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Seasonal distribution of seahorses employing a social reproductive strategy and supporting for a closed fishing season

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The unique morphology and life history characteristics make seahorses excellent flagship species for marine ecosystems. The ecological rarity of seahorses has made it difficult to address fundamental demographic processes of seahorses, such as seasonal migration and ecological characteristics. This 3-year investigation evaluated, for the first time, the seasonal distribution of a wild seahorse, *Hippocampus mohnikei*, and its ecological significance. The results revealed a significant difference in the abundance of wild *H. mohnikei* populations between warm and cold seasons, indicating that seahorses might undertake a seasonal inshore-offshore migration every year. A total of 42 794 samples were taken during the 3-year survey in the Laizhou Bay, and migratory seahorses had the highest reproductive rates from July to September each year, suggesting that seahorse reproduction might be an important trigger for variation in distribution and migration. Moreover, water temperature might also affect the temporal and spatial abundance of migratory seahorses. There was no genetic subdivision among the different geographical populations of *H. mohnikei* along China's coast. In conclusion, this study confirmed that large-scale seasonal seahorse migrations occur periodically and that reproduction is one of the important drivers, suggesting that closed-season fishing of breeding grounds is essential and applicable for seahorse conservation.

Keywords: genetic divergence, *Hippocampus mohnikei*, migration, population, reproduction.

Introduction

Seahorses (genus *Hippocampus*) are flagship species in a wide range of marine ecosystems, but some species are overexploited for traditional Chinese medicine (TCM) and by the aquarium trade (Thangaraj and Lipton, 2007; Koldewey and Martin-Smith, 2010; Vincent *et al.*, 2011). The genus *Hippocampus* is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and the status of most species is listed as Data Deficient (IUCN, 2016). However, on the Red List of Threatened Species, several *Hippocampus* species are currently listed as Vulnerable or Endangered (IUCN, 2016). Seahorses have much in common with other small marine fish species, both in terms of the threats they face and our general lack of knowledge regarding their biology (Foster and Vincent, 2004). Existing information about the life history characteristics of seahorses includes their sparse distributions, low mobility, small home ranges, low fecundity, and mate fidelity, all of which contribute to their vulnerability to overfishing, pollution, and habitat damage (Lourie *et al.*, 2016; Foster and Vincent, 2004; Vincent *et al.*, 2011; Lin *et al.*, 2012). Additional knowledge about the distribution and migration patterns of seahorses would have important implications for fisheries management and biodiversity conservation (Foster and Vincent, 2004).

Migration is one of the most important behaviours in most fish species; the adult fish of many species periodically migrate to shallow near-shore waters to spawn, whereas juveniles or immature groups swim offshore to feed and overwinter (Conover and Murawski, 1982). Seahorses have been commonly recognized as native species that do not typically undergo migration because of their low mobility, although newborns have a planktonic stage that facilitates population dispersion (Foster and Vincent, 2004; Curtis and Vincent, 2006; Caldwell and Vincent, 2013). However, there are a few seahorse studies that suggest migration characteristics. For instance, seahorses can take small migrations in search of proper habitat, food or holdfasts (Caldwell and Vincent, 2013; Correia, 2015). Seasonal migration behaviour has been inferred in several seahorse species, including *Hippocampus capensis*, *Hippocampus erectus*, *Hippocampus mohnikei*, and *Hippocampus whitei*, but no *in situ* survey data have supported the hypothesis so far (Whitfield, 1995; Foster and Vincent, 2004; Choi *et al.*, 2012; Boehm *et al.*, 2015). Owing to unclear knowledge about the life history of seahorses, many conservation protocols concerned with the seasonal variation of the reproductive behaviour and migrations remain controversial (Whitfield, 1995; Choi *et al.*, 2012).

Genetics can be used to test the current status of population structure, to estimate the movement of individuals between local populations, and to characterize the spatial distribution of genetic variation (Lowe and Allendorf, 2010; Eggert *et al.*, 2014). Migration might improve gene flow frequency and thus prevent genetic subdivision among different geographic populations (Hellberg, 2009). Analyses of the genetic structure and connections among seahorses can be meaningful for the estimation of species distributions and migration patterns (Lourie *et al.*, 2005; Teske *et al.*, 2007). Moreover, population genetic studies can contribute to the identification of evolutionarily significant units and management units to inform evidence-based conservation decision-making regarding endangered species, such as seahorses (Teske *et al.*, 2004; Zhang *et al.*, 2014).

There are diverging opinions regarding the hypothesized occurrence of offshore or near-shore migration in seahorses and about their relatives in the Syngnathidae family (Kanou and Kohno, 2001); however, data about seahorse migrations and their ecological drivers are lacking (Foster and Vincent, 2004). To investigate whether migration exists in a seahorse population and why it occurs, well-protected or unexploited seahorse stocks should be investigated. Although some studies have focused on seahorse resources for many years, only a few have investigated migration owing to the high cost of conducting such studies.

Hippocampus mohnikei is a small seahorse species that is widely distributed in shallow waters in the northwestern Pacific Ocean (Lourie *et al.*, 2016). *Hippocampus mohnikei* is widely distributed in Hokkaido, Matsushima Bay, Tokyo Bay, and the southern coastal waters of Korea and China (Kanou and Kohno, 2001; Otsuka *et al.*, 2009; Choi *et al.*, 2012; Zhang *et al.*, 2014); it also occurs in Southeast Asia as well as in the Indian Ocean (Thangaraj and Lipton, 2007; Aylesworth *et al.*, 2016). Although all seahorse species have been threatened by marine pollution or habitat destruction, the wild *H. mohnikei* population has experienced a relatively low degree of exploitation owing to the small size of the individuals and their low market value for TCM (Wen *et al.*, 2013). Our primary survey also determined that the abundance of *H. mohnikei* was much higher than that of other seahorse species, suggesting that *H. mohnikei* could be a good model species to determine the distribution and migration of seahorses. A series of field investigations and laboratory analyses in the present study aimed to answer the following questions: does seasonal migration exist in this wild seahorse population? If so, what is the ecological function of the migration? What is the genetic structure of this species given its migration patterns?

