

Novel life-history data for threatened seahorses provide insight into fishery effects

J. M. LAWSON*†, S. J. FOSTER*, A. C. O. LIM‡§||, V. C. CHONG‡§
AND A. C. J. VINCENT*

*Project Seahorse, Fisheries Centre, The University of British Columbia, Vancouver, BC, V6T 1Z4 Canada, ‡Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia, §Institute of Ocean and Earth Science, University of Malaya, Kuala Lumpur 50603, Malaysia and ||Save Our Seahorses Malaysia, No. 2, Jalan 6/24, Seksyen 6, Selangor 46000, Malaysia

(Received 3 April 2014, Accepted 5 August 2014)

Life-history variables for three incidentally captured species of seahorse (Kellogg's seahorse *Hippocampus kelloggi*, the hedgehog seahorse *Hippocampus spinosissimus* and the three-spot seahorse *Hippocampus trimaculatus*) were established using specimens obtained from 33 fisheries landing sites in Peninsular Malaysia. When samples were pooled by species across the peninsula, sex ratios were not significantly different from unity, and height and mass relationships were significant for all species. For two of these species, height at physical maturity (H_M) was smaller than the height at which reproductive activity (H_R) commenced: *H. spinosissimus* ($H_M = 99.6$ mm, $H_R = 123.2$ mm) and *H. trimaculatus* ($H_M = 90.5$ mm, $H_R = 121.8$ mm). For *H. kelloggi*, H_M could not be estimated as all individuals were physically mature, while $H_R = 167.4$ mm. It appears that all three *Hippocampus* spp. were, on average, caught before reproducing; height at 50% capture (H_C) was $\geq H_M$ but $\leq H_R$. The results from this study probe the effectiveness of assessment techniques for data-poor fisheries that rely heavily on estimates of length at maturity, especially if maturity is poorly defined. Findings also question the sustainability of *H. trimaculatus* catches in the south-west region of Peninsular Malaysia, where landed specimens had a notably smaller mean height (86.2 mm) and markedly skewed sex ratio (6% males) compared with samples from the south-east and north-west of the peninsula.

© 2014 The Fisheries Society of the British Isles

Key words: assessment; *Hippocampus*; incidental capture; Malaysia; maturity; Syngnathidae.

INTRODUCTION

The incidental capture of marine organisms has become a critical point of concern in fisheries management, as basic biological data for incidentally captured animals are often absent or sparse. The majority of studies on incidental capture have been focused on large or charismatic animals (Hall, 1997), yet the vast majority of animals that are incidentally caught are small fishes, maturing at lengths of <20 cm and weighing <100 g (Alverson, 1994; Kelleher, 2005). These smaller fish species may be discarded or retained and those that are retained are seldom monitored, making species-specific

†Author to whom correspondence should be addressed. Tel.: +1 604 368 1739; email: j.lawson@fisheries.ubc.ca

assessment of fishing mortality and abundance challenging (Hall, 1997; Foster & Vincent, 2010).

In response to a growing need to evaluate data-poor fisheries, life-history based approaches have been developed to discern the effects of exploitation on marine fishes. Length measurements such as maximum body size (Dulvy & Reynolds, 2002) and size at maturity (Hutchings & Reynolds, 2004) have proven to predict intrinsic vulnerability to extinction in marine fishes effectively, with larger and later maturing fish species having a greater intrinsic vulnerability than those that are smaller and mature earlier (Winemiller, 2005). Froese (2004) used length at maturity to develop a data-poor method to assess overfishing, by applying three simple indicators to catch data. Catch data can also provide insight into population-level effects of fishing. For example, sex bias in commercial catches predicts a higher rate of population decline and a greater time needed for recovery following collapse (Rowe & Hutchings, 2003). Although most of the data-poor assessment techniques are aimed at using sparse information to make an assessment of a fishery, they still often require information such as fishing mortality and size or age at maturity, which may not be confidently known for some species.

Hippocampus spp. provide examples of incidentally captured small fishes where basic life history is available for very few of the species that are being affected by fisheries. Currently, 75% of *Hippocampus* spp. are listed as data deficient on the International Union for Conservation of Nature (IUCN) Red List (www.iucnredlist.org), meaning that limited data have prevented a reliable assessment of extinction risk for these species (IUCN, 2013). Those studies that have investigated *Hippocampus* spp. life histories characterized them as having early sexual maturity, high natural mortality, a short life span and rapid growth relative to other teleosts (Curtis & Vincent, 2006; Harasti *et al.*, 2012). Yet, other traits such as complex social interactions, including parental care and monogamous mating patterns, may make *Hippocampus* spp. more likely to be overfished (Sadovy, 2001; Foster & Vincent, 2004). Discovering and reconciling the mixture of *Hippocampus* spp. life-history traits are critical to understanding the effects of their incidental capture.

It is estimated that the international trade in *Hippocampus* spp., fuelled by demand for traditional Chinese medicine, the aquarium trade and curios, obtains 95% of its *Hippocampus* spp. from incidental capture (Vincent *et al.*, 2011). The 2002 addition of *Hippocampus* spp. to Appendix II of the Convention on International Trade in Endangered Species (CITES) has generated formal data on legal and reported international trade (Vincent *et al.*, 2013). Yet, little is known about the role of incidental capture in such international trade or how it is affecting *Hippocampus* spp. populations. It is known, however, that the five data-poor species of *Hippocampus* spp. that account for 91% of the reported international trade are frequently obtained through incidental capture (Vincent *et al.*, 2011; UNEP-WCMC, 2012).

This study focuses on Malaysia, a country that historically exported four of the five *Hippocampus* spp. that dominate international trade, and was the fifth largest global seahorse-exporting nation between 2004 and 2011 as reported to CITES (UNEP-WCMC, 2012). A trade survey carried out during 1998–1999 estimated that the Malaysian trawl fishery alone obtained 1 million *Hippocampus* spp. (*c.* 2900 kg) annually through incidental capture (Perry *et al.*, 2010). Previous studies on *Hippocampus* spp. in Peninsular Malaysia have focused on reporting species distribution (Choo & Liew, 2004; Lim *et al.*, 2011) and examining trade routes (Choo & Liew,

2005; Perry *et al.*, 2010), but none have published life-history information on the three focal species of this study.

This study had two goals; the first was to generate critical information on *Hippocampus* spp. life history that could aid in employing assessment models for data-poor fisheries. The second was to apply this novel information to *Hippocampus* spp. incidental capture in Peninsular Malaysia, with the aim of identifying vulnerable *Hippocampus* spp. populations. Results from this study will be applicable to surrounding south-east Asian nations, whose non-selective fisheries catch the same *Hippocampus* spp.

