of Southeast Asian civets (Viverridae) and mongooses (Herpestidae). In *Small Carnivores: Evolution, Ecology, Behaviour, and Conservation* (eds San D.L., Sato J.J., Belant, J.L., Somers, M.J.), 393–427. Wiley Online Library.

Johnson, A., Vongkhamheng, C. & Saithongdam, T. (2009) The diversity, status and conservation of small carnivores in a montane tropical forest in northern Laos. *Oryx*, **43**, 626–633.

Kamler, J.F., Minge, C., Rostro-García, S., Gharajehdaghipour, T., Crouthers, R., In V., Pay C., Pin C., Sovanna P. & Macdonald, D.W. (2021) Home range, habitat selection, density, and diet of golden jackals in the Eastern Plains Landscape, Cambodia. *Journal of Mammalogy*, **102**, 636–650.

Kamler, J.F., Thatdokkham, K., Rostro-García, S., Bousa, A., Caragiulo, A., Crouthers, R., In V., Pay C., Pin C., Prum S. & Macdonald, D.W. (2020) Diet and prey selection of dholes in evergreen and deciduous forests of Southeast Asia. *The Journal of Wildlife Management*, **84**, 1396–1405.

MacKenzie, D.I., Nichols, J.D., Lachman, G.B., Droege, S., Andrew Royle, J. & Langtimm, C.A. (2002) Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, **83**, 2248–2255.

MacKenzie, D.I., Nichols, J.D., Royle, J.A., Pollock, K.H., Bailey, L. & Hines, J.E. (2017) *Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence*. Academic Press, London, UK.

MacKenzie, D.I. & Royle, J.A. (2005) Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology*, **42**, 1105–1114.

Meredith, M. & Ridout, M. (2018) Package 'overlap'. In *Estimates of Coefficient of Overlapping for Animal Activity Pattern*s. Https://cran.rroject.org/web/packages/overlap/overlap [accessed 19 January 2024]

Niedballa, J., Sollmann, R., Courtiol, A. & Wilting, A. (2016) camtrapR: an R package for efficient camera trap data management. *Methods in Ecology and Evolution*, **7**, 1457–1462.

Nuttall, M.N., Griffin, O., Fewster, R.M., McGowan, P.J.K., Abernathy, K., O'Kelly, H., Nut M., Sot V. & Bunnefeld, N. (2022) Long-term monitoring of wildlife populations for protected area management in Southeast Asia. *Conservation Science and Practice*, **4**, e614.

O'Kelly, H.J., Evans, T.D., Stokes, E.J., Clements, T.J., Dara, A., Gately, M. & Watson, J. (2012) Identifying conservation successes, failures and future opportunities: assessing recovery potential of wild ungulates and tigers in eastern Cambodia. *PLoS One*, **7**, e40482.

Penjor, U., Macdonald, D.W., Wangchuk, S., Tandin, T. & Tan C.K.W. (2018) Identifying important conservation areas for the clouded leopard *Neofelis nebulosa* in a mountainous landscape: Inference from spatial modeling techniques. *Ecology and Evolution*, **8**, 4278–4291.

Penjor, U., Tan C.K.W., Wangdi, S. & Macdonald, D.W. (2019) Understanding the environmental and anthropogenic correlates of tiger presence in a montane conservation landscape. *Biological Conservation*, **238**, 108196.

Pin C., Ngoprasert D., Gray, T. N., Savini, T., Crouthers, R., & Gale, G.A. (2018) Utilization of waterholes by globally threatened species in deciduous dipterocarp forest of the Eastern Plains Landscape of Cambodia. *Oryx*, **54**, 572–582.

Pin C., Phan C., Kamler, J.F., Rostro-Garcia, S., Penjor U., In V., Crouthers, R., Macdonald, E.A., Chou S. & Macdonald, D.W. (2022) Density and occupancy of leopard cats across different forest types in Cambodia. *Mammal Research*, **67**, 287–298.

Pin C., Phan C., Prum S. & Gray, T.N.E. (2013) Structure and composition of deciduous dipterocarp forest in the Eastern Plains Landscape, Cambodia. *Cambodian Journal of Natural History*, **2013**, 27–34.

R Core Team (2020) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rostro-García, S., Kamler, J.F., Crouthers, R., Sopheak, K., Prum S., In V., ... & Macdonald, D.W. (2018) An adaptable but threatened big cat: density, diet and prey selection of the Indochinese leopard (*Panthera pardus delacouri*) in eastern Cambodia. *Royal Society Open Science*, **5**, 171187.

Rostro-García, S., Kamler, J.F., Minge, C., Caragiulo, A., Crouthers, R., Groenenberg, M., Gray, T.N., In V., Pin C., Sovanna P., Kéry, M. & Macdonald, D.W. (2021) Small cats in big trouble? Diet, activity, habitat use, and occupancy of jungle cats and leopard cats in threatened dry deciduous forests, Cambodia. *Ecology and Evolution*, **11**, 4205–4217.

Rostro-García, S., Kamler, J. F., Sollmann, R., Balme, G., Augustine, B.C., Kéry, M., Crouthers, R., Gray, T.N.E., Groenenberg, M., Prum S. & Macdonald, D.W. (2023) Population dynamics of the last leopard population of eastern Indochina in the context of improved law enforcement. *Biological Conservation*, **283**, 110080.

Sollmann, R., Mohamed, A., Samejima, H. & Wilting, A. (2013) Risky business or simple solution—relative abundance indices from camera-trapping. *Biological Conservation*, **159**, 405–412.

Suzuki A., Thong S., Tan S. & Iwata A. (2017) Camera trapping of large mammals in Chhep Wildlife Sanctuary, northern Cambodia. *Cambodian Journal of Natural History*, **2017**, 63–75.

Timmins, R., Duckworth, J., Chutipong, W., Ghimirey, Y., Willcox, D., Rahman, H., Long, B. & Choudhury, A. (2016a) *Viverra zibetha*, large Indian civet. The IUCN Red List of Threatened Species 2016, e.T41709A45220429.

Timmins, R., Duckworth, J.W., WWF-Malaysia, Roberton, S., Gray, T.N.E., Willcox, D.H.A., Chutipong, W. & Long, B. (2016b) *Viverra megaspila*, large-spotted civet. The IUCN Red List of Threatened Species 2016, e.T41707A45220097.

Tordoff, A.W., Timmins, R.J., Maxwell, A., Huy K., Lic V. & Khou E.H. (2005) *Biological Assessment of the Lower Mekong Dry Forests Ecoregion*. WWF Greater Mekong Programme, Phnom Penh, Cambodia.

Vehtari, A., Gelman, A. & Gabry, J. (2015) Efficient implementation of leave-one-out cross-validation and WAIC for evaluating fitted Bayesian models. *ArXiv preprint*, 1507.04544.

Vehtari, A., Gelman, A. & Gabry, J. (2016) Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. *Statistics and Computing*, **27**, 1413–1432.

Wohlfart, C., Wegmann, M. & Leimgruber, P. (2014) Mapping threatened dry deciduous dipterocarp forest in South-east Asia for conservation management. *Tropical Conservation Science*, **7**, 597–613.

Willcox, D.H.A., Tran Q.P., Hoang M.D. & Nguyen T.T.A. (2014) The decline of non-*Panthera* cat species in Vietnam. *Cat News*, **S8**, 53–61.

Using aquatic insect communities (Arthropoda: Insecta) to assess water quality in Kob Srov Lake, Phnom Penh, Cambodia

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មូលន័យសង្ខេប

សត្វល្អិតទឹកជាសូចនាករជីវសាស្ត្រដ៏មានសារៈសំខាន់ក្នុងការបង្ហាញពីគុណភាពទឹក។ យើងបានធ្វើការសង្កេតបម្រែបម្រួលរបាយនៃសត្វល្អិតទឹក និងទំនាក់ទំនងរបស់វាជាមួយនឹងលក្ខណៈរូប-គីមីនៃប៉ារ៉ាម៉ែត្រទឹកដើម្បីវាយតម្លៃគុណភាពទឹកនៅបឹងកប់ស្រូវ រាជធានីភ្នំពេញ។ សំណាញ់ដៃ និងអន្ទាក់សិប្បនិម្មិតត្រូវបានប្រើដើម្បីប្រមូលសត្វល្អិតទឹកពីទីតាំងសិក្សាចំនួន ៧ កន្លែងនៅបឹងកប់ស្រូវ។ អនុគមន៍លីនេអ៊ែរ (Multiple linear regression) ត្រូវបានប្រើដើម្បីសិក្សាពីទំនាក់ទំនងរវាងចំនួនអំបូរ ចំនួនឯកត្តៈ និងភាពសម្បូរបែបនៃសត្វល្អិតទឹកដោយប្រើតម្លៃប៉ារ៉ាម៉ែត្រលក្ខណៈរូប-គីមីនៃទឹក។ តម្លៃសន្ទស្សន៍ជីវសាស្ត្រ (Biological Monitoring Working Party index) និងតម្លៃមធ្យមក្នុងអំបូរមួយត្រូវបានប្រើដើម្បីចាត់ថ្នាក់គុណភាពទឹកនៅតាមទីតាំងសិក្សា។ យើងបានប្រមូលសត្វល្អិតទឹកសរុបចំនួន ២,២៨៣ ក្បាល ស្ថិតក្នុង ១៨ អំបូរ និង ៦ លំដាប់។ សត្វល្អិតទឹកដែល មានចំនួនច្រើនជាងគេនៅទីតាំងសិក្សាទាំងអស់គឺ Chironomids (Diptera) (មានលើសពី ៧៧% នៃចំនួនសំណាកសត្វល្អិតទឹកសរុប)។ សត្វល្អិតទឹកដែលមានចំនួនតិចជាងគេគឺស្ថិតក្នុងលំដាប់ Lepidoptera។ ចំនួនអំបូរ ចំនួនឯកត្តៈ និងភាពសម្បូរបែបនៃសត្វល្អិតទឹកមានភាពខុសគ្នានៅទីតាំងទាំង ៧ កន្លែង ហើយវាមានទំនាក់ទំនងទៅនឹងប៉ារ៉ាម៉ែត្ររូប-គីមីជាក់លាក់។ ចំនួនអំបូរ ចំនួនឯកត្តៈ និងភាពសម្បូរបែបនៃសត្វល្អិតទឹកមានទំនាក់ទំនងវិជ្ជមានជាមួយអុកស៊ីសែនរលាយក្នុងទឹក ខណៈដែលចំនួនអំបូរ និងចំនួនឯកត្តៈមានទំនាក់ទំនងអវិជ្ជមានជាមួយសីតុណ្ហភាពទឹក។ គុណភាពទឹកមានកម្រិតពីមិនល្អទៅមធ្យមនៅក្នុងទីតាំងផ្សេងៗគ្នានៃបឹង។ លទ្ធផលនៃការសិក្សារបស់យើងបានបង្ហាញថា ការធ្វើឱ្យប្រសើរឡើងនូវកម្រិតអុកស៊ីសែនរលាយក្នុងទឹក និងសីតុណ្ហភាពទឹកនឹងធ្វើឱ្យប្រសើរឡើងនូវគុណភាពទឹក និងបង្កើនប៉ូពុយឡាស្យុងសត្វល្អិតទឹកនៅក្នុងបឹងកប់ស្រូវ។

Abstract

Aquatic insects are effective bioindicators of water quality. We investigated the spatial distribution of aquatic insects and their relationships with physico-chemical water parameters to evaluate water quality in Kob Srov Lake, Phnom Penh. Hand nets and artificial substrate traps were employed to sample aquatic insects at seven sites across the lake.

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Multiple linear regressions were applied to explore associations between the taxonomic richness, abundance and diversity of aquatic insects with physico-chemical parameters, whereas Biological Monitoring Working Party index values and average scores per taxon were used to rank water quality at study sites. We collected 2,283 aquatic insects belonging to 18 families in six orders. Chironomids (Diptera) dominated all study sites (representing >77% of all individuals sampled), whereas lepidopterans were the least represented order. Patterns of taxonomic richness, abundance and diversity varied across the lake and were related to specific physico-chemical parameters. Taxonomic richness, abundance and diversity were positively associated with dissolved oxygen, whereas the first two metrics were negatively associated with water temperature. Water quality ranked from poor to moderate at different locations across the lake. Our results suggest management efforts focusing on enhancing dissolved oxygen levels and water temperature would improve water quality and support aquatic insect populations in Kob Srov Lake.

Keywords Aquatic insects, Biological Monitoring Working Party, Diptera, water quality.

Introduction

Aquatic ecosystems are threatened by various human-induced factors including chemicals from agricultural, industrial and mining activities, as well as sewage water discharged from households into the water bodies (Dudgeon, 2000; Yule & Yong, 2004; Gopal, 2005). Agricultural practices, particularly the use of chemical fertilizers, pesticides and livestock manures are the main sources of water pollutants such as heavy metal ions (Rashid *et al*., 2023). These pollutants have resulted in water contamination and waterborne diseases including typhoid fever, cholera, and diarrhoea (Cairns & Pratt, 1993), consequently affecting the health of humans and other organisms. As a result, various methods including the use of aquatic insects have been developed to analyse water quality impairment and assess the health status of aquatic ecosystems (Rosenberg & Resh, 1993; Xu *et al*., 2014).

Aquatic insects occur in both terrestrial and mainly aquatic habitats (mostly freshwater habitats) including lotic systems (e.g., springs, streams and rivers) and lentic systems (e.g., lakes, ponds, wetlands and bogs) (Starr & Wallace, 2021; Vilenica *et al*., 2022). Aquatic insects have one or more life cycle stages which mostly occur in water in their egg and larval forms and migrate to terrestrial habitats in their adult stages (Dijkstra *et al*., 2013; Starr & Wallace, 2021; Vilenica *et al*., 2022). They are one of the most abundant and speciose groups, comprising approximately 130,000 species across 12 orders, representing more than 60% of freshwater species (Mayhew, 2007; Dijkstra *et al*., 2013). Some species are very vulnerable and sensitive to pollution, such as Ephemeroptera (mayflies), Trichoptera (caddisflies), Plecoptera (stoneflies) and Odonata (dragonflies & damselflies) (Lindenmayer *et al*., 2000; Bonada *et al*., 2006; Shafie *et al*., 2017), whereas others such as Diptera can survive in very polluted waters (Cummin & Merrit, 1996; Hepp *et al*., 2013; Arimoro *et al*., 2018). These groups are important components of both aquatic and terrestrial ecosystems, serving as primary consumers, detritivores, predators and pollinators (Dijkstra *et al*., 2013; Vilenica *et al*., 2022). They also play a vital role in nutrient cycling and contribute significantly to the aquatic-to-terrestrial transfers of food and energy (Dijkstra *et al*., 2013; Starr & Wallace, 2021).

Due to their sensitivity to environmental conditions, aquatic insects are widely employed as bioindicators to assess ecosystem health (Arimoro & Ikomi, 2008; Chowdhury *et al*., 2023). They are useful for assessing or monitoring anthropogenic stress on ecosystems, including sewage discharge, agricultural runoff, recreational activities, land clearing and urban development over extended periods (Cairns & Pratt, 1993). It has been reported that aquatic insects offer more accurate insights into the dynamics of water bodies or river systems than chemical data (Shafie *et al*., 2017). As such, many indices have been developed to monitor water quality, including diversity indices (e.g., Simpson diversity, Shannon diversity) and scoring system (i.e., Biological Monitoring Water Party (BMWP) (Armitage *et al*., 1983) and Belgian Biotic Index (de Pauw & Vanhooren, 1983).

Aquatic insects in freshwater bodies have been extensively researched, but remain relatively poorly studied in stable freshwater systems such as lakes. Few papers have investigated water quality in the Lower Mekong Basin in Cambodia to date. Those that have include studies on the spatial heterogeneity of macro-invertebrates (Sor *et al*., 2017) and aquatic insect communities (Sor *et al*., 2021). Other studies have examined aquatic insect diversity and its relationship to water quality in urban ponds in Phnom Penh (Chhy *et al*., 2019), alongside studies on aquatic Ephemeroptera (Chhorn *et al*., 2020) and Coleoptera (Doeurk *et al*., 2022) in relation to freshwater quality in southwest Cambodia. Additionally, research has been undertaken on aquatic Hemiptera (Zettel *et al*., 2017) and Polyphaga checklist (Freitag *et al*., 2018).

Although water quality was recently examined in Kob Srov Lake (KSL) in the northern area of Phnom Penh in terms of heavy metal contaminations (Kev *et al*., 2019), this has yet to be assessed in relation to aquatic insect communities. The latter is necessary for enhancing understanding of the ecological context and implications of water pollution at the site. We consequently aimed to assess water quality in KSL using aquatic insect communities as bioindicators. To this end, we first examined the spatial distribution of aquatic insects in the lake, then quantified relationships between aquatic insect diversity and selected chemical parameters. Finally, we assessed water quality in the lake using the BMWP system. Our overall purpose was to provide baseline information to local inhabitants and authorities about water quality in KSL and the need for improvement of wastewater management strategies in surrounding areas.

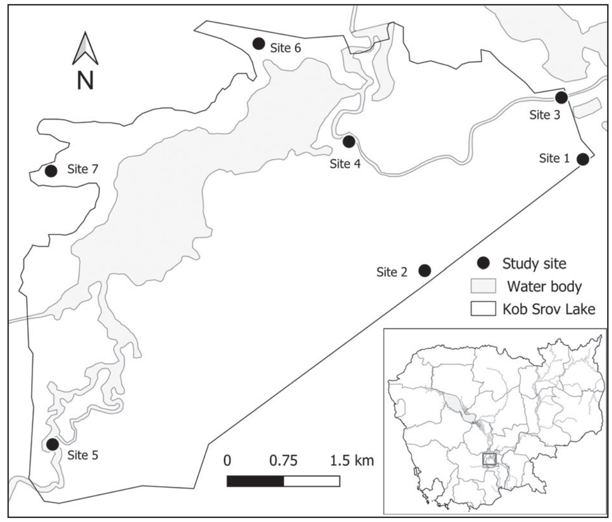

Fig. 1 Location of seven study sites at Kob Srov Lake, Phnom Penh, Cambodia.

Methods

Study area

This study was conducted in the KSL in the northern area of Phnom Penh City (11.638969°N, 104.818686°E; Figs. 1–2), Cambodia. Kob Srov Lake encompasses approximately 26 km^2 and is located in the Sen Sok and Posenchey Districts of the city. While the lake is artificial and was created to protect Phnom Penh from flooding during in the wet season, it has recently also received wastewater from the western side of the city and been subjected to agricultural and other human activities which have degraded water quality. The lake is an important reservoir, not just because it receives excess water from Phnom Penh but because it stores a large amount of agricultural wastewater. Further, the lake supplies water to people around the lake for crop production, aquaculture, livestock and fishing activities and also serves as a biofilter to clean water (Kev *et al*., 2019) before draining into the Tonle Sap River at Prek Pnov.

Sampling design & data collection

Aquatic insects and water quality data were collected from seven sites in the KSL from 10 to 16 March 2018 (Figs. 1–2). Three samples were collected from each site, resulting in 21 samples for the study. Collection of a single sample took about 20 minutes, including measurement of water parameters and collection of aquatic insects.

Aquatic insects were sampled using two methods, namely hand nets and artificial substrate traps. The hand nets had an opening area of 30 x 30 cm, a depth of 92 cm and a mesh size of 1 mm and was used to collect insects in water or reed vegetation along the shoreline. Artificial substrate traps were employed to sample insects in deeper waters, with a single trap left at each study site for approximately four weeks. Following collection, insect samples were placed on a white tray in the field and rinsed with water for sorting and screening. These were then transferred with forceps into labelled containers containing 75% or 80% ethanol. In the laboratory, insects were sorted and identified to the family level using taxonomic keys (Dudgeon, 1999; Yule & Yong, 2004). Larger aquatic insects were examined by naked eye, whereas smaller insects were examined using an Olympus SZ51 dissecting microscope.

Five physico-chemical parameters were employed for our water quality assessment, namely dissolved oxygen (DO, as %), potential hydrogen (pH), turbidity (TBD, as FNU [Formazin Nephelometric Units]), water temperature (WT, in °C) and electrical conductivity (CON, in mS/m [molloSiemens per metre]). These were measured at each study site using a HI-7609829 multiparameter portable water quality meter (Hanna Instruments Ltd., Bedfordshire, UK) at water depths ranging from 0.1 – 0.5 m.

Data analyses

We used three metrics to quantify the aquatic fauna of our study sites: taxonomic richness, abundance and

Fig. 2 Indicative images of study sites at Kob Srov Lake, Phnom Penh, Cambodia.

Shannon-Wiener's diversity index (H). The latter H values were computed using the 'vegan' package (Oksanen *et al*., 2022) in R (R CoreTeam, 2022) and are based on taxonomic richness and abundance (Kerkhoff, 2010; Hamid & Rawi, 2017). Bubble plots were used to depict spatial distribution in these metrics across the seven sampling sites in the lake.

We employed multiple linear regression to investigate the relationships between the above metrics and environmental parameters. In our model, insect richness, abundance and Shannon-Wiener's diversity index were dependent variables, whereas physico-chemical factors were employed as explanatory variables e.g., DO, pH, TBD, WT and CON. Prior to model construction, dependent variables were log-transformed to remove the effects of outliers and ensure data normality, whereas independent variables were normalized (by making the margin sum of squares equal to one; ranging from 0 to 1) to ensure the same scale across variables. We assumed that the logarithmic transformation enables normal data distribution. Afterwards, a stepwise variable selection approach based on Akaike Information Criterion (AIC) was employed to remove unimportant variables using the 'stepAIC()' function of the 'MASS package (Venables & Ripley, 2002) in R (R CoreTeam, 2022). The influence of each environmental parameter on the distribution of the taxonomic richness, abundance and H was assessed using standardized regression coefficients. Model performance was assessed using coefficients of determination (R-square). The models were created using 'lm()' function of the 'stats' package in R (R CoreTeam, 2022).

The BMWP system was used to score each aquatic insect family present in our seven study sites in KSL. Scores for individual families reflect their pollution tolerance based on knowledge of distribution and abundance. Pollution-intolerant families have high BMWP scores, whereas pollution-tolerant families have low scores (Armitage *et al*., 1983; Sivaramakrishnan, 1992). More specifically, scores ranging from 0–40, 41–70 and 71–100+ indicate very poor to poor (heavily polluted to polluted), moderate (moderately impacted) and good to very good water quality (clean but slightly impacted to unpolluted), respectively (Chesters, 1980; Mustow, 2002). The Average Score Per Taxon (ASPT) represents the average tolerance of all taxa within the community and is calculated by dividing the BMWP score by the number of families in a sample (Walley & Hawkes, 1996). More specifically, ASPT values ranging from 1–2.49 (water quality class III–IV), 2.5–3.99 (III), 4.0–5.49 (II–III), 5.5–6.99 (II), 7.00–7.99 (I–II) and 8.0–10.0 (I) indicate 'poor', 'fairly poor', 'moderate', 'fairly good', 'good' and 'very good' water quality, respectively (Mustow, 2002). Thus, a high ASPT value denotes clean sites with a relatively large number of high-scoring taxa (Sivaramakrishnan, 1992).

Results

Community composition of aquatic insects

We recorded a total of 2,283 aquatic insects belonging to 18 families and six orders during sampling (Annex 1). These were dominated by Diptera (80.11%), followed by Hemiptera (12.44%), Odonata (5.52%), Coleoptera (0.96%), Ephemeroptera (0.79%) and Lepidoptera (0.18%). At a family level, members of Chironomidae were most dominant taxa (77.31%), followed by members of the Micronectidae (8.60%), Libellulidae (3.64%), Ceratopogonidae (2.67%), Belostomatidae (1.88%), Notonectidae

Table 1 Physico-chemical parameters recorded at sampling sites in Kob Srov Lake, Phnom Penh. Values are given as mean, min–max (based on three samples collected at each site).

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Dissolved oxygen (%)	59.7, 40.1–70.6	124.36, 50–167.1	24, 19.6–32.3	59.73, 55.4–65.6	78.33, 60.5– 90	83.6, 79.5–88.3	75.7, 71.5–79.6
pH	9.71, 9.64–9.82	8.63, 6.33–10.25	7.17, 7.11–7.21	7.46, 7.36–7.52	7.77, 7.49–7.99	7.52, 7.49–7.55	7.57, 7.46–7.65
Turbidity (FNU)	124.73, 86.2–161	478.03, 41.1–878	270.66, 200–349	45.56, 44.3–46.7	132.53, 98.6–189	224.66, 116–406	99.6, 97.6–103
Water temperature (°C)	27.76, 27.61–28.03	31.79, 30.67–33.65	29.81, 29.45–30.16	28.31, 28.28–28.33	30.03, 27.33–31.7	29.71, 29.47–29.94	28.14, 27.83–28.46
Electrical conductivity (mS/m)	197.66, 194–202	293.66, 210–348	247.66, 246–251	169.66, 169–170	150.66, 148–153	160, 159–161	163, 163–163

(1.58%), Coenagrinonidae (1.49%), Hydrophilidae (0.96%), Caenidae (0.44%), Protoneuridae (0.35%), Baetidae (0.35%), Gerridae (0.31%), Crambidae (0.18%), Syrphidae (0.09%), Stratiomyidae (0.04%), Gomphidae (0.04%), Veliidae (0.04%) and Nepidae (0.04%). Physico-chemical variables recorded at sampling sites are summarized in Table 1.

Spatial distribution of aquatic insects

Significant variation was observed in the taxonomic (family) richness, abundance and diversity of aquatic insects between sites (Fig. 3). More specifically, taxonomic richness was higher in the southeastern (site 1), south-central (site 2) and southwestern (site 5) portions of the lake, and lower in the northwestern (site 7) and north-central (site 6) areas of the lake. No aquatic insects were observed in the northeastern (site 3) and central (site 4) portions of the lake. Further, the southeastern (site 1) and south-central (site 2) areas of the lake supported signficantly higher insect abundance. Finally, the southwestern (site 5), northwestern (site 7) and north-central (site 6) areas of the lake hosted the highest levels of diversity, followed by the southeastern (site 1) and south-central (site 2) areas.

Aquatic insect & water quality associations

Our multiple linear regression models yielded adjusted R^2 values of 0.310, 0.480 and 0.170 for taxonomic richness, abundance and Shannon-Wiener's diversity, respectively (Table 2). Dissolved oxygen was positively related to taxonomic richness, abundance and Shannon-Wiener's diversity, whereas water temperature was negatively correlated with taxonomic richness and abundance.

Table 2 Standardized regression coefficients for taxonomic richness (SR), abundance (AB) and Shannon-Weiner's index values (H) for aquatic insects modelled against water parameters in Kob Srov Lake, Phnom Penh. Model performance for each is indicated by adjusted R^2 values. Asterisks indicate significance at $p<0.05$.

Variables	TR	AB	H
Dissolved oxygen	2.729*	2.897*	2.319*
pH	1.794	1.931	-
Turbidity	-	1.246	-
Water temperature	-2.715*	-3.544*	-
Conductivity	-	1.413	-
Adjusted R^2	0.310	0.480	0.170

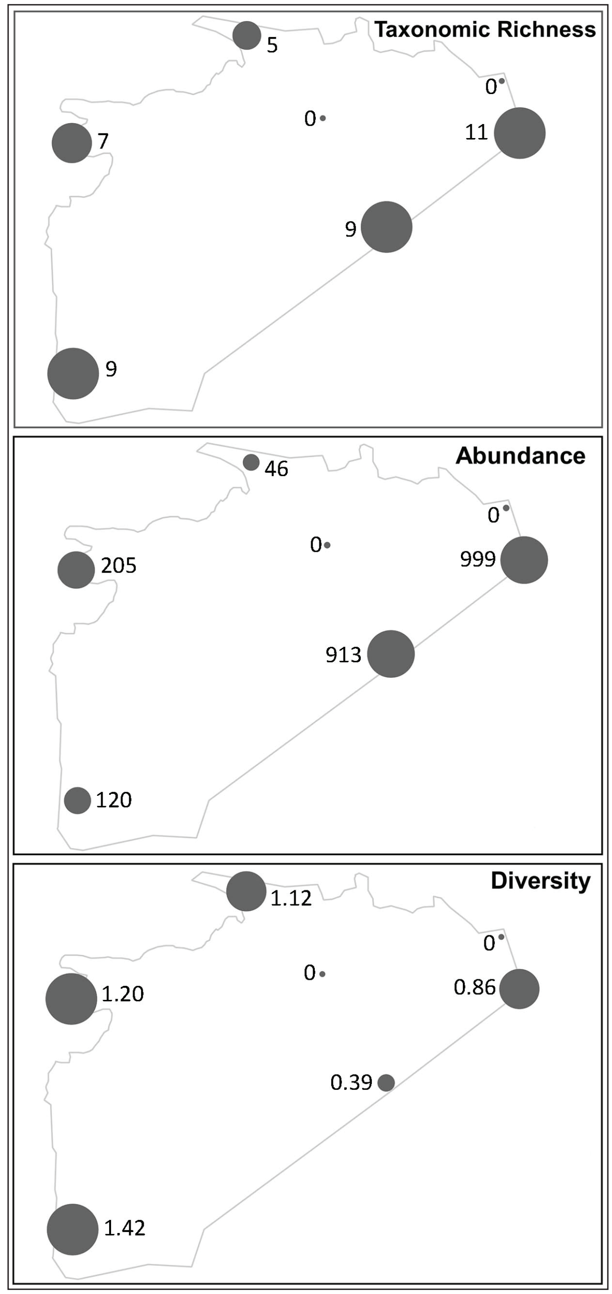

Fig 3 Bubble plots indicating taxonomic richness, abundance and Shannon-Wiener diversity index values recorded at sampling sites in Kob Srov Lake, Phnom Penh.

Water quality in Kob Srov Lake

On the basis of BMWP and ASPT scores, water quality ranked as moderate (albeit decreasingly so) in the southeastern (site 1), south-central (site 2), southwestern (site 5) and north-central (site 6) portions of the lake, whereas it ranked as fairly poor in the northwestern portion (site 7) (Table 3). As before, the northeastern (site 3) and central

Table 3 Water quality scores and associated rankings for sampling sites in Kob Srov Lake, Phnom Penh.

Sampling Site	Biological Monitoring Working Party	Average Score Per Taxon	Water Quality Class	Water Quality Category
Site 1	57	4.07	II-III	Moderate
Site 2	52	4.0	II-III	Moderate
Site 3	-	-	-	-
Site 4	-	-	-	-
Site 5	46	4.18	II-III	Moderate
Site 6	32	4.0	II-III	Moderate
Site 7	30	3.75	III	Fairly poor

(site 4) portions of the lake did not achieve biotic scores for water quality as no aquatic insects were recorded in these areas.

Discussion

Our study contributes to a better understanding of aquatic insects and water quality of Kob Srov Lake. We recorded 18 families belonging to six orders of aquatic insects and found that patterns of aquatic insect taxonomic richness, abundance and diversity varied across the lake. These three metrics were positively correlated with dissolved oxygen, whereas taxonomic richness and abundance were negatively correlated with water temperature, and water quality ranked as moderate to poor in different areas of the lake.

Similar to studies of aquatic insects inhabiting freshwater ecosystems elsewhere (Balian et al., 2008; Sarikar & Vijaykumar, 2022), members of Diptera and Hemiptera were the most abundant taxa in our study. This is unsurprising because Diptera is typically the largest taxonomic group in representing half of all aquatic insects (Dijkstra *et al*., 2013). Members of Coleoptera and Lepidoptera were the least abundant taxa in our study, which is consistent with reports identifying these as the smallest taxonomic groups of aquatic insects (Dijkstra *et al*., 2013).

More specifically, Chironomidae were the most dominant family in our study, accounting for over 77% of insects. These were ubiquitous across our sampling sites and evidently tolerant of differing environmental conditions, as suggested by Popoola & Otalekor (2011). Standing and low current waters and mud or sandy areas with high fine particle sizes are known to support higher diversity and abundance of chironomids (Doisy & Rabeni, 2001; Kubendran & Ramesh, 2016) and they are commonly found in high turbidity and polluted waters (Armitage *et al*., 1995; Johnson, 1995). This is consistent with the dominance of the group at sampling site 2, which was characterised by such conditions.

Heterogeneous patterns of taxonomic richness, abundance and diversity were observed across the lake. Taxonomic richness was high along the southern periphery of the lake where various aquatic vegetation (water grass, small trees and water hyacinth) was observed. This likely provides favourable conditions for aquatic insects to shelter and forage, as well as refuges from predators (Andersson, 2014), allowing them to complete life cycles and providing feeding and nursing grounds. In contrast, the northern area of the lake hosted lower aquatic insect richness and abundance, possibly due to less aquatic vegetation and microhabitat, whereas no insects were found in the northeastern and central portions of the lake. This may be attributable to significant water disturbance and pollution as well an absence of vegetation in these areas.

The physico-chemical parameters we measured offer good indications for determining aquatic insect communities. We found dissolved oxygen was positively related to taxonomic richness, abundance and diversity. This is consistent with previous studies that found that aquatic insect abundance was positively correlated with dissolved oxygen (Yahaya & Suleiman, 2017; Chhy *et al*., 2019). We also found that water temperature was negatively related to taxonomic richness and abundance. Water temperature appears to be an important factor impacting the ecosystem changes (Arim *et al*., 2007) and rising temperatures contribute to declines in the density and richness of aquatic insects (Vannote & Sweeney, 1980; Glazier, 2012). Dissolved oxygen levels also decrease when water temperature increase, likely due respiration and other processes such as organic matter degradation (Ebenebe *et al*., 2016). Higher temperature may cause the water column to become more biologically active, which could lead to species in more tolerant groups (such as Diptera, Hemiptera and Odonata) thriving over less tolerant groups (e.g., Trichoptera and Ephemeroptera) (Ngodhe *et al*., 2014). Our data suggests that sensitive taxa require higher levels of dissolved oxygen for respiration, whereas tolerant organisms are capable of aerobic biodegradation of organic matter in lower oxygen aquatic environments. Further, most dipterans, hemipterans and odonates were found in water temperatures between 27°C and 34°C. This may attributable that these being able to shift their metabolic processes from aerobic to predominantly anaerobic at high temperatures (Hamburger *et al*., 1994).

Our data suggests that water quality in Kob Srov Lake ranges from moderate in the southeastern, south-central, southwestern and north-central lake portions to fairly poor in the northwestern lake portions. The latter is likely due in part due to the fact that this area was dominated by tolerant taxa. Water quality ranked least in the northeastern and central portions due to the complete absence of aquatic insects. This may be attributable to disturbance from transport or sewage discharge from Phnom Penh, as these can lead to water degradation (Angelidis *et al*., 1995; Yang *et al*., 2004; Okumagba & Ozabor, 2014).

It is important to note that our results do not reflect the current status of Kob Srov Lake, but rather the conditions six years ago. Some parts of the lake have been experienced increased urbanization since this time. Irrespectively, our study highlights the important roles of aquatic insect diversity in association with dissolved oxygen and water temperate in indicating water quality in the lake. They also suggest measures are needed to improve poor water quality in certain areas of the lake for human and agriculture consumption. Management of wastewater will undoubtedly be important in this regard. Further assessments of water quality during both the dry and wet seasons with more sample sites would be of interest to evaluate seasonal variations in aquatic insects and water quality. Additionally, identification of aquatic insects to species level is advised to elucidate community structure and generate a checklist of aquatic insects inhabiting the lake and surrounding areas.

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References

Andersson, J. (2014) *Aquatic insect community structure in urban ponds: effects of environmental variables*. MSc thesis, Uppsala University, Sweden.

Angelidis, M.O., Markantonatos, P.G. & Bacalis, N.C. (1995) Impact of human activities on the quality of river water: The case of Evrotas River catchment basin, Greece. *Environmental Monitoring and Assessment*, **35**, 137–153.

Arim, M., Bozinovic, F. & A. Marquet, P. (2007) On the relationship between trophic position, body mass and temperature: reformulating the energy limitation hypothesis. *Oikos*. DOI 10.1111/j.0030-1299.2007.15768.x

Arimoro, F.O., Auta, Y.I., Odume, O.N., Keke, U.N. & Mohammed, A.Z. (2018) Mouthpart deformities in Chironomidae (Diptera) as bioindicators of heavy metals pollution in Shiroro Lake, Niger State, Nigeria. *Ecotoxicology and Environmental Safety*, **149**, 96–100.

Arimoro, F.O. & Ikomi, R.B. (2008) Ecological integrity of upper Warri River, Niger Delta using aquatic insects as bioindicators. *Ecological Indicators*, **9**, 455–461.

Armitage, P.D., Moss, D., Wright, J.F. & Furse, M.T. (1983) The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Research*, **17**, 333–347.

Armitage, P.D., Pinder, L.C. & Cranston, P.S. (1995) *The Chironomidae: Biology and Ecology of Non-biting Midges*. Springer, New York, USA.

Balian, E. V., Segers, H., Lévèque, C. & Martens, K. (2008) The freshwater animal diversity assessment: an overview of the results. *Hydrobiologia*, **595**, 627–637.

Bonada, N., Prat, N., Resh, V.H. & Statzner, B. (2006) Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual Review of Entomology*, **51**, 495–523.

Cairns, J. & Pratt, J.R. (1993) A history of biological monitoring using benthic macroinvertebrates. In *Freshwater Biomonitoring and Benthic Macroinvertebrates* (eds D.M. Rosenberg & V.H. Resh), pp. 10–27. Chapman & Hall, New York, USA.

Chesters, R.K. (1980) *Biological Monitoring Working Party. The 1978 National Testing Exercise*. Technical Memorandum 19. Water Data Unit, Department of Environment, Reading, UK.

Chhorn S., Chan B., Sin S., Doeurk B., Chhy T., Phauk S. & Sor R. (2020) Diversity, abundance and habitat characteristics of mayflies (Insecta: Ephemeroptera) in Chambok, Kampong Speu Province, southwest Cambodia. *Cambodian Journal of Natural History*, **2020**, 61–68.

Chhy T., Soth S., Nheb S. & Sor R. (2019) Diversity of aquatic insect families and their relationship to water quality in urban ponds in Phnom Penh, Cambodia. *Cambodian Journal of Natural History*, **2019**, 113–120.

Chowdhury, S., Dubey, V.K., Choudhury, S., Kumar, N., Semwal, A. & Kumar, V. (2023) Insects as bioindicator: a hidden gem for environmental monitoring. *Frontiers in Environmental Science*, **11**, 1–16.

Cummin, K. & Merrit, M.R. (1996) An introduction to the aquatic insects of North America. *Journal of the North American Benthological Society*, **15**, 401–403.

de Pauw, N. & Vanhooren, G. (1983) Method for biological quality assessment of watercourses in Belgium. *Hydrobiology*, **100**, 153–168.

Dijkstra, K.B., Monaghan, M.T. & Pauls, S.U. (2013) Freshwater biodiversity and aquatic insect diversification. *Annual Review of Entomology*, **59**, 143–163.

Doeurk B., Chhorn S., Sin S., Phauk S. & Sor R. (2022) Diversity, distribution and habitat associations of aquatic beetles (Order Coleoptera) in Chambok, southwest Cambodia. *Cambodian Journal of Natural History*, **2022**, 90–98.

Doisy, K.E. & Rabeni, C.F. (2001) Flow conditions, benthic food resources, and invertebrate community composition in a low-gradient stream in Missouri. *Journal of the North American Benthological Society*, 20, 17–32.

Dudgeon, D. (1999) *Tropical Asian Streams: Zoobenthos, Ecology and Conservation*. Hong Kong University Press, Hong Kong.

Dudgeon, D. (2000) Riverine biodiversity in Asia: a challenge for conservation biology. *Hydrobiologia*, **418**, 1–13.

Ebenebe, C. I., Ihouma, J., Ononye, B. & Ufele, A. (2016) Effect of physicochemical properties of water on aquatic insect communities of a stream in Nnamdi. *International Journal of Entomology Research*, **1**, 2455–4758.

Freitag, H., Doeurk B., Chhorn S., Khin C., Sin S., Ehlers, S., Voges, J., Garces, J.M., Phauk S. (2018) Aquatic Polyphaga (Insecta: Coleoptera) from Kampong Speu Province, Cambodia. *Cambodian Journal of Natural History*, **2018**, 90–100.

Glazier, D.S. (2012) Temperature affects food-chain length and macroinvertebrate species richness in spring ecosystems. *Freshwater Science*, **31**, 575–585.

Gopal, B. (2005) Does inland aquatic biodiversity have a future in Asian developing countries? *Hydrobiologia*, **542**, 69–75.

Hamid, S.A. & Rawi, C.S.M. (2017) Application of aquatic insects (Ephemeroptera, Plecoptera and Trichoptera) in water quality assessment of Malaysian headwater. *Tropical Life Sciences Research*, **28**, 143–162.

Hepp, L.U., Restello, R.M., Milesi, S.V., Biasi, C. & Molozzi, J. (2013) Distribution of aquatic insects in urban headwater streams. *Acta Limnologica Brasiliensia*, **25**, 1–9.

Johnson, K.J. (1995) The Thienemann lecture: the indicator concept in freshwater biomonitoring. In *Chironomids: From Genes to Ecosystems* (ed P.S. Cranston), pp. 11–27. CSIRO Publishing, Canberra, Australia.

Kev S., Kruy L., Kreng S., Lim S. & Phan K. (2019) Assessing heavy metals contamination in Kob Srov Lake of Phnom Penh. *The Bulletin of Cambodian Chemical Society*, **9**, 47–50.

Kubendran, T. & Ramesh, M. (2016) Composition and distribution of aquatic insect communities in relation to water quality in two freshwater streams of southern western Ghats, India. *Journal of Entomology and Zoology Studies*, **4**, 689–695.

Lindenmayer, D.B., Margules, C.R. & Botkin, D.B. (2000) Indicators of biodiversity for ecologically sustainable forest management. *Conservation Biology*, **14**, 941–950.

Mayhew, P.J. (2007) Why are there so many insect species? Perspectives from fossils and phylogenies. *Biological Reviews*, **82**, 425–454.

Mustow, S.E. (2002) Biological monitoring of rivers in Thailand: use and adaptation of the BMWP score. *Hydrobiologia*, **479**, 191–229.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, H.H., Wagner, H. (2022) *Vegan: Community Ecology Package (R package version 2.6-4)*. Https://www.researchgate.net/publication/360782912_vegan_community_ecology_package_version_26-2_April_2022.

Okumagba, P.O. & Ozabor, F. (2014) The effects of socio-economic activities on River Ethiope. *Journal of Sustainable Society*, **3**, 1–6.

Ngodhe, S.O., Raburu, P.O. & Achieng, A. (2014) The impact of water quality on species diversity and richness of macroinvertebrates in small water bodies in Lake Victoria Basin, Kenya. *Journal of Ecology and The Natural Environment*, **6**, 32–41.

Popoola, K.O.K. & Otalekor, A. (2011) Analysis of aquatic insects' communities of Awba Reservoir and its physico-chemical properties. *Research Journal of Environmental and Earth Sciences*, **3**, 422–428.

R CoreTeam (2022) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rashid, A., Schutte, B.J., Ulery, A., Deyholos, M.K., Sanogo, S., Lehnhoff, E.A. & Beck, L. (2023) Heavy metal contamination in agricultural soil: environmental pollutants affecting crop health. *Agronomy*, **13**, 1521.

Rosenberg, D. & Resh, V.H. (1993) *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman & Hall, New York, USA.

Sarikar, S. & Vijaykumar, K. (2022) Monitoring of water quality using aquatic insects as biological indicators in Bhosga reservoir, Karnataka, India. *Advances in Zoology and Botany*, **10**, 82–92.

Shafie, M.S.I., Wong A.B.H., Harun, S. & Fikri, A.H. (2017) The use of aquatic insects as bio-indicator to monitor freshwater stream health of Liwagu River, Sabah, Malaysia. *Journal of Entomology and Zoology Studies*, **5**, 1662–1666.

Sivaramakrishnan, K.G. (1992) *Composition and Zonation of Aquatic Insect Fauna of Kaveri and its Tributaries and the Identification of Insect Fauna as Indicator of Pollution*. Department of Environment Project Report, India.

Sor R., Boets, P., Chea R., Goethals, P.L.M. & Lek S. (2017) Spatial organization of macroinvertebrate assemblages in the Lower Mekong Basin. *Limnologica*, **64**, 20–30.

Sor R., Ngor P.B., Soum S., Chandra, S., Hogan, Z.S. & Null, S.E. (2021) Water quality degradation in the Lower Mekong Basin. *Water*, **13**, 1–18.

Starr, S.M. & Wallace, J.R. (2021) Ecology and biology of aquatic insects. *Insects*, **12**, 51.

Vannote, R. L. & Sweeney, B. W. (1980) Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *The American Naturalist*, **115**, 667–695.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*. Springer, New York, USA.

Vilenica, M., Vuataz, L. & Yanai, Z. (2022) Ecology and Conservation Challenges. *Diversity*, **14**, 10–13.

Walley, W.J. & Hawkes, H.A. (1996) A computer-based reappraisal of the biological monitoring working party scores using data from the 1990 river quality survey of England and Wales. *Water Research*, **30**, 2086–2094.

Xu M., Wang Z., Duan X. & Pan B. (2014) Effects of pollution on macroinvertebrates and water quality bio-assessment. *Hydrobiologia*, **729**, 247–259.

Yahaya, M.M. & Suleiman, B. (2017) Assessment of water quality of Kware Lake using aquatic insects as bioindicators. *International Journal of Science & Technoledge*, **5**, 136–142.

Yang S.L., Shi Z., Zhao H.Y., Li P., Dai S.B. & Gao A. (2004) Research Note: effects of human activities on the Yangtze River suspended sediment flux into the estuary in the last century. *Hydrology and Earth System Sciences*, **8**, 1210–1216.

Yule C. & Yong H. (2004) *Freshwater of the Malaysian Region*. Academy of Science Malaysia, Kuala Lumpur, Malaysia.

Zettel, H., Phauk S., Kheam S. & Freitag, H. (2017) Checklist of the aquatic Hemiptera (Heteroptera: Gerromorpha and Nepomorpha) of Cambodia, with descriptions of new species of *Microvelia* Westwood, 1834 and *Ranatra* Fabricius, 1790. *Aquatic Insects*, **38**, 21–48.

Annex 1 Abundance of aquatic insect families recorded in Kob Srov Lake, Phnom Penh

Order/Family	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Diptera							
Chironomidae	740	843			40	28	114
Ceratopogonidae	1				4		56
Stratiomyidae	1						
Syrphidae		2					
Odonata							
Coenagrinonidae		34					
Protoneuridae		3			5		
Libellulidae	8	5			56	9	5
Gomphidae					1		
Hemiptera							
Notonectidae	36						
Micronectidae	172				4	3	17
Veliidae						1	
Nepidae	1						
Gerridae	1	6					
Belostomatidae	30	13					

Annex 1 Cont'd

Order/Family	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Coleoptera							
Hydrophilidae	5	5			3		9
Ephemeroptera							
Caenidae					4	5	1
Baetidae		2			3		3
Lepdipotera							
Crambidae	4						

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