Material and methods

Field investigation

We investigated the wild seahorse *H. mohnikei* in 17 study locations along the coast of China for three years (2012–2015) to evaluate the abundance of seahorse stocks (Figure 1, Supplementary Table S1). We investigated each location twice a year, once during the warm season (from May to October) and once during the cold season (from November to April). At all of the locations, port visits and interviews with fishermen and biologists were also conducted to obtain baseline data before the start of the investigation. All of the research locations were investigated during the same month and in the same sea areas during the yearly survey. Three research groups participated in this investigation during the two seasons, and each group consisted of two researchers and two fishermen. The total area surveyed per site covered $\sim$10–30 km^2, and random travelling routes in the surveyed areas were adopted to evaluate the abundance of seahorses per site, considering the patchy distribution of *H. mohnikei* (Choi *et al.*, 2012). All survey areas were less than 30 km from the coast, where the water depth was less than 40 m (Supplementary Table S1). A seining net (length × width = 100 m × 5 m; mesh size of 10 mm) trawled with two small boats (2–3 tons, 13–20 km hr^{-1}, depending on the local fishermen and weather conditions) was used to collect the seahorses, and the actual working time at each site was 6 hr day^{-1}. Finally, the numbers of seahorses caught per day at each site were used to evaluate the abundance of the local seahorses in this study; this survey method was modified from Choi *et al.* (2012).

According to our previous survey, *H. mohnikei* is most abundant in the Laizhou Bay of the Bohai Sea; therefore, to evaluate the potential temporal variation in the number of *H. mohnikei*, we performed a continuous daily seining net investigation at Qinglingpu (37.31°N 119.88°E) in the Laizhou Bay from May 2012 to May 2015 (Figure 1). The daily seahorse catch yield per boat was recorded. The body height (BH) and wet weight (WW) were measured according to the methods described by Lourie *et al.* (2016). The seawater temperature of the study area was recorded by nearby hydrometeorological buoys.

Characteristics related to reproduction

Adult males were separated from females based on the presence of a brood pouch (Otsuka *et al.*, 2009). Seahorses were regarded as young if they were smaller than the smallest seahorse with a brood pouch (BH = 49 mm) (Choi *et al.*, 2012). Pregnant males were identified by the presence of a distended and full brood pouch (Foster and Vincent, 2004). The sex ratio (males:females) and the percentage of pregnant males (numbers of pregnant males divided by total males) were also calculated during sampling. The effective brood size (number of offspring born) was determined by taking a subset of pregnant males back to the laboratory and enumerating the neonates after they were released from the brood pouch. Furthermore, we cultured wild seahorses from the Laizhou Bay in indoor ponds (5 m × 10 m × 1 m), from July to September 2012, for behavioural observation. The courtship and aggressive behaviour of the collected seahorses in the pond were observed for two hours (9:00 am to 11:00 am) in

