# The use of non-invasive sampling to estimate long-term abundance of *Hippocampus kuda* in the Koh Sdach Archipelago, Cambodia

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## មូលន័យសង្ខេប

ប្រជុំកោះស្ដេចត្រូវបានរៀបចំជាទីតាំងបន្ទាប់ សម្រាប់ដាក់ជាតំបន់គ្រប់គ្រងជលផលសមុទ្រនៅប្រទេសកម្ពុជា។ នៅពេលដែលការ ប្រកាសខិតជិតមកដល់ វាចាំបាច់ណាស់ក្នុងការកំណត់អត្តសញ្ញាណប្រភេទជីវៈចម្រះក្នុងសមុទ្រដែលកំពុងរងការគំរាមកំហែង ដើម្បី ជាមូលដ្ឋានក្នុងការកំណត់តំបន់គ្រប់គ្រងនៅក្នុងប្រជុំកោះនេះ។ ទោះយើងដឹងហើយថាប្រជាជនកម្ពុជាភាគច្រើនពឹងផ្អែកលើវិស័យជល ផលដើម្បីបម្រើដល់សន្តិសុខស្យៀង និងជាប្រភពប្រាក់ចំណូលមូលដ្ឋានក៏ដោយ ក៏ការសិក្សាស្រាវជ្រាវអំពីសមុទ្រ ដើម្បីជួយដល់ការ អភិរក្សសមុទ្រប្រកបដោយប្រសិទ្ធភាពនៅតែមានភាពខ្វះខាត។ ចាប់ពីឆ្នាំ២០១៤ ដល់ ២០២១ យើងបានធ្វើការតាមដាននៅតាម ទីតាំងសិក្សាចំនួនប្រាំកន្លែងនៅក្នុងប្រជុំកោះនេះ ដើម្បីសង្កេតពីនិន្នាការប៉ូពុយឡាស្យងនៃសត្វសេះសមុទ្រ Hippocampus kuda (Teleostei: Syngnathidae) ដែលត្រវបានគេដកហ្វូតលើសកម្រិតតាមរយៈការកំណត់ជាប្រភេទដែលត្រវប្រមូល ការមិនបាន កំណត់ប្រភេទឧបករណ៍នេសាទជាក់លាក់ និងការបាត់បង់ទីជម្រក។ យើងបានរកឃើញថា ដង់ស៊ីតេទូទៅនៃប្រភេទសត្វនេះមាន កម្រិតទាប (0.000៨ SE ±0.000១ ឯកត្ត:/ម<sup>២</sup>) ហើយមិនមានភាពខុសគ្នាពីកន្លែងមួយទៅកន្លែងមួយទេ។ ភាពលប់របស់វាហាក់ដូច ជានៅថេរដោយមិនមានការផ្លាស់ប្តូរគួរឱ្យកត់សម្គាល់ទេក្នុងអំឡុងពេលសិក្សា។ យើងបានកំណត់ទីតាំងសិក្សាដែលមានលក្ខណៈ បរិស្ថានផ្សេងៗគ្នា ចាប់ពីតំបន់មានខ្សាច់ទទេ រហូតដល់វាលស្មៅសមុទ្រ ហើយការសិក្សានេះបង្ហាញថា H. kuda អាចសម្របខ្លួន បានខ្ពស់ ជាពិសេសអាចរស់នៅនិងប្រើប្រាស់ទីជម្រកទូទៅជុំវិញខ្លួនបាន ហើយក៏អាចបម្លាស់ទីទៅកាន់តំបន់ដែលវាចូលចិត្តផង នេះបង្ហាញពីសត្តានុពលនៃភាពធន់របស់ប៉ូពុយឡាស្យងនៃប្រភេទ ធៀបនឹងប្រភេទសត្វដែលមានតម្រវការទីជម្រកជាក់ ដែរៗ លាក់។ ការសិក្សាស្រាវជ្រាវអំពីបម្រែបម្រលដង់ស៊ីតេនៃប៉ូពុយឡាស្យងតាមរដូវកាល គឺចាំបាច់ដើម្បីកំណត់ព័និន្នាការបម្រែបម្រល ព្រមទាំងអថេរមួយចំនួនទៀតដូចជាគុណភាពទឹក និងជម្រើសនៃទម្ររ ត្រវតែមានការសិក្សាបន្ថែមទៀតដើម្បីស្វែងយល់អំពីទំនោរនៃ ការប្រើប្រាស់ជម្រករបស់សេះសមុទ្រ H. kuda នៅប្រជុំកោះស្ដេច។

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## Abstract

The Koh Sdach Archipelago has been designated as the next location for a marine fisheries management area in Cambodia. As the final phase of the proclamation draws near, it is essential to identify threatened populations of marine species to guide the design of appropriate zones for different forms of management within the archipelago. Despite much of Cambodia's population being reliant upon the fisheries industry for food security and primary income, there is a paucity of marine research to facilitate effective conservation. We undertook monitoring at five sites in the archipelago between 2014 and 2021 to investigate population trends of *Hippocampus kuda* (Teleostei: Syngnathidae), a heavily exploited seahorse species which is susceptible to overexploitation due to targeted collection, non-selective fishing gear and habitat loss. Our findings indicate that overall densities of the species are low ( $0.0008 \pm 0.0001$  individuals/m<sup>2</sup>) and did not differ significantly between survey sites. Its abundance appeared to be relatively stable with no significant temporal changes noted during the study period. As our study sites comprised a variety of environmental characteristics ranging from bare sand substrate to seagrass meadows, this suggests *H. kuda* is a highly adaptive habitat generalist capable of utilizing its surroundings or migrating to more preferable areas. This highlights the potential resilience of the population compared to species with more specific requirements. Research on how population densities fluctuate seasonally is required to determine recruitment trends and additional variables such as water quality and holdfast choice must be studied to further understand the habitat preferences of *H. kuda* within the Koh Sdach Archipelago.

**Keywords** habitat generalist, *Hippocampus kuda*, non-invasive sampling, underwater visual census.

## Introduction

Cambodia's coastal ecosystems underpin food security and offer valuable resources which increasingly play important roles in the Kingdom's socio-economic development, facilitating advances in infrastructure and attracting international tourists (RGC, 2016). Despite their importance, the paucity of peer-reviewed marine research in the country is stark, with only a few studies primarily focussing upon the biophysical characteristics of coral reef health to date (Chou et al., 2003; Savage et al., 2014; Thorne et al., 2015). For cryptic syngnathids, there are no published data related to their demographic characteristics, which hinders understanding of their population trends and limits conservation effort. This knowledge gap could be critical given the low biomass of fish in many areas of Cambodia, this being indicative of overexploited ecosystems, with the factors responsible for such declines posing an equal threat to seahorse populations (Glue et al., 2020).

Seahorses (*Hippocampus* spp.) belonging to the Syngnathidae are iconic marine fishes with significant ecological, economical and medicinal values (Vincent *et al.*, 2011). Their emblematic morphology has resulted in their adoption as flagship species for conservation of marine and estuarine areas and they are important predators of bottom-dwelling species (Bologna, 2007; Vincent *et al.*, 2011; Correia *et al.*, 2018). However, seahorses are threatened by a myriad of factors including incidental capture in fisheries, exploitation for use in traditional medicines, collection for sale as curios and ornamental

displays and degradation of essential habitats (Vincent *et al.*, 2011). Their vulnerability is primarily due to lifehistory traits such as low mobility, small home ranges and mate fidelity, coupled with their tendency to inhabit shallow areas where anthropogenic disturbances are often recurrent (Lourie *et al.*, 1999; Vincent *et al.*, 2011; Gristina *et al.*, 2015). These factors render seahorses particularly susceptible to population declines and have led to inclusion of the entire *Hippocampus* genus in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Several species are also classified as globally threatened on the International Union for Conservation of Nature (IUCN) Red List (Pollom *et al.*, 2021).