MATERIALS AND METHODS

STUDY SPECIES

This study focuses on three *Hippocampus* spp. in Peninsular Malaysia: Kellogg's seahorse *Hippocampus kelloggi* Jordan & Snyder 1901, the hedgehog seahorse *Hippocampus spinosissimus* Weber 1913 and the three-spot seahorse *Hippocampus trimaculatus* Leach 1814, that were identified according to Lourie *et al.* (2004). All three species are geographically distributed throughout the Indian and western Pacific Oceans and are associated with soft-bottom and coral habitats (Foster & Vincent, 2004). Knowledge of the life-history variables for these three species was either completely unknown (*H. kelloggi*) or partially described (*H. spinosissimus*: Meeuwig *et al.*, 2006; *H. trimaculatus*: Meeuwig *et al.*, 2006; Murugan *et al.*, 2009, 2011). Together, the three species made up 75% of trade volumes that were reported to CITES from 2004 to 2011 (annual range by number of individuals): *H. trimaculatus* (1.1–2.5 million), *H. spinosissimus* (1.0–2.5 million) and *H. kelloggi* (0.75–1.4 million; UNEP-WCMC, 2012).

FISHERIES-DEPENDENT SURVEYS

Catches were surveyed for *Hippocampus* spp. at 31 landing sites found along the west coast of Peninsular Malaysia (from 06° 06' N; 100° 17' E to 01° 19' N; 103° 26' E) and at two landing sites on the south-east coast of Peninsular Malaysia (from 02° 26' N; 103° 49' E to 02° 39' N; 103° 36' E). The north-east coast of Peninsular Malaysia was not visited during this study due to logistical constraints. Sites were visited from May to August 2013 as shown in Fig. 1(a) (mapped using ESRI ArcGIS 9.3; www.esri.com). The landing sites surveyed in this study ranged from small fishing villages to large, government operated ports.

During the first month of surveys in May 2013, 31 landing sites (all located on the west coast of Peninsular Malaysia) were visited, with *Hippocampus* spp. encountered in 22 of these sites [Fig. 1(a)]. Fishers often had stockpiles of dried *Hippocampus* spp., and all *Hippocampus* spp. that were encountered were measured. Although these stockpiles were treated as random samples of the fished population, fishers may be more likely to retain large *Hippocampus* spp. that are higher in value (Perry *et al.*, 2010). During this initial survey period, 361 stockpiled *Hippocampus* spp. from 26 fishers at 22 landing sites were measured. Fishers reported that these *Hippocampus* spp. had been collected within the previous two-year period (median = 9 months, $n = 26$ fishers). Fishing gears used by these fishers included bottom trawl nets, bag nets, tidal shrimp nets (gombang Y-nets), drift nets and purse seines.

Fishers were interviewed to identify landing sites where *Hippocampus* spp. were frequently caught. Those who claimed to catch *Hippocampus* spp. were asked if they would be willing to collect *Hippocampus* spp. as part of this research project. In total, 10 fishers agreed to assist with this project: five fishers (from four landing sites) collected dried *Hippocampus* spp., and five fishers (from five landing sites) agreed to collect freshly dead *Hippocampus* spp. in 95% ethanol. Participating fishers were visited at 1 month intervals from June to August 2013 to measure *Hippocampus* spp. collections [Fig. 1(a)]. These monthly visits to participating fishers resulted in a total of 486 *Hippocampus* spp. (112 in ethanol and 374 dry).

Seahorse collectors (who purchased *Hippocampus* spp. from fishers) were interviewed at three landing sites [Fig. 1(a)]. Four seahorse collectors allowed measurement of 307 dried

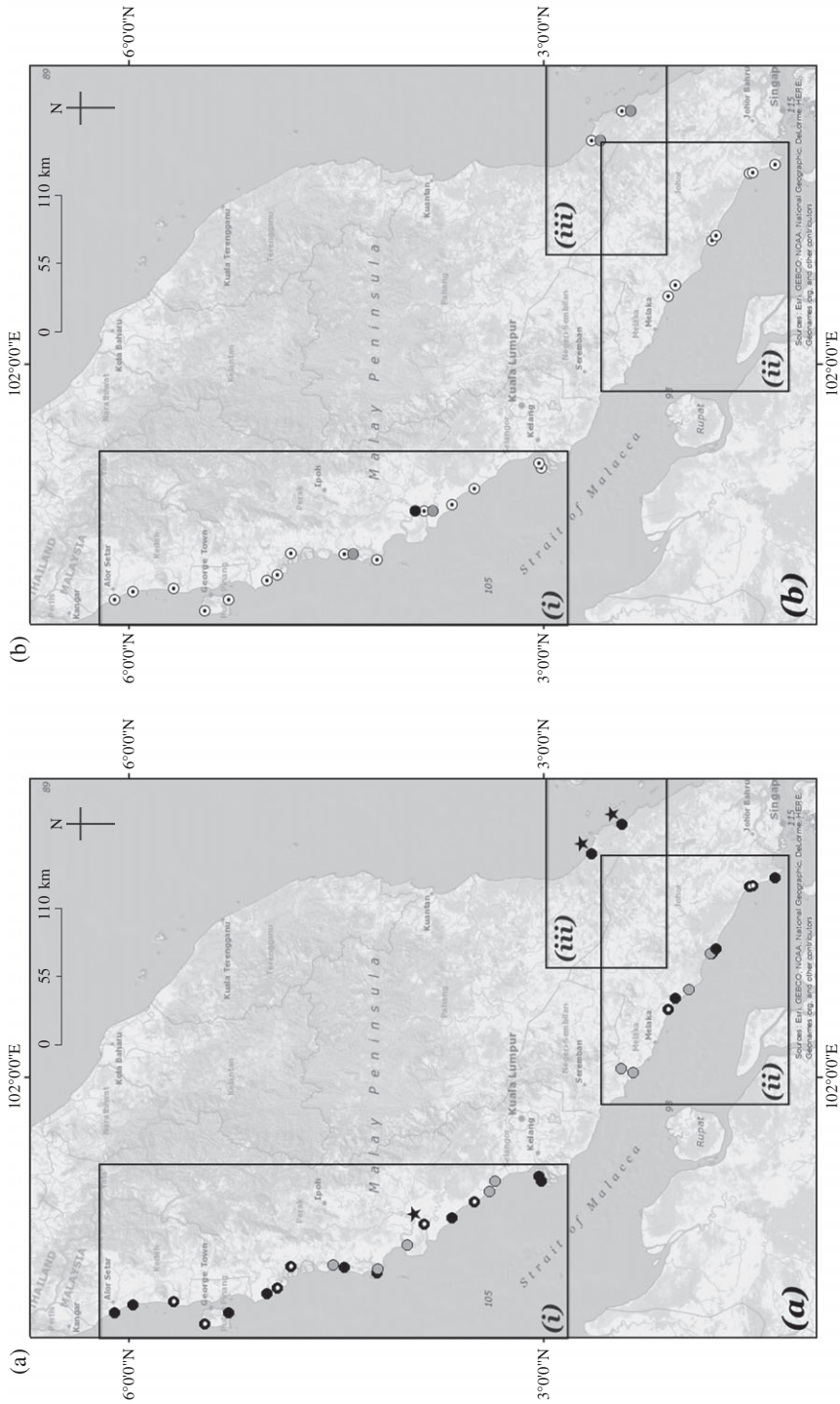


FIG. 1. (a) All the landing sites visited throughout Peninsular Malaysia, indicating the presence (●) or absence (○) of *Hippocampus* spp. samples from fishers at these sites. Also indicated are the landing sites sampled monthly (○) and sites where additional samples were obtained from *Hippocampus* spp. collectors (★). (b) The distribution of *Hippocampus kelloggi* (●), *Hippocampus spinosissimus* (○) and *Hippocampus trimaculatus* (○) found in samples. Boxes indicate (i) the north-west, (ii) south-west and (iii) south-east regions, as described throughout the analysis. Maps were generated using ESRI ArcGIS 9.3.

Hippocampus spp. in total. Three of these collectors were located at two landing sites on the east coast of Peninsular Malaysia, a region that was only visited in July 2013 and was not visited monthly. The fourth collector was located on the west coast, and was visited monthly from June to August.

Data collection involved recording the height, mass, sex, reproductive state and maturity of each individual *Hippocampus* spp. This study measured *Hippocampus* spp. height, the length measurement taken from the tip of the coronet to the tip of the outstretched tail (Lourie, 2003). *Hippocampus* spp. mass was taken using a Chestnut Tools (<http://chestnuttools.com/>) pocket scale that was accurate to the nearest 0.1 g. Male *Hippocampus* spp. were visually distinguished from females by the presence of a brood pouch or, for juvenile males, the presence of a darkened oval zone where a brood pouch was developing (Boisseau, 1967).

For mature males, reproductive status was assigned based on categories developed by Perante *et al.* (2002) as: stage 0, pouch empty and taut; stage 1, pouch slightly distended or newly empty; stage 2, heavily pregnant, where the pouch was very much distended. Animals that were physically mature (had a brood pouch), however, were not necessarily reproductively active (engaging in reproduction), as noted in an *in situ* study by Harasti *et al.* (2012). To examine this difference, males of reproductive stages 0, 1 and 2 were considered to be physically mature, but only those of reproductive stages 1 and 2 were considered to be reproductively active Morgan & Vincent (2013).

As female maturity state can only be determined by dissecting ovaries in freshly dead or preserved specimens (Foster & Vincent, 2004), and sampling obtained only six females in ethanol, females were necessarily assumed to mature at the same size as males.

Given the logistical constraints of this study period, it was assumed that all three species displayed a year-round breeding season, as with the majority of tropical *Hippocampus* spp. and described by Foster & Vincent (2004). Indeed, studies on *H. spinosissimus* and *H. trimaculatus* in Vietnam also confirmed year-round breeding seasons, but with peaks in reproductive activity at certain times of the year (Meeuwig *et al.*, 2006).

DATA ANALYSES

Samples that were acquired were either dried or preserved in 95% ethanol. In order to account for shrinkage that occurs during the drying process, the heights of preserved samples were mathematically converted to dried heights using equations provided in the study of Nadeau *et al.* (2009).

The height at physical maturity (H_M) was determined by fitting a logistic regression to the proportion of mature males (reproductive stages 0, 1 and 2) out of all observed males in a given 10 mm height class to calculate the 50% transition point (King, 2007). The logistic regression was fitted using a non-linear least-squares search function in the R statistical platform (R Development Core Team; www.r-project.org). Similarly, height at reproductive activity (H_R) was determined by fitting a logistic regression to the proportion of reproductively active males (reproductive stages 1 and 2) in a given 10 mm size class against all males in that size class (Morgan & Vincent, 2013).

Minimum, maximum and mean heights were determined for each *Hippocampus* spp. examined in this study. The relationship between mass and height was also determined for each species by fitting a ln-linear regression to these data. To test for evidence of sexual dimorphism, mean heights were compared between sexes using the Mann–Whitney *U*-test, as samples were not normally distributed (*H. kelloggi*: Shapiro–Wilk normality test, $W = 0.9489$, $P < 0.001$; *H. spinosissimus*: Shapiro–Wilk normality test, $W = 0.9940$, $P < 0.05$; *H. trimaculatus*: Shapiro–Wilk normality test, $W = 0.9423$, $P < 0.001$). Height and mass relationships were examined using analysis of covariance (ANCOVA) with sex or region as the covariate, as ln-transformed sample distributions were approximately normal. Comparisons between the sexes were carried out for mature animals only. Where maturity was ambiguous, a category of maturity was assigned retroactively based on estimated height at physical maturity for males (H_M) of each species. Sex ratio was calculated as the proportion of mature individuals that were males to the proportion of mature individuals that were females. A χ^2 -test was used to identify if the ratio was significantly different from unity. Data analyses were carried out using the R statistical platform (R Development Core Team).

Height at first capture (H_C), the point at which a given *Hippocampus* spp. had a 50% chance of being retained when it encountered a fishing net (Sparre *et al.*, 1989), was calculated by fitting a logistic regression to the proportion of individuals caught in a given 10 mm height class compared with the size class where most of the individuals were retained. These analyses were completed using a non-linear least-squares search function in the R statistical platform (R Development Core Team).

PATTERNS ACROSS SPACE AND TIME

Spatial patterns were examined among the regions identified in Fig. 1 where data permitted (mapped using ESRI ArcGIS 9.3), and temporal patterns were examined using only those samples for which the month of capture was known (June, July or August). As sample sizes allowed, spatial and temporal patterns were analysed with respect to mean height, height and mass relationship, sex ratio, H_C and H_M . Spatial patterns could only be examined for *H. spinosissimus* and *H. trimaculatus*, as all *H. kelloggi* samples were obtained from a single location. Spatial and temporal analysis was completed using Kruskal–Wallis tests to compare mean heights, height–mass relationships, H_C and H_M . χ^2 -tests were used to compare sex ratios. All data analyses were carried out using the R statistical platform (R Development Core Team). Tests were considered significant when P values were <0.05 .