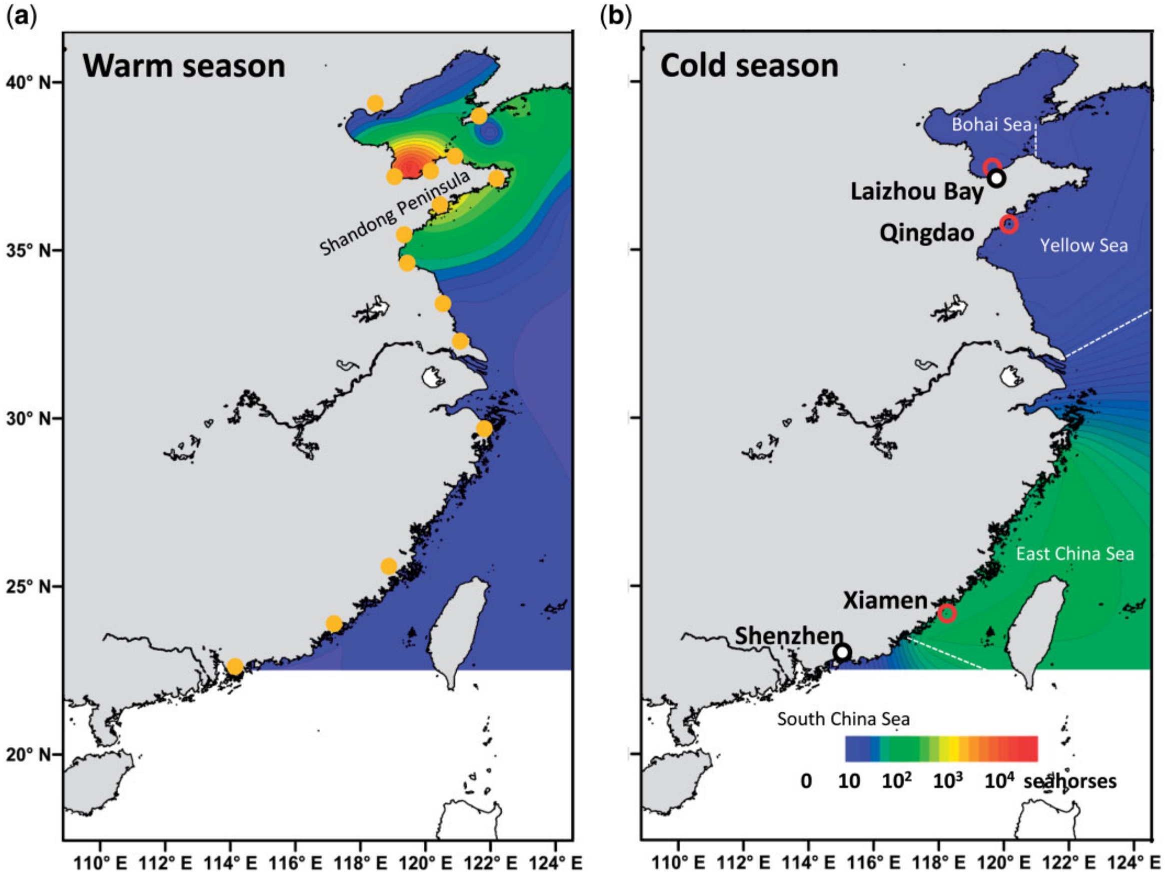

Figure 1. Relative abundance (seahorses collected per season) of *Hippocampus mohnikei* along the coastline of China during the warm season (from May to October) and the cold season (from November to April). Yellow circles indicate the survey and sampling locations; black empty circles indicate the locations of the culture experiments; solid red circles indicate the three sites where samples were collected for genetic analysis.

the morning, based on the methods from Faleiro *et al.* (2008). The courtship activity was defined as the percentage of male and female seahorses performing a heterosexual mating dance among all of the collected seahorses, whereas aggressive activity referred to the percentage of seahorses dancing with the same sex.

Variation in growth among low- and high-latitude populations

Hippocampus mohnikei has a wide distribution along China's coast, and this study compared the growth and feeding performances of low- and high-latitude seahorse populations from September 2012 to May 2013. The high-latitude population consisted of 600 seahorses collected from Laizhou Bay, whereas the low-latitude population consisted of 600 seahorses collected from the Fujian and Guangdong coastlines. All of the seahorses in the two populations were sexually mature in equal ratios. Both seahorse populations were kept indoors in six concrete ponds (3 m × 5 m × 1 m), with a density of 100 ind. pond^{-1} at the Laizhou Seahorse Center (Laizhou, China, 37.21°N 119.91°E) and the Shenzhen Seahorse Center (Shenzhen, China, 22.27°N 113.46°E). Seawater was pumped directly from the nearby sea, and the culture temperature was maintained at a similar level as the local ambient seawater temperature. The seahorse husbandry management procedures were the same at the two research centres, and the water temperature was recorded daily throughout the experiment. Seahorses at both sites were fed the same diets, which included live adult *Artemia* and *Mysis*, and the daily food intake was recorded by individual seahorses with daily feeding amounts divided by the number of seahorses in each pond. The BH, WW and condition factor $[(K = \mathrm{WW}\ (\mathrm{g})/[\mathrm{BH}\ (\mathrm{cm})]^3 \times 100)]$ of the seahorses were calculated at the end of the experiment.

Population genetics

A total of 182 seahorses were sampled from 3 locations to investigate the population genetics of *H. mohnikei*. We used TIANamp Marine Animal DNA Kit (Tiangen, Dalian, China) for DNA extraction from partial fin clip from the seahorses. Partial mitochondrial cytochrome *b* (*Cytb*) gene (779 bp) was amplified by employing the seahorse-specific forward (5′-CTACCTGCACCATCAAATATTTC-3′) and reverse (5′-CGGAAGGTGAGTCCTCGTTG-3′) primers following the methodology of Zhang *et al.* (2014). The mitochondrial *COI* gene (633 bp) was amplified by employing new primers, forward 5′-TCAACTAATCACAAAGACATCGGCAC-3′ and reverse 5′-ACTTCGGGGTGCCCAAAGAATC-3′. All of the sequences were submitted to GenBank (accession numbers: KX688906–KX689045, KX689046–KX689227). Genetic diversity indices were calculated using MEGA 6.0

(Tamura *et al.*, 2013). We also tested the neutrality of the markers using Arlequin 3.1, employing Tajima's D and Fu's Fs, as well as Pairwise mismatch distributions (Tajima, 1989; Fu, 1997; Excoffier and Lischer, 2010). The haplotype diversity (h) and nucleotide diversity (p) for each population were estimated using DnaSP 5.10.00 software (Librado and Rozas, 2009), and an unrooted median-joining haplotype network was constructed using the Network 4.6.1.0 software package (Bandelt *et al.*, 1999). The single nucleotide polymorphisms of the mitochondrial sequences were identified and analysed using a Bayesian clustering approach to estimate the number of populations (K) in STRUCTURE version 2.3 (Pritchard *et al.*, 2000).

Statistical analysis

Statistical analyses were conducted using SPSS 19.0 (Statistical Program for the Social Sciences 19.0, IBM, USA) and SigmaPlot 10.0 (version 10.0, Systat Software, Inc., USA). A one-way analysis of variance (ANOVA) was used to assess the differences in the BH, WW, growth rate, and condition factors of the seahorses among the treatments at a confidence level of $\alpha = 0.05$. If the ANOVA results were significant, the different mean values were compared using the *post hoc* least significant differences (Duncan test).

Results

Variation in seahorse distribution and abundance

During the warm seasons (from May to October) from 2012 to 2015, *H. mohnikei* seahorses were widely distributed in the shallow waters along China's coastline, primarily along the high-latitude coast (Figure 1). There were dramatic differences in seahorse distribution and abundance between the warm and cold seasons (Figure 1). Laizhou Bay had the most abundant populations of seahorses, with more than 10 000 seahorses detected during the warm season. The seahorses were also highly abundant adjacent to this area. In contrast, no seahorses were found along the high-latitude coasts (>35°N), and seahorses were only found at relatively lower abundance in the low-latitude area (<35°N) during the cold season (from November to April; Figure 1, Supplementary Table S1).

Seasonal variation in the seahorse population in Laizhou Bay

A continuous, large-scale survey conducted from May 2012 to May 2015, consisting of 42 794 seahorses from 79 catches in the Laizhou Bay, indicated that the abundance of wild seahorse stocks varied markedly (Figure 2a). The survey revealed that the cumulative catch yield of seahorses in the Laizhou Bay reached the 50th percentile of the total annual catch yield one month sooner in the 2012 season, compared with 2013 and 2014 (Figure 2b). There were no significant differences in catch yield among the three years (ANOVA, $F_{2,76} = 1.226$, $p = 0.299$), and no seahorses were found in winter (Figure 2a and b). An obvious periodic temporal pattern in the abundance of wild seahorse stocks was exhibited over the three years (Figure 2a). Moreover, the seawater temperature was positively correlated with seahorse abundance ($\rho = 0.543$, $p < 0.001$), and most of the seahorses were caught when the temperature of the local seawater was greater than 20 °C (Figure 2c).

Characteristics of the summer population

A body structure analysis indicated that the summer seahorse population in the Laizhou Bay ranged from 37 to 76 mm in BH and 0.30 to 1.20 g in WW. The standard-length frequency histograms of the seahorse populations suggested that the size distributions of the landed seahorses were unimodal, with the same peaks in BH, which ranged from 54 to 60 mm, and WW, which ranged from 0.6 to 0.7 g (Figure 3).

The seahorses in the Laizhou Bay population displayed both courtship and aggressive behaviours, which lasted from July to September and peaked before August in 2012. A total of 10–44% of the collected seahorses participated in courtship (Figure 4a), and aggressive behaviour was exhibited earlier than courtship behaviour (generally 1 or 2 weeks earlier). Pregnant seahorses were found in high numbers in July and August, and the average percentage of pregnant males was 34.7 ± 14.6%, with a peak value of 58.4% (Figure 4b). A total of 42 clutches of newborns were used to evaluate brood size, and the mean brood size of the pregnant males was 186 ± 140 ind. per brood (ranging from 20 to 654 ind.) with a median value of 153 ind. per brood (Figure 4c).

Growth variation in low- and high-latitude populations

After nine months of local husbandry, a remarkable increase in the BH of *H. mohnikei* occurred in both the low- and high-latitude populations, but there was no significant difference in BH between the two treatments (75.0 ± 5.1 mm in the low-latitude vs. 77.0 ± 3.5 mm in the high-latitude, $t = -1.163$, $df = 34$, $p = 0.253$, Figure 5b). Seahorses in the low-latitude location (warm water) exhibited higher growth rates in both WW (1.50 ± 0.31 g; $t = -7.300$, $df = 34$, $p < 0.01$) and the condition factor (K; 3.3 ± 0.5; $t = -10.961$, $df = 34$, $p < 0.01$) compared with those in the high-latitude location (cold water) after 9 months of growth (Figure 5a and c). The feeding behaviour of the seahorses was also significantly affected by water temperature in this study ($\rho = 0.811$, $p < 0.001$); when the temperature was above 20 °C, the food intake by the seahorses in the warm-water groups was $\sim 0.40 \pm 0.01\ \mathrm{g \cdot d^{-1} \cdot g^{-1}}$. When the temperature decreased below 12 °C, the food intake by seahorses in the cold-water groups dropped sharply to $0.03 \pm 0.01\ \mathrm{g \cdot d^{-1} \cdot g^{-1}}$, and most seahorses stopped swimming (Figure 5d).

Population genetics

The results indicate that the gene diversity (h) of *H. mohnikei* ranged from 0.9785 to 0.9803, and the nucleotide diversity (π) ranged from 0.0345 to 0.0381. Among the 1412 bp nucleotide sequence, there were 132 variable sites, and 56 parsimony informative sites were found at these variable sites (Supplementary Table S2). A Bayesian cluster analysis estimated two parental populations ($K = 2$) of *H. mohnikei* from three geographical populations, and the three populations did not show any distinct structure (Figure 6a). An Analysis of molecular variance (AMOVA) revealed that there was no geographic subdivision among the three populations ($\Phi_{ST} = 0.00116$, $p = 0.333$).

A total of 133 unique haplotypes were identified from 182 seahorse samples, and the haplotype diversity was 0.978 ± 0.007. The median-joining network among the haplotypes of the *H. mohnikei* populations presented a star-like distribution, and two shared haplotypes representing 13.85 and 4.90% of the individuals were identified (Figure 6b). The partial mitochondrial genes all exhibited a unimodal mismatch distribution (Figure 6c).

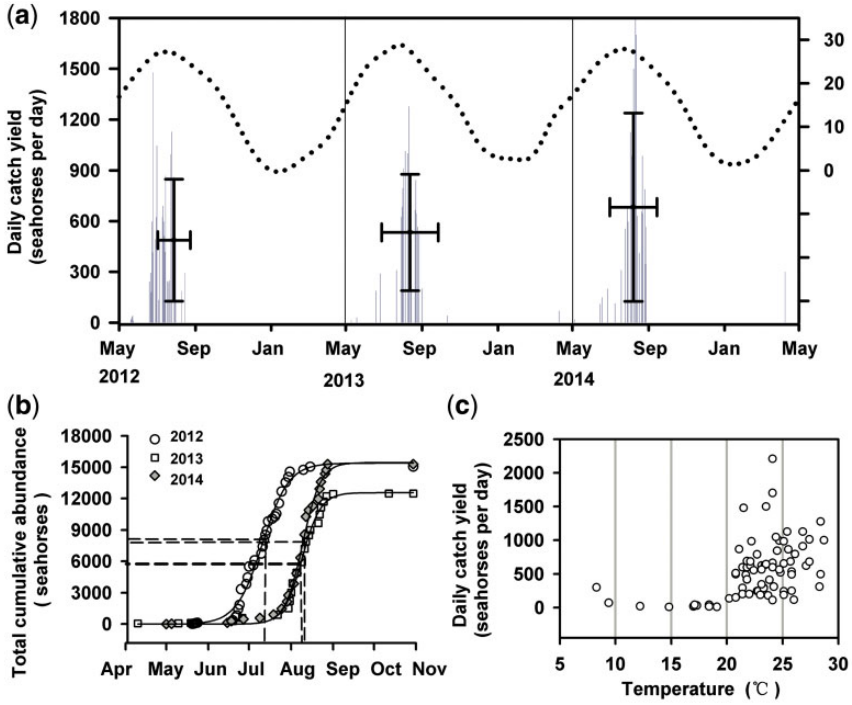

Figure 2. The variation in the daily catch yield of *Hippocampus mohnikei* seahorses (a) and the total cumulative abundance (b) in Laizhou Bay from May 2012 to April 2015; (c) the daily catch yields were positive relative to temperature ($\rho = 0.543$, $p < 0.001$). The dotted line in (a) represents the temperature of the seawater, and the grey areas indicate the warm seasons (May to October). The short dashed lines in (b) indicate the 50th percentile of total cumulative abundance.

Tajima's D and Fu's F_S statistics from a neutrality test were both significantly negative for all three populations (Supplementary Table S2).

Discussion

The present study is the first to investigate the geographic distribution and seasonal abundance of *H. mohnikei* along the coast of China. Our survey on seahorse abundance suggested that *H. mohnikei* abundance varied remarkably in lower and higher latitude sea areas. *Hippocampus mohnikei* in lower latitude sea areas, such as the East China Sea, could be found all year long, in accordance with the reports of *H. mohnikei* in Southeast Asia (Aylesworth *et al.*, 2016). In contrast, *H. mohnikei* in the north China seas occurred in the near shore shallow waters only during the warm seasons, from May to October. The continuous 3-year investigation in Laizhou Bay strongly supports that seasonal variation of seahorse abundance occurred every year. This result is consistent with reports of seahorses in Tokyo Bay, Matsushima Bay and South Korea (Kanou and Kohno, 2001; Otsuka *et al.*, 2009; Choi *et al.*, 2012).

The present findings suggest that substantial seasonal migration might occur among seahorses in high latitude areas. Seahorses are considered prime examples of sedentary marine fish (Foster and Vincent, 2004), and sedentary animals can remain within a small area because they can access all of their needed resources at that location (Caldwell and Vincent, 2013). Sedentary fish should be more sensitive to habitat change than more mobile fish (Caldwell and Gergel, 2013). However, temperature, food availability, and shelter in high latitude areas along the China coast, such as in Laizhou Bay, varied a lot in different seasons. In summer, the bottom seawater temperature could be above 25 °C, whereas it decreased to below 5 °C in Laizhou Bay in winter (Song, 2009). Zooplankton, the diet of seahorses, also varied significantly in their species composition and abundance (Bi *et al.*, 2000). Therefore, it is difficult for *H. mohnikei* to remain in shallow areas throughout the year. Furthermore, considering that seahorses had limited daily movement, the adults were most likely to migrate seasonally to deeper waters nearby for overwintering, but not likely to move a long distance from higher cold latitudes to lower warm latitudes. Similar migrations were inferred for several other seahorse species, including *H. erectus, H. guttulatus, H. whitei* and *H. hippocampus*, although there are no specific data to date (Foster and Vincent, 2004). Similar to these seahorses, a wild population of the pipefish *Syngnathus fuscus* has been found to migrate along a particular route; this species inhabits estuaries from the spring through the fall and moves into near-shore continental shelf waters in September–November before returning to estuaries in March–April (Lazzari and Able, 1990).

The life history traits and behaviours of seahorses suggest that their appearance in Laizhou Bay in summer coincided with their breeding stages, and most of the seahorses collected during the warm season were mature adults. Positive courtship and aggressive behaviours further substantiate that the *H. mohnikei* were in their breeding season. This seasonal breeding phenomenon in *H. mohnikei* is similar to that of an investigation of the ratio of mature oocytes in Matsushima Bay (Otsuka *et al.*, 2009), as well as a study of the early life history in Tokyo Bay (Kanou and Kohno, 2001).

A total of 42 794 seahorses (599–649 seahorses$\cdot$d^{-1}) were collected during the research in Laizhou Bay, which was far more than has been collected in any previous seahorse survey (Baum *et al.*, 2003; Curtis and Vincent, 2006). It is likely that a large

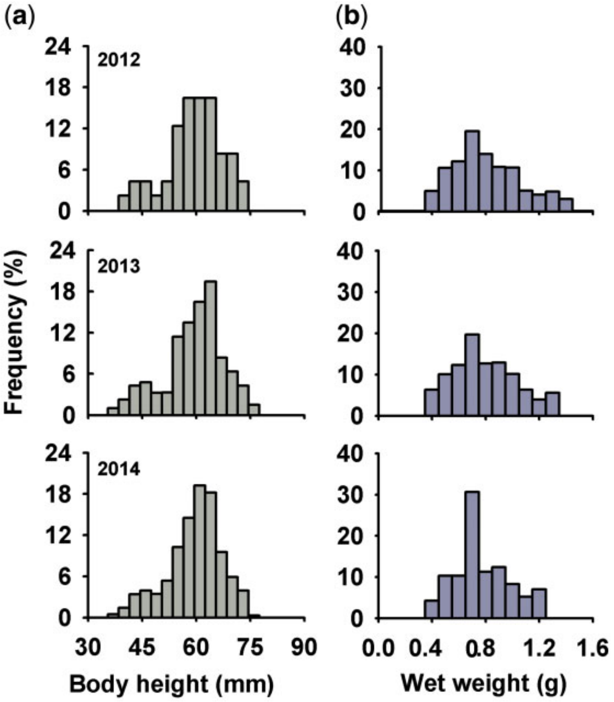

Figure 3. Size–frequency histograms and body weight histograms of *Hippocampus mohnikei* seahorses during the summer of 2012–2014.

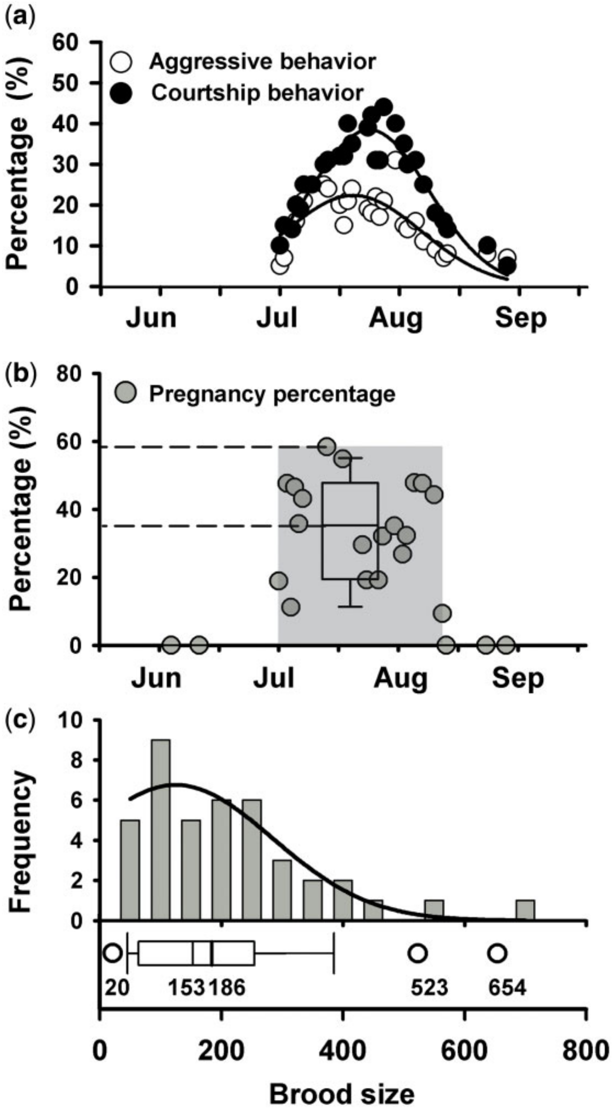

Figure 4. Reproductive characteristics of *Hippocampus mohnikei*. (a) Temporal variation in mating and aggressive behaviours; (b) temporal variation in the percentage of pregnant seahorses (pregnant males/total males); (c) brood size ($n = 42$).

seahorse population has been aggregating in Laizhou Bay periodically in the summer. Considering the high percentage of breeding individuals in the stock, Laizhou Bay might be one of the most important breeding grounds for *H. mohnikei*. The discovery of aggregation behaviours in *H. mohnikei* is novel and crucial to understanding its life history and reproduction strategy, as most seahorses are expected to have limited dispersal, low density and site fidelity (Foster and Vincent, 2004). However, we have not obtained detailed evidence of diffused and clumped distribution data and need more *in situ* surveys to draw a strong conclusion.

Another interesting result in the present study is that the wild stocks of *H. mohnikei* maintained their conventional sex roles with equal sex ratio (males/females) and with larger males than females (Supplementary Figure S1), which conflicts with previous reports (Otsuka *et al.*, 2009; Choi *et al.*, 2012). A possible explanation of the confusing results might be that some immature males without apparent brood pouches were identified as females by mistake or an insufficient sample size led to false conclusions in previous studies. The more aggressive behaviour that was observed in males proved that *H. mohnikei* exhibit conventional sex roles in mating competitions that is similar to other seahorses (Clutton-Brock and Vincent, 1991; Vincent, 1994).

Food availability was considered to be the one of the factors affecting the distribution of the seahorses (Lourie *et al.*, 2005). Zooplankton, such as *Oithona davisae*, *Calanoida*, and *Penilia avirostris*, were identified as the main prey for juvenile and young *H. mohnikei* in Tokyo Bay, and the adults mainly fed on larger prey, such as gammarid and caprellid amphipods (Kanou and Kohno, 2001; Kwak *et al.*, 2004). The nutrient-rich waters from the coastal areas and appropriate temperatures during the warm season contributed to high primary productivity and a high abundance of zooplankton, which supply sufficient prey taxa for both adult and juvenile seahorses in Laizhou Bay during the breeding season (Bi *et al.*, 2000).

Water temperature might be an essential and direct factor triggering the migration of wild seahorse stocks. Field survey figured determined that most seahorses were collected during the warm season when the seawater temperature was higher than 20 °C (Figure 2c). Few seahorses were found in the shallow waters at higher latitudes of China during the winter (December–February, 5–12 °C), which is consistent with a previous study of Tokyo Bay (Kanou and Kohno, 2001), whereas some seahorses were found throughout the year in low latitude areas, where the lowest temperatures ranged from 13 to 16 °C (Ju *et al.*, 2013). Furthermore, the results of the culture experiment demonstrated that seahorses in cold water almost completely cease to swim or feed, and their growth rates significantly decreased, in accordance with the reports in other temperate seahorse species such as *H. erectus*

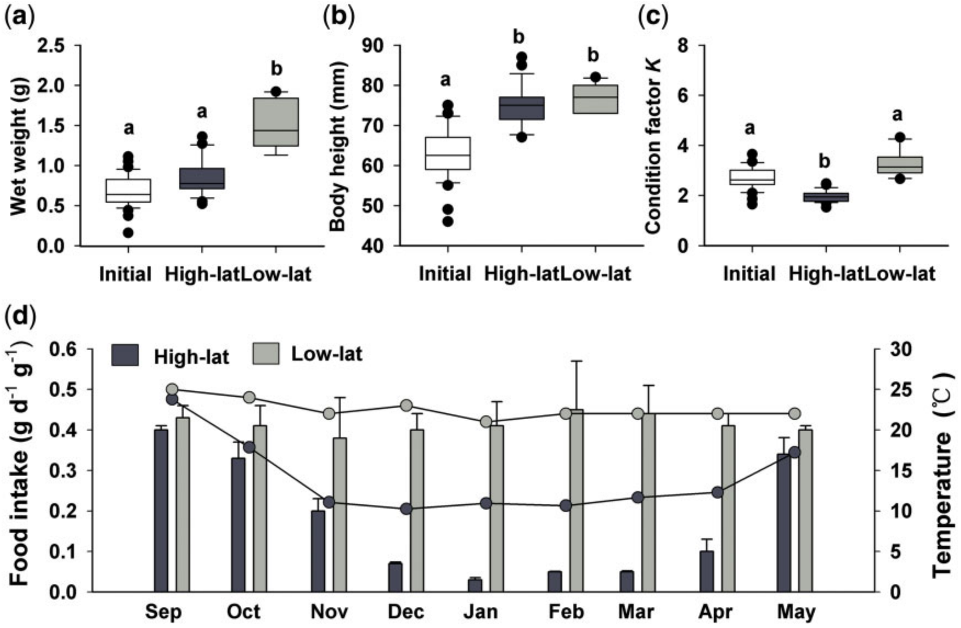

Figure 5. Comparison of growth performance; (a) wet weight (WW); (b) body height (BH); (c) condition factor (K); and (d) food intake (bars) by *Hippocampus mohnikei* seahorses in low- and high-latitude populations and water temperature (lines) from September 2012 to May 2013. Condition factor (K)=WW (g)/[BH (cm)]^3×100. Different superscripts indicate a significant difference among the treatments ($p < 0.05$).

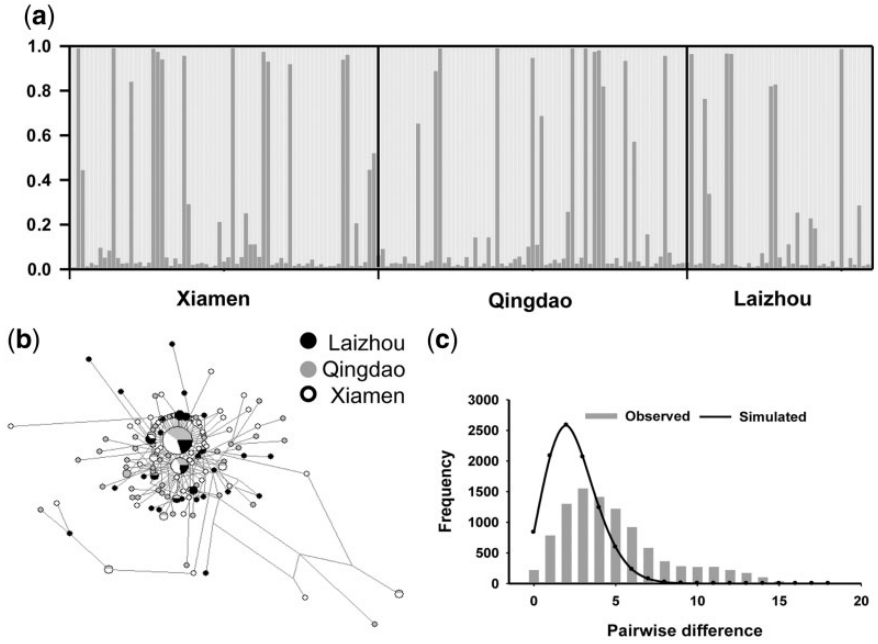

Figure 6. The population genetic structures of *H. mohnikei* seahorses from three different locations based on partial mitochondrial genes (*Cytb* and *COI*). (a) Bayesian cluster analysis using the program STRUCTURE 2.3.4. Each line represents an individual within a population, and populations are grouped together on the *x*-axis. The *y*-axis shows the probabilities of an individual being assigned to either cluster. (b) Statistical parsimony network showing the mutational steps among *H. mohnikei* haplotypes. The sizes of the circles are proportionate to the number of individuals with that haplotype. (c) Mismatch distributions of the *H. mohnikei* population.

(Lin *et al.*, 2008, 2009). As a result, seahorses might have to escape from low temperature stress through migration.

Information about species genetic diversity is essential for the management and protection of wild fish resources. Although *H. mohnikei* is widely distributed along China's coast and in other areas (Zhang *et al.*, 2014; Aylesworth *et al.*, 2016), there were no significant subdivisions among the three geographical populations located along China's coast. Furthermore, the haplotype diversity was high compared with the nucleotide diversity in *H. mohnikei*. This phenomenon may be the result of more recent range expansion events in seahorses (Lourie and Vincent, 2004); similar results have been reported in other teleost fish (Tinti *et al.*, 2002; Song *et al.*, 2008). A Bayesian cluster analysis based on partial mitochondrial genes indicated that the three geographical populations contained a high degree of admixture, which reflects a lack of genetic heterogeneity. Zhang *et al.* (2014) suggested that the long-distance passive dispersal of *H. mohnikei*, driven by water circulation in the Yellow Sea and longshore current, led to a high level of gene flow and contributed to the homogeneity of *H. mohnikei* populations. A single-centre, star-like distribution showed that all three seahorse populations shared the same common haplotype groups, which is a typical genetic pattern of a population that has undergone recent population expansion. AMOVAs also indicated the lack of geographic subdivision among the three *H. mohnikei* populations in this study. The lack of genetic structure in the Bohai Sea, the Yellow Sea, and the East China Sea may reflect recent range expansion after the last glacial maximum with insufficient time to attain a migration-drift equilibrium (Han *et al.*, 2008). However, limited genetic sampling based on mitochondrial DNA and five nuclear loci indicated no subdivision among the different populations of lined seahorses, *H. erectus* (Boehm *et al.*, 2013), whereas population genomics utilizing RADseq datasets revealed a significant subdivision, suggesting that the local population has historically persisted off the coast of Virginia, rather than provisionally from the Gulf of Mexico (Boehm *et al.*, 2015). Therefore, more population genomic evidence might be necessary to identify the population structure of *H. mohnikei* before coming to a conclusion. Additionally, further studies may be required to confirm the distribution and dispersal patterns of *H. mohnikei* based on more *in situ* investigations, such as mark-recapture studies (Correia, 2015).

The present findings also facilitate understanding of the relationship between seahorse habitat and climate change, as one study indicated that climate change is expected to negatively affect inshore marine habitats and their fauna, including seahorses (Vincent *et al.*, 2011). A combination of flooding and high littoral water temperatures resulted in massive mortality of *H. capensis* (Russell, 1994), and ocean acidification has also had a pronounced negative impact on *H. guttulatus* seahorses (Faleiro *et al.*, 2015). Given the poor swimming skills and small home ranges of seahorses, migration to more suitable areas has been thought to be somewhat restricted (Vincent *et al.*, 2011). Accordingly, previous studies only address seahorses' physiological ability to cope with climate change (Lin *et al.*, 2007; Aurélio *et al.*, 2013; Faleiro *et al.*, 2015; Mascaro *et al.*, 2016). The present findings indicate that water temperature plays an essential role in the distributions of *H. mohnikei.* Generally, the food/diet abundance and habitat environment might be easily impacted by water temperature (Thangaraj and Lipton, 2007), and both are key factors that affect a seahorse's life history. Therefore, the study of seahorse behavioural and physiological responses to climate change should be an important focus in the future.

The present study is of considerable importance in conjunction with seahorse conservation programs. The protection of seahorses has been highly prioritized owing to their vital ecological position, high economic value, and endangerment (Vincent *et al.*, 2011). In recent years, shrimp aquaculture and shellfish farming have been established in some near-shore areas that are inhabited by seahorse species (Jin *et al.*, 2013); therefore, excessive aquaculture along the coastline might affect original stocks of wild seahorses. The implementation of conservation strategies should help maintain wild stocks of rare fish, including seahorses (Olden *et al.*, 2007). Seahorses are often considered to be an ecological flagship species (Shokri *et al.*, 2009), and it will be more feasible to protect wild seahorse stocks if their seasonal migration routes are well known. Moreover, because seahorse breeding grounds can also be confirmed, a closed fishing season should be established in these areas every year, which might be an effective strategy for seahorse conservation.

Conclusion

The International Union for Conservation of Nature (IUCN) Red List categorizes *H. mohnikei* as "data deficient" because a large portion of its putative range has not been determined (IUCN, 2016). The present study confirmed the seasonal distribution and abundance of *H. mohnikei* along China's coast as well as the potential existence of seasonal migration among wild seahorse stocks. Furthermore, the large-scale social reproductive behaviour of seahorses is one of the important factors in seasonal distribution and abundance, further suggesting the existence of migration in seahorse populations. This finding also suggests that the Laizhou Bay might be an important natural breeding ground; therefore, it would be helpful for seahorse conservation if a closed fishing season was established in this area during the breeding season. Furthermore, genetic differentiation among geographical populations was indistinct, suggesting that marine protected area strategies that aim to preserve breeding grounds would potentially be effective for seahorse conservation.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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References

Aurélio, M., Faleiro, F., Lopes, V., Pires, V., Lopes, A., Pimentel, M., Repolho, T. *et al.* 2013. Physiological and behavioral responses of temperate seahorses (*Hippocampus guttulatus*) to environmental warming. Marine Biology, 160: 2663–2670.

Aylesworth, L., Lawson, J. M., Laksanawimol, P., Ferber, P., and Loh, T. L. 2016. New records of the Japanese seahorse *Hippocampus mohnikei* in Southeast Asia lead to updates in range, habitat and threats. Journal of Fish Biology, 88: 1620–1630.

Bandelt, H. J., Forster, P., and Rohl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16: 37–48.

Baum, J., Meeuwig, J., and Vincent, A. 2003. Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. Fishery Bulletin, 101: 721–731.

Bi, H., Sun, S., and Gao, S. 2000. The ecological characteristics of the zooplankton community in the Bohai Sea. species composition and community structure. Acta Ecologica Sinica, 20: 716–721.

Boehm, J. T., Waldman, J., Robinson, J. D., and Hickerson, M. J. 2015. Population genomics reveals seahorses (*Hippocampus erectus*) of the Western Mid-Atlantic Coast to be residents rather than Vagrants. PLoS ONE, 10: e0116219.

Boehm, J. T., Woodall, L., Teske, P. R., Lourie, S. A., Baldwin, C., Waldman, J., and Hickerson, M. 2013. Marine dispersal and barriers drive Atlantic seahorse diversification. Journal of Biogeography, 40: 1839–1849.

Caldwell, I. R., and Gergel, S. E. 2013. Thresholds in seascape connectivity: influence of mobility, habitat distribution, and current strength on fish movement. Landscape Ecology, 28: 937–1948.

Caldwell, I., and Vincent, A. J. 2013. A sedentary fish on the move: effects of displacement on long-snouted seahorse (*Hippocampus guttulatus* Cuvier) movement and habitat use. Environmental Biