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Cover image: Female Malayan sun bear (*Helarctos malayanus*) at a wildlife release station in Koh Kong Province, 2016 (© Jeremy Holden/Wildlife Alliance). An account of three sun bears monitored after their release in the southern Cardamom Mountains is given by Marx *et al.* in this issue (p. 42–50).

News

First study of wildlife poisoning practices in Preah Vihear Province

Wildlife poisoning is an increasing concern for conservation in Cambodia, but little is known about this practice. With support from the Ministry of Environment, we conducted the first comprehensive study of wildlife poisoning which was published this year in the *Oryx* journal. The study was conducted in 2017 across 12 villages within the Chheb and Kulen Promtep wildlife sanctuaries in the Northern Plains landscape in Preah Vihear Province. We used a mixed-methods approach including interviews with 57 key informants, 24 focus group discussions, and a questionnaire survey of 462 respondents based on the Theory of Planned Behaviour framework.

We found that wildlife poisoning is widespread, occurring in nine of the 12 villages studied. Prevalence varied from just a few households to approximately 30% of each village, according to informants. Hunters place carbamate pesticides (known locally as 'termite poisons') with rice or fish near waterholes during the dry season to harvest wild meat for consumption at home. To avoid health risks, they remove the head and internal organs of harvested animals before eating. Despite this, we recorded many negative impacts of poisoning on wildlife, the environment, and people. For example, several reports of poisoned cattle were recorded. As a result, most residents strongly disapprove of wildlife poisoning, and some villages have acted against it, by warning offenders or organising community meetings.

Wildlife poisoning is a major threat to endangered wildlife and human health and must be urgently addressed by national authorities and local communities. Cambodian law regulating or banning various carbamate pesticides must be enforced, and regulations on the sale of pesticides should be imposed to ensure clear labelling and education about their safe use. Local authorities should also engage with community leaders on the issue. For example, instituting a reporting hotline would enable communities to respond to poisoning incidents.

The full details of our study are available in English at <https://doi.org/10.1017/S0030605319001492> and in Khmer at <https://doi.org/10.6084/m9.figshare.12146181.v1>

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Launch of national action plan to conserve Cambodian elephants

Asian elephants *Elephas maximus* are widely regarded as a flagship, keystone and umbrella species for conservation due to their cultural significance, important role in ecosystems and large area requirements. The species is regarded as Endangered because its global population has declined by an estimated 50% in the past 60–75 years while its range has been reduced by almost 90%. Populations of wild elephants in Cambodia have also decreased dramatically and are now believed to number 400–600 animals, most of which occur in the Cardamom Mountains Landscape and Eastern Plains Landscape, with much smaller numbers fragmented across several areas including Prey Lang Wildlife Sanctuary, Virachey National Park and Chheb Wildlife Sanctuary. Given ongoing threats posed by habitat loss and fragmentation, human-elephant conflict and poaching, coupled with stochastic and genetic vulnerabilities due to the small size of remaining populations, concerted actions are urgently required to avoid extinction of the species in Cambodia.

Following years of dedicated research and consultations with a wide variety of stakeholders including the Cambodian Elephant Conservation Group, the Ministry of Environment has launched a ten-year (2020–2029) action plan to conserve wild elephants in Cambodia. The goal of the action plan is to provide a policy framework and management mechanism for stakeholders to reduce threats to the long-term survival of elephants nationally. To this end, it proposes a variety of activities across seven strategic areas: 1) reduction of habitat loss, 2) improved habitat connectivity, 3) strengthened law enforcement, 4) prevention of wild captures, 5) mitigation of human-elephant conflict, 6) improved awareness and 7) dedicated research efforts.

Implementation of the action plan will require coordination of stakeholders nationally and regionally, adequate resources, and landscape-level approaches to secure the remaining viable sub-populations and

promote their recovery. The adoption of the first national strategy for conservation of Asian elephants in Cambodia presents a unique opportunity to renew momentum in this regard. Bilingual copies (Khmer & English) of the action plan are available at <https://www.fauna-flora.org/projects/elephant-conservation-cambodia>

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Local community protects an important population of Endangered *Bos javanicus* in Kampong Speu Province

Local community members have been protecting ≈1,000 ha of forest in Thpormg District in Kampong Speu Province without outside support since 2003. This area of forest, known as the Prambei Mom Community Forest, supports ca. 50 individuals of the Endangered banteng *Bos javanicus* (Fig. 1) and is now surrounded by plantations. Due to local forest loss, most of the surviving wildlife in the area has retreated into the few small patches of natural forest that remain in the wider landscape. Wildlife Alliance began working at the site in 2018 when it was requested by the community to help an adult bull banteng that was caught in a snare at the site.

Despite patrols by local community rangers, hunting continues at the site, mostly through the use of snares (Fig. 2). Safari-style hunting parties have also taken place and on one occasion in 2018, a Wildlife Alliance team was present and apprehended one offender. Since this time, safari-style hunting has ceased at the site and numbers of snares within the forest have been greatly reduced, although they are still being removed from neighbouring plantations by the community rangers.

The Forestry Administration, Wildlife Alliance and several Cambodian businesses are now providing support to the community. For instance, construction of an official building at the site was recently funded by Oknha Ly Yung Phat. Camera trap surveys by Wildlife Alliance indicate at least six banteng calves were born within the forest in 2020, and have confirmed the presence of other species including green peafowl *Pavo muticus*, southern red muntjac *Muntiacus muntjak*, golden jackal *Canis aureus*, wild pig *Sus scrofa* and yellow-throated marten *Martes flavigula*. Following the death of a snared

banteng at the site in February 2020, there have been no subsequent incidents of snared wildlife and ongoing camera trapping has yet to show evidence of further injured animals. We plan to obtain an accurate estimate of banteng numbers in the area using camera traps and drones during the 2020/2021 dry season and hope this information will help efforts to ensure the safety of this important population of endangered animals.

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Fig. 1 Herd of banteng in Prambei Mom Community Forest, Kampong Speu Province, 2019 (© J.C. Eames).



Fig. 2 Snared banteng cow in Prambei Mom Community Forest, showing an injured leg (© Try S.).

Short Communication

First record of *Cheironotus parryi* Grey, 1848 (Coleoptera: Eucirinae) in Cambodia

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Within the family Scarabaeidae, the subfamily Eucirinae is regarded as under-studied and its status and phylogenetic placement remain uncertain (Young, 1989; Smith *et al.*, 2006; Šípek *et al.*, 2011). The group comprises 16 species (Young, 1989; Muramoto, 2008) divided among three genera: *Propomacrus* Newman, 1837 (four species), occurring in East Asia (Japan, China and Korea), East Europe and the Middle East (Iran, Syria, Turkey, Cyprus and Balkan Peninsula); *Euchirus* Burmeister & Schaum, 1840 (two species), distributed in the Philippines and Indonesia; *Cheironotus* Hope, 1841 (ten species), found in continental Asia. Species within the latter genus are usually associated with densely forested highlands with mature broadleaved trees, alluvial forests and vegetative growth next to small streams and rivers, all these having an abundance of trees with cavities required for survival of the immature stages and adults (Šípek *et al.*, 2011).

The larvae of *Cheironotus* spp. feed on the decaying wood parts of living trees (Young, 1989). Under laboratory conditions, the eggs hatch after three weeks, and the first instar lasts for about a month. The second instar lasts between 21 and 170 days, while the last instar can last more than 200 days, or even over a year in certain cases (Šípek *et al.*, 2011). The larvae feed mainly on large pieces of decayed wood and make deep burrows into soft wood. Their pupal chamber is realized with wooden debris coagulated around the larvae. The nymphal stage is short (two or three weeks). Adult beetles remain buried inside the substrate and are mainly active from dusk onwards

(Šípek *et al.*, 2011). They feed mostly on ripe fruits or tree sap. Males are active for two or three weeks while females live longer and start laying eggs soon after their emergence. The full life cycle lasts between one and two years (Šípek *et al.*, 2011).

We collected a single large male specimen of *C. parryi* in Phnom Kulen National Park, Svay Leu District, Siem Reap Province (Fig. 1–2). Phnom Kulen National Park is

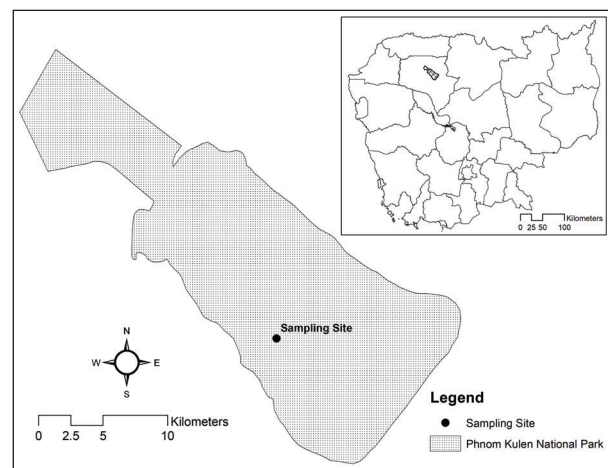


Fig. 1 Location of the first record of *Cheironotus parryi* in Phnom Kulen National Park and Cambodia.

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Fig. 2 Live adult male of *Cheironotus parryi* Grey, 1848 from Phnom Kulen National Park.

located in the Southern Indochina Dry Evergreen Forest Ecoregion (WWF, 2020) and covers 37,350 ha. The park encompasses lowland areas and sandstone hills that climax in two plateaus ca. 450 m above sea level (Phauk *et al.*, 2013). Habitat types present include evergreen and semi-evergreen forests on the hillsides and plateaus, while lowland areas are dominated by dry dipterocarp forest (Neou *et al.*, 2008). Although not the southernmost known location for the species, our record represents the first for Cambodia. The species was collected during an insect inventory conducted by the Cambodian Entomology Initiatives (CEI) team on 8 July 2015 and was accidentally captured with a sweep net around 1900 hrs, close to the ranger station within the park (13°33.870'N, 104°06.447'E). The specimen, measuring 56 mm, matches the description of *C. parryi* by Young (1989) and Ek-Amnuay (2008) and is deposited in the entomology collection of the CEI at the Royal University of Phnom Penh (Accession code: CEI-004121). Its pronotum bears



Fig. 3 A) Dorsal habitus of the male *Cheironotus parryi* Grey, 1848 (CEI-004121). B) Details of male genitalia.

a deep median groove with a greenish reflection and the characteristically-shaped long-apical process on the front tibia (Fig. 3). Prior to our record, the taxon was known to occur Myanmar (Mandalay District), India (Assam State, Himachal Pradesh State, Nagaland State, Sikkim State, Sikkim-Bhutan border, Uttar Pradesh State), Laos (Ban Pak Neun district, Khammouane “plateau”) and Thailand (Ban Chiang Dao, Doi Pui, Nakhon Ratchasima) (Ek-Amnuay, 2008; Young, 1989). It was also recorded in Nam Cat Tien National Park in Vietnam (Bezděk & Spitzer, 1996) and appears to inhabit lowland seasonal forests including *Lagerstroemia* tree species (Spitzer *et al.*, 1991).

While the occurrence of Euchiridae is often considered as a bio-indicator of pristine, old and well-established tropical forests (Young, 1989; Šípek *et al.*, 2011), the broader situation in Cambodia presents a concern in possessing one of the fastest deforestation rates in the world. Between 1965 and 2016 for instance, the country

reportedly lost almost one-quarter of its forest cover (Forest Administration, 2010; WWF, 2013). As such, potentially suitable habitats for *C. parryi* could disappear in the near future. From the conservation point of view, further investigations should be conducted in potential habitats for this rare species in the northwest and eastern part of the country.

Material examined: CEI-004121, 1 ♂ “Cambodia, Siem Reap Province, Phnom Kulen National Park; 13°33.870'N, 104°06.447'E (WGS84); 08.vii.2015; sweep net; Phauk, Kheam, Chhum, Sour, Ly, Heang, Lorn, Hok.”

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Stump size and resprouting ability: responses to selective cutting in a sandy dry dipterocarp forest, central Cambodia

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មូលនិយសរង្វេប

ព័ត៌មានមូលដ្ឋានគ្រឹះនៃការដុះលូតលាស់ព្រៃឈើឡើងវិញគឺមានសារៈសំខាន់ណាស់ក្នុងការផ្តល់នូវគោលការណ៍ណែនាំសម្រាប់ការប្រើប្រាស់ព្រៃឈើប្រកបដោយនិរន្តរភាព។ នៅក្នុងលក្ខខណ្ឌនេះ លទ្ធភាពនៃការដុះពន្លកឡើងវិញរបស់ដើមឈើគឺជាលក្ខណៈសំខាន់នៃប្រភេទដាក់លាក់ណាមួយក្នុងការបន្តទៅនឹងធម្មជាតិ និងផលវិបាកពីសកម្មភាពមនុស្ស។ នៅក្នុងការសិក្សាស្រាវជ្រាវនេះ យើងផ្តោតទៅលើលទ្ធភាពដុះពន្លកឡើងវិញរបស់ប្រភេទដើមឈើមួយប្រភេទដែលត្រូវបានគេកាប់ជាទូទៅគឺប្រភេទដើមឈើត្បែង (*Dipterocarpus obtusifolius*) នៅក្នុងអំបូរ Dipterocarpaceae ស្ថិតនៅភូមិភាគកណ្តាលនៃប្រទេសកម្ពុជា។ គោលបំណងនៃការសិក្សាស្រាវជ្រាវរបស់យើងគឺធ្វើការពិនិត្យលើការប្រើប្រាស់ព្រៃឈើប្រកបដោយនិរន្តរភាពដែលស្ថិតនៅទីតាំងព្រៃឈើជ្រុះស្លឹក តំបន់ដីខ្សាច់ (sandy dry dipterocarp) ដែលភាគច្រើនកាន់កាប់ និងសំបូរទៅដោយប្រភេទឈើត្បែង។ ក្នុងគោលបំណងនេះ យើងបានត្រួតពិនិត្យលើទំហំគល់ដើមឈើ ដែលបង្ហាញអំពីលទ្ធភាពដុះពន្លកឡើងវិញរបស់ប្រភេទឈើនៅក្នុងទីតាំងសិក្សា។ ទំហំគល់ឈើត្រូវបានប្រើប្រាស់សម្រាប់ព្យាករណ៍អំពីវត្តមាន ឬអវត្តមាននៃការដុះពន្លកឡើងវិញរបស់ប្រភេទឈើ ហើយវាកើតឡើងច្រើនសម្រាប់ដើមឈើដែលមានទំហំដើមតូច។ ទំហំដើមឈើអតិបរមាត្រូវបានគេប្រើប្រាស់ធ្វើជាអុស(មានអង្កត់ផ្ចិត ៣០ស.ម) គឺមានទំហំធំពេកក្នុងការរំពឹងឱ្យដុះពន្លកឡើងវិញ។ យើងក៏បានវិភាគអំពីប្រជាសាស្ត្ររបស់ដើមឈើដែលមានអង្កត់ផ្ចិត(នៅកម្ពស់ដើមទ្រូង) ធំជាង ៥.០ ស.ម ដោយយោងទៅតាមការរាប់ដើមឈើក្នុងរយៈពេល១៦ឆ្នាំ(២០០៣-២០១៩) នៅតំបន់សិក្សានេះ។ អត្រាដំណុះកូនឈើឡើងវិញមានកម្រិតខ្ពស់ក្នុងអំឡុងឆ្នាំ២០១៤-២០១៩ ដែលនេះហាក់បីដូចជាបណ្តាលមកពីស្តុកបុប្ផនៃក្រុមដើមឈើមិនទាន់ពេញវ័យ(juvenile trees) មានចំនួនច្រើន តែមិនមែនមកពីការដុះពន្លកកូនឈើឡើងវិញបន្ទាប់ពីព្រឹត្តិការណ៍កាប់បំផ្លាញឈើនោះទេ។ លើសពីនេះ នាពេលអនាគតឆាប់ៗនេះ យើងនឹងមិនមានការរំពឹងថានឹងអាចប្រមូលគ្រាប់ពូជឈើក្នុងបរិមាណច្រើនបានទេ ដោយសារដើមត្បែងធំៗដែលអាចផលិតគ្រាប់ពូជបាន សឹងតែទាំងអស់ត្រូវបានគេកាប់ដើមធ្វើអុសដុត។ លទ្ធផលនៃការសិក្សារបស់យើងអាចបញ្ជាក់ថា ការដកហូតយកឈើធ្វើអុសដុតអាចជាកត្តាគំរាមកំហែងខ្ពស់បំផុតក្នុងការប្រើប្រាស់ព្រៃឈើជ្រុះស្លឹក តំបន់ដីខ្សាច់ ដែលស្ថិតក្នុងតំបន់សិក្សានេះ។

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Abstract

Basic information on forest regeneration is necessary to provide guidelines for sustainable forest use. In this context, the resprouting ability of trees is an important species-specific character for adaptation to natural and anthropogenic disturbances. In this study, we focused on resprouting ability of a commonly logged tree species, *Dipterocarpus obtusifolius* (Dipterocarpaceae) in central Cambodia. Our aim was to examine the sustainability of forest use in a sandy dry dipterocarp forest i.e., an open forest community dominated by *D. obtusifolius*. For this purpose, we determined the stump sizes showing resprouting ability for species in the study forest. Stump size significantly predicted the presence/absence of resprouting and this was much more common in smaller stems. The maximum tree size used for fuel wood (30 cm in diameter) was too large to expect resprouting. We also analysed the demography of trees with diameters at breast height > 5 cm based on a 16-year chronological tree census (2003–2019) in the study forest. The relatively high recruitment rate during 2014–2019 was likely achieved by a stock of juvenile trees and not by resprouting caused by tree-cutting events. Moreover, a large supply of seeds cannot be expected in the near future because almost all reproductive *D. obtusifolius* trees have been logged for firewood. Our results suggest that fuel wood extraction may be the greatest threat to sustainable use of sandy dry dipterocarp forests in our study region.

Keywords Conservation, *Dipterocarpus obtusifolius*, forest degradation, fuel wood, resprouting ability, selective cutting, sustainable management.

Introduction

The resprouting ability of trees is an important species-specific character for adaptation to natural disturbances (Bellingham & Sparrow, 2000). Various studies in tropical forests have examined this ability in response to hurricanes (Bellingham *et al.*, 1994; Zimmerman *et al.*, 1994; Jimenez-Rodríguez *et al.*, 2018), fire (Paciorek *et al.*, 2000; Mlambo & Mapaure, 2006; Lawes *et al.*, 2011; Nguyen *et al.*, 2019), and slash-and-burn agriculture (Miller & Kauffman, 1998). From the perspective of conservation, information on resprouting ability is fundamental to practical applications.

Human population growth is currently causing deforestation pressure in Cambodia. The country has attracted the attention of the REDD+ programme (i.e., reducing emissions from deforestation and forest degradation and the role of conservation, sustainable management of forests and enhancement of forest carbon stocks in developing countries) because it is regarded as a “hot spot” for deforestation and forest degradation (FAO, 2010; FAO, 2020). As a result, quantitative studies have been conducted on the sustainability of forest use in Cambodia e.g., on wood fuel consumption (Top *et al.*, 2004a) and the gain-loss approach quantifying carbon gains from annual increases and losses in biomass caused by natural and anthropogenic processes (Sasaki, 2006; Sasaki *et al.*, 2013, 2016; Kiyono *et al.*, 2017).

Basic information on forest stands and their regeneration is necessary to provide guidelines for sustainable forest use in Cambodia (Ito *et al.*, 2016). Such information must be presented for individual forest types,

appropriately stratified by degradation processes. In this study, we focused on resprouting ability of a commonly logged tree species, *Dipterocarpus obtusifolius* Teijsm. ex Miq. (Dipterocarpaceae). Our aim was to examine the sustainability of forest use in an open forest community dominated by *D. obtusifolius*, referred to as sandy dry dipterocarp forest. For this purpose, we determined the stump size showing resprouting ability for tree species in a sandy dry dipterocarp forest in central Cambodia.

Methods

Our study was conducted in Kampong Thom Province (12.8°N, 105.5°E; elevation: 70 m). The climate of the area is seasonally tropical, and the months from November through April are dry. Mean annual temperature is 27 °C and annual rainfall (mean ± SD) is 1542 ± 248 mm (2000–2010; NIS, 2012). Our study sites were located on extensive quaternary sedimentary rock. The soils present are classified as Acrisols, but have albic and arenic features that suggest a closer relationship with Arenosols (Toriyama *et al.*, 2007a).

Our study forest was a sandy dry dipterocarp forest (Ito *et al.*, 2017). Sandy dry dipterocarp forests are characterised by a strong dominance of *Dipterocarpus obtusifolius* (Dipterocarpaceae; Khmer: “Tbeng”), which favour sandy or gravelly soils or laterites (Smitinand *et al.*, 1980). This forest type has been variously referred to as “forêt claire à *Dipterocarpus obtusifolius*” (Vidal, 1960), *D. obtusifolius* on sand or grey soil (“à *D. obtusifolius*, sur sable ou terre grise”; Rollet, 1972), *D. obtusifolius* commu-

nity (Baltzer *et al.*, 2001), and *D. obtusifolius* stand type (Hiramatsu *et al.*, 2007). Sandy dry dipterocarp forests are most characteristic of areas east of the Mekong River in Cambodia in sites with thin sandy soils over laterites (Rundel, 1999). In Kampong Thom Province, northeast of Tonle Sap Lake, they often occur as scattered forest patches among evergreen forests in sites with deep sandy soils that are subject to seasonal flooding (Hiramatsu *et al.*, 2007).

The Cambodian Forestry Administration has classified national forest cover into four major types: evergreen, semi-evergreen, deciduous, plus a mixture of other forms such as forest re-growth, inundated forests, stunted forests, mangrove forests and forest plantations (Forestry Administration, 2011). Deciduous forests comprising dry mixed deciduous forests and dry dipterocarp forests are predominant in Cambodia and account for 24.7% of its land area (Forestry Administration, 2011). Dry dipterocarp forests are characterized by a dominance by a small number of deciduous species of Dipterocarpaceae, such as *D. intricatus*, *D. tuberculatus*, *Shorea obtusa* and *S. siamensis* (Rundel, 1999). They have also been subdivided into four forms, each with different combinations of soil type and dominant dipterocarp species (Rollet, 1972). Although our sandy dry dipterocarp study forest is classified as one form of dry dipterocarp forest among deciduous forests, it has not shown clear deciduousness, only displaying irregular and incomplete leaf shedding of component tree species (Ito *et al.*, 2007). For example, the leaf longevity of *D. obtusifolius* in the forest often exceeds one year (E. Ito & Tith B., unpublished data) and never results in a leafless crown.

Sandy dry dipterocarp forests often exhibit low species richness (Hiramatsu *et al.*, 2007; Ito *et al.*, 2017), annual growth rates and carbon increments (Ito *et al.*, 2017). They have open structures with 40–70% canopy cover (Rundel, 1999; Hiramatsu *et al.*, 2007; Forestry Administration, 2011), are associated with ground fires (Rundel, 1999; Hiramatsu *et al.*, 2007), have nutrient-poor sandy soils (Rollet, 1972; Toriyama *et al.*, 2007a,b) and experience seasonal flooding and drought conditions (Rollet, 1972; Rundel, 1999; Baltzer *et al.*, 2001; Araki *et al.*, 2007). *Dipterocarpus obtusifolius* predominates and is an ecologically plastic and stress tolerant species (Rundel, 1999). It is also fire resistant in having the ability to resprout after fire like other deciduous dipterocarps (*D. tuberculatus*, *S. obtusa* and *S. siamensis*) (Nguyen *et al.*, 2019).

We established a permanent sample plot (30 × 80 m) to investigate stand structure and dynamics in sandy dry dipterocarp forest at the study site in 2003 (Hiramatsu *et al.*, 2007; Ito *et al.*, 2017). Field surveys were conducted

in 2003, 2008, 2009, 2010, 2011, and 2012 (pre-logging) and in 2014 and 2019 (post-logging) to investigate tree growth and demography. Based on a 2012 census undertaken before illegal logging occurred at the site, the tree density and basal area of stems with a diameter at breast height (DBH) ≥ 5 cm were 408 stems ha⁻¹ and 12.3 m² ha⁻¹, respectively (Ito *et al.*, 2017). The plot had one dominant dipterocarp species, *D. obtusifolius* (accounting for 50% of stand basal area and 60% of stand tree number), which was associated with *Gluta laccifera* (Pierre) Ding Hou (Anacardiaceae, 35% and 6%, respectively) (Hiramatsu *et al.*, 2007). The forest lacked auxiliary deciduous species such as *D. tuberculatus*, *S. obtusa*, *S. siamensis*, *Pterocarpus macrocarpus* (Fabaceae) and *Xylia xylocarpa* (Fabaceae), which usually occur together in dry dipterocarp or deciduous dipterocarp forests (Royal Forest Department, 1962; Hiramatsu *et al.*, 2007; Tani *et al.*, 2007; Pin *et al.*, 2013). Edaphic limitations are potential factors limiting species richness (Hiramatsu *et al.*, 2007) and the ground surface was waterlogged several times in the middle of the rainy season in 2005 (August through September, Araki *et al.*, 2007). The ground vegetation includes *Xyris complanata* R.Br. (Xyridaceae) and insectivorous plants (*Drosera* sp., Droseraceae and *Nepenthes* sp., Nepenthaceae), which suggest low-nutrient edaphic conditions (Hiramatsu *et al.*, 2007).

We investigated the resprouting ability of individual trees that had suffered illegal cutting in and around the permanent sample plot. To this end, the heights and diameters of the remaining stumps were measured in December 2019. Diameter was measured at the upper surface of the stumps. We recorded the presence/absence of resprouting stems on the stumps, the number of resprouting stems, and the place where each resprouting stem emerged (basal sprouting from the ground around the stump or sprouting from the upper side of the stump). Individuals of *D. obtusifolius* whose main stems were broken by the toppling of other trees were also investigated.

A nominal logistic regression model was used to generate prediction equations for the relationship between stump size and presence/absence of resprouting using a dataset of all tree species and a dataset for *D. obtusifolius* only. One-way ANOVA was used to test for differences in the height or diameter of resprouting on logged stumps for *D. obtusifolius*. Tukey HSD tests were used to distinguish differences in the place of resprouting. Statistical analysis was conducted using *JMP* statistical software vers. 10.0 (SAS Institute Inc., Cary, NC, USA). The threshold for significance applied in all tests was $P < 0.05$.

Results

During the chronological tree census (2003–2019), a total of 47 tree individuals were cut or had their stems broken by anthropogenic activities (Table 1). Illegal tree cutting within the plot progressed in stages, as follows.

During 2003–2012, there were no cutting events in our study plot. The site sometimes experienced fire, but no tree (>5 cm DBH) died as a result. Logging occurred during the 2012–2014 census. Stumps were found on 8 February 2014 and we estimate that these were cut between December 2013 and January 2014 due to the freshness of the logged stumps and because logging in the study area usually takes place during the dry season. Relatively large individuals of *D. obtusifolius* and *G. laccifera* (>38 cm DBH) were cut, probably for their timber (Table 1). Small trunks (<26 cm DBH) were also cut and discarded on site (Table 1). These may have been felled for fuel or used to test chainsaw performance. Further details on tree cutting during this period are reported by Ito *et al.* (2017). During the 2014–2019 census, the remaining large individuals of *D. obtusifolius* and *G. laccifera* were completely cut (Table 1). Most small trees were also cut during this period. On 28 February 2019, we found that the smaller trees had been gathered in one location at the site and estimate that these were cut between December 2018 and February 2019.

We investigated the resprouting ability of 47 trees. These comprised 45 trees that had been logged and two *D. obtusifolius* with broken stems (Table 1). Most of the investigated trees were *D. obtusifolius* ($n=36$) and the

remaining species are shown in Table 1. Resprouting was observed in 15 trees in total (Table 1), most of which were also *D. obtusifolius* ($n=13$). The remaining two individuals were *Calophyllum calaba* var. *bracteatum* (Calophyllaceae) and *Parinari anamensis* (Chrysobalanaceae). Resprouting occurred in trees ranging from 4.8 to 26.9 cm in stump diameter (Fig. 1). Stump size significantly predicted the presence/absence of resprouting in all trees investigated ($P=0.0006$) and *D. obtusifolius* ($P=0.0228$). Among 26 individuals of *D. obtusifolius* that had a stump diameter less than 26.9 cm, 42.3% showed resprouting ability. Nominal logistic regression models indicated that >50% of the *D. obtusifolius* stumps with a diameter less than 16.1 cm also retained resprouting ability (Fig. 2).

The median number of resprouts per tree was two, and there was no clear trend for smaller stumps to generate a greater number of sprouts. The highest number of sprouts on a single stump was 24, followed by 13. Dominant–inferior relationships were clear among the resprouts however, with only one or two resprouts being vigorous. Basal sprouting was found in eight trees, and stem sprouting was found in four trees. One exceptional individual had a single vigorous resprout which occurred on a stem and a total of 12 small resprouts which occurred on the base.

There was a significant difference in the height of logged stumps with basal sprouting and those with stem sprouting for *D. obtusifolius* (ANOVA, $P=0.0043$, Fig. 3), but no significant difference was found in stump diameter between the two. The addition of the explanatory

Table 1 Selective tree cutting during 2012–2014 and 2014–2019 in the permanent sample plot in central Cambodia. Numerical values in square brackets represent resprouting trees. Stem diameter (cm) is given as mean and range in parentheses.

Tree species (Family)	Cutting period				Total <i>n</i>
	2012–2014		2014–2019		
	<i>n</i>	Stem diameter	<i>n</i>	Stem diameter	
<i>Dipterocarpus obtusifolius</i> Teijsm. ex Miq. (Dipterocarpaceae)	6 [3]	23.8 (11.4–42.3) [16.5 (11.4–26.0)]	30 [10]	25.4 (7.6–55.5) [20.9 (4.8–26.9)]	36 [13]
<i>Gluta laccifera</i> (Pierre) Ding Hou (Anacardiaceae)	3 [0]	57.4 (54.3–61.8) [—]	2 [0]	65.1 (63.3–66.8) [—]	5 [0]
<i>Calophyllum calaba</i> L. var. <i>bracteatum</i> (Wight) P.F. Stevens (Calophyllaceae)	1 [1]	9.5 [9.5]	0 [0]	— [—]	1 [1]
<i>Parinari anamensis</i> Hance (Chrysobalanaceae)	1 [1]	4.9 [4.9]	3 [0]	26.7 (7.2–46.0) [—]	4 [1]
<i>Syzygium oblatum</i> (Roxb.) Wall. ex A.M. Cowan & Cowan (Myrtaceae)	0 [0]	— [—]	1 [0]	31.2 [—]	1 [0]
Total	11 [5]		36 [10]		47 [15]

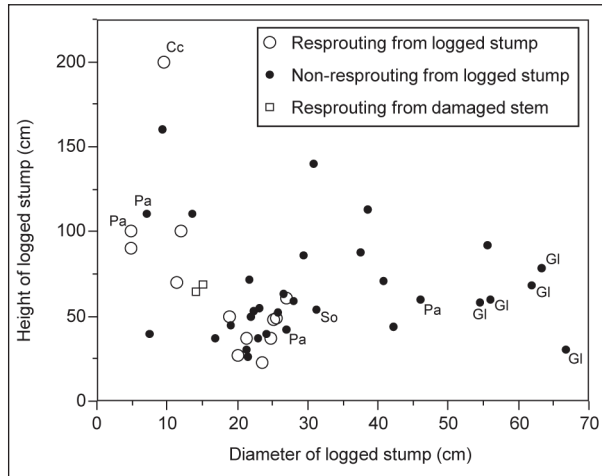


Fig. 1 Relationship between diameter and height of logged stumps. Abbreviations indicate the presence/absence of resprouting stems. Species other than *Dipterocarpus obtusifolius* are GI=*Gluta laccifera*, C=*Calophyllum calaba* var. *bracteatum*, Pa=*Parinari anamensis*, So=*Syzygium oblatum*.

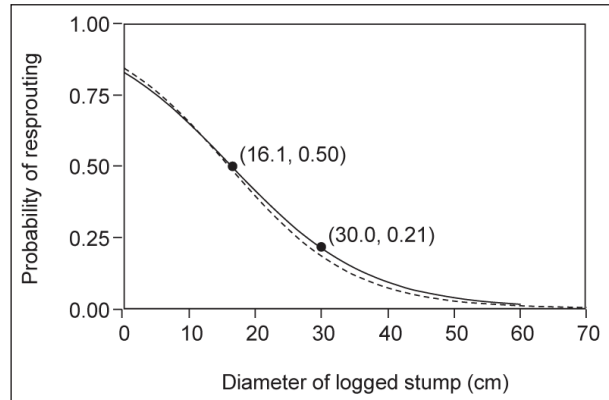


Fig. 2 Estimated probability of resprouting according to the stump diameter using nominal logistic regression. Dashed and straight lines indicate estimates for all species and *D. obtusifolius*, respectively.

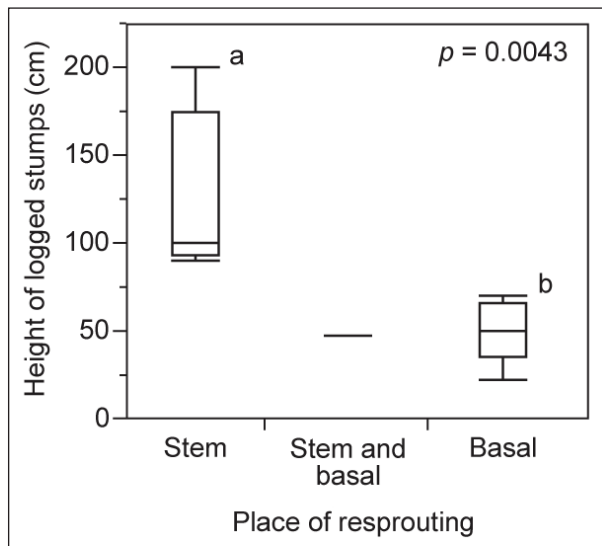


Fig. 3 Height of logged stumps by location of resprouting stem of *Dipterocarpus obtusifolius*. Data are presented as box-and-whisker plots (median, 25% and 75% quartiles, range). Columns labelled with different letters differ significantly.

factors of stump height and interaction of stump size and height did not significantly improve the nominal logistic regression models predicting the presence/absence of resprouting.

The distribution of diameters for *D. obtusifolius* shifted markedly during our censuses (Fig. 4). What began as a relatively flat distribution (Figs. 4A, 4B) changed to an

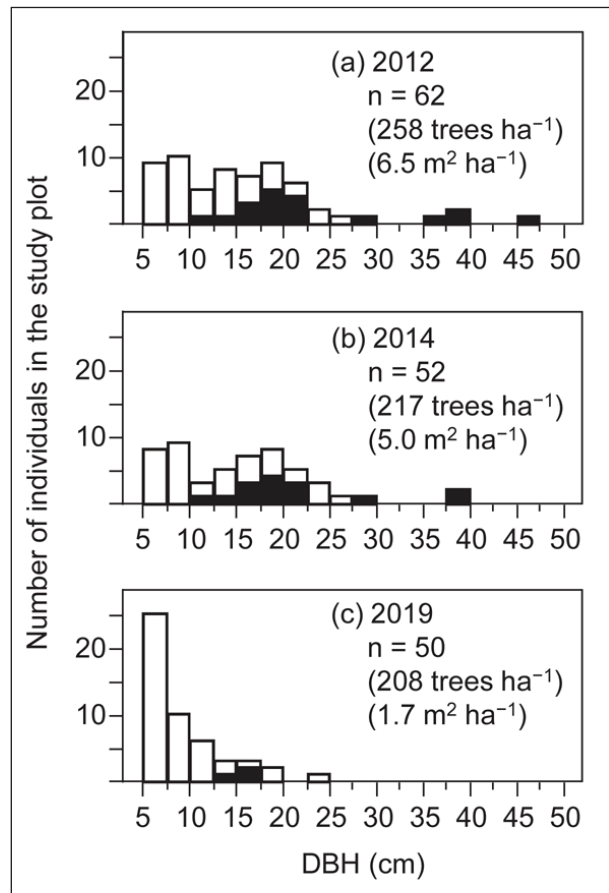


Fig. 4 Frequency distribution of DBH of *Dipterocarpus obtusifolius* in study plot: A) 2012 pre-logging census; B) 2014 post-timber logging census; C) 2019 post-firewood logging census. Black and white columns indicate individuals with confirmed and unconfirmed flowering and/or fruiting, respectively, prior to the 2009 census.

L-shaped distribution (Fig. 4c). Tree density and basal area also decreased greatly, from 258 trees ha⁻¹ and 6.5 m²ha⁻¹ during the 2012 census to 208 trees ha⁻¹ and 1.7 m² ha⁻¹ during the 2019 census (Fig. 4).

Recruitment of *D. obtusifolius* occurred during the 2014–2019 censuses at a rate of 29 trees per plot. This was clearly greater than the recruitment of six trees per plot in the 11 years covered by the 2003–2014 censuses. Of the 29 recruitments observed in 2014–2019, only five were derived from resprouting.

Discussion

Following the selective logging recorded during our 2014 census (Ito *et al.*, 2017), considerable cutting of small-diameter trees occurred during the 2014–2019 censuses (Table 1). The former was possibly for timber, whereas the latter was probably for firewood. *Dipterocarpus obtusifolius* is preferred for fuel wood (Top *et al.*, 2004b; San *et al.*, 2012) and was targeted among the smaller trees. These were also gathered in one location, presumably to dry and lighten the wood prior to transport.

Consistent with previous studies in continental Southeast Asia (Baker *et al.*, 2009) and a meta-analysis of literature (Vesk, 2006), we found that resprouting was much more common in smaller stems (Figs. 1,2). Although sandy dry dipterocarp forest is often affected by fires (Rundel, 1999; Hiramatsu *et al.*, 2007), the maximum diameter of resprouting stumps in our study was 26.9 cm (Fig. 1), whereas Baker *et al.* (2009) documented resprouting after fire in trees whose diameters ranged from 1 to 50 cm. Top *et al.* (2004b) reported that the maximum diameter of trees used for fuel wood in our study area was 30 cm and our data suggests that the probability of *D. obtusifolius* resprouting at this diameter is 21% (Fig. 2). However, caution is required here because we were not able to directly confirm resprouting for stumps with diameters greater than 26.9 cm (Fig. 1). In addition, the height of logged stumps did not influence the presence/absence of resprouting (Fig. 3). This suggests that resprouting ability may not be enhanced by managing felling heights.

Sist *et al.* (2003) recommended a procedure for setting cutting limits based on tree DBH during the reproduction stage of target species. In the case of *D. obtusifolius*, DBH at 50% and 90% of tree reproduction have been estimated as 18.8 cm (95% CI=16.2–23.9 cm) and 27.1 cm (95% CI=22.7–42.5 cm) respectively (Ito *et al.*, 2016). Official guidelines state a cutting limit of 45 cm DBH for *D. obtusifolius* (MAFF, 2005). If logging at our study site adhered to these guidelines, a reproductive tree popula-

tion could have persisted in the forest. However, given that trees cut during the 2014–2019 censuses averaged 21.5 cm DBH, it is likely that the size criterion for fuel-wood cutting was too low to meet the recommendation of Sist *et al.* (2003). For instance, substantial densities of reproductive trees were recorded during the 2009 census in our study plots (83 stems ha⁻¹; Ito *et al.*, 2017), but almost all reproductive trees were subsequently logged by 2019 (Fig. 4c) and the remainder were relatively small (ca. 16 cm in diameter; see also Fig. 4c). As such, a large supply of seeds cannot be expected in the near future and if further cutting were to occur before the current population of young trees begin reproduction, regeneration from seedlings would be very difficult.

A relatively high recruitment rate was observed during 2014–2019. This was likely due to a stock of juvenile trees with diameters less than 5 cm rather than resprouting caused by tree-cutting. Only 1–2 stems typically sprout from a logged stump and some small-diameter trees did not show resprouting ability (Fig. 1, 2). As a consequence, it is likely too optimistic to expect that resprouting could compensate for clear-cutting and thereby regenerate the forest. Top *et al.* (2004b) suggested that agricultural expansion may be the main cause of deforestation in Kampong Thom Province, rather than fuel wood extraction. However, the scattered sandy dry dipterocarp forests in the province are not a high priority for agricultural development because they are established on seasonally flooded and low-nutrient lands (Toriyama *et al.*, 2007a). As such, fuel wood extraction may pose the largest threat to sustainable use of sandy dry dipterocarp forests in the province.

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Release of rescued Malayan sun bears *Helarctos malayanus* in the Southern Cardamom Mountains, Cambodia

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មូលនិយមសង្ខេប

កម្មវិធីដោះលែងសត្វព្រៃឲ្យត្រឡប់ចូលព្រៃវិញគឺជាការចាំបាច់ ព្រោះចំនួនសត្វព្រៃដែលរឹបអូសបានពីការជួញដូរ និងការចិញ្ចឹមដោយខុសច្បាប់កាន់តែមានចំនួនច្រើនឡើងៗ ដើម្បីគាំទ្រដល់កម្មវិធីនេះ យើងបានបរិយាយពីបទពិសោធន៍ដែលទទួលបានពីការដោះលែងសត្វខ្លាឃ្មុំតូច (*Helarctos malayanus*) នៅតំបន់ជួរភ្នំក្រវាញ ភាគខាងត្បូងនៃប្រទេសកម្ពុជា។ បន្ទាប់ពីការស្តារនីតិសម្បទា និងការបន្ស៊ាំ សត្វខ្លាឃ្មុំតូចចំនួន៣ក្បាលត្រូវបានដោះលែងនៅក្នុងឱកាស២ផ្សេងៗគ្នា។ មុនពេលដោះលែង ខ្លាឃ្មុំទាំងនោះត្រូវបានគេចិញ្ចឹមតាំងពីវាតូចៗ។ ការតាមដានដោយឧបករណ៍ GPS ដែលបំពាក់នៅនឹងក បានបង្ហាញថា ខ្លាឃ្មុំទាំង៣មានសមត្ថភាពរស់ដោយខ្លួនឯងបាន និងចេះគេចពីមនុស្ស បន្ទាប់ពីលែងឲ្យចូលទៅក្នុងព្រៃវិញ។ ទោះជាយ៉ាងណា ខ្លាឃ្មុំទាំង៣បានជួបនឹងបញ្ហាគឺ ២ក្បាលត្រូវបានជាប់អន្ទាក់ម្តងទៀត និង១ក្បាលទៀតត្រូវបានសម្លាប់ដោយសត្វព្រៃ។ លទ្ធផលរបស់យើងបង្ហាញថា ខ្លាឃ្មុំតូចអាចទទួលបានជំនាញសម្រាប់ការរស់នៅ ហើយការចិញ្ចឹមក្នុងទ្រុងមិនមែនជាឧបសគ្គដល់ភាពជោគជ័យនៃការដោះលែងឲ្យចូលព្រៃវិញទេ ប្រសិនបើសត្វទាំងនោះត្រូវបានគេបន្ស៊ាំនៅក្នុងព្រៃដែលមានទំហំធំ ដែលជំរុញឲ្យវាកើតមានអាកប្បកិរិយាធម្មជាតិ និងការកាត់បន្ថយការឃើញមនុស្ស មុននឹងធ្វើការដោះលែងឲ្យវាចូលព្រៃវិញទេ។ តាមបទពិសោធន៍ ការអភិវឌ្ឍកម្មវិធីដោះលែង យើងគួរពិចារណាទៅលើកត្តាបរិបូរណ៍ និងវត្តមានប្រភេទខ្លាឃ្មុំតូចដែលមាននៅក្នុងទីតាំងដោះលែង។

Abstract

Well-conceived programmes for releasing wildlife are essential due to the growing numbers of animals confiscated from the illegal wildlife and pet trade. To support the development of such programmes, we describe experiences gained from the release of Malayan sun bears *Helarctos malayanus* in the Southern Cardamom Mountains of southwest Cambodia. Following rehabilitation and acclimatisation, three sun bears were released on two different occasions. Prior to their release, the bears had been in captivity since infancy. Post-release monitoring with GPS collars showed that all three bears were capable of sustaining themselves unassisted and avoided human interactions after their release. However, all three encountered problems which later resulted in their recapture or death: two were caught in snares and one was killed by a wild resident. Our results demonstrate that sun bears can acquire the skills necessary for survival and that captivity need not be a barrier to successful release if the animals are provided with large forested enclosures that encourage ‘natural’ behaviours and human contact is minimised prior to release. Our experiences also emphasize the importance of considering hunting pressure and presence of conspecifics at release sites when developing release programmes.

Keywords Acclimatisation, conspecifics, monitoring, rehabilitation, snare, soft-release, sun bear.

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Introduction

The rehabilitation and release of captive animals into their historical ranges has long been considered a conservation strategy for zoos to repopulate 'silent' forests or bolster wild populations of scarce species (Kleiman, 1989; Wilson & Stanley Price, 1991; Beck *et al.*, 1994; IUCN/SCC, 2013). For a release to have a conservation and animal welfare benefit, proper protocols must be conducted, including site selection, health checks, behavioural assessment and selection of appropriate candidates, rehabilitation and acclimatization at the release site, and supplementary feeding and monitoring post-release for as long as necessary (IUCN/SCC, 2013). Release programmes therefore require long-term management and financial commitment and must never be conducted as a means of discarding animals considered surplus to requirements, which will compromise good husbandry practises and risk undermining the conservation goal of supporting wild populations (Kleiman, 1989; Huber, 2010). Documentation of the outcomes of reintroduction programmes is also crucial to develop species-specific reintroduction guidelines, particularly for species with a long history of failed release attempts (Wilson & Stanley Price, 1991; van Manen & Pelton, 1997; Clark *et al.*, 2002; Clark, 2009; Crudge *et al.*, 2019).

Species in the Ursidae present a challenge for release efforts due to their extensive home ranges, ability to adapt to captivity and humans, and the volume of survival skills cubs learn from their mothers during their early development (Fredriksson, 2005; van Dijk, 2005). Bears that have been hand-reared or spent prolonged periods in captivity are more likely to be unafraid of humans, lack necessary survival skills and become nuisance animals once released (Alt & Beecham, 1984; Stiver *et al.*, 1997; Fredriksson, 2005; Clark, 2009; Huber, 2010). Conservation translocations of North American and European bear species have been extensively reported, with reintroductions in Europe occurring as early as the 1930s (*Ursus americanus*: Alt & Beecham, 1984; Stiver *et al.*, 1997; Eastridge & Clark, 2001; Clark, 2009; *U. arctos*: Buchalczyk, 1977; Jonkel *et al.*, 1980; Clark *et al.*, 2002; Preatoni *et al.*, 2005; Huber, 2010). Thus far however, reports on the outcomes of Malayan sun bear *Helarctos malayanus* releases have been limited (Fredriksson, 2005; Abidin *et al.*, 2018).

Malayan sun bears are the smallest member of the Ursidae, weighing between 30 to 65 kg. The species is predominantly terrestrial, but climbs well and is arrhythmic: active both day and night (Augeri, 2005). It is also omnivorous, foraging for a wide range of different foods including fruit, roots, insects and other forms of animal protein. Reportedly the least studied of the bear

species (Servheen, 1999), sun bears have been recorded in lowland tropical primary and secondary dipterocarp forests throughout Southeast Asia (Wong *et al.*, 2004; Nazeri *et al.*, 2014; Abidin *et al.*, 2018), although population estimates are lacking throughout their range. The species is considered Vulnerable (Scotson *et al.*, 2017) due to declining numbers as a result of habitat loss and hunting for use in the pet trade, food delicacies and traditional medicines (Mills & Servheen, 1994; Scotson *et al.*, 2017).

In Cambodia, snares are the most common hunting method. Made from easily sourced and affordable materials, snares are indiscriminate and extremely damaging to terrestrial wildlife, including sun bears (O'Kelly *et al.*, 2018; Heinrich *et al.*, 2020). Sun bears are also targeted due to their value on the black market, because hunters can sell a single animal to wildlife traders for 2,500 USD (Wildlife Alliance, unpublished data, Chi Phat Commune, Koh Kong Province). Although national legislation exists to protect wildlife in Cambodia from such exploitation, these laws are poorly enforced in most areas (Gray *et al.*, 2017).

The Wildlife Rapid Rescue Team (WRRT) was established in 2001 to combat the illegal wildlife trade in Cambodia. The WRRT is an official government task force which comprises seven Military Police and four Forestry Administration officials and is supported technically and financially by the non-governmental organisation Wildlife Alliance (Gray *et al.*, 2017). Between 2001 and 2019, the WRRT confiscated 111 sun bears from illegal trafficking or pet trade (WRRT, unpublished data) and transferred these to the Phnom Tamao Wildlife Rescue Centre (PTWRC) in Takeo Province, Cambodia. Approximately 140 rescued Malayan sun bears and Asiatic black bears *Ursus thibetanus* are managed at PTWRC by an Australian charity, Free the Bears. Because demand for bears and their parts in the illegal wildlife trade continues, housing a growing number of confiscated sun bears that will require lifetime care at such centres is neither practical nor a conservation goal. As such, well-conceived and planned release programmes using confiscated animals are essential and will become even more critical in the future (Griffith *et al.*, 1989).

The purpose of the present paper is to support the development of such programmes. To this end, we describe experiences gained from the release of three Malayan sun bears in accordance with the IUCN Reintroduction Guidelines (IUCN/SCC, 2013), including the soft-release protocols employed, challenges faced, lessons learned, and actions undertaken to mitigate possible issues in future releases.

Methods

We undertook the release of three sun bears in south-west Cambodia on two separate occasions, releasing two female bears in 2012 and a single male in 2019. All three bears were rescued from the illegal wildlife trade as cubs and spent at least four years in captivity prior to their release, including time in the acclimatization enclosure at the release site.

Wildlife release station

Our wildlife release station (11°22'12.2"N, 103°30'26.3"E) is situated on the edge of the Southern Cardamom Mountains, in Tatai Wildlife Sanctuary (ca. 1,443 km²), Koh Kong Province. This location was chosen as it balanced the need for remoteness with the need for accessibility to provide supplies for the camp and animals and was established in 2019 as a permanent site for releasing native species rescued from the illegal wildlife trade or born in captivity at PTWRC. The station is situated in an area of predominately evergreen forest and is surrounded by hills less than 100 m in elevation. The nearest human settlement to the release station is Chi Phat village (Chi Pat Commune), approximately 8 km to the southeast, whereas the nearest military patrol station, Stung Proat, is located 7 km to the south.

Preliminary field surveys in 2008 indicated that the area contained sufficient resources for sun bears and limited competition from conspecifics, because local wildlife populations had been severely reduced by rampant illegal logging and hunting. This conclusion was based on villager reports, direct observations and checklists of species that were obtained through camera-trapping at the site (Reimer & Walter, 2013). In response, a community-based eco-tourism project was initiated in Chi Phat Commune in 2007 to provide alternative livelihoods for hunters and illegal loggers. The activities of this project and patrols undertaken by seven military police stations reduced wildlife hunting in the area considerably and as a result of these changes, the site was regarded as suitable for our first release of bears in 2012.

Hunting pressure in the forests surrounding the release station was further reduced by the creation of a community-based anti-poaching unit in 2013. The unit comprised 11 local community members who were tasked with patrolling the forest, removing snares, interrupting illegal activities and recording wildlife movements. This undoubtedly improved the safety of the area for wildlife, which was also suggested by subsequent camera trap records of previously unrecorded species including clouded leopard *Neofelis nebulosa* and dhole *Cuon alpinus*. As a result of these developments and

because sun bears were not encountered in the area, it was deemed appropriate for our second release in 2019.

Study animals

The sun bears selected for our first release were two females, named Sloat and Sopheap. These were confiscated as cubs (approximately four months old) by the WRRT in December 2008 from Kampong Speu Province and brought to PTWRC for care and rehabilitation. Tests conducted for tuberculosis were negative, and the results of blood chemistry tests were normal for the species. The two females were cared for at PTWRC for approximately four years and were housed in a large, natural, open-topped enclosure. Handling and human interactions were kept to a minimum during feeding and cleaning, and were otherwise restricted to health checks. We transported the two females to the release station in May 2011 and moved the pair from their travel cages into a 'bear house' which comprised two dens (each measuring 3 m × 3 m × 3 m) to acclimatise for two weeks before then giving them access to an open-topped forest enclosure.

The candidate for our second release was a male sun bear, named Tela. Tela was rescued as a cub (approximately four months old) by the WRRT in 2014 from a petrol station in Monduliri Province. Veterinary staff present during the confiscation decided to bring the cub immediately to the wildlife release station, because he was deemed healthy and exhibited fear and aggression towards people, indicating he would be suitable for future release. To gradually introduce Tela to Sopheap and provide him with access to the outside while keeping the two bears separate, we fenced off a small portion of the enclosure outside the bear house with chain-link fencing and installed electrified wires.

Rehabilitation & acclimatisation

All three bears were kept in an open-topped forest enclosure (measuring 100 m × 100 m × 3 m) at the wildlife release station. The enclosure is situated approximately 200 m from the campsite which services the station and encompasses a section of forest containing large trees and natural and artificial ponds. On arrival at the release station, the bears were released from their travel cages into the bear house next to the enclosure to enable treatment, monitoring and acclimatisation before their introduction to the enclosure. We installed five strands of electric wires at vertical intervals of 0.4 m along the enclosure fence, with insulators attached to fence posts. As with the small section fenced for Tela, the electrified wires were serviced by a solar panel that fed direct current into a car battery for storage, and this in turn was connected to an

energizer (Speedrite) which converted and regulated the voltage of alternating current in the wires to ensure this would not injure the sun bears while conditioning them to avoid the fencing. The bears quickly learned to avoid the fence after one or two instances of contact.

This natural environment enabled the bears to acquire appropriate behaviour such as climbing and nest-building in the trees and foraging for roots, insects and termites. We kept human interaction to a minimum, with food either lowered into the enclosure using a remote pulley system or quickly placed inside the enclosure by staff, who then retreated behind a hide to observe the physical health of the bears. The time devoted for acclimatisation depended on the responses of each animal and continued until they were deemed to be sufficiently familiar with their new environment. This was considered the case when the bears explored and utilised the entire enclosure area without exhibiting stereotypical behaviours such as pacing or self-harming, coupled with the expression of 'natural' behaviours such as avoiding animal care staff, foraging independently within the enclosure and climbing, building nests and sleeping in the trees. The release of the bears was timed to coincide with periods when there was sufficient food (i.e. fruiting trees, frogs and termites) in the forest for them to forage. Sopheap and Sloat spent 13 months in the acclimatisation enclosure prior to their release. Tela was released after five years in the enclosure as he arrived to the release station as a four month old cub and we wanted to ensure that forest protection activities undertaken in 2013 had significantly reduced the risks posed by snares before attempting another release.

Post-release monitoring & supplementary feeding

Sopheap and Sloat were fitted with a G2110E - Iridium GPS collars (Advanced Telemetry Systems Inc., Minnesota, USA) which weighed 825 g. Tela was fitted with a Tellus Iridium GPS collar (Followit AB, Lindesburg, Sweden) which weighed 800 g. Both types of collars had VHF and GPS monitoring functions. We programmed the collars on Sopheap and Sloat to record a GPS coordinate every two hours, whereas the collar on Tela was programmed to record his position every six hours. These were attached one month before their expected release to allow the animals time to adjust and ensure the collars were working effectively without causing unnecessary discomfort such as ulcers or tick accumulation. To attach the collars, the animals were sedated by veterinary staff who administered intramuscular injections of Zoletil and Metedomide (4 mg/kg + 0.03 mg/kg) using a blow-pipe, and monitored their physiological parameters throughout sedation. Following collar attach-

ment, the bears were revived using Atipamazol (0.4 mg/kg) which was injected intramuscularly. Sopheap and Sloat were fitted with their collars on 31 May 2012 and Sopheap adapted to her collar much faster than Sloat, who fought hers for hours. Tela was fitted with a collar at the end of January 2019, and took a few days to adjust.

Following rehabilitation and acclimatisation, we encouraged the bears to leave the release station by opening the main gate of the enclosure and a slide-door on its eastern side in the late afternoon. One camera trap was placed outside the main enclosure gate to monitor the timing of their departure and subsequent events if they returned. The doors to the enclosure remained open to allow the bears to return if they wished and were closed one month after their departure as they did not re-enter the enclosure or return for supplementary food provided during this period. The latter was placed outside the main enclosure gate in the morning and afternoon for the entire month and comprised the same diet provided to the bears during acclimatization. On departing the enclosure, the bears were tracked daily until signals were no longer received from their GPS collars. This information was supplemented by occasional reports from villagers who observed the bears in the forest.

Results

Sopheap & Sloat

On being allowed to access the outdoor enclosure at the release station (two weeks after their arrival), Sopheap and Sloat immediately began to climb trees and forage for termites and frogs. They learned how to build nests in the trees, pulling the branches inwards to form a platform. After one month in the outdoor enclosure, they consistently avoided staff, fleeing when keepers approached the enclosure.

We opened the enclosure gates on 11 June 2012 at 1600 hrs. Sopheap departed three days afterwards, whereas Sloat left almost four weeks later, on 4 July. The bears did not return to their enclosure, nor did they take any of the supplementary food provided outside it. However, we suspect that they may have taken food left on a separate platform for a family of previously released binturongs, when the structure was found broken in mid-July. Following this, the two bears separated and ranged widely (Fig. 1). Sopheap displayed a preference for the fragmented habitats of acacia plantations and grasslands on the fringe of the forest due east of the enclosure. After spending a brief period around the camp area and adjacent forest, Sloat ventured north into denser forest (Fig. 1). Staff and villagers entering the forest to collect non-

timber forest products reported brief sightings of the bears as both avoided humans. Sloat was more elusive and warier of humans compared to Sopheap.

Sopheap's collar stopped providing GPS positions on 2 August (49 days post-departure) and Sloat's did likewise on 15 August (42 days post-departure). Both bears were then manually tracked using the VHF function of their collars. Although it was not possible to locate them every day due to weather conditions and limited resources, both bears were found on most days and were monitored for health based on body condition which was observed from a distance with binoculars. On 11 August (58 days post-departure), Sopheap was seen to be clearly injured by a snare noose around her right front leg. As such, she was located and recaptured at the same location the following day and returned to the bear house at the release station for treatment. On 26 August (53 days post-departure), we learned that Sloat had also been snared and managed to recapture her the same day, similarly returning her to the bear house for treatment.

Sopheap recovered well from her injuries and was moved to the outdoor enclosure at the end of August (Fig. 2; present issue cover), when Sloat was recaptured and taken to the bear house. Sloat's wound was more severe; the snare had cut to the bone and she had lost all the skin and much of the muscle from her foot, such we considered amputating the injured paw. However, following treatment every five days, including sutures and bandaging, she recovered by the end of September and was released back into the outdoor enclosure. Both bears avoided each other once reunited. On 11 March 2013, Sloat was found dead among the trees in the outdoor enclosure. The subsequent necropsy did not reveal any obvious cause of death and all organs appeared to be in good health, although she was thin.

Tela

Forest protection activities were intensified at the release site after Sopheap and Sloat were caught in snares in 2012. These activities included increased operations by the WRRRT in the surrounding area, and in 2013, the creation of a permanent police patrol team based in Chi Phat village and establishment of a community-based anti-poaching unit.

Tela was brought directly to the acclimatization enclosure at WRS following his confiscation in 2014. The section of the main enclosure we had separated with fencing to gradually introduce Tela to Sopheap proved ineffective, as Tela promptly broke through this on his release from the bear house. After an initial period of mistrust, the two bears became tolerant of each other

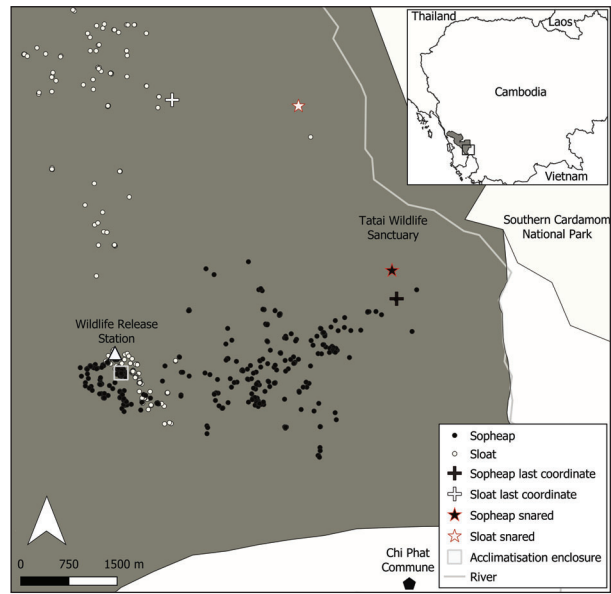


Fig. 1 Locations of female Malayan sun bears (Sopheap and Sloat) after their release in Tatai Wildlife Sanctuary, south-west Cambodia, June–August, 2012.



Fig. 2 Female sun bear (Sopheap) in open-topped enclosure at the wildlife release station, Tatai Wildlife Sanctuary, January 2019 (© Jeremy Holden/Wildlife Alliance).

and Sopheap adopted the cub as her own within weeks, allowing Tela to suckle for comfort and calling to him when they were separated. The pair were frequently found resting in the branches of large trees in the more open, southern end of the enclosure in the mornings. Sopheap became less afraid of people in the acclimatization enclosure over the years compared to her behaviour

in the forest after her release. In contrast, Tela remained extremely nervous and always avoided humans, fleeing whenever staff approached the enclosure. As a result, we constructed a hide to allow us to observe his physical health during feeding times.

Despite their friendship, we decided not to re-release Sopheap with Tela due to her increasing age and preferences she showed for the open areas and acacia plantation in the direction of the village during her first release (rather than the dense forests in the opposite direction). On 23 February 2019, we confined Sopheap to the bear house and opened the outside enclosure door during the afternoon. Tela exited the enclosure on 25 February and initially ventured into dense forests to the north, after which he subsequently preferred areas to the southwest. He also travelled close to our forest patrol station bordering the river in Stung Proat (Fig. 3) and sometimes moved long distances (up to 5 km) in a single night.

Following his release, we were not able to observe Tela in the forest due to his wary disposition. Because the coordinates provided by his GPS collar indicated he was travelling large distances however, we assumed he was alive and in good health. On occasion, local people gathering non-timber forest products in the forest reported sightings of Tela, which were typically of him rapidly retreating into the forest. Tela's collar ceased transmitting coordinates on 19 June 2019 (114 days post-departure), after which we continued to track him manually, using the VHF transmitter.

On 9 July, Tela's position southwest of the release station was located and his intact body was found near this area the following day (134 days post-departure). The surrounding area was flattened and his collar had been bitten through. We conclude he was most likely killed in a fight with a wild bear for several reasons: i) no other large carnivores that could overcome a bear occur in this forest area to our knowledge; ii) the signs of struggle and that nothing had been eaten or removed from his body suggest his death was not caused by a predator or a human; iii) his physical condition at the time of death was good, indicating that an inability to forage for food was unlikely to be the cause.

Discussion

Although our three sun bears displayed appropriate 'natural' behaviours in the wild (e.g., climbing, nest-building, foraging and human avoidance), their releases were unsuccessful, albeit for different reasons. The first two bears released (Sopheap and Sloat) were caught in snares and so were recaptured for treatment. Sopheap

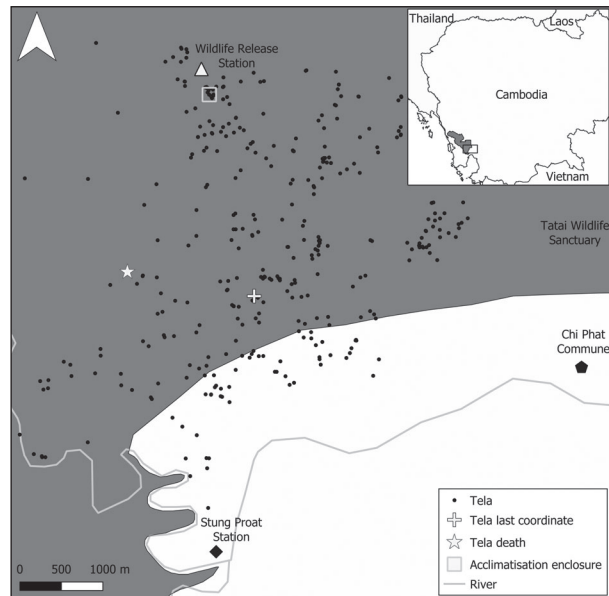


Fig. 3 Locations of male Malayan sun bear (Tela) after his release in Tatai Wildlife Sanctuary, February–July, 2019.

recovered from the experience and remains in captivity, whereas Sloat died seven months after her recapture. The third bear released (Tela) died less than half a year later and was probably killed by a wild male bear.

Each release provided valuable lessons for future attempts. For example, despite the work undertaken to protect and improve the safety of the forests before the first release, experiences from the release indicated that further efforts were needed to ensure the security of wildlife around the release station and Chi Phat Commune. Once the problem of snares was addressed however, the challenge of conspecifics arose. In this context, Tela's death, which was likely caused by a wild bear which previously could not survive in the area due to hunting and snares, actually suggests that our increased protection has benefitted the area and its wildlife. More specifically, we believe that our elimination of these threats has facilitated the reappearance of wild sun bears, although this in turn means the release station is no longer a suitable site for releasing captive male bears. Thus, while our release attempts failed, our wider conservation programme could be considered successful, in leading to the reappearance of previously extirpated species.

The length of time the bears spent in captivity did not appear to influence their behaviour after release. At the time of recapture, or in Tela's case, death, all three bears were in good physical condition, indicating they were successfully foraging for themselves. In addition, although caught in snares, Sopheap and Sloat

avoided farmland, villages and humans. In other bear releases, animals in prolonged captivity with constant human contact have become 'nuisance bears', which has resulted in their death or recapture (Alt & Beecham, 1984; Fredriksson, 2005). Although none of our bears entered human habitation, Sopheap did travel in open areas and the disturbed acacia plantation in the direction of Chi Phat Commune. As such, we did not release her with Tela in 2019, as we believed there was a risk she might enter farms or villages.

We suspect our three sun bears exhibited behaviours similar to those of wild bears due to the rehabilitation protocols we employed, particularly the provision of a large forested enclosure which encouraged 'natural' behaviour, and minimal human contact (Stiver *et al.*, 1997). Although nest-building behaviour is documented for the species, it remains unclear how much of this behaviour is learned or instinctual (Wong *et al.*, 2004; Hall & Swaisgood, 2009). As Sopheap and Sloat foraged, climbed trees and built nests in the acclimatization enclosure, despite having been in captivity since they were young cubs, these may be instinctive behaviours for sun bears. Feeding and foraging behaviour have also been observed as instinctive in sun bears rehabilitated for release in Indonesia (Fredriksson, 2005). However, our protocols differed from previous sun bear releases, some of which have proven successful, where young bears were walked in the forest by carers and returned to their enclosure each evening, until the animals themselves decided not to return (Fredriksson, 2005). In contrast, our experience in rehabilitating many different animal species indicates that if kept naturally in an appropriate setting that allows captive animals to sufficiently fine tune their survival skills, 'natural' behaviour is instinctive (Marx, 2008; Marx & Bunthoeun, 2014; Leroux *et al.*, 2019). Added to which, provision of food after release provides animals with the support they need as they perfect the art of survival, should this be required. The three bears we released were no exception to our experiences with other species.

Where possible, bears should not be released into areas where they will have to compete with resident conspecifics (Fredriksson, 2005; van Dijk, 2005). As such, our release station is no longer a suitable site for releasing male sun bears due to the presence of wild bears in the surrounding forests. When the release site was initially selected, the loss of sun bears and other large mammals due to heavy hunting pressure meant it was suitable for releasing many species, including bears. In the years following increased protection, we assumed the area remained well below its carrying-capacity for sun bears because sightings of scat were limited and camera-traps

did not reveal any evidence of resident bears in the area. We noted however that Tela sometimes travelled long distances (up to 5 km) in a single day, compared to the average of 2 km recorded by Wong *et al.* (2004) for six wild bears. We considered the possibility that these distances, combined with Tela's preference for the acacia plantation at the forest boundary (despite reduced food availability and increased human activity), might be attempts to avoid a wild bear, but had no definite evidence that this was the case. Release of female sun bears could be considered in future, as existing populations may be more tolerant to released female bears than male competitors (Clark, 2009; Fredriksson, 2005). However, if the wild bear population is recovering in the forests surrounding the release station as a result of our protective activities there, further releases of sun bears might be unnecessary and selection of a new release site more suitable.

It is vital that practitioners disseminate the results of wildlife release efforts to ensure that past mistakes are not repeated and improve success rates. Careful consideration of a variety of relevant factors is essential prior to release, particularly the selection of release sites which should be free of pressures such as hunting and land conversion. Our release of sun bears demonstrates that with careful selection of candidate animals and thorough rehabilitation protocols, sun bears that have been in captivity since infancy can sustain themselves and revert to life in the forest without difficulty. As such, reintroduction of captive animals could be a great asset to bolster wild populations where these have declined or become extirpated, provided responsible protocols that incorporate the IUCN Reintroduction Guidelines are followed. Notwithstanding this, effective protection efforts might be all that is required in many areas to enable the return of wild animals, including sun bears.

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Cetacean monitoring methods: using Irrawaddy dolphins to compare land-based surveys and acoustic sampling in Kep, Cambodia

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ការត្រួតពិនិត្យតាមដានមានសារៈសំខាន់ណាស់ ដើម្បីធានាថាប៉ូពុយឡាស្យុងបាឡែននៅតែមានសុខភាពល្អ ប្រឈមទៅនឹងការគំរាមកំហែងនៃប្រព័ន្ធអេកូឡូស៊ីសមុទ្រ ជាពិសេសសម្រាប់ប្រទេសកំពុងអភិវឌ្ឍ។ វិធីអង្កេតដីគោកគឺជាវិធីបែបប្រពៃណីសម្រាប់ការត្រួតពិនិត្យបាឡែន វាសមស្របខ្លាំងនៅពេលដែលថវិកា ពេលវេលា និងធនធានផ្សេងៗមានកំណត់។ បច្ចុប្បន្ន ឧបករណ៍ចាប់សំឡេងគឺជាបច្ចេកទេសមួយទៀតសម្រាប់ការត្រួតពិនិត្យបាឡែន ហើយអាចត្រូវបានប្រើដើម្បីរកវត្តមានរបស់ពួកវាដោយមិនចាំបាច់មានវត្តមានមនុស្សជាប្រចាំ។ យើងបានវិភាគទិន្នន័យនៃសត្វផ្សោតអ៊ុកាឌី (*Orcaella brevirostris*) ដែលប្រមូលបានក្នុងអំឡុងខែសីហា ឆ្នាំ២០១៨ និងមិថុនា ឆ្នាំ២០១៩ នៅខេត្តកែប ដើម្បីប្រៀបធៀបអំពីអត្រានៃការរកឃើញដោយការអង្កេតដីគោក និងការប្រើឧបករណ៍ចាប់សំឡេង (C-POD)។ យើងក៏បានស៊ើបអង្កេតផងដែរពីឥទ្ធិពលនៃលក្ខណៈអាកប្បកិរិយារបស់ក្រុមផ្សោត(ព្រឹត្តិការណ៍អាកប្បកិរិយា ស្ថានភាពអាកប្បកិរិយា របៀបហែលទឹក ប្រភេទក្រុម និងទំហំក្រុម) ទៅលើអត្រានៃការរកឃើញ (detection rates)។ ទិន្នន័យផ្តល់ដោយវិធីសាស្ត្រត្រួតពិនិត្យនីមួយៗ ត្រូវបានផ្ទៀងផ្ទាត់គ្នាដើម្បីកំណត់ថាតើកំណត់ត្រាផ្សោតត្រូវបានរកឃើញដោយវិធីសាស្ត្រមួយឬវិធីសាស្ត្រទាំងពីរ និងកំណត់ពីភាពខុសគ្នាដាច់ខាតទៅលើអត្រារកឃើញនៃវិធីសាស្ត្រទាំងពីរ។ លើកលែងតែព្រឹត្តិការណ៍អាកប្បកិរិយាលើកកន្ទុយ (fluke-up) លក្ខណៈទាំងឡាយដែលបានឃើញ មិនមានទំនាក់ទំនងជាមួយនឹងអត្រារកឃើញទេ។ ចំនួននៃការសង្កេតសម្រាប់ព្រឹត្តិការណ៍លើកកន្ទុយគឺអាស្រ័យផងដែរទៅលើវិធីសាស្ត្រសង្កេតដែលបានប្រើ។ យើងពិចារណាពីចំណុចខ្លាំងនិងខ្សោយនៃវិធីសាស្ត្រនីមួយៗ និងផ្តល់យោបល់ថា ការប្រើវិធីអង្កេតទាំងពីរប្រមាណគឺសាកសមបំផុតសម្រាប់ត្រួតពិនិត្យក្រុមបាឡែន ។

Abstract

Monitoring is crucial to ensure that cetacean populations remain healthy due to the threats facing marine ecosystems and current knowledge gaps, especially in developing countries. Land-based surveys are a traditional method for monitoring cetaceans which are practical when budget, time and other resources are limited. Passive acoustic monitoring has recently emerged as another technique for monitoring cetaceans and can be used to detect them without constant human presence. We analysed data collected on Irrawaddy dolphins *Orcaella brevirostris* between August 2018 and June 2019 in Kep Province to compare rates of detection by land-based surveys and passive acoustic sampling with a continuous porpoise detector (C-POD). We also investigated if the characteristics of dolphin groups sighted (behav-

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ioral events, behavioural states, swim styles, group types and group sizes) affected detection rates. Data provided by each monitoring method were cross-referenced to determine if dolphin records were detected by one or both methods and found a significant difference in their detection rates. With the exception of fluke-up behavioural events, the characteristics of sightings were not correlated with detection rates. The number of observations for fluke-up events was also dependent on the observation method used. We consider the strengths and weaknesses of each method, and suggest that a combination of both will be most suitable for monitoring Irrawaddy dolphins.

Keywords Cetacean, C-POD, Irrawaddy dolphins, Kep Archipelago, land-based survey, passive acoustic monitoring, research methods.

Introduction

Population monitoring is required to develop and inform appropriate conservation strategies for cetaceans due to declines in their population sizes and lack of reliable baseline data for developing countries (Aragones *et al.*, 1997; Smith *et al.*, 2016). Survey goals, site geography, human resources, and available budgets, time and equipment must be considered when selecting a monitoring method (Aragones *et al.*, 1997). Land-based surveys have been found to be one of the most practical methods when budgets, time and equipment are limited (Aragones *et al.*, 1997; Morete *et al.*, 2018). They also have disadvantages however, which include observer bias and surveys being limited to daylight hours and suitable environmental conditions (Evans & Hammond, 2004).

The use of passive acoustic monitoring (PAM) devices to study delphinids (oceanic dolphins) has increased in recent years (Verfuss *et al.*, 2018). One such device, a continuous porpoise detector (C-POD; Chelonia Ltd, Cornwall, UK) is moored to seabed for the purpose of continuously recording the echolocation clicks produced by delphinids to navigate their environments and hunt prey (Au, 1993; Tyack, 1997; Chelonia Ltd, 2014a). As such, C-PODs have been used to monitor and study cetaceans for conservation purposes in variety of ecosystems from the arctic to the tropics. For example, they have been used to evaluate the status of harbour porpoises in Baltic Proper (Gallus *et al.*, 2012), to study the distribution of beluga whales and killer whales in Cook Inlet, Alaska (Lammers *et al.*, 2013), to track vaquita population declines in Mexico (Jaramillo-Legorreta *et al.*, 2016), and explore the relationship between Burmeister's porpoise and fishing by-catch in Peru (Clay *et al.*, 2018). Like other acoustic sampling devices however, they have drawbacks which include a limited detection range (no further than 1 km), with detection also confined to echolocation signals that are directed towards the device.

Irrawaddy dolphins *Orcaella brevirostris* are an Endangered cetacean species whose populations are declining (Minton *et al.*, 2018), largely due to anthropogenic threats resulting from bycatch and habitat degradation (Smith & Jefferson 2002; Reeves *et al.*, 2003; Smith *et al.*, 2004; Perrin *et al.*, 2005; Smith *et al.*, 2008; Peter *et al.*, 2016). The species is found in lakes, estuaries, shallow coastal waters, and large rivers in Southeast Asia (Perrin *et al.*, 1995, 1996; Ponnampalam *et al.*, 2013). Marine populations of Irrawaddy dolphins in Cambodia have received increased research attention in recent years (Beasley & Davidson, 2007; Smith *et al.*, 2016; Tubbs *et al.*, 2019, 2020). Established in 2017, the Cambodian Marine Mammal Conservation Project (CMMCP) is the first long term project dedicated to research and conservation of marine mammals in the country's southern Kep Province. Through weekly land- and boat-based surveys and PAM, the CMMCP has found that Irrawaddy dolphins are the only cetacean species present in the area and generated information on their distribution, behaviour and seasonal variation (Tubbs *et al.*, 2020). Because these surveys were undertaken in the same area, this provided an opportunity to compare the efficacy of land-based surveys and acoustic sampling with C-PODs. This was of interest because although many studies have used either land-based surveys or C-PODs to monitor cetaceans, few have employed both methods simultaneously or compared their utility to our knowledge.

The present paper investigates the relative efficiency of these methods for detecting Irrawaddy dolphins (hereafter 'dolphins') using data collected in Kep Province between August 2018 and June 2019. We also investigate if the characteristics of dolphin groups sighted (behavioural events, behavioural states, swim styles, group type and group size) affected the rate of observations by either method. As such, we provide a critical analysis of the efficiency of the two methods, with the view that this may be used to facilitate selection of appropriate methods for future cetacean research in developing countries.

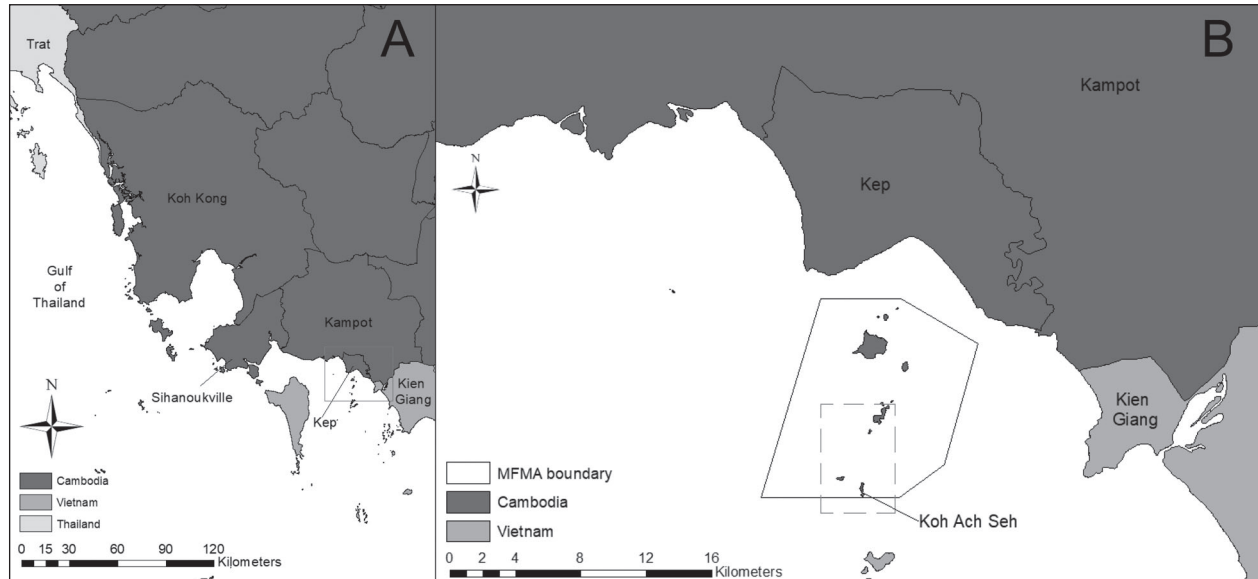


Fig. 1 Location of A) Kep Province on the coast of Cambodia and B) islands within the Kep Archipelago, including the study site (Koh Ach Seh) and Kep Marine Fisheries Management Area (MFMA). From Tubbs & Keen (2019).

Methods

Study site

Our study was undertaken in the Kep Archipelago of Kep Province, one of the four coastal provinces on Cambodia's 435 km long coastline (Fig. 1). The archipelago comprises 13 islands surrounded by marine waters <12 m in depth which include habitats such as coral reefs, seagrass beds and mangrove forests. It also includes a Marine Fisheries Management Area—Cambodia's equivalent of a marine protected area—and is considered as an important area for marine mammals (IUCN-MMPATF, 2020). Our land-based survey site and acoustic monitoring station were located on the east side of the Koh Ach Seh within the archipelago (Figs. 1–2).

Land-based surveys

Weekly land-based surveys, each lasting approximately three hours, were undertaken by the CMMCP between 1 August 2018 and 12 June 2019. These took place from an east-facing observation platform, 21 m above sea level (Fig. 2). During each survey, two surveyors used 8 x 42 Bushnell binoculars to continuously search for dolphin groups, while two additional surveyors rested. All surveys were conducted when the Beaufort Sea state was ≤ 3 . For each dolphin sighting, a group number was assigned and the time was recorded. Sightings of dolphins that were separated by >15 minutes were assumed to be

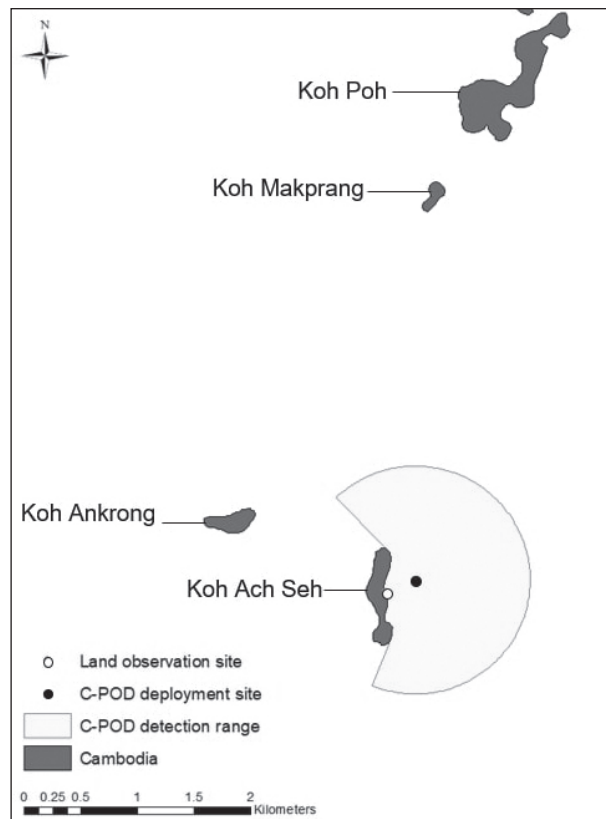


Fig. 2 Location of the land-based survey and acoustic sampling (including C-POD detection range) within the southern islands of the Kep Archipelago, Cambodia.

of different groups. Data was then collected on a variety of behavioural states, group types, swim styles and group sizes for a one-minute period (Table 1). Categories and definitions for these characteristics followed Lusseau (2003, 2006), Parra (2006), Akkaya-Bas *et al.* (2015) and Tubbs *et al.* (2020).

Acoustic sampling

We deployed a single C-POD approximately 100 m east of Koh Ach Seh (10°21'31"N, 104°19'22"E; Fig. 2) between 1 August 2018 and 12 June 2019 (Table 2). The

device was moored 3.5 m below the sea surface and 1 m above the seabed and detected echolocation clicks between 20–160 kHz within the 1 km range of the device. Information recorded on echolocation clicks was stored on a SD card and included frequency, bandwidth, ICI (inter-click interval), NCyc (number of cycles), intensity, time and date. The sensitivity of the C-POD was adjusted to the low setting to avoid an excess of background noise in recordings, and the limit for records was set to the maximum of 4,096 per minute. The device was retrieved from the mooring site once each month to remove biofouling and replace its batteries and SD card.

Table 1 Characteristics of dolphin groups recorded during land-based surveys in the Kep Archipelago, Cambodia.

Behavioural event	
Fluke-up	Individual raises only its tail fluke above the water surface.
Behavioural states	
Diving	Individuals disappear from surface for between 30 seconds and several minutes. Individuals show no obvious progressional movement and resurface within 100 m from where they disappeared.
Travelling	Individuals move with a constant speed in a certain direction, with a diving interval of 3–5 seconds.
Travel-diving	Individuals disappear from surface for between 30 seconds and several minutes. Individuals make progressional movement, reappear at distance from their starting location.
Surface-feeding	Individuals show active, rapid directional changes just under the surface. Splashes may be present.
Resting	Individuals are drifting at the surface, disappearing and reappearing in the same location.
Group types	
Tight	Individuals are spread out less than 5 m apart from each other.
Far	Individuals are spread out more than 5 m apart from each other.
Mixed	Group is a mixture of Tight and Far.
Alone	One single individual present.
Swim styles	
Line	Individuals swim in a line, head to tail. The line can be straight or offset.
Circular-diving	Individuals create a circular formation by appearing in turns at the surface after each other.
Spread	The group is spread out, individuals do not swim close to each other.
Team	The group is split up into smaller independent teams.
Cluster	Individuals are clustered with no directional movement.
Front	Individuals swim in a line, side by side. The line can be straight or offset.
Kettled	Individuals are clustered at the surface and water appears to be boiling. Splashes may be present.
Alone	One single individual is present.
Group sizes	
Small	A group with 1–3 individuals.
Medium	A group with 4–8 individuals.
Large	A group with more than 9 individuals.

Table 2 Deployment and retrieval dates for the acoustic monitoring device (C-POD) employed in the study.

No.	Deployment date	Retrieval date
1	1 August 2018	16 August 2018
2	16 August 2018	13 September 2018
3	13 September 2018	22 October 2018
4	22 October 2018	23 November 2018
5	28 November 2018	18 December 2018
6	18 December 2018	21 January 2019
7	21 January 2019	18 February 2019
8	18 February 2019	20 March 2019
9	27 March 2019	18 April 2019
10	18 April 2019	13 May 2019
11	13 May 2019	3 June 2019
12	3 June 2019	12 June 2019

Table 3 Dates of land-based survey and acoustic sampling data employed in analyses.

No.	Date	No.	Date
1	1 August 2018	12	7 February 2019
2	3 August 2018	13	21 February 2019
3	14 August 2018	14	7 March 2019
4	15 August 2018	15	20 March 2019
5	27 August 2018	16	20 April 2019
6	4 September 2018	17	1 May 2019
7	17 October 2018	18	2 May 2019
8	6 November 2018	19	22 May 2019
9	30 November 2018	20	23 May 2019
10	5 December 2018	21	4 June 2019
11	7 December 2018	22	12 June 2019

Data processing & analysis

We used C-POD software and the associated *KERNO classifier* (Chelonia Ltd, 2014b) to sort our detections into four categories: narrow-band high frequency (NBHF), which typically represent porpoises; other cetaceans, cetacean clicks that were not NBHF; sonar, signals from

boats; unclassified, distinct from the other classes. These were assigned to quality groups (doubtful, low, medium, high) and only NBHF and other cetacean clicks of high and medium quality were included in analysis.

Data from a total of 55 hours and 36 minutes of land-based surveys undertaken over 22 days were directly compared with data generated by the C-POD for the same periods (Table 3). As such, data recorded by the C-POD outside of the land-based survey periods was not included in analysis. Data provided by each method were treated as separate samples when calculating their detection rates. *Rstudio* vers. 4.0.3 (R Core Team, 2020) was used for all data analysis.

Each dolphin group sighted during a land-based survey was counted as a single observation and the timing of each land-based observation was cross-referenced against the C-POD data to determine whether the group was also recorded acoustically or not. In analysis, an observation was counted if it was recorded by at least one of the two sampling methods. Data on the characteristics of dolphin sightings (behavioural events, behavioural states, swim styles, group types and group sizes) were used to investigate differences in detection rates between the two survey methods. For this purpose, the behavioural characteristics of acoustic records were assumed to be the same as those of visual (land-based) observations that occurred simultaneously.

A Chi-square test was used to test for differences in the number of records produced by land-based surveys and C-POD sampling. The number of records produced by each method were also compared to assess differences in the frequencies of behavioural characteristics detected by either method. For this purpose, the number of observations was taken as the number of records registered by both land surveys and C-POD, whereas frequencies represented the number of times land surveys and C-POD registered a given behavioural characteristic in a single record.

Results

Over the course of the study, dolphins were detected on a total of 26 separate occasions. Twenty-two of these occasions were registered by land-based surveys and nine were registered in acoustic sampling. Five occasions were simultaneously detected by both methods (Fig. 3).

Dolphin records were highest in August 2018 for both methods, followed by May 2019 (Fig. 3). Records from land-based surveys were higher in most months

compared to acoustic sampling, aside from December 2018 (equal number of records) and January 2019 (no records). No acoustic records were made in September 2018, March 2019 and April 2019.

Detection rates

The total number of observations produced by land-based surveys was significantly greater than acoustic sampling ($X^2=13.499$, $DF=1$, $P=0.0002387$).

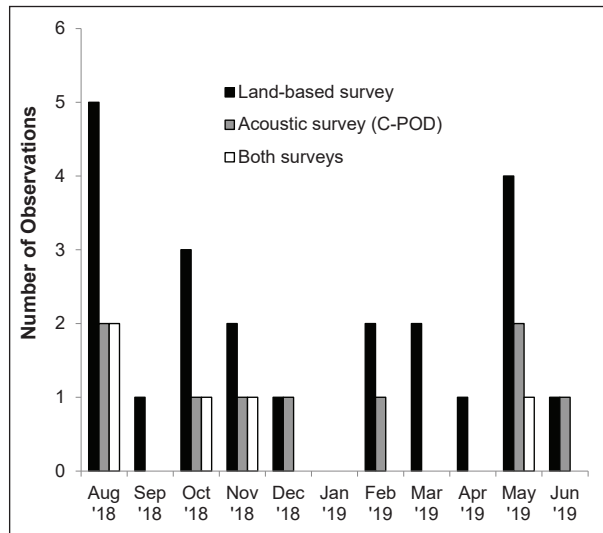


Fig. 3 Monthly dolphin observations produced by the two survey methods from August 2018 to June 2019.

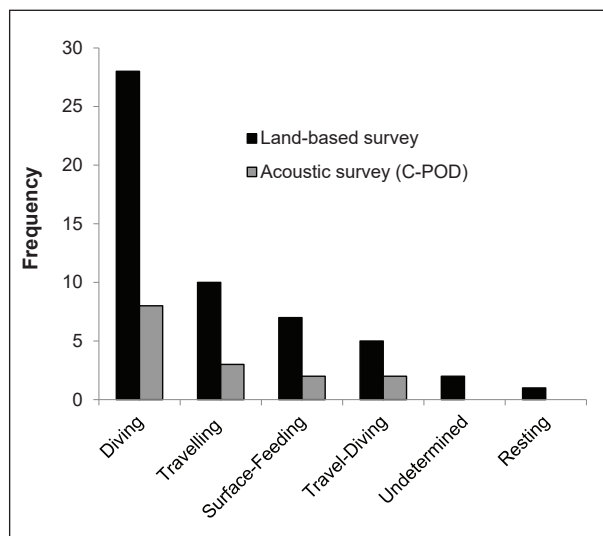


Fig. 4 Frequencies of behavioural states exhibited by dolphins recorded by the two survey methods.

Behavioural characteristics

‘Fluke-up’ behavioural events were registered 66 times during our land-based surveys, 15 of which occurred during observations also registered in acoustic sampling.

Similarly, our land-based surveys observed all other categories of behavioural states (Table 1) more frequently than acoustic sampling (Fig. 4). ‘Diving’ represented the behavioural state most frequently registered during visual and acoustic observations, with a collective total of 36 observations, followed by ‘travelling’ (13 observations), ‘surface-feeding’ (nine), and ‘travel-diving’ (seven).

Our land-based surveys observed all categories of group type more frequently than acoustic sampling (Fig. 5). The ‘tight’ category was most commonly recorded by both methods, with a collective total of 37 observations, followed by ‘alone’ (15 observations).

All but two categories of swim styles were observed more frequently in land-based surveys compared to acoustic sampling (Fig. 6), the exceptions being the ‘team’ and ‘circular-diving’ categories (both equal). The ‘line’ swim style was the most frequently observed category, with a collective total of 16 observations, followed by ‘alone’ (15 observations), ‘cluster’ (14) and ‘spread’ (12).

Visual (land-based) records of dolphins were more frequent than acoustic records when their groups were small or medium sized (Fig. 7), whereas large groups were observed equally frequently.

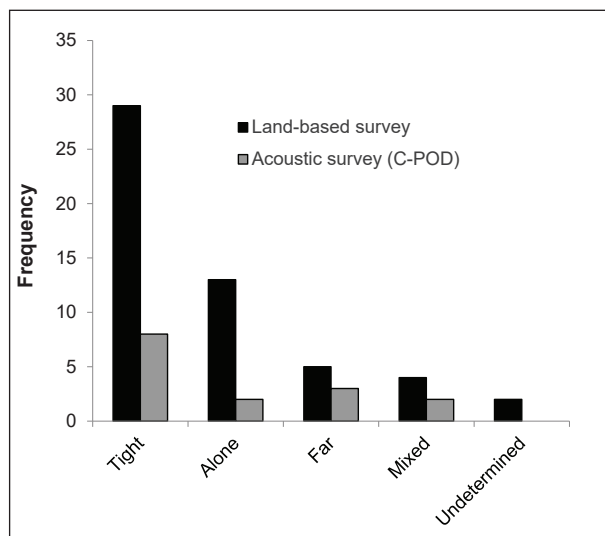


Fig. 5 Frequencies of dolphin group types recorded by the two survey methods.

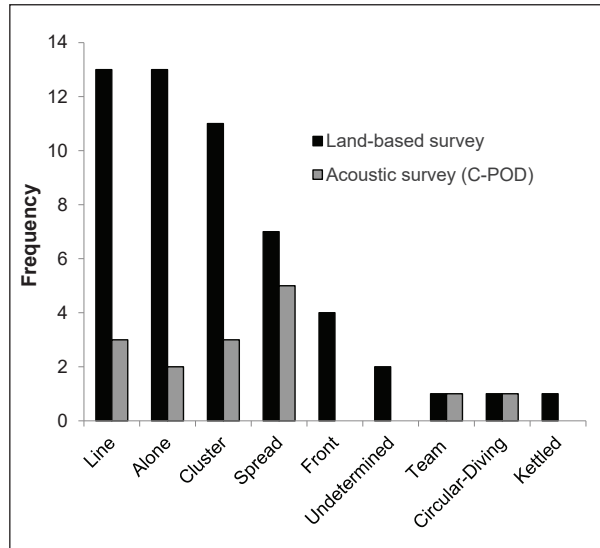


Fig. 6 Frequencies of dolphin swim styles recorded by the two survey methods.

Discussion

We found that land-based surveys generated more observations of dolphins compared to acoustic sampling. This might be due to the limited detection range of our acoustic device, since C-PODs can only detect echolocation clicks that are produced within 1 km of the device and travelling its direction, whereas greater distances could be surveyed with binoculars in our land-based surveys. Additionally, as the sensitivity of our C-POD was set to low to avoid excessive background noise in recordings and maximise battery life and digital storage, this could have contributed to under-sampling.

'Fluke-up' events and 'diving' behaviour were recorded more often in land-based surveys than acoustic sampling. Both have been associated with foraging behaviour (Smith *et al.*, 1997; Casipe *et al.*, 2013) and as cetaceans use echolocation during foraging to determine prey location (Johnson *et al.*, 2004; Madsen *et al.*, 2007), acoustic sampling should detect dolphins during this activity. That this did not always prove the case in our study might again be due to the lower detection range of the C-POD or because there were occasions when echolocation signals produced by dolphins foraging in the area were not directed towards the device.

The disparities between the frequencies of different dolphin group sizes recorded by land-based and acoustic sampling was greatest for small groups, followed by

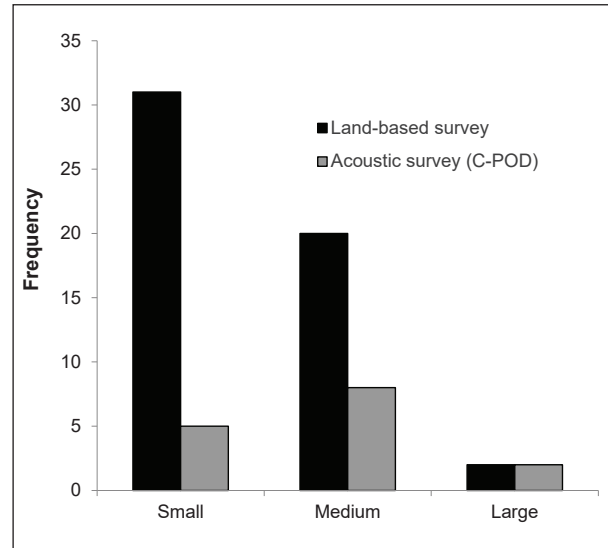


Fig. 7 Frequencies of dolphin group sizes recorded by the two survey methods.

medium-sized groups. As such, these were more likely to be detected by land-based surveys, whereas large groups were equally likely to be observed by both methods. We attribute this to the likelihood that large groups of dolphins generate more echolocation signals, thereby improving their likelihood of detection in acoustic sampling. This would be consistent with our finding that the number of observations of dolphins in the 'alone' category of group types and swim styles was much greater in our land-based surveys. This collectively suggests that C-PODs are more efficient at detecting large groups compared to small groups or individual dolphins, whereas land-based surveys are more effective than C-PODs at detecting either of the latter.

While our study provides new insights on the relative efficiency of land-based survey and acoustic sampling methods for monitoring dolphins, it does have limitations. For example, we assumed that dolphin groups simultaneously registered by both methods were the same group. This could potentially have led to over-representation in the number of observations counted for both methods and under-representation in the overall number of observations. Future research to discern the acoustic characteristics associated with specific behaviours could potentially contribute to overcoming the former challenge.

Land-based surveys are a traditional and widely-used method for monitoring cetaceans worldwide.

With appropriate binoculars, they allow large areas to be surveyed from a stationary position and have been especially used to study cetacean behaviour (Giacoma *et al.*, 2013; Keen *et al.*, 2020). Being based on visual observation, they do have limitations however in that they can only be undertaken during daylight hours and suitable weather conditions. In contrast, acoustic sampling devices such as C-PODs have revolutionized cetacean studies in allowing researchers to remotely study the animals at any time of day or night, irrespective of weather conditions (Roberts & Read, 2014). As mentioned before however, they are limited by detection range (up to 1 km in the case of C-PODs) and can generally only detect echolocation signals directed towards the device. They can also only detect dolphins when these are actively echolocating (such that more passive activities will be under-sampled), whereas their effectiveness for behavioural studies has yet to be fully determined. Despite these drawbacks however, acoustic devices such as C-PODs have great potential for overcoming the inherent limitations of visual surveys, particularly for situations where human resources are scarce.

Our study provides new insights into the relative strengths and weaknesses of land-based surveys and acoustic sampling for studying cetaceans which will hopefully aid researchers in designing future studies. Further studies are needed to confirm and elucidate our findings however, as well as to assess their applicability to other cetacean species and geographical regions. For instance, research to determine the appropriate density of sampling stations required to ensure effective detection in studies that combine land-based survey and acoustic sampling methods would be particularly useful.

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Diversity, abundance and habitat characteristics of mayflies (Insecta: Ephemeroptera) in Chambok, Kampong Speu Province, southwest Cambodia

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មូលនិយមសង្ខេប

Mayflies (Ephemeroptera) គឺជាលំដាប់មួយនៃសត្វល្អិតទឹក។ ក្នុងដំណាក់កាលជាកូនញាស់ ជីវិតរបស់វាមានទំនាក់ទំនងជាមួយនឹងប្រភេទទីជម្រកផ្សេងៗក្នុងប្រព័ន្ធអេកូឡូស៊ីទឹកសាប។ យើងបានធ្វើការសិក្សាពីនានាភាពនៃចំនួនប្រភេទ ចំនួនឯកត្តៈ និងទំនាក់ទំនងទីជម្រករបស់ Mayflies ក្នុងស្ទឹងមួយ ដែលមិនធ្លាប់មានការសិក្សាពីមុនមក គឺស្ទឹងនៃតំបន់ទេសចរណ៍ធម្មជាតិចំបក់ក្នុងខេត្តកំពង់ស្ពឺ ភាគខាងត្បូងឈៀងខាងលិចនៃប្រទេសកម្ពុជា។ Mayflies ចំនួន១២ប្រភេទ ស្ថិតក្នុង៥អំបូរ ត្រូវបានរកឃើញ។ ចំនួនឯកត្តៈនៃអំបូរ Teloganodidae មានវត្តមានច្រើនជាងគេនៅក្នុងស្ទឹងចំបក់ ដែលមានចំនួន៥២ក្បាល។ បន្ទាប់មកគឺឯកត្តៈនៃអំបូរ Heptageniidae (៣៥ក្បាល), Baetidae (៣៥ក្បាល), Leptophlebiidae (១៨ក្បាល) និង Caenidae (៩ក្បាល)។ យើងរកឃើញថា ចំនួនប្រភេទ តម្លៃនានាភាព (Shannon-Wiener) និងចំនួនឯកត្តៈ មានការកើនឡើងចាប់ពីផ្នែកខាងលើរហូតដល់ផ្នែកខាងក្រោមនៃខ្សែទឹក។ ផ្អែកលើការវិភាគតាមអនុគមន៍លីនេអ៊ែរ ចំនួននិងតម្លៃដែលបានរកឃើញនៅតាមទីតាំងសិក្សានីមួយៗមានទំនាក់ទំនងអវិជ្ជមានជាមួយនឹងកម្រិតកកក្នុងទឹក និងរយៈកម្ពស់ ប៉ុន្តែវាក៏មានទំនាក់ទំនងជាវិជ្ជមានផងដែរជាមួយនឹងបរិមាណគម្របព្រៃប្រស្សី និងវត្តមានថ្មបាតស្ទឹងដែលមានទំហំមធ្យម (៦៥-២៥០ មីលីម៉ែត្រ)។ នៅតាមទីជម្រកក្នុងទីតាំងខ្សែទឹកខាងក្រោម លម្អិតមានលក្ខណៈយឺតៗ កម្រិតកកក្នុងទឹកទាប និងមានគម្របព្រៃជុំវិញ ដែលជាលក្ខខណ្ឌសមប្រកប (ឧ. ជាទីជម្រក និងប្រភពអាហារដ៏ល្អ) សម្រាប់ Mayflies។ លទ្ធផលនៃការសិក្សានេះផ្តល់នូវមូលដ្ឋានគ្រឹះដ៏សំខាន់សម្រាប់ការស្រាវជ្រាវបន្ថែមពីនានាភាព និងរបាយការណ៍របស់ Mayflies នៅក្នុងទីតាំងសិក្សា និងកន្លែងផ្សេងៗទៀតនៃប្រទេសកម្ពុជា។

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Abstract

Mayflies (Ephemeroptera) are an aquatic order of insects whose larval stages are generally associated with different habitat types in freshwater ecosystems. We investigated the species diversity, abundance and habitat associations of mayflies along a previously unstudied freshwater stream in the Chambok area of Kampong Speu Province, southwest Cambodia. Twelve mayfly taxa belonging to five families were recorded. Members of the Teloganodidae dominated the study stream with 52 individuals, followed by Heptageniidae (35 individuals), Baetidae (35), Leptophlebiidae (18) and Caenidae (9). We found that the taxonomic richness, Shannon-Wiener's diversity and abundance of mayflies increased from upstream to downstream sections of the stream. Based on linear regression models, these patterns were negatively associated with water turbidity and altitude, but positively associated with bamboo cover and availability of medium-sized stone substrates (64–250 mm) at sampling sites. Habitats at the downstream sampling sites were characterised by slow-flowing water, lower water turbidity and surrounding forest cover, all of which are typically considered to provide suitable conditions (e.g., good shelter and food sources) for mayflies. Our results provide a useful baseline for further investigations on the diversity and distribution of mayflies in the study site and elsewhere in Cambodia.

Keywords Ephemeroptera, Shannon-Wiener diversity, environmental parameter, habitat quality, water quality.

Introduction

Mayflies (Ephemeroptera) are a small order of insects which are characterised by their distinct nymph morphology: seven pairs of abdominal gills, three caudal filaments and mouthparts (Barber-James *et al.*, 2008). The order is an ancient group of aquatic insects which is composed of approximately 440 genera belonging to 40 families with 3,330 described species (Sartori & Brittain, 2015). Mayflies are longer in the aquatic nymph stage compared to their winged stage (subimago & imago) and undergo a series of ecdysis as they grow (Sartori & Brittain, 2015). They commonly possess soft-bodies, which are small to medium-sized, and bear gills along the sides of the abdomen and two or three segmented caudal filaments (Borror *et al.*, 1989). A variety of morphological adaptations also define their functional feeding behaviours and specific microhabitat preferences (Sartori & Brittain, 2015).

Mayfly nymphs inhabit lentic and lotic freshwater habitats (Khoo, 2004), although their diversity and abundance are usually higher in the latter (Barber-James *et al.*, 2008). They commonly forage on particulate organic matter, macrophytes, algae, and periphyton on substrates (i.e. stones, silt and aquatic vegetation) (Sartori & Brittain, 2015) and their local species diversity depends greatly on habitat characteristics such as temperature, water quality, altitude, food availability and water flow velocity (Brittain & Sartori, 2009). For instance, high species richness has been found in shallow littoral stream habitats with slow current velocities and clean water (Sartori & Brittain, 2015; Vilenica *et al.*, 2018). Conversely, the diversity of mayfly nymphs is lower in deeper streams with high sediment loads and water pollution (Extence *et al.*,

2011; Sartori & Brittain, 2015). Due to their sensitivity, mayflies are regarded as useful bio-indicators of water quality, particularly good quality waters with high levels of dissolved oxygen (Bauernfeind & Moog, 2000). Partly as a result, many studies have investigated the relationship between environmental factors (e.g., physical and chemical variables) and mayfly species richness, abundance and assemblage composition (Minshall *et al.*, 1985; Hawkins *et al.*, 1997; Khoo, 2004; Finn & Poff, 2005; Gustafson, 2008; Ross *et al.*, 2008; Arimoro & Muller, 2010).

Numerous studies have shown that mayfly diversity and abundance are threatened by anthropogenic activities including watercourse alterations (including small dams or weirs along mountain streams), habitat fragmentation, forest degradation and water pollution (Bauernfeind & Moog, 2000; Benstead & Pringle, 2004; Lange *et al.*, 2018). However, understanding of their status in countries such as Cambodia is currently limited by a lack of studies. Because so few studies have been undertaken to date, the same knowledge gap also applies to many other aquatic insect groups in the country. Those that have include site-based assessments of the diversity of aquatic Polyphaga (Freitag *et al.*, 2018), a national checklist for aquatic Hemiptera (Zettel *et al.*, 2017), studies of relationships between water quality and aquatic insects in Phnom Penh city (Chhy *et al.*, 2019) and the distribution patterns of macroinvertebrates in the Lower Mekong Basin, including Cambodia (Sor *et al.*, 2017). As such, investigations of mayflies and their habitat associations are needed to improve basic understanding of the group in Cambodia. To this end, we investigated the species diversity, abundance and habitat associations of

mayflies along a previously unstudied freshwater stream in southwest Cambodia.

Methods

Study site

Our study was conducted on an elevational gradient along a freshwater mountain stream in the Chambok Community-Based Ecotourism Site, which is located in Chambok Commune, Phnom Sruoch District, Kampong Speu Province, southwest Cambodia. Sampling was undertaken in three undisturbed locations at higher elevations of the stream (site codes S01–S03), four locations at intermediate elevations (S04–S07), and three locations in lower elevation areas characterised by bamboo forest and patches of agriculture (S08–S10) (Table 1). One of the sampling sites (S04) included a waterfall and semi-natural pool which is regularly visited by tourists and used by local people for its clean water.

Field sampling

Sampling was undertaken on one occasion at each of the ten locations selected from 13–15 August 2018. Mayfly samples were collected in different areas of the stream at each location i.e. along both banks and in the mid-stream area. Each area was sampled for 30 minutes by sweeping hand-held nets with a 0.1 mm mesh size in the water and brushing the surfaces of microhabitats and substrates (e.g., stones, dead wood and leaves) into nets.

Table 1 Sampling locations, elevations and dates in Chambok, Kampong Speu Province, southwest Cambodia.

Site	Latitude, Longitude	Elevation (m)	Sampling Date
S01	11.21251°N, 104.06108°E	425	13 August
S02	11.21277°N, 104.06143°E	394	13 August
S03	11.21313°N, 104.06123°E	391	13 August
S04	11.21534°N, 104.06124°E	293	14 August
S05	11.2159°N, 104.06173°E	241	14 August
S06	11.22039°N, 104.06267°E	197	14 August
S07	11.22071°N, 104.06356°E	150	15 August
S08	11.2214°N, 104.06427°E	133	15 August
S09	11.22246°N, 104.06448°E	117	15 August
S10	11.22307°N, 104.06499°E	116	15 August

The contents of nets were frequently transferred into a white tray for sorting and to avoid overloading of nets. This material was then slowly rinsed and all mayflies present were gently removed by hand with forceps and preserved in labelled plastic vials containing 97% ethanol. These specimens were later sorted in the laboratory and identified to genus level using keys provided by MRC (2006), Khoo (2004) and Sartori *et al.* (2008).

Water temperature, conductivity and turbidity were measured at each sampling location using a HI 7609829 Multiparameter, Portable Water Quality Meter (Hanna Instruments Ltd., Bedfordshire, UK). Additional variables recorded included altitude, water depth, stream width and flow velocity. The percentage cover of different stream substrates in each sampling location was measured. Three categories of substrate were employed for this purpose, based on the size of stones present: 'large', stones >250 mm; 'medium', stones 64–250 mm; 'small', stones 2–64 mm. Land cover (i.e. forest, agriculture, residential areas, orchards, bamboo and shade) were measured using the AusRivAS (Parsons *et al.*, 2002) and RHS protocols (Raven *et al.*, 1998). The results of these measurements are provided in Table 2.

Statistical analyses

We used three measures to analyse the mayfly fauna of our study sites: taxonomic richness, Shannon-Wiener's diversity and abundance. Shannon-Wiener's diversity (H) was calculated using the *vegan* package of R (Oksanen *et al.*, 2015). Multiple linear regression (MLR) models were first used to test for associations between environmental variables and mayfly diversity and abundance, after which stepwise selection was employed to retain the most important variables. Each response variable (taxonomic richness, abundance and diversity) was regressed (univariate regression) against each of the remaining significant environmental variables. The response of taxonomic richness, diversity and abundance to environmental factors was assessed using the standardized regression coefficient, whereas the performance of regression was evaluated using the coefficient of determination (adjusted R²). Probability values of <0.05 were considered significant. All statistical analyses were performed in R software vers. 3.6.2 (R Core Team, 2019).

Results

We collected and identified a total of 149 mayfly nymphs belonging to 12 taxa, 10 genera and five families. Members of the Teloganodidae dominated the study stream with 52 individuals, followed by Heptageniidae (35 individuals), Baetidae (35), Leptophlebiidae (18) and Caenidae

Table 2 Environmental variables recorded at sampling sites in Chambok, Kampong Speu Province, southwest Cambodia.

Environmental variable	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
Forest (%)	70	100	80	80	60	70	60	20	20	10
Agriculture (%)	0	0	0	0	0	0	0	45	50	40
Bamboo (%)	30	0	20	20	40	30	40	35	30	50
Shade (%)	75	80	95	75	95	70	92	40	40	60
Dead wood (%)	1	2	2	1	2	2	2	2	1	1
Large stones (>250mm) (%)	60	50	45	65	45	48	55	65	55	35
Medium stones (64–250mm) (%)	15	25	15	25	15	20	20	10	25	25
Small stones (2–64mm) (%)	10	15	10	5	20	15	7	10	7	25
Sand (<2mm) (%)	10	8	10	3	10	10	5	10	3	10
Silt/clay (%)	5	2	20	2	10	7	13	5	10	5
Water depth (m)	0.37	0.47	0.24	0.44	0.42	0.40	0.45	0.46	0.29	0.24
Stream width (m)	6.6	5.6	10.7	20.5	9.8	13.5	8.8	6.2	6.6	8.0
Velocity (m/s)	1.27	1.50	2.45	2.10	1.95	1.90	2.36	1.03	3.90	2.75
Altitude (m)	425	394	391	293	241	197	150	133	117	116
Conductivity (EC.uS.cm)	14	14	14	12	13	13	11	12	13	14
Total dissolved solid (ppmTds)	7	7	7	6	7	7	6	6	6	7
Turbidity (FNU)	19.9	12.2	24.0	11.6	10.9	11.2	12.4	11.7	10.3	10.2
Temperature (°C)	23.71	23.77	23.70	23.69	24.07	24.23	24.42	24.42	24.24	24.19

(9). The twelve taxa identified comprised *Dudgeodes* sp. (52 individuals; Fig. 1), *Cinygmima* sp. 1 (6), *Cinygmima* sp. 2 (12), *Thalerosphyrus* sp. (15; Fig. 1), *Asionurus* sp. (2), *Liebebiella* sp. 1 (28), *Liebebiella* sp. 2 (3), *Labiobaetis* sp. (2), *Platybaetis* sp. (2), *Choroterpes* sp. (2), *Isca* sp. (16) and *Caenoculis* sp. (9).

Taxonomic richness, diversity & abundance

The distribution of taxonomic richness, diversity and abundance of mayflies across our sampling sites is shown in Fig. 2. In summary, all three values increased from upstream to downstream sections of the stream, although mayfly abundance was relatively low at two of the downstream sampling sites (S07 and S08).

Habitat associations

Four environmental variables were significantly associated with our response variables in the MLR models and stepwise selection process. Water turbidity and altitude were negatively associated with taxonomic richness.

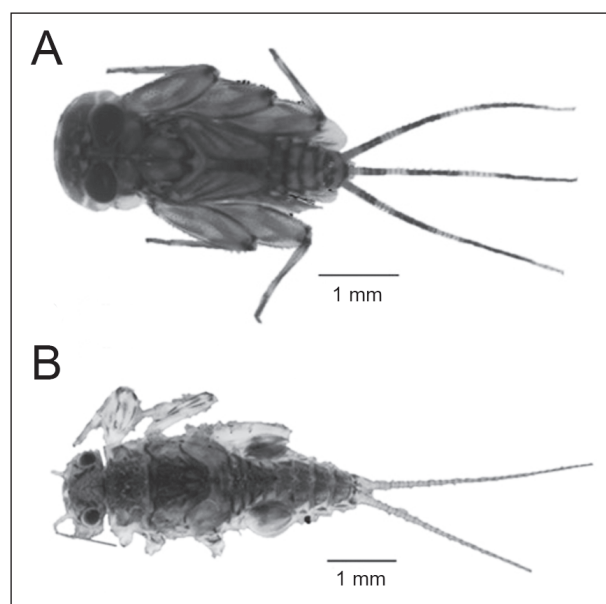


Fig. 1 Two mayfly taxa recorded during the study in Chambok, Kampong Speu Province, southwest Cambodia: A) *Thalerosphyrus* sp., B) *Dudgeodes* sp.

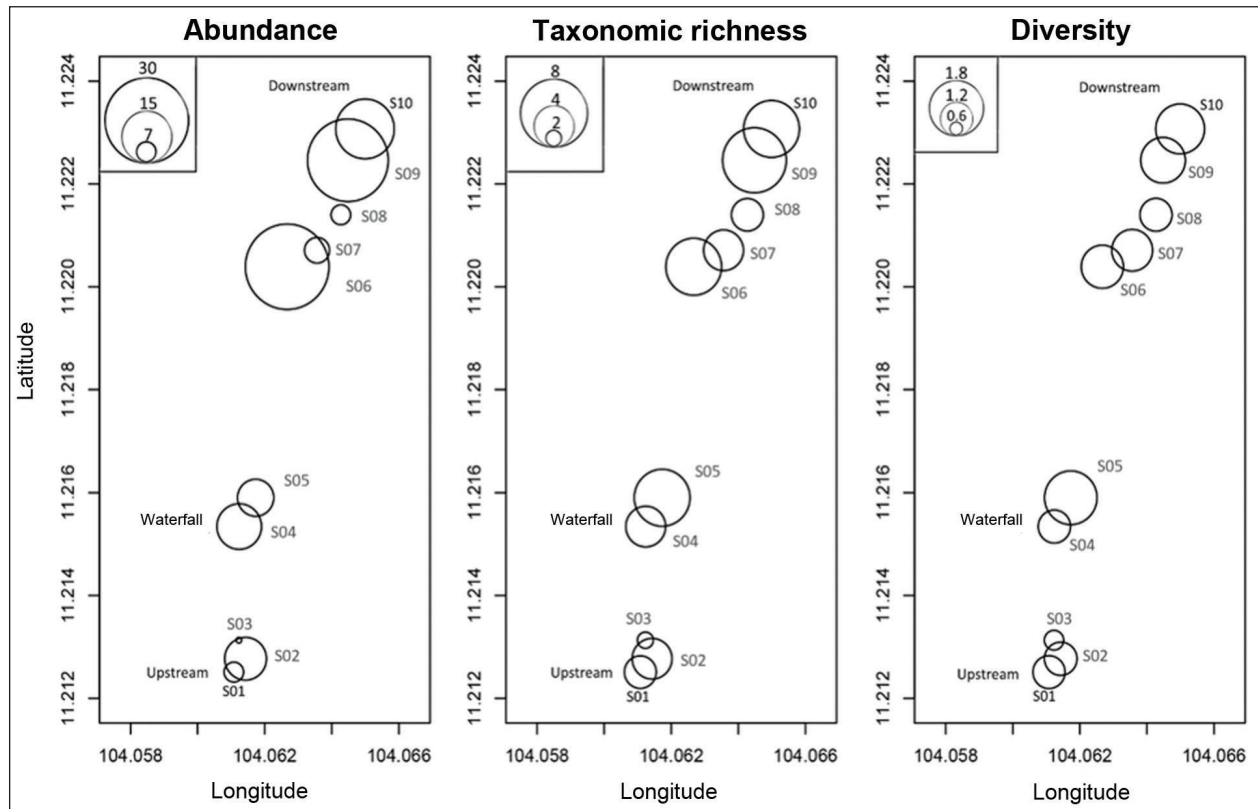


Fig. 2 Bubble plots showing the distribution of mayfly abundance, taxonomic richness and Shannon-Wiener's diversity along the study stream in Chambok, Kampong Speu Province, southwest Cambodia.

ness, diversity and abundance (Figs. 3A-B), whereas the percentage cover of bamboo and medium-sized stone substrates were positively associated with diversity and abundance (Figs. 3C-D).

Discussion

Ours is the first study of mayflies in the Chambok area of Cambodia, although we only recorded 12 taxa arranged in 10 genera and five families. These figures are relatively low compared to other studies of aquatic insects, water quality and habitat characteristics (Bauernfeind & Moog, 2000; Siegloch *et al.*, 2008; Sor *et al.*, 2017; Chhy *et al.*, 2019). *Dudgeodes* sp. (Teloganodidae) was the dominant taxon in our study, being abundant at all of our sample sites. This may be due to the fact that it can apparently survive low and high turbidity environments, being found in waters at Chambok with turbidity values of 10.2 FNU and 24.0 FNU, respectively. Members of the genus also occur in relatively undisturbed habitats surrounded by secondary forest and highly disturbed areas including settlements (Garces *et al.*, 2020). *Dudgeodes* species have also been found in fast-flowing waters with sand or

gravel substrates (Sartori *et al.*, 2008) and studies outside the region have found that Teloganodidae are the dominant family (Harrison & Agnew, 1962). In contrast, *Caenoculis* sp. (Caenidae) was the least common taxon in our study and only occurred at five sampling sites characterised by high vegetation cover and medium water flows. Members of the genus may be indicative of good quality or slightly polluted waters (Alhejoj *et al.*, 2014).

Habitat associations

We found that the taxonomic richness, Shannon-Wiener's diversity and abundance of mayflies increased from upstream to downstream sections of our study stream. Previous studies have shown that water flow rates, turbidity and forest cover are important drivers of mayfly distributions (Ogbogu & Akinya, 2001; Rueda *et al.*, 2002; Buss & Salles, 2007) and that slow-flowing and less turbid waters, with medium-sized stone substrates and surrounding forest cover may provide more suitable micro-habitats for mayflies (Siegloch *et al.*, 2008; Sartori & Brittain, 2015; Vilenica *et al.*, 2018). All of these factors likely reflect our finding of high mayfly diversity

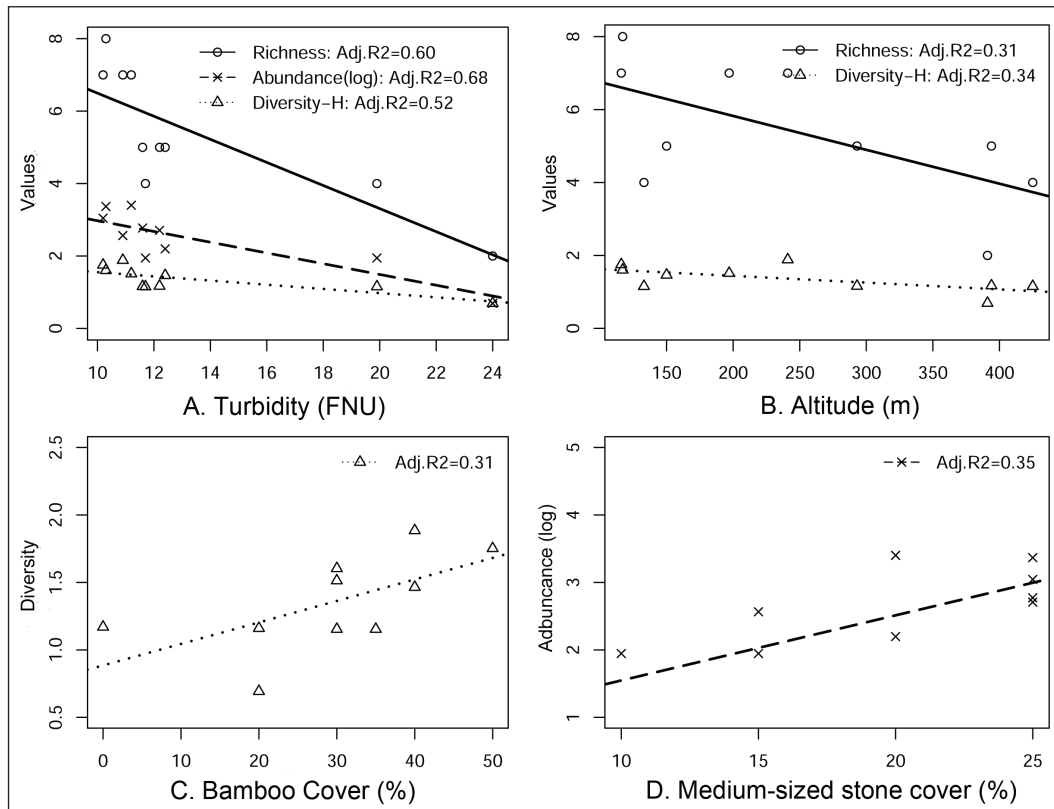


Fig. 3 Linear regression models of environmental variables significantly associated with the taxonomic richness, diversity and abundance of mayflies at the study site. Adj.R2 = Adjusted R-square (coefficient of determination).

in downstream sampling sites (S06–S10), where average water turbidity was 11.16 ± 0.93 FNU, medium-sized stone cover was $20 \pm 6.12\%$ and bamboo cover was $37 \pm 8.37\%$. This was supported by our regression models. Bamboo forest cover may provide good habitats and food sources for insects (Siegloch *et al.*, 2008; Sor *et al.*, 2017), whereas medium-sized stones might provide suitable ecological niches for foraging (Siegloch *et al.*, 2008). However, one exception was found in the form of low mayfly abundance at S07 and S08, although their taxonomic richness and diversity were relatively comparable to other downstream sites. Further investigations are warranted to explore our observations at these sites.

The diversity of mayflies was somewhat lower in the upstream portions of our study stream where our sampling sites were characterised by large stone substrates, fast-flowing waters and high turbidity. High levels of turbidity resulting from greater sediment loads in fast-flowing waters can reduce the availability of certain food sources (e.g., macrophytes, algae and diatoms) (Sartori & Brittain, 2015) for mayflies and our regression models suggest that water turbidity is negatively associated with mayfly diversity. Upstream

locations also naturally occur at higher altitudes where most adult female mayflies typically lay their eggs, which subsequently get washed downstream (Robinson, 2005). As such, this factor may have contributed to the higher mayfly diversity we observed in the downstream sections of the stream.

While our results provide useful insights into factors influencing the taxonomic richness, diversity and abundance of mayflies in Chambok, they are based on a single sampling event and so could primarily reflect the prevailing conditions at that time. As such, additional sampling with replication should be undertaken over time to assess whether the patterns we observed are indeed typical for the mayfly fauna of Chambok. It would also be interesting to explicitly test if our finding that the percentage cover of medium-sized stone substrates is positively associated with mayfly diversity holds true on a larger temporal and spatial scale. If found to be the case, this could have practical implications for restoration and management of degraded stream habitats. Taken as a whole, our study provides a useful baseline for further investigations of the diversity and distribution of mayflies in Chambok and elsewhere in Cambodia.

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Diet preferences of insectivorous bats (Mammalia: Chiroptera) in Chambok, Kampong Speu Province, Cambodia

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មូលនិយមសង្ខេប

សត្វប្រៀវដើរតួនាទីយ៉ាងសំខាន់ក្នុងការកំចាត់សត្វល្អិតចង្រៃ។ ដោយហេតុថា ការស្រាវជ្រាវពីបំណែកសត្វល្អិតក្នុងលាមកសត្វប្រៀវនៅមានកម្រិតនៅឡើយ យើងបានប្រមូលសំណាកលាមកប្រៀវពីតំបន់ទេសចរណ៍ធម្មជាតិចំបក់ ក្នុងខេត្តកំពង់ស្ពឺ ដើម្បីធ្វើការសិក្សា និងប្រៀបធៀបពីប្រភេទអាហារដែលវាចូលចិត្ត។ សំណាកលាមកចំនួន២៩៤គ្រាប់ ត្រូវបានប្រមូលពីសត្វប្រៀវ ៥ប្រភេទ (*Rhinolophus shameli*, *R. pusillus*, *Hipposideros pomona*, *H. cf. larvatus* និង *Megaderma spasma*) ដែលក្នុងចំណោមនោះ ១២០គ្រាប់ត្រូវបានយកទៅពិនិត្យជាមួយមីក្រូទស្សន៍។ សំណាកលាមករបស់ប្រៀវទាំងអស់មានបំណែករបស់សត្វល្អិតចំនួន៩៧ដាប់ ហើយបំណែកមួយចំនួនទៀតជារបស់ពពួកចៃ និងកណ្តុរ។ លំដាប់សត្វល្អិតដែលសត្វប្រៀវចូលចិត្តស៊ីច្រើនបំផុតគឺ Lepidoptera និង Coleoptera និងបន្ទាប់មកគឺ Heteroptera, Hymenoptera, Homoptera និង Diptera។ ចំណែកដទៃទៀតដែលសត្វប្រៀវមិនសូវចូលចិត្តស៊ីមាន Isoptera, Trichoptera និង Orthoptera និងពពួកចៃ និងកណ្តុរ។ លទ្ធផលបានបង្ហាញទៀតថា សត្វប្រៀវ rhinolophid និង hipposiderid ចូលចិត្តស៊ីសត្វល្អិតក្នុងបរិមាណខុសៗគ្នាក្នុងកម្រិតជឿជាក់ រីឯប្រៀវប្រភេទ *M. spasma* មានចំណូលចិត្តស៊ីសត្វល្អិតក្នុងកងតូចៗ។ ការសិក្សាស្រាវជ្រាវបន្ថែមពិតជាចាំបាច់ដើម្បីបង្កើនចំណេះដឹងអំពីចំណូលភាពនៃចំណីអាហាររបស់សត្វប្រៀវទាំងអស់ដែលមានវត្តមាននៅកម្ពុជា ពីព្រោះសត្វប្រៀវចូលរួមយ៉ាងសំខាន់ក្នុងការផ្តល់សេវាកម្មប្រព័ន្ធអេកូឡូស៊ីដែលទាក់ទងនឹងសេដ្ឋកិច្ច។

Abstract

Bats provide economically significant ecosystem services in insect pest suppression. As little is known regarding the diets of insectivorous bats in Cambodia, we sampled these in the Chambok area of Kampong Speu Province in August 2018 so as to describe and compare their specific diet preferences. Our field survey yielded 294 faecal pellets produced by five insectivorous species (*Rhinolophus shameli*, *R. pusillus*, *Hipposideros pomona*, *H. cf. larvatus* and *Megaderma spasma*), 120 of which were examined by microscopy. These samples contained fragments representing nine insect

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orders, and included fragments of mites (Acari) and Rodentia. The most common orders in terms of their percentage frequency of occurrence were Lepidoptera and Coleoptera, followed by Heteroptera, Hymenoptera, Homoptera and Diptera, whereas the least common were Rodentia, Acari, Isoptera, Trichoptera and Orthoptera. Comparisons based on the percentage volume of the most common orders indicated significant differences in the amounts consumed by rhinolophid and hipposiderid bats, whereas *M. spasma* showed a preference for small vertebrates. Further studies are warranted to develop understanding of the diet preferences of Cambodian bats, not least because these likely give rise to economically significant ecosystem services.

Keywords Ecosystem services, frequency percentage, frequency volume, *Hipposideros*, *Megaderma*, *Rhinolophus*.

Introduction

Insectivorous bats provide economically significant ecosystem services in insect pest consumption (Boyles *et al.*, 2011). For example, the value of these services to agriculture in the continental USA has been estimated at approximately US\$ 22.9 billion /year, including the reduced costs of pesticides that are not needed to suppress the insects consumed by bats (Boyles *et al.*, 2011). In Thailand, populations of the wrinkle-lipped free-tailed bat *Mops plicatus* (Molossidae) are regarded as important pest control agents because they may consume as many as 55 tons of insects per night, a significant portion of which comprises major agricultural pests (Leelapaibul *et al.*, 2005).

Analysis of bat faeces can provide valuable information on the insect taxa consumed by bat species. These have shown that insect orders commonly consumed by insectivorous bats include Homoptera, Coleoptera, Heteroptera, Lepidoptera, Diptera, Hymenoptera, Neuroptera, Trichoptera, Odonata and Araneae (Leelapaibul *et al.*, 2005; Oliveira *et al.*, 2015). Some studies have shown that certain bat species mostly consume soft-bodied insects such as moths, whereas other species are less specific in their preferences (Bogdanowicz *et al.*, 1999; Ghazali & Dzeverin, 2013; Weterings & Umponstira, 2014).

At least 80 bat species including 68 animalivorous taxa are presently known to occur in Cambodia (Kingsada *et al.*, 2011; Neil Furey, unpublished data), although this figure is relatively low compared to neighbouring countries such as Lao PDR with ≈ 100 bat species, Thailand with ≈ 146 species and Vietnam with ≈ 125 species (Soisook 2011; Kruskop 2013; Thomas *et al.*, 2013; Karapan *et al.*, 2016; Neil Furey, unpublished data). Aside from a single study on the cave nectar bat *Eonycteris spelaea* (Pteropodidae) (Hoem *et al.* 2017), nothing has been published regarding the diet preferences of bats inhabiting the country to date. To address this gap, we sampled insectivorous bats in the Chambok area of southwest Cambodia in August 2018 with the aim of documenting and comparing their diet preferences.

Methods

Study area

Our study was undertaken in the Chambok Community-Based Ecotourism Site (hereafter 'Chambok') in Kampong Speu Province, southwest Cambodia. Chambok occupies a total area of 8,257 ha and borders Kirirom National Park (Lonn, 2013). The site includes three areas under different forms of community-based management: 1) community forestry areas established by the Forest Administration in 2005, which cover 286 ha and border the national park; 2) community protected areas created by the Ministry of Environment in 2002, which occupy 758 ha within the national park; and 3) community-based ecotourism areas created by the Ministry of Environment in 2003, which extend over 161 ha inside the national park (FAO, 2012). Four villages with a total population of 3,670 people are located within Chambok (Chambok Dangcum, Beng, Krangchek and Thmei). The natural vegetation of the Chambok landscape includes bamboo, degraded semi-evergreen forests and grasslands with deciduous forest.

Field sampling

We employed two four-bank harp traps and one mist net to sample seven sites in Chambok in August 2018. Six of these sites (S01–S06) were located within the interior of semi-evergreen forests at the site, whereas one site (S07) was situated in bamboo forest adjacent to agricultural land (Table 1). Our harp traps and mist net were employed from 18:00 to 21:00 hrs on a single occasion at each site. All insectivorous bats captured in the harp traps and net were gently removed by hand and retained in individual cloth bags until the following morning when their faeces were collected. Before release, each bat was identified to species based on its external morphology and standard measurements for the group using Francis (2008). The faecal pellets produced by each bat overnight were transferred from their individual cloth bags with soft forceps into appropriately labelled vials containing

70% ethanol and the bags used to retain individual bats were thoroughly cleaned before each reuse.

Faecal analysis

Insectivorous bats chew their prey into small fragments, the less digestible portions of which are passed into their faeces. The faecal samples obtained from bats in Chambok were processed by randomly selecting and placing one pellet at a time on a petri dish containing a few drops of glycerol and teasing this apart with a pair of needles under an Olympus SZ51 microscope. All insect fragments present in each faecal pellet (e.g., legs, wings, antennae) were then mounted onto slides for observation under an Olympus CX23 microscope and photographed using an OPTIKA Microscope P6 Pro Camera. These were sorted according to size and identified to order level using McAney *et al.* (1997), Pokhrel & Budha (2014) and Ponmalar & Vanitharani (2014).

Data analysis

Following identification, we visually estimated the percentage volume of each insect order in each faecal pellet by placing the relevant fragments on paper with six 1 x 1 cm grid squares (Fig. 1). In each instance, the number of squares employed for this purpose depended on the quantity of material for a given order in each faecal pellet. We then calculated the percentage frequency (PF) and percentage volume (PV) of each insect order using the following formulae (Kunz & Parsons, 2009):

$$PF = \frac{\text{number of fecal pellets in which food items present}}{\text{total number of fecal pellets}} \times 100$$

$$PV = \frac{\text{sum of the individual volume}}{\text{total volumes of samples}} \times 100$$

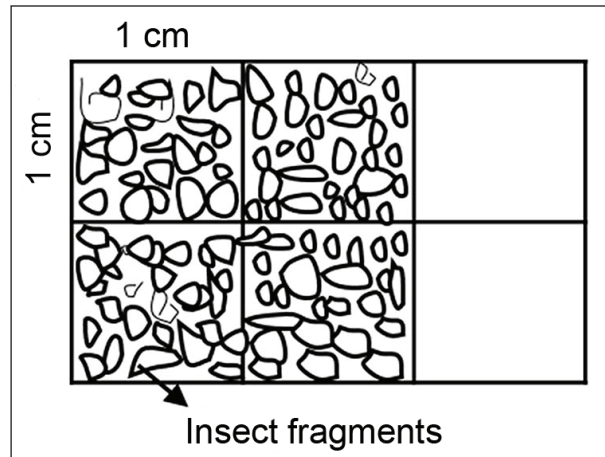


Fig. 1 Grid scheme used to visually estimate the percentage volume of a given animal order in faecal samples.

As such, PF represents the average occurrence of each food type (insect order) consumed, whereas PV represents the average percentage volume of each food type. We employed PV values to classify each food type into one of four categories following Shetty & Sreepada (2013): 1) PV >20% representing ‘preferred food’; 2) PV 5–20% representing ‘frequent food’; 3) PV 1–5% representing ‘supplementary food’; 4) PV <1% representing ‘incidental food’. Where more than 10 faecal pellets were obtained for a bat species, Kruskal Wallis tests were used to test for significant differences in the PV values of each food type between bat species. Probability values <0.05 were regarded as significant and all statistical tests were undertaken using *IBM SPSS Statistics for Windows* vers. 21.0 (IBM, New York, USA).

Table 1 Sampling sites in Chambok, Kampong Speu Province, southwest Cambodia.

Site	Latitude, Longitude	Elevation (m)	Sampling Date	Habitat
S01	11°21'29.40"N, 104°06'09.0"E	407	13 August 2018	Semi-evergreen forest, ≈60 m from a stream
S02	11°21'39.6"N, 104°06'07.2"E	371	13 August 2018	Semi-evergreen forest along a stream
S03	11°21'45.5"N, 104°06'07.9"E	374	14 August 2018	Semi-evergreen forest with bamboo
S04	11°21'59.9"N, 104°06'13.7"E	266	15 August 2018	Mixed bamboo and semi-evergreen forest, ≈400m from a cave bat colony
S05	11°22'06.9"N, 104°06'25.0"E	203	15 August 2018	Mixed bamboo and semi-evergreen forest
S06	11°22'10.2"N, 104°06'33.1"E	177	16 August 2018	Mixed bamboo and semi-evergreen forest
S07	11°22'22.1"N, 104°06'41.4"E	123	16 August 2018	Bamboo forest and agriculture land use

Results

We captured 58 individuals of five bat species belonging to three families: *Rhinolophus shameli*, *R. pusillus* (Rhinolophidae), *Hipposideros pomona*, *H. cf. larvatus* (Hipposideridae) and *Megaderma spasma* (Megadermatidae) (Fig. 2; Table 2). These provided a total of 294 faecal pellets, 120 of which were randomly selected for analysis (Tables 2 & 3).

The faecal samples included a total of nine insect orders (Coleoptera, Lepidoptera, Heteroptera, Homoptera, Diptera, Orthoptera, Hymenoptera, Trichoptera and Isoptera), as well as mites (Acari) and rodent fragments (Rodentia) (Fig. 3; Table 3). Insect fragments identified in faecal pellets produced by *R. pusillus* included eight insect orders, whereas those produced by *R. shameli*, *H. pomona* and *H. cf. larvatus* comprised six insect orders and one mite order. Food types observed in faeces produced by *M. spasma* differed greatly in including just two insect orders and fragments of a rodent (Table 3).

The most commonly eaten insect orders in terms of percentage frequency were Lepidoptera and Coleoptera, followed by Heteroptera, Hymenoptera, Homoptera and Diptera, whereas the least common were Rodentia,

Table 2 Sample sizes of bat species and faecal pellets obtained in Chambok, August 2018.

Species	<i>n</i>	Male	No. ♂ pellets	Female	No. ♀ pellets
<i>Rhinolophus shameli</i>	25	4	41	21	77
<i>R. pusillus</i>	19	10	67	9	38
<i>Hipposideros pomona</i>	5	-	-	5	58
<i>H. cf. larvatus</i>	8	4	-	4	11
<i>Megaderma spasma</i>	1	-	-	1	2
Total	58	18	108	40	186

Acari, Isoptera, Trichoptera and Orthoptera (Table 3). Based on percentage volume, *R. shameli* apparently preferred three insect orders (Lepidoptera, Coleoptera and Hymenoptera, with PV values of 33.3%, 31% and 20%, respectively), whereas *R. pusillus* preferred a single insect order (Lepidoptera, PV=38%) (Table 4, Fig. 4). In



Fig. 2 Bat species captured in August 2018 in Chambok: A) *Rhinolophus shameli*, B) *R. pusillus*, C) *Hipposideros pomona*, D) *H. cf. larvatus*, E) *Megaderma spasma* (© Phauk S.).

contrast, *H. pomona* preferred Heteroptera (55.7%), while *H. cf. larvatus* preferred Heteroptera (54.4%) and Coleoptera (35.6%). Food types consumed on a supplementary or incidental basis included the orders Orthoptera, Trichoptera, Isoptera and Acari. Not surprisingly, the percentage volume of the most common six insect orders differed significantly between four bat species (all except *M. spasma* which was excluded from comparisons due to low sample sizes) (Fig. 4; Table 4).

Discussion

Our study represents the first published information on the diets of insectivorous bats in Cambodia. All of the

bat species we sampled are currently considered Least Concern (IUCN, 2020) and typically regarded as cave-roosting taxa, although some also roost in anthropogenic sites (e.g., temples, mines or houses) or hollow trees (Francis, 2008).

We found the most common insect orders consumed by bats in Chambok in terms of percentage frequency were Lepidoptera and Coleoptera, followed by Heteroptera, Hymenoptera, Homoptera and Diptera, whereas the least common were Rodentia, Acari, Isoptera, Trichoptera and Orthoptera. Comparisons based on percentage volume indicated significant differences in the relative amounts of six insect orders consumed by rhinolophid and hipposiderid bats, whereas *M. spasma* showed a pref-

Table 3 Percentage frequency of food types consumed by bat species in Chambok. Abbreviations: Col=Coleoptera, Lep=Lepidoptera, Het=Heteroptera, Hym=Hymenoptera, Hom=Homoptera, Dip=Diptera, Ort=Orthoptera, Tri=Trichoptera, Iso=Isoptera, Aca=Acari, Rod=Rodentia.

Species	No. of pellets	Col	Lep	Het	Hym	Hom	Dip	Ort	Tri	Iso	Aca	Rod
<i>Rhinolophus shameli</i>	40	78	90	53	68	20	30	-	-	-	5	-
<i>R. pusillus</i>	47	77	94	43	49	55	77	2	6	-	-	-
<i>Hipposideros pomona</i>	20	25	100	95	60	85	-	-	-	45	5	-
<i>H. cf. larvatus</i>	11	73	45	91	18	9	18	-	-	-	18	-
<i>Megaderma spasma</i>	2	100	100	-	-	-	-	-	-	-	-	100

Table 4 Percentage volume of food types consumed by bat species in Chambok. Abbreviations for each type are provided in Table 3 above. Asterisks indicate significant differences in values for specific insect orders between bat species (** $P < 0.01$, *** $P < 0.001$).

Species	No. of pellets	Col ***	Lep ***	Het ***	Hym **	Hom ***	Dip ***	Ort	Tri	Iso	Aca	Rod
<i>Rhinolophus shameli</i>	40	31.0 ±5.3	33.3 ±4.7	11.4 ±2.5	20.0 ±3.7	2.3 ±1.1	1.7 ±0.5	-	-	-	0.3 ±0.2	-
<i>R. pusillus</i>	47	14.2 ±2.4	38.0 ±3.9	11.0 ±3.4	10.3 ±1.8	17.2 ±2.8	8.9 ±1.8	0.2 ±0.2	0.3 ±0.2	-	-	-
<i>Hipposideros pomona</i>	20	4.9 ±2.6	12.2 ±2.1	55.7 ±4.4	6.3 ±1.9	18.6 ±2.7	-	-	-	2.3 ±0.8	0.1 ±0.1	-
<i>H. cf. larvatus</i>	11	35.6 ±11	3.6 ±1.6	54.4 ±12.8	2.1 ±1.4	2.5 ±2.5	1.2 ±0.9	-	-	-	0.7 ±0.5	-
<i>Megaderma spasma</i>	2	4.0 ±1	7.5 ±0.5	-	-	-	-	-	-	-	-	88.5 ±1.5

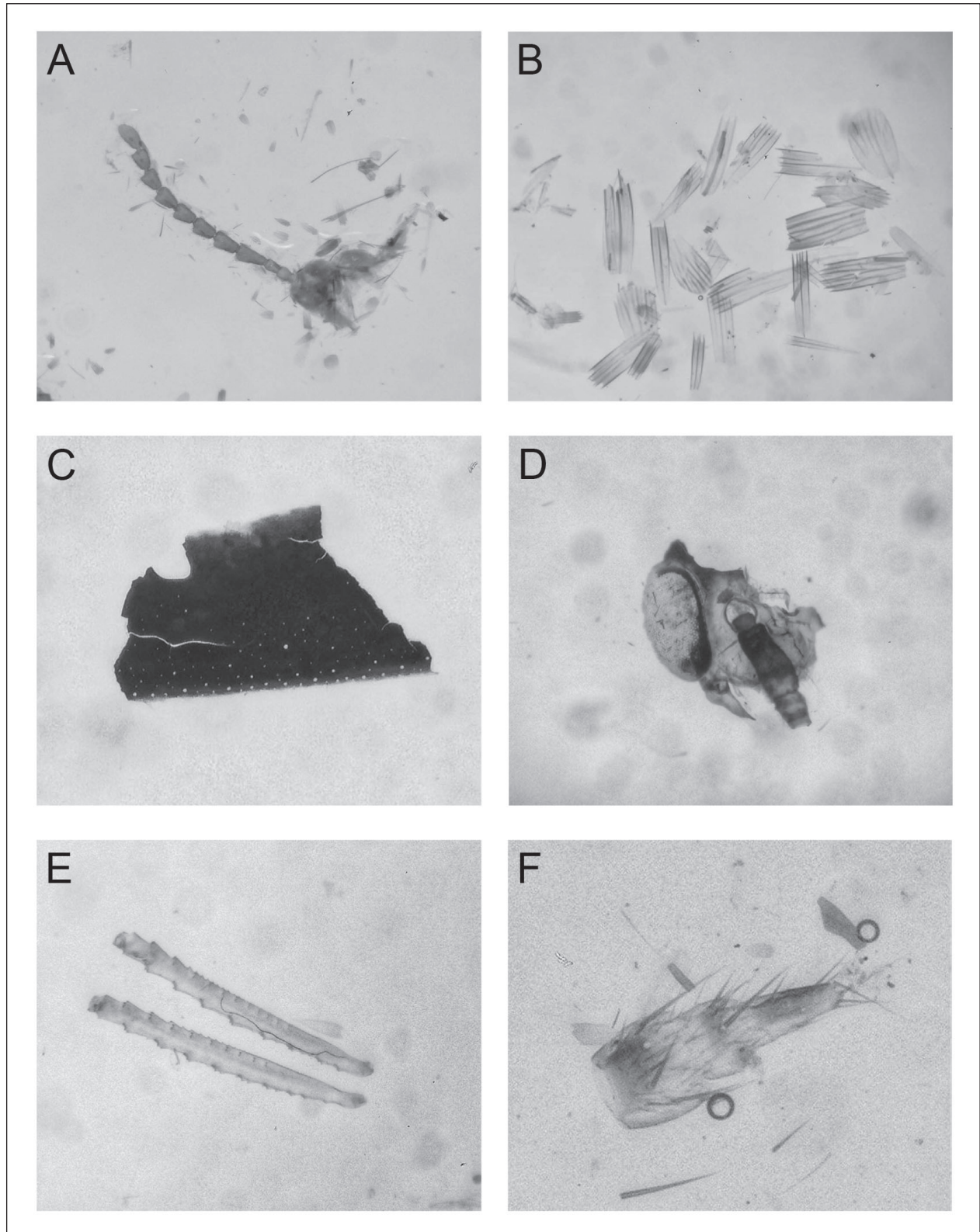


Fig. 3 Examples of insect fragments commonly found in bat faecal samples from Chambok: A) Coleoptera—antennae, B) Lepidoptera—wing scales, C) Heteroptera—forewing, D) Hymenoptera—head, E) Homoptera—hind limbs, F) Diptera—abdomen.

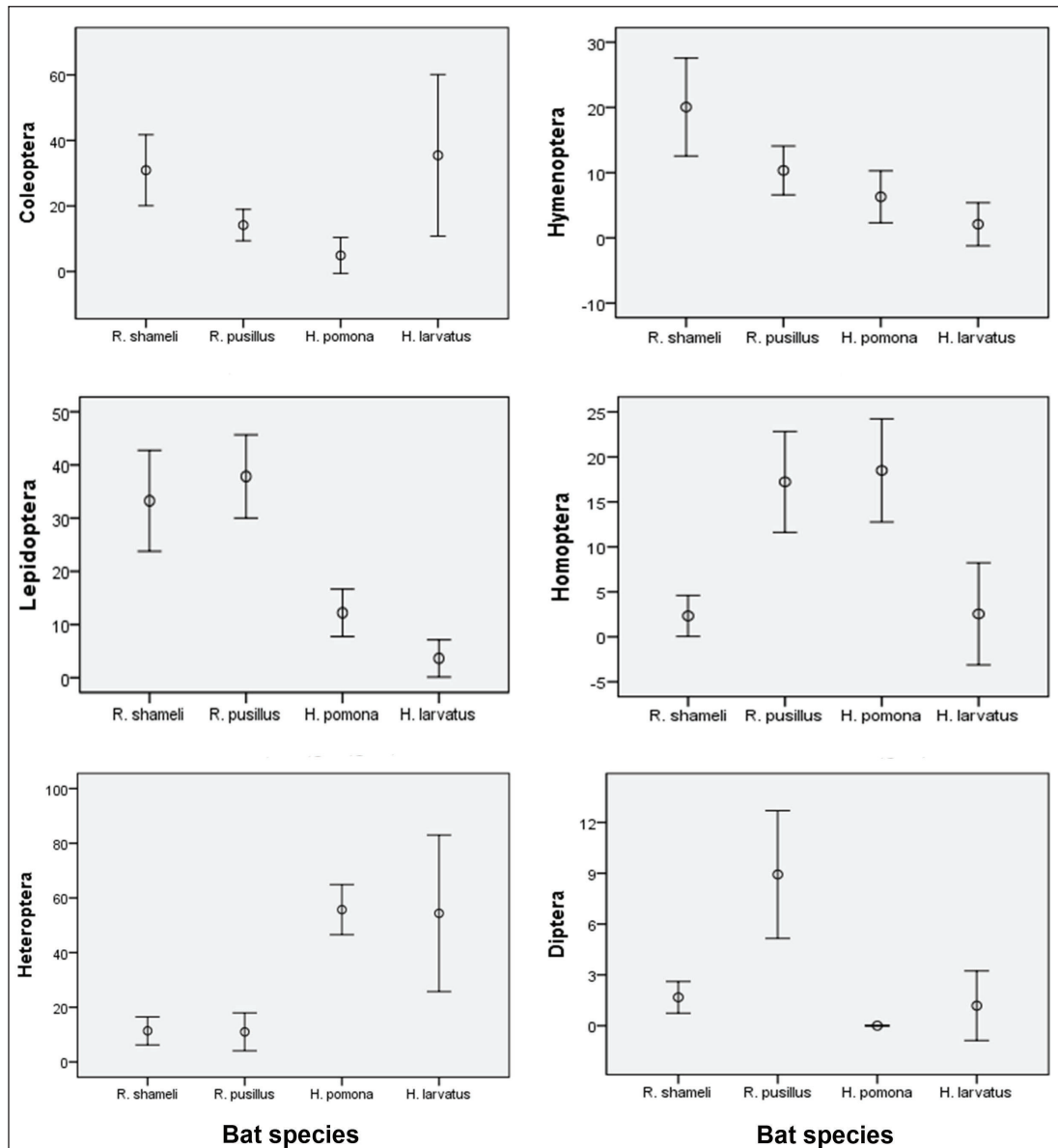


Fig. 4 Percentage volume (mean \pm standard error) of six insect orders in faecal samples of four bat species in Chambok.

erence for small vertebrates (Rodentia). In general terms, these differences could be due to variations in the life history and ecology (e.g., body size and foraging behaviour) of each species (Bogdanowicz *et al.*, 1999; Weterings & Umponstira, 2014).

Our data suggest that populations of *R. shameli* in Chambok may prefer lepidopteran, coleopteran and hymenopteran insects, whereas *R. pusillus* may prefer lepidopterans (all PV values $\geq 20\%$). Rhinolophid bats are generally small-bodied animals which predisposes them to prey upon smaller, softer-bodied insects (Ponmalar & Vanitharani, 2014). Studies elsewhere have found that

members of the genus exhibit a preference for Lepidoptera, small Coleoptera and Diptera (Bogdanowicz *et al.*, 1999; Fukui *et al.*, 2009; Weterings & Umponstira, 2014).

The two hipposiderids we captured at Chambok (*H. pomona* and *H. cf. larvatus*) showed a marked preference for heteropterans (PV values $>50\%$) and *H. cf. larvatus* also frequently consumed coleopterans (PV=35.6%). Although the two species differ in body size (*H. cf. larvatus* being larger than *H. pomona*), both taxa produce high-frequency echolocation signals whose call durations are shorter than those emitted by *R. shameli* and *R. pusillus* (Phauk *et al.*, 2013). Previous studies have

found that larger hipposiderids such as *H. armiger* and *H. diadema* also consume heteropterans and coleopterans (Pavey & Burwell, 1997; Weterings *et al.*, 2015).

Somewhat interestingly, fragments of mites (Acari) occurred in faecal samples produced by three of our bat species (*R. shameli*, *H. pomona* and *H. cf. larvatus*). Mites are parasitic invertebrates which occur on bats (Whitaker *et al.*, 1983; Baker & Craven, 2003) and have been found in faecal samples produced by *Hypsugo cadornae* (Vespertilionidae) in Thailand (Weterings *et al.*, 2015). They also parasitize coleopterans (Abbot & Dill, 2001; Almane & Elnov, 2009). Additionally, in containing fragments of Rodentia, our data for *M. spasma* is consistent with previous studies suggesting the taxon consumes small vertebrates (Balete, 2010; Vanitharani *et al.*, 2015). More broadly, the species is thought to forage on large and hard-bodied insects, lizards and other small vertebrates (Francis, 2008).

Our study provides a useful baseline for future research on the diets of insectivorous bat species in the Chambok area. They also demonstrate that these frequently feature insect orders which include important pest species for agriculture (e.g., Lepidoptera, Coleoptera and Heteroptera; Sallam, 2001; Thongdara *et al.*, 2009; Rabitsch, 2010) and to a lesser extent, disease vectors for humans (Diptera). As such, we recommend further studies to develop understanding of the diet preferences of Cambodian bats, not least because these likely give rise to economically significant ecosystem services.

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Instructions for Authors

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- Tanaka S. & Ohtaka A. (2010) Freshwater Cladocera (Crustacea, Branchiopoda) in Lake Tonle Sap and its adjacent waters in Cambodia. *Limnology*, **11**, 171–178.

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- MacArthur, R.H. & Wilson, E.O. (1967) *The Theory of Island Biogeography*. Princeton University Press, Princeton, USA.
- Rawson, B. (2010) The status of Cambodia’s primates. In *Conservation of Primates in Indochina* (eds T. Nadler, B. Rawson & Van N.T.), pp. 17–25. Frankfurt Zoological Society, Frankfurt, Germany, and Conservation International, Hanoi, Vietnam.

Reports:

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Theses:

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