Overexploitation, primarily by non-selective fisheries, has been identified as the leading threat to syngnathids, followed by habitat loss (Pollom et al., 2021). However, these threats are rarely isolated from the cumulative factors driving population declines. Anthropogenic influences are increasing at unprecedented rates and rapidly changing coastal areas around the world with the combined effects of development, destructive fishing techniques and unsustainable tourism causing severe habitat degradation (Crain et al., 2009). Associated factors including excessive boat traffic, nutrient enrichment, deterioration of water quality, and increased sedimentation, which typify Cambodian waters, evidently threaten marine communities and may well be leading to population patchiness (Browne et al., 2012; Correia, 2015; Claassens & Hodgson, 2018). The fact that habitat alterations have negative effects on seahorse species is

well documented (Vincent, 1996). For instance, significant declines in populations of long-snouted seahorse *H. guttulatus* have occurred in seagrass meadows damaged by seining (Curtis *et al.*, 2007) and the abundance of dwarf seahorse *H. zosterae* has been reduced by marina construction adjacent to seagrass habitats (Mason-Jones *et al.*, 2010). As such, understanding the population parameters of species threatened with extinction is paramount to their conservation and this in turn is intrinsically linked to protection of their preferred habitats (Brown 1984; Harasti *et al.*, 2014).

Determining the causes of population fluctuations is crucial for effective natural resource management (Kareiva, 1987; Clark, 2010; Correia, 2021). To achieve progress, conservation programmes require demographic data encompassing extant geographic distribution, habitat use, taxon-specific life-history characteristics and the relative influence of environmental disturbances in shaping populations (Thrush & Dayton, 2002; De Raedemaecker et al., 2010; Correia, 2015; Shapawi et al., 2015; Woodall et al., 2015). This information is typically utilized in three ways: in the design and implementation of habitat management areas; in communicating the scope and severity of threats; and in monitoring changes in the status of protected species. The latter is especially necessary for measuring success in halting biodiversity loss and the effectiveness of management areas (Bailie et al., 2004). It is imperative that conservation efforts focus on protecting hotspots that promote species density, particularly in areas where anthropogenic threats may have masked the magnitude of loss (Jackson et al., 2001; Pandolfi et al., 2003; Correia, 2015).

Progress is being made towards large-scale protection of ocean habitats in Cambodia with the proclamation of two marine protected areas (MPAs) since 2016. The Koh Sdach Archipelago (KSA) is the third location designated for development of a Marine Fisheries Management Area (MFMA) (FFI, 2020)—a national term for a type of multiple-use MPA. Within the archipelago, Koh Sdach village has opted to actively contribute to management of their marine resources through Cambodia's community fisheries (CFi) system. In many areas of the country, CFis are employed as legally recognised communitybased organisations which are central in the design and management of MPAs (Roig-Boixeda et al., 2018). As a consequence, it is essential to understand the status of threatened marine species within the KSA to guide zonation processes and develop strategies that will maintain ecosystem functionality and promote the recovery of fisheries sustaining the local economy.

Five species of seahorse have been documented in Cambodia to date, namely *H. spinosissimus*, *H. kuda*, *H. mohnikei, H. trimaculatus* and *H. comes* (MCC, 2022). Among these, *H. kuda* is reportedly the most common in the Kingdom (Kuda Divers, 2021). With pressures mounting on the Cambodian marine environment (NESAP, 2016), we undertook a baseline assessment of the population dynamics of *H. kuda* within the KSA to assist its long-term conservation. Our study focussed on the distribution, abundance and population parameters of the species in five locations across the KSA, including data on sex, torso lengths and holdfast preferences. It was also based on non-invasive sampling techniques and designed to provide insights into population dynamics which can lead to more accurate estimations of responses to disturbance, survival and migration.

## Methods

#### Study species

*Hippocampus kuda* is widely distributed throughout the Indo-Pacific region and is abundant in the Gulf of Thailand (Panithanarak *et al.*, 2010), although it is currently classified as Vulnerable (Aylesworth, 2014) (Fig. 1). The species is thought to be a habitat generalist having been recorded in several inshore environments including mangroves, seagrass beds and estuaries (Lourie *et al.*, 1999; Kuang & Chark, 2004; Ambo-Rappe *et al.*, 2021) and is one of the most heavily-traded seahorse species in many Southeast Asian countries (Job *et al.*, 2002).



**Fig. 1** *Hippocampus kuda,* Koh Sdach Archipelago (© Roger Bruget).

However, few studies have investigated the population dynamics of *H. kuda* in-situ to our knowledge, although laboratory research has employed the species as a model organism (Choo & Liew, 2003; Kuang & Chark, 2004; Ambo-Rappe *et al.*, 2021).

#### Study sites

The KSA comprises a collection of rocky islands in the Kiri Sakor District of Koh Kong Province, approximately 60 km southeast of Cambodia's border with Thailand (10°55'N, 103°5'E) (Fig. 2). The islands are predominantly uninhabited with the exception of Koh Sdach, which according to government census, is the most populated island on the Cambodian coast. Approximately 4,000 people inhabit Koh Sdach, over half of which are economically dependent on the fishing industry (FFI, 2020).

Our research was conducted at five sites in the KSA which were surveyed by citizen science organizations at irregular intervals between December 2014 and April 2021. The five sites were selected based upon their differing environmental characteristics and were representative of potential seahorse habitat (Table 1).

#### Underwater visual census

We surveyed *H. kuda* using underwater visual census techniques based on the iSeahorse methodology (iSeahorse, 2014). The starting position and compass direction of each transect survey were randomly selected at the site and a GPS was used to obtain UTM coordinates for the start and end point of transects. Search times



**Fig. 2** Survey site locations in the Koh Sdach Archipelago, Cambodia (Mainland, Koh Totung, Koh Chan, Koh Sdach, Koh Smach).

lasted approximately 60 minutes but varied depending on water conditions, as did the area searched. Search areas were calculated ex-situ and utilized the start and end coordinates to obtain their lengths in metres. As each surveyor searched the square metre directly in front of them, the search area was multiplied by the number of participants to determine total survey area.

For each seahorse sighted, photographs were taken with a ruler placed as close as possible to allow later measurement of biometric data. The species, sex and torso length of each individual were subsequently recorded on land (iSeahorse, 2014). Species were identified using information provided by Kuiter (2009) and for *H. kuda* included its characteristically long crown

**Table 1** Details of sampling locations for underwater visual censuses of *Hippocampus kuda* in the Koh Sdach Archipelago,arranged from North to South.

Site	Substrate	Depth (m)	Habitat characteristics		
Mainland	Sand & Silt	2.4–3.4	Bare sand substratum with seasonal sparse seagrass ( <i>Halophila ovalis</i> ); high boat traffic, high presence of discarded fishing gear (ropes, monofilament trammel nets), abandoned mooring piers.		
Koh Totung	Sand	3.9–5.7	Predominantly seagrass meadow ( <i>H. ovalis, Halodule uninervis &amp; Cymodocea serrulata</i> ) with seasonal fluctuations in density; Macroalgal mats (largely <i>Dictyota</i> sp.) dominate in May-June; little anthropogenic debris or disturbance.		
Koh Chan	Sand	5.6–9.0	Sandy bottom; strong currents; trawler zone; anchor hotspot as a refuge boats during unfavourable weather.		
Koh Sdach	Sand	4.7–6.9	Sandy bottom; high boat traffic and watersport activities, active gill and trammel a present during surveys, some anthropogenic debris; the only site with a large settl ment and no solid waste or sewage management systems operating on the island		
Koh Smach	Sand & Silt	4.0–5.7	Seasonal sparse seagrass ( <i>H. ovalis</i> ); development on the island began in 2018 leading to high turbidity and silt substratum predominating in 2021		

filaments, square head profile over the eye and smooth form. Seahorses with a full brood pouch were considered pregnant males and noted as such. Following Lourie *et al.* (1999), sex was assigned based on the presence (male) or absence (female) of a brood pouch. Environmental variables were also recorded, including the depth of occurrence, water temperature and holdfast at first sighting. Alongside data collected on transect surveys, 38 individuals were identified through roving sightings and although these were excluded from density calculations, their sex, torso length, and holdfast information were included in our dataset for analysis.

#### Statistical analyses

Descriptive statistics are reported as means ± standard error, unless stated otherwise. Comparative analyses of population density between sites and time periods were performed using generalized linear models (GLMs) which assumed a normal distribution with a log function. The data were transformed to normalize the distribution prior to model selection and summarised in Pearson chi-squared matrices to identify collinear variables as well as variables correlated with seahorse densities. Multiple regressions were carried out for seahorse densities between sampling sites and seasons (wet [May-October] vs. dry [November-April]) and the data were separated into two survey periods (2014-2017 and 2018-2021) to determine temporal fluctuations before and after major land use changes occurred in the study region. Successive GLMs to test torso length, holdfast and site were compared with previous models using either the 'ANOVA' or 't-test' function of the 'bioinfokit' Python package followed by Tukey-Kramer tests to identify differences in the amount of variance.

### Results

A total of 160 *H. kuda* were sighted over the course of 260 surveys completed during our study period. Three other seahorse taxa were also encountered during our surveys (*H. mohnikei*=33 individuals, *H. trimaculatus*=14, *H. spinosissimus*=6), but are not considered further in this study.

Hippocampus kuda had an overall density of 0.0008 (± 0.0001) individuals/m<sup>-2</sup> across our study area. Densities per site ranged from 0.0002 (± 0.00007) individuals/m<sup>-2</sup> (Koh Chan) to 0.0015 (± 0.0004) individuals/m<sup>-2</sup> (mainland site) (Table 2), although there were no significant differences in mean density between locations (f=1.58, df=99, p=0.18). Sightings were greater during the dry season  $(0.0009 \pm 0.0002 \text{ individuals/m}^{-2})$  compared to the wet season (0.0005  $\pm$  0.0002 individuals/m<sup>-2</sup>), although there were no significant differences between seasons (f=0.29, df=99, p=0.59) or specific months (f=1.41, df=249, p=0.17) (Fig. 3A). The more recent surveys in the KSA (2018-2021) revealed greater seahorse densities (0.00083  $\pm$  0.00017 individuals/m<sup>-2</sup>) compared to the 2014–2017 surveys (0.0007  $\pm$  0.0001 individuals/m<sup>-2</sup>). This was due to an increase at the mainland site, but no H. kuda were sighted at Koh Chan and minor declines occurred at all other sites in 2018-2021 (Fig. 4). However, the differences between the two periods and survey sites were not significant (f=0.04; df=99; p=0.84).

Overall, torso lengths of male *H. kuda* (49.00 ± 2.36 mm) were significantly larger than female (28.50 ± 1.21 mm) (*t*=7.58, df=47, *p*=1.084x10<sup>-9</sup>) (Fig. 5), although they did not differ significantly between sites (*f*=0.86; df=192; *p*=0.49). The torso lengths of most individuals were between 13.97 mm and 47.69 mm, with a minimum of 8 mm and a maximum of 86 mm.

**Table 2** Survey area, number of individuals and densities of *Hippocampus kuda* recorded at sampling sites within the Koh Sdach Archipelago.

Site	Surface area (m <sup>2</sup> )	Number of individuals recorded	Mean density (individuals/m <sup>-2</sup> )	Mean density (individuals/km <sup>-2</sup> )
Mainland	43,037	41	$0.0015 \pm 0.0004$	$1.4538 \pm 0.40318$
Koh Totung	86,496	57	$0.0009 \pm 0.00016$	$0.9255 \pm 0.16146$
Koh Chan	60,941	14	$0.0002 \pm 0.00007$	$0.2854 \pm 0.07010$
Koh Sdach	75,924	34	$0.0007 \pm 0.00017$	$0.6535 \pm 0.16701$
Koh Smach	33,692	14	$0.0006 \pm 0.00023$	$0.6266 \pm 0.22825$
Koh Sdach Archipelago	30,090	160	$0.0008 \pm 0.0001$	$1.1080 \pm 0.0937$

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**Fig. 3** Monthly densities (A) and sex ratios (B) of *Hippocampus kuda* within the Koh Sdach Archipelago from December 2014 to April 2021. Density data are given as mean (symbols)  $\pm$  standard errors (bars).

Eighty-one percent of individuals whose sex was confirmed during the survey (n=163) were female, giving a male:female ratio of 1:4.26 (Fig. 3B). The dataset for sexes was insufficient for reliable comparisons between sites. Only 15 of seahorses recorded appeared to be pregnant males with swollen brood pouches and there was no noticeable seasonal pattern in their occurrence. The mean torso length of pregnant males was 51.92 ± 3.30 mm.

A large proportion of seahorses were not grasping any holdfast when recorded (36.55%). For individuals that were, 36.55% were associated with plant materials, 14.21% with fishing gear and 4.06% with invertebrates. The relative proportions of items employed as holdfasts varied between survey locations (Fig. 6) and reflected the abundance of site-specific benthic substrata. Macroalgae were the most commonly used holdfast at Koh Sdach (18.18%), whereas fishing gears (43.48%) were most frequently used at the mainland site, and seagrass (32.94%) was preferred at Koh Totung.



**Fig. 4** Density of *Hippocampus kuda* recorded during two survey periods at five sites within the Koh Sdach Archipelago. Symbols represent means and bars represent standard errors.



**Fig. 5** Pooled frequency distribution of torso lengths for *Hippocampus kuda* within the Koh Sdach Archipelago (*n*=198).

## Discussion

Ours is the first study to provide population parameters for the Vulnerable *H. kuda* seahorse in Cambodian waters. Population densities in the KSA are low at 0.0008 ± 0.0001 individuals/m<sup>-2</sup> compared to similar generalists including *H. reidi* in Brazil (with 0.026 individuals/m<sup>-2</sup>: Rosa *et al.* 2007), *H. zosterae* in Florida, USA (0.080 individuals/m<sup>-2</sup>: Mason-Jones *et al.* 2010) and *H. hippocampus* in the Ria



Fig. 6 Proportion of holdfasts utilized by *Hippocampus kuda* in sample sites and Koh Sdach Archipelago as a whole. Sample sizes are given in parentheses.

Formosa Lagoon of Portugal (0.008 individuals/m<sup>-2</sup>: Correia, 2015). The limited availability of historical data for *H. kuda* in Cambodia and elsewhere in the world precludes comparisons of its abundance across its range. Our five survey locations were representative of a range of habitat characteristics and environmental variables within the KSA. As these sites were thought to be representative of seahorse habitats, our results provide insights into the drivers of seahorse paucity in the archipelago.

Our results suggest that the distribution of *H. kuda* in the KSA is not determined by habitat type and that the species occurs in a variety of dissimilar environments. Considering its distribution by site, H. kuda appears to be a habitat-generalist which occupies bare and complex environments in similar densities. This was contrary to our initial expectations as Kuang & Chark (2004) found that *H. kuda* preferred vegetation substrates in Malaysia, where individuals were consistently associated with Enhalus acroroides or Halimeda beds. In our study, the highest densities of H. kuda were recorded at the mainland site, where the predominant benthos comprises sand, with sparse, short Halodule ovalis only present for a portion of the year. It has been suggested that individuals encountered in habitats with bare substrates may be temporarily exploiting open habitats while transiting between preferred complex microhabitats or that these may have been displaced from their home ranges by currents (Curtis & Vincent, 2005). Additionally, the high energy conditions that occur during the southwest monsoon are likely to displace some seahorses from their territories but may also facilitate the recruitment of others (Choo & Liew, 2003). In our case, sightings of *H. kuda* repeatedly occurred across a gradient of seagrass meadows, macroalgal mats and near-shore open areas. This supports the notion that populations of the species in the KSA are capable of adapting to dynamic environments and are either exploiting their surrounding habitats or transiting between habitats. In general, species with broader habitat requirements are likely to show more resilience to disturbance compared to those with more specialized needs (Foster & Vincent, 2004).

Water quality and physical disturbance have been identified as possible factors in seahorse population declines (Martin-Smith & Vincent, 2005) and oceanographic and climatic conditions are known to influence *H. kuda* densities in Malaysia (Choo & Liew, 2003). The heavy rains that occur in Cambodia during the wet season from May to October are responsible for changes to its coastal ecosystems, with turbulent and low visibility waters typifying the KSA. During this period, siltation and runoff are high, with excess dissolved nutrients likely leading to reduced water quality (Krishna *et al.*, 2016). In addition, coastal developments began in mainland areas adjacent to the archipelago in 2008 and infrastructure development accelerated on its islands in 2018, providing significant potential for transforming the marine environment (Nguyen, 2019). Some syngnathids respond poorly to associated pollutants and eutrophication which can manifest as disease (Koldewey & Martin-Smith, 2010) and while all of the individuals we observed appeared to be healthy, their vulnerability to stress cannot be ruled out despite their ability to tolerate poor quality environments. Reduced water visibility is also noteworthy as this has long been recognized as detrimental for seahorses. Since visual acuity is vital for feeding, reduced visibility can significantly reduce prey capture rates which may have broad implications for the reproductive success and survival of seahorses (James & Heck, 1994; Vincent et al., 2011; Correia et al., 2015a). While the waters of the archipelago are calm during the dry season (November to April), boat traffic increases markedly during this period, particularly at our mainland site and Koh Sdach. Boat noise has been defined as contributing to decreased populations of H. capensis (Claassens & Hodgson, 2018) and H. erectus has been shown to exhibit a physiological stress response to loud noise which leads to reduced body mass (Anderson et al., 2011). Indeed, distress has the potential to result in local extirpation of seahorse populations if poor conditions are continuous (Correia, 2021). However, some evidence also suggests that *H. zosterae* actively migrate from adverse shallow habitats towards deeper waters in search of more suitable surroundings (Mason-Jones et al., 2010). As the density of H. kuda did not appear to fluctuate significantly between seasons or over the wider study period, despite development activities in the KSA, this demonstrates the plasticity of the species and raises the possibility that individuals may be able to relocate from habitats they cannot endure to some extent.

Seahorses often display high site fidelity with small home ranges and low mobility (Kvarnemo et al., 2000; Foster & Vincent, 2004). However, their low density in the KSA and the temporal gaps between sightings of seahorses in successive surveys at the same locations suggests that its populations are likely mobile. Should individuals be dispersing however, this could potentially lead to their isolation, risking further declines in abundance due to fragmentation and reduced reproductive success of local populations (Correia et al., 2018). A myriad of anthropogenic stressors occur in the KSA and given the recently documented preference of H. kuda towards greater habitat complexity (Ambo-Rappe et al., 2021), these render it likely that populations of the species can tolerate fluctuations in environmental conditions. As such and similar to other Hippocampus spp. in Portugal (Correia et al., 2018), H. kuda could be occupying alternate, less preferable areas within the KSA which ultimately become their selected habitats.

We found that holdfast choice in the KSA clearly reflected the available benthos at our survey sites. Individuals encountered at the mainland site primarily employed fishing gear (e.g., discarded nets, rope, line) as a holdfast, which might be due to the diminished availability of natural structures mandating that they become holdfast generalists. This preference may also be a consequence of the artificial structures hosting fewer predators and competitors than seagrass meadows, in addition to being food rich (Morgan & Vincent, 2007; Correia, 2015). At the majority of our sites, the individuals we observed did not utilize any holdfast at all, even when in the presence of expected choices. This could be attributable to these habitats serving as temporary settlements while H. kuda move towards preferred environments. Given their limited mobility, seahorses are reliant on holdfasts for anchoring, particularly in areas that may be strongly influenced by hydro-dynamics (Harasti et al., 2014; Aylesworth et al., 2015). In the Ria Formosa lagoon of Portugal, a highly dynamic area where strong currents characterize the environment, stable holdfasts are vital in ensuring seahorse populations are not swept away from their preferred habitats and *H. guttulatus* has been found to grasp close to the base of structures to minimize instability (Correia et al., 2015b). At all of our survey sites, a number of individuals were observed clinging to drift macroalgae. Algal biomass is a key refugia utilized by juvenile and subadult H. hippocampus in Gran Canaria (Otero-ferrer, 2015) and as we encountered H. kuda adults attached to these fronds, it could be that the algae provides a viable method of dispersal rather than security. If found to the case, this possibility would be consistent with reports for Syngnathus fuscus and H. zosterae in the USA (Able et al., 2002; Mason-Jones et al., 2010) and further establish H. kuda as a mobile species.

Artificial structures have been employed to combat the degradation of natural ecosystems by creating alternative habitats. This has been successful in aiding several *Hippocampus* species by providing opportunities for predator avoidance, hunting and reproduction (Hellyer *et al.*, 2011; Correia *et al.*, 2015b; Otero-Ferrer *et al.*, 2015; Simpson *et al.*, 2020). As anthropogenic debris is utilized by *H. kuda* at multiple sites in the KSA, artificial structures could potentially be beneficial in preferred habitats within the region that have been degraded and lack holdfasts. Strap-like leaf forms of seagrass have been found to be important for *H. kuda* in other studies (Kuang & Chark, 2004; Ambo-Rappe *et al.*, 2021) and further research into holdfast choices in the KSA will aid in determining if preferences exist within its populations of the species.

We found male *H. kuda* had significantly longer torso lengths than females across all sites. Total height has also

been found to be significantly longer in male H. capensis, with longer tails and shorter heads observed (Bell et al., 2003; Claassens & Hodgson, 2018). Longer tails are common to Syngnathidae and believed to allow individuals with larger caudal pouches to either securely grasp holdfasts or assist tail-wrestling (Vincent, 1990). It is uncommon for size between sexes to significantly differ (Otero-Ferrer et al., 2015). Although reproductive success is not always linked to size of an individual, larger

individuals have been shown to yield increased young (Vincent & Giles, 2003; Rosenqvist & Berglund, 2011). Kvarnemo et al. (2007) established that sexual selection can be substantial and act on males in certain species that can be both monogamous and polygynandrous. As such, larger males may be an adaptive approach utilized in mobile, low-density populations (which are probable in the KSA) where meetings are infrequent and time for assessing potential mates is limited.

Adult sex ratios vary greatly among Hippocampus spp. with female-biased sex ratios, such as in the present study, typical of H. abdominalis in Australia (Martin-Smith & Vincent, 2005), H. hippocampus in the Macaronesian Islands (Otero-Ferrer et al., 2015) and H. erectus in the USA (Teixeira & Musick, 2001). A pair of seahorses were not sighted at any point during our surveys and this could suggest a lack of mate fidelity and evidence of polygynandry within the population. One possible explanation for the excess of females is an increased mortality rate for the opposite sex-since males are larger they are more visible and therefore more frequently predated upon (Claassens & Hodgson, 2018). Populations with heavilyskewed sex ratios have shown reduced reproductive success (Kvarnemo et al., 2007) and understanding the preferences of H. kuda in the KSA could be of high importance to the future of populations there.

It is important to outline the key limitations of our study. The scope of our work was restricted by the capacity of researchers in the citizen science organisations who assisted our fieldwork, with temporal gaps also existing in the data. This meant our analyses of seasonality were limited by decreased activity during the monsoon season and further understanding of reproductive activity and recruitment was not possible. Our sampling was biased towards Koh Totung and the mainland site which were surveyed orders of magnitudes more than others due to factors including reduced swell and better visibility. As such, the condensed survey effort in some areas may have contributed towards the low abundance recorded or even the notable absences at certain sites. While these constraints reduced the likelihood of identifying significant trends or population predictability, there was still sufficient data to complete our purposes of providing a

baseline of data that can be employed for localised, sitespecific protection of H. kuda.

Successful protection and management of threatened species are only possible with up to date information on populations and life history traits. Ours is the first study to describe the ecology of H. kuda in the coastal environment of Cambodia. Our findings indicate that population densities are low but further research on environmental variables is required to define the specific factors responsible for seahorse demographics. Ecological monitoring plays a vital role in determining when a system has been altered from a desired state as well as informing the success of conservation actions (Legg & Nagy, 2006; Claassens & Harasti, 2020). As such, our research provides a reference point regarding the status of H. kuda within the KSA and continued research will confirm the effectiveness of zonations proposed for the MFMA. Our observations also highlight the potential mobility of H. kuda and provide evidence the species may be a habitat and holdfast generalist capable of adapting to changing environments. This plasticity may well have ensured the survival of local populations despite ongoing threats in the KSA. Ongoing research will be vital to confirm population densities and determine if monthly fluctuations occur in these. This information will aid in determining recruitment trends and amplify existing knowledge which can support measures that provide a foundation for biodiversity recovery (Glue et al., 2020).

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