RESULTS

No significant difference in mean height was observed when preserved sample heights were mathematically converted to dry-equivalent sample heights for *H. spinosissimus* (Mann–Whitney U -test, $W = 250\,345$, $P > 0.05$) or for *H. trimaculatus* (Mann–Whitney U -test, $W = 21\,173$, $P > 0.05$). Therefore, both dried and preserved samples were pooled in subsequent analysis. All samples of *H. kelloggi* were dried, so conversion was not necessary.

DISTRIBUTION OF HIPPOCAMPUS SPP. IN CATCHES

Hippocampus spp. were encountered at 24 of the 33 landing sites surveyed in this study [Fig. 1(a)], with varying distribution by species [Fig. 1(b)]. *Hippocampus trimaculatus* was the most commonly sampled species of *Hippocampus* spp. encountered in all 23 landing sites with *Hippocampus* spp. [Fig. 1(b)]. The second most commonly encountered species was *H. spinosissimus*, which was sampled from four landing sites: two on the south-east coast and two on the south-west coast [Fig. 1(b)]. *Hippocampus kelloggi* was found at just one landing site on the west coast of the peninsula.

MATURITY

Estimates for height at 50% physical maturity (H_M) were obtained for *H. spinosissimus* and *H. trimaculatus* [Table I and Fig. 2(b), (c), respectively], but not for *H. kelloggi* as only one of the sampled males was immature [Table I and Fig. 2(a)]. Height at 50% reproductive activity (H_R) was modelled for all three species (Table I and Fig. 2).

GEAR RETENTION

Height at 50% capture (H_C) was largest for *H. kelloggi*, smallest for *H. trimaculatus* and intermediate between these two species for *H. spinosissimus* (Table I and Fig. 3). A histogram of all sampled heights with H_C indicated is presented in Fig. 4.

TABLE I. Values for height at 50% capture (H_C), height at 50% physical maturity (H_M) and height at 50% reproductive activity (H_R) for sampled male *Hippocampus kelloggi* ($n = 48$), *Hippocampus spinosissimus* ($n = 77$) and *Hippocampus trimaculatus* ($n = 197$). These data were obtained from May to August 2013 across Peninsular Malaysia. Also included are the γ values associated with the logistic curve, reported with 95% C.I.

	<i>H. kelloggi</i>		<i>H. spinosissimus</i>		<i>H. trimaculatus</i>	
	Mean	95% C.I.	Mean	95% C.I.	Mean	95% C.I.
H_C (mm)	132.5*	127.3–137.7	106.0*	101.1–110.9	82.5*	82.1–82.9
γ	0.14**	0.04–0.25	0.10**	0.05–0.15	0.29*	0.26–0.32
H_M (mm)	N/A	N/A	99.6*	94.2–105.0	90.5*	90.2–90.8
γ	N/A	N/A	0.09**	0.04–0.14	0.16*	0.16–0.17
H_R (mm)	167.4*	148.8–186.0	123.2*	117.0–129.4	121.8*	116.1–127.5
γ	0.04**	0.01–0.06	0.08***	0.04–0.11	0.06***	0.04–0.08

N/A, not applicable as all individuals were mature. P values from logistic regressions: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

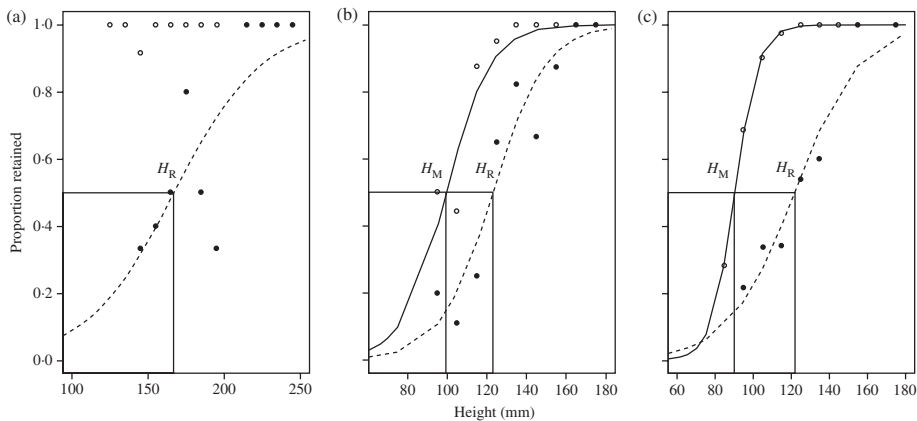


FIG. 2. Logistic regression curves showing height at 50% male maturity (H_M) and height at 50% male reproductive activity (H_R) for (a) *Hippocampus kelloggi*, (b) *Hippocampus spinosissimus* and (c) *Hippocampus trimaculatus* obtained from May to August 2013 across Peninsular Malaysia. For *H. kelloggi*, only H_R could be fitted to a logistic regression as all observed individuals were mature.

MEAN HEIGHT

Mean height varied among all species, and between sexes for two of the species sampled in this study; *H. kelloggi* had the largest mean height and *H. trimaculatus* had the smallest (Table II). Mean height was significantly larger for sampled males than females for both *H. spinosissimus* and *H. trimaculatus*, but no significant difference in sampled mean height was found between sexes for *H. kelloggi* (Table II).

Spatial and temporal differences in mean height were also found. Mean height of the samples obtained in this study differed across regions for *H. trimaculatus*, but not for *H. spinosissimus*. Mean height of *H. trimaculatus* samples were smallest in the south-west region when compared with the north-west and south-east regions (Table III). Both *Hippocampus kelloggi* and *H. spinosissimus* showed significant differences in mean height

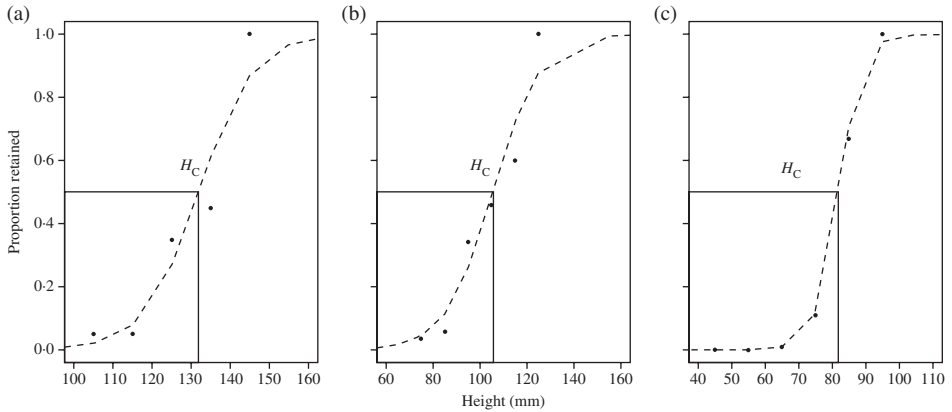


FIG. 3. Logistic regression curves showing the height at first capture (H_C), the point at which a given *Hippocampus* spp. has a 50% chance of being retained in a fishing net, for (a) *Hippocampus kelloggi*, (b) *Hippocampus spinosissimus* and (c) *Hippocampus trimaculatus* obtained from May to August 2013 across Peninsular Malaysia.

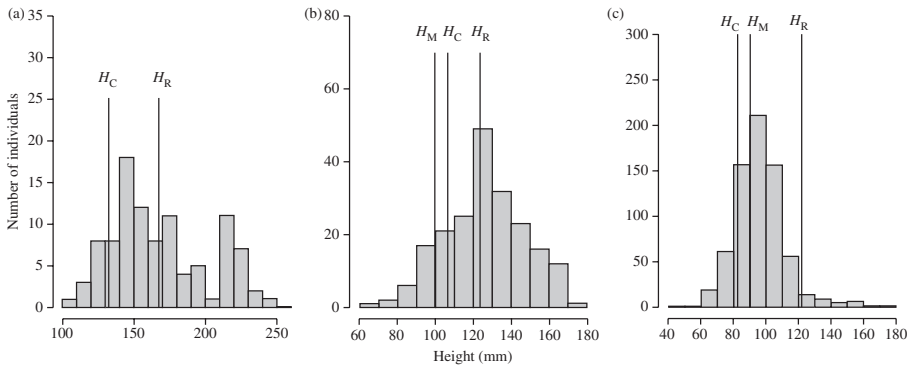


FIG. 4. Total height frequency histograms for (a) *Hippocampus kelloggi*, (b) *Hippocampus spinosissimus* and (c) *Hippocampus trimaculatus* obtained from May to August 2013 across Peninsular Malaysia. Bins containing height at 50% physical maturity (H_M), height at 50% reproductive activity (H_R) and height at first capture (H_C) are indicated. H_M was not included for *H. kelloggi* as all observed individuals were mature.

across the three sampling months. For *H. kelloggi*, the mean height of samples collected in June was significantly smaller than the mean heights observed in July and August, and for *H. spinosissimus* the mean height of samples collected in August was significantly smaller than those collected in June and July. For *H. trimaculatus*, differences could only be compared between June and July because of sample size limitations, and no significant difference was found (Table III).

SEX RATIO

The ratios of sampled mature males to females for *H. kelloggi*, *H. spinosissimus* and *H. trimaculatus* were not significantly different from unity (Table II). Only *H. trimaculatus* showed a spatial pattern in sex ratio, with more mature females

TABLE II. Mean \pm S.D. height, maximum and minimum height, from the mean (mm) and sample size (number of individuals) for mature males (M), mature females (F) and all samples (including juveniles) for *Hippocampus kelloggi*, *Hippocampus spinosissimus* and *Hippocampus trimaculatus*. These data were obtained from May to August 2013 across Peninsular Malaysia. *P* values and test statistics from Mann–Whitney *U*-tests comparing mean heights of mature males with mature females are reported, as well as sampled sex ratios (males to females) using mature animals only. Reported *P* values for sex ratios were obtained using a χ^2 -test

	Sex	Mean \pm S.D. height (mm)	Minimum height (mm)	Maximum height (mm)	Sample size	Height <i>P</i> value and test statistic	Sex ratio
<i>H. kelloggi</i>	All	169.3 \pm 34.4	101	246	100		0.48 (<i>P</i> > 0.05)
	M	171.9 \pm 32.3	124	246	48	<i>P</i> > 0.05	
	F	167.5 \pm 36.6	101	230	52	<i>W</i> = 1133	
<i>H. spinosissimus</i>	All	126.4 \pm 20.9	70	175	205		0.44 (<i>P</i> > 0.05)
	M	135.4 \pm 16.5	101	175	77	<i>P</i> < 0.05	
	F	129.4 \pm 16.7	100	169	97	<i>W</i> = 2956	
<i>H. trimaculatus</i>	All	96.8 \pm 15.8	48	175	688		0.47 (<i>P</i> > 0.05)
	M	107.0 \pm 12.8	91	175	197	<i>P</i> < 0.001	
	F	103.8 \pm 13.7	91	164	224	<i>W</i> = 16 921	

sampled than mature males in the south-west region of Peninsular Malaysia (6% males, d.f. = 1, $\chi^2 = 24.5$, *P* < 0.001).

HEIGHT–MASS RELATIONSHIPS

All samples were pooled by species to examine the ln-linear relationship between height and dry mass, and the relationship was significant for all three species (Table IV). Sexes were pooled for each species because the height–mass relationship was found not to differ between the sexes (*H. kelloggi* ANCOVA, *F* = 1087, d.f. = 2, 95, *P* > 0.05; *H. trimaculatus* ANCOVA, *F* = 50.46, d.f. = 2, 183, *P* > 0.05; *H. spinosissimus* ANCOVA, *F* = 192.1, d.f. = 2, 116, *P* > 0.05). Although significant, height only predicted 59% of the variation in mass for *H. trimaculatus* when all samples were pooled. This was due to the poor condition of some of the dried samples, and was improved when analysis was carried out using preserved animals only. For *H. spinosissimus* only, *Hippocampus* spp. sampled from the north-west coast were significantly heavier than those from the south-east coast [north-west coast slope and 95% C.I. = 3.2 (3.0–3.5), $r^2 = 0.92$, *P* < 0.001; v. south-east coast slope and 95% C.I. = 2.8 (2.6–3.1), $r^2 = 0.87$, *P* < 0.001; ANCOVA, *F* = 4.565, d.f. = 1, 181, 0.05 > *P* > 0.01].

DISCUSSION

This study is the first to examine potential effects of the incidental capture for the heavily traded *Hippocampus* spp. *H. kelloggi*, *H. spinosissimus* and *H. trimaculatus*, and among the first to estimate certain *in situ* life-history variables for these three

TABLE III. Temporal (June, July and August) and spatial [north-west (NW), south-west (SW) and south-east (SE) regions] mean heights, sample sizes (n) and associated 95% C.I. for *Hippocampus kelloggi*, *Hippocampus spinosissimus* and *Hippocampus trimaculatus*. These data were obtained from May to August 2013 across Peninsular Malaysia. Significant spatial and temporal differences in mean height were calculated from Kruskal–Wallis or Mann–Whitney U -tests

	<i>H. kelloggi</i>		<i>H. spinosissimus</i>		<i>H. trimaculatus</i>	
	Mean (mm) (n)	95% C.I.	Mean (mm) (n)	95% C.I.	Mean (mm) (n)	95% C.I.
Temporal						
June	154.7*** (59)	147.0–162.3	129.8 (80)	125.5–134.0	97.4 (180)	95.3–99.5
July	198.5 (22)	185.9–211.2	137.2 (32)	131.9–142.4	98.4 (140)	96.3–100.5
August	176.7 (17)	165.8–187.7	110.9*** (24)	101.4–120.4	N/A	N/A
Spatial						
NW	N/A	N/A	126.0 (161)	122.8–129.2	97.5*** (513)	96.5–98.6
SW	N/A	N/A	N/A	N/A	86.2*** (149)	84.5–88.1
SE	N/A	N/A	128.0 (44)	121.6–134.4	141.7*** (26)	134.9–148.6

N/A, no samples. ***, $P < 0.001$.

TABLE IV. Relationship between height (H , mm) and mass (M , nearest 0.1 g) as fitted by $M = aH^b$ (where a is the intercept and b is the slope of the line) for *Hippocampus kelloggi*, *Hippocampus spinosissimus* and *Hippocampus trimaculatus*. Samples were pooled from May to August 2013, and were obtained across Peninsular Malaysia. P -values indicate significance of r^2 values

	Sample condition	d.f.	b	a	r^2	P
<i>H. kelloggi</i>	Dry	96	3.15	6.56×10^{-7}	0.958	<0.001
<i>H. spinosissimus</i>	Dry	182	3.14	7.80×10^{-7}	0.867	<0.001
<i>H. trimaculatus</i>	Dry	666	2.41	1.35×10^{-5}	0.590	<0.001
<i>H. trimaculatus</i>	Ethanol	67	3.55	1.39×10^{-7}	0.842	<0.001

species. When samples were pooled throughout Peninsular Malaysia, capture occurred at approximately the same height as physical maturity and sex ratios were equal for all species, which might have suggested little reason for concern. It was worrying, however, that height at first capture was less than height at actual reproductive activity for all species. Additionally, regional analyses raised concern for *H. trimaculatus*, the only species sampled from the south-west of the peninsula, as these samples had traits that are often associated with overfished populations, including a highly skewed sampled sex ratio and a smaller mean body size.

Comparing size at maturity with size at which 50% of specimens are retained in fishing gear is a common data-poor assessment method for teleosts (Rueda & Defeo, 2003; Foster & Vincent, 2005, 2010; Kuparinen *et al.*, 2009). For *Hippocampus* spp., however, physical maturity is not always indicative of behavioural or physiological maturity (Cai *et al.*, 1984; Harasti *et al.*, 2012; Morgan & Vincent, 2013). Height at first capture was either similar to (*H. spinosissimus* and *H. trimaculatus*) or greater

than (*H. kelloggi*) height at physical maturity for all species. This could suggest that, on average, these species are able to reproduce before becoming vulnerable to capture. When height at first capture was compared with height at reproductive activity, however, all three species were captured well before (c. 20–40 mm) they actually started reproducing and therefore were unable to contribute to the next generation. In another *Hippocampus* spp., 20 mm of growth corresponded to 1.5 months, a time period that is probably critical for pair bonding (Morgan & Vincent, 2013). Therefore, the common practice of using external indicators of maturity for *Hippocampus* spp. (*H. kelloggi*: Lourie *et al.*, 1999; *H. trimaculatus*: Murugan *et al.*, 2009; *H. spinosissimus*: Nguyen & Do, 1996) would potentially underestimate fishery effects. Fishery effects would also be underestimated if fishers discard smaller, potentially less valuable individuals (Perry *et al.*, 2010), such a practice if it existed would also lead to an overestimation of height at 50% capture.

This distinction between physical maturity and reproductive activity is important as size at maturity plays a critical role in many data-poor assessment techniques. For example, Froese (2004) created three indicators that rely solely on an estimate of length at maturity to assess a catch for the per cent that is: (1) over length at maturity, (2) within an optimal length–weight range and (3) considered to be mega-spawners. Using physical maturity to assess the first three of these indicators (1) for *H. trimaculatus* catch in Peninsular Malaysia would indicate that 61% of the catch was mature, but only 5% would be considered mature when using height at reproductive activity instead. This is concerning, as the majority of the catch may be removed during an important pair-bonding period (Morgan & Vincent, 2013). In contrast, for *H. spinosissimus*, 85% of the catch was above height at physical maturity, and 59% above height at reproductive activity, which is far less concerning. This example shows that applying these indicators to the sampled *Hippocampus* spp. catch from Malaysia provides different insights into the health of the stock.

Variations in life history and sample distribution were also useful for identifying vulnerable *Hippocampus* spp. populations in Peninsular Malaysia. While the distribution of species in samples from this study was consistent with results of a fisheries-dependent survey conducted in 2001 in Peninsular Malaysia (Choo & Liew, 2003), the results of both studies are nonetheless surprising. *Hippocampus spinosissimus* was not encountered in the south-west even though *H. trimaculatus* was, and these two species are thought to share habitat and depth preferences (Choo & Liew, 2003, 2004). The reason why *H. spinosissimus* was absent from the south-west is unclear. While spatial differences in sample distribution cannot be equated with true species distribution due to biases in fishing effort and behaviour, the distribution of most *Hippocampus* spp. is so poorly understood (Foster & Vincent, 2004) that information obtained through fisheries-dependent surveys can still provide valuable insight for *Hippocampus* spp.

As described previously, peninsular-wide catches of *H. trimaculatus* raised concern when assessed using height at reproductive activity. Additional concern was raised by a strong female-biased sex ratio in catches, and a smaller mean height of samples in the south-west compared with the north-west and north-east regions of the peninsula. A female-biased sex ratio has been found in other fished populations of *Hippocampus* spp. (*i.e.* *Hippocampus erectus* Perry 1810; Baum *et al.*, 2003), and in *Hippocampus* spp. sampled from areas with poor environmental conditions (*i.e.* *H. erectus* in Chesapeake Bay; Teixeira & Musick, 2001). Previous studies on the incidental capture

of *H. trimaculatus* have reported both equal sex ratios (Vietnam; Meeuwig *et al.*, 2006), and female-biased sex ratios (India; Murugan *et al.*, 2011). Skewed ratios could also be explained by a violation of the assumption that females mature at the same size as males, but then skewed ratios would be expected for all regions, and not just in one region as was the case for *H. trimaculatus* in this study. Skewed sex ratios may also be associated with spatial segregation between males and females in the population, thus affecting catchability, or it may be indicative of disruption to the breeding population through fishery effects (Baum *et al.*, 2003). Heavy fishing pressure has been shown to reduce mean size in other fish species by selecting for individuals that reach maturity earlier (Hutchings & Baum, 2005), and such fishing pressure may be responsible for the smaller mean height of *H. trimaculatus* observed in the south-west region. These results suggest potential for disruption to the breeding population of *H. trimaculatus* in south-west Peninsular Malaysia, as a highly skewed sex ratio reduces the amount of potential mates (Rowe & Hutchings, 2003), and brood size has been shown to increase with male body size in other species of *Hippocampus* spp. (Teixeira & Musick, 2001).

A lack of time-series data from long-term monitoring makes it impossible to conclusively determine if *Hippocampus* spp. in the south-west are overfished, yet the results from this study can provide valuable information for future assessment. The south-west region of Malaysia has been undergoing rapid development, and damage to coastal ecosystems owing to development is of top concern to the local communities (Lim *et al.*, 2011). These results provide direction to *Hippocampus* spp. research efforts in the region. In order to develop robust assessments of these populations, future efforts should focus on estimating abundance and fishing mortality (Walters & Martell, 2004) and the spatial distribution of each species.

The three heavily traded *Hippocampus* spp. examined in this study demonstrate that, in the face of limited data, simple catch information can be used to estimate life-history variables and identify populations that may be overfished. While this study provides the first robust *in situ* estimates of life-history variables for *H. kelloggi*, and is among the first to do so for *H. spinosissimus* and *H. trimaculatus*, the methods described are applicable to many species of small, incidentally captured fishes. Small fishes are often considered to be less vulnerable to overfishing (Jennings *et al.*, 1999), yet this study and others demonstrate that these fishes too can show signs of overfishing (Foster & Vincent, 2010). The results in this study also showed that overfishing assessments for these species can differ based on how length at maturity is defined, a variable that is often the cornerstone of data-poor assessment methods. Ideally, length at maturity should always be defined as the point where reproductive activity begins. This is especially true for rare or endangered species where maturity is often determined externally [*e.g.* skates and rays: Estalles *et al.* (2011); fin whales: Aguilar & Lockyer (1987); leatherback turtles: Stewart *et al.* (2007)].

This is a contribution from Project Seahorse. This study was made possible through collaborations with the Institute of Earth and Ocean Science at the University of Malaya, and was supported by grants from The Explorers Club and the University of British Columbia's Biodiversity Research Integrative Training and Education (BRITE) programme. Support provided by the John G. Shedd Aquarium in Chicago, Guylian Chocolates Belgium and an anonymous donor was also critical in advancing the goals of this project. A special thanks to Y. S. Leong who assisted not only in sample and data collection, but was also critical in helping translate conversations with fishers, and in many other aspects of field support. Lastly, this contribution recognizes C. C. Kuang, who laid the foundations of seahorse research in Malaysia before his untimely death in 2013.

References

- Aguilar, A. & Lockyer, C. H. (1987). Growth, physical maturity, and mortality of fin whales (*Balaenoptera physalus*) inhabiting the temperate waters of the northeast Atlantic. *Canadian Journal of Zoology* **65**, 253–264.
- Alverson, D. L. (1994). A global assessment of fisheries bycatch and discards. *FAO Fisheries Technical Paper* **339**.
- Baum, J. K., Meeuwig, J. J. & Vincent, A. C. J. (2003). Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. *Fishery Bulletin* **101**, 721–731.
- Boisseau, J. (1967). Régulations hormonales de l'incubation chez un vertébré mâle: recherches sur la reproduction de l'Hippocampe. PhD Thesis, Bordeaux, University of Bordeaux, France.
- Cai, N., Xu, Q., Yu, F., Wu, X. & Sun, G. (1984). Studies on the reproduction of the seahorse *Hippocampus trimaculatus*. *Studia Marina Sinica* **23**, 83–92.
- Choo, C. K. & Liew, H. C. (2003). Spatial distribution, substrate assemblages and size composition of sea horses (Family Syngnathidae) in the coastal waters of Peninsular Malaysia. *Journal of the Marine Biological Association of the United Kingdom* **83**, 271–276.
- Choo, C. K. & Liew, H. C. (2004). A record of seahorse species (Family Syngnathidae) in East Malaysia, with notes on their conservation. *Malayan Nature Journal* **56**, 409–420.
- Choo, C. K. & Liew, H. C. (2005). Exploitation and trade in seahorses in Peninsular Malaysia. *Malayan Nature Journal* **57**, 57–66.
- Curtis, J. M. R. & Vincent, A. C. J. (2006). Life history of an unusual marine fish: survival, growth and movement patterns of *Hippocampus guttulatus* Cuvier 1829. *Journal of Fish Biology* **68**, 707–733. doi: 10.1111/j.1095-8649.2006.00952.x
- Dulvy, N. K. & Reynolds, J. D. (2002). Predicting extinction vulnerability in skates. *Conservation Biology* **16**, 440–450.
- Estalles, M., Coller, N. M. & Perier, M. R. (2011). Skates in the demersal trawl fishery of San Matías Gulf, Patagonia: species composition, relative abundance and maturity stages. *Aquatic Living Resources* **24**, 193–199. doi: 10.1051/alr/2011119
- Foster, S. J. & Vincent, A. C. J. (2004). Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology* **65**, 1–61. doi: 10.1111/j.1095-8649.2004.00429.x
- Foster, S. J. & Vincent, A. C. J. (2005). Enhancing sustainability of the international trade in seahorses with a single minimum size limit. *Conservation Biology* **19**, 1044–1050. doi: 10.1111/j.1523-1739.2005.00192.x
- Foster, S. J. & Vincent, A. C. J. (2010). Using life-history information to assess potential effects of shrimp trawling on small fishes. *Journal of Fish Biology* **76**, 2434–2454. doi: 10.1111/j.1095-8649.2010.02631.x
- Froese, R. (2004). Keep it simple: three indicators to deal with overfishing. *Fish and Fisheries* **5**, 86–91.
- Hall, M. A. (1997). On bycatches. *Reviews in Fish Biology and Fisheries* **6**, 319–352.
- Harasti, D., Martin-Smith, K. & Gladstone, W. (2012). Population dynamics and life history of a geographically restricted seahorse, *Hippocampus whitei*. *Journal of Fish Biology* **81**, 1297–1314. doi: 10.1111/j.1095-8649.2012.03406.x
- Hutchings, J. A. & Reynolds, J. D. (2004). Marine fish population collapses: consequences for recovery and extinction risk. *BioScience* **54**, 297–309. doi: 10.1641/0006-3568(2004)054[0297:MFPCCF]2.0.CO;2
- Hutchings, J. A. & Baum, J. K. (2005). Measuring marine fish biodiversity: temporal changes in abundance, life history and demography. *Philosophical Transactions of the Royal Society B* **360**, 315–338. doi: 10.1098/rstb.2004.1586
- Jennings, S., Reynolds, J. D. & Polunin, N. V. C. (1999). Predicting the vulnerability of tropical reef fishes to exploitation with phylogenies and life histories. *Conservation Biology* **13**, 1466–1475.
- Kelleher, K. (2005). Discards in the world's marine fisheries. *FAO Fisheries Technical Paper* **470**.
- King, M. (2007). *Fisheries Biology, Assessment and Management*, 2nd edn. Oxford: John Wiley & Sons.

- Kuparinen, A., Kuikka, S. & Merilä, J. (2009). Estimating fisheries-induced selection: traditional gear selectivity research meets fisheries-induced evolution. *Evolutionary Applications* **2**, 234–243. doi: 10.1111/j.1752-4571.2009.00070.x
- Lim, A., Chong, V. C., Wong, C. S. & Choo, C. K. (2011). Diversity, habitats and conservation threats of syngnathid (Syngnathidae) fishes in Malaysia. *Tropical Zoology* **24**, 193–222.
- Lourie, S. A. (2003). Measuring seahorses. *Project Seahorse Technical Report No 4*. Vancouver, BC: Project Seahorse, Fisheries Centre, The University of British Columbia.
- Lourie, S. A., Pritchard, J. C., Casey, S. P., Truong, S. K., Hall, H. J. & Vincent, A. C. J. (1999). The taxonomy of Vietnam's exploited seahorses (family Syngnathidae). *Biological Journal of the Linnean Society* **66**, 231–256.
- Lourie, S. A., Foster, S. J., Cooper, E. W. T. & Vincent, A. C. J. (2004). *A Guide to the Identification of Seahorses*. Washington, DC: Project Seahorse and TRAFFIC North America.
- Meeuwig, J. J., Hoang, D. H., Si Ky, T., Job, S. D. & Vincent, A. C. J. (2006). Quantifying non-target seahorse fisheries in central Vietnam. *Fisheries Research* **81**, 149–157.
- Morgan, S. K. & Vincent, A. C. J. (2013). Life-history reference points for management of an exploited tropical seahorse. *Marine and Freshwater Research* **64**, 185–200. doi: 10.1071/MF12171
- Murugan, A., Dhanya, S., Sreepada, R. A., Rajagopal, S. & Balasubramanian, T. (2009). Breeding and mass-scale rearing of three spotted seahorse, *Hippocampus trimaculatus* Leach under captive conditions. *Aquaculture* **290**, 87–96. doi: 10.1016/j.aquaculture.2009.01.033
- Murugan, A., Dhanya, S., Sarcar, A. B., Naganathan, V., Rajagopal, S. & Balasubramanian, T. (2011). Fishery biology, demography of the three spotted seahorse, *Hippocampus trimaculatus* inhabiting Gulf of Mannar region, southeast coast of India. *Indian Journal of Geo-Marine Sciences* **40**, 411–423.
- Nadeau, J. L., Curtis, J. M. R. & Lourie, S. A. (2009). Preservation causes shrinkage in seahorses: implications for biological studies and for managing sustainable trade with minimum size limits. *Aquatic Conservation: Marine and Freshwater Ecosystems* **19**, 428–438. doi: 10.1002/aqc.1002
- Nguyen, V. L. & Do, H. H. (1996). Biological parameters of two exploited seahorse species in a Vietnamese fishery. In *Proceedings of the 1st International Conference in Marine Conservation* (Morton, B., ed), pp. 449–464. Hong Kong: Hong Long University Press.
- Perante, N. C., Pajaro, M. G., Meeuwig, J. J. & Vincent, A. C. J. (2002). Biology of a seahorse species *Hippocampus comes* in the central Philippines. *Journal of Fish Biology* **60**, 821–837. doi: 10.1006/jfbi.2002.1878
- Perry, A. L., Lunn, K. E. & Vincent, A. C. J. (2010). Fisheries, large-scale trade, and conservation of seahorses in Malaysia and Thailand. *Aquatic Conservation: Marine and Freshwater Ecosystems* **20**, 464–475.
- Rowe, S. & Hutchings, J. A. (2003). Mating systems and the conservation of commercially exploited marine fish. *Trends in Ecology and Evolution* **18**, 567–572. doi: 10.1016/j.tree.2003.09.004
- Rueda, M. & Defeo, O. (2003). Linking fishery management and conservation in a tropical estuarine lagoon: biological and physical effects of an artisanal fishing gear. *Estuarine, Coastal and Shelf Science* **56**, 935–942. doi: 10.1016/S0272-7714(02)00298-6
- Sadovy, Y. (2001). The threat of fishing to highly fecund fishes. *Journal of Fish Biology* **59**, 90–108. doi: 10.1006/jfbi.2001.1760
- Sparre, P., Ursin, E. & Venema, S. C. (1989). Introduction to tropical fish stock assessment. *FAO Fisheries Technical Paper* **306**.
- Stewart, K., Johnson, C. & Godfrey, M. H. (2007). The minimum size of leatherbacks at reproductive maturity, with a review of sizes for nesting females from the Indian, Atlantic and Pacific Ocean basins. *Herpetological Journal* **17**, 123–128.
- Teixeira, R. L. & Musick, J. A. (2001). Reproduction and food habits of the lined seahorse, *Hippocampus erectus* (Teleostei: Syngnathidae) of Chesapeake Bay, Virginia. *Revista Brasileira de Biologia* **61**, 79–90.
- Vincent, A. C. J., Foster, S. J. & Koldewey, H. J. (2011). Conservation and management of seahorses and other Syngnathidae. *Journal of Fish Biology* **78**, 1681–1724. doi: 10.1111/j.1095-8649.2011.03003.x

- Vincent, A. C. J., Sadovy de Mitcheson, Y. J., Fowler, S. L. & Lieberman, S. (2013). The role of CITES in the conservation of marine fishes subject to international trade. *Fish and Fisheries* (in press). doi: 10.1111/faf.12035
- Walters, C. J. & Martell, S. J. D. (2004). *Fisheries Ecology and Management*. Princeton, NJ: Princeton University Press.
- Winemiller, K. O. (2005). Life history strategies, population regulation, and implications for fisheries management. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 872–885.

Electronic References

- IUCN (2013). *IUCN Red List of Threatened Species. Version 2013.2*. Available at <http://www.iucnredlist.org/> (last accessed 31 March 2014).
- UNEP-WCMC (2012). *CITES Trade Database*. Available at <http://www.unep-wcmc.org/citestrade/trade.cfm/> (last accessed 31 March 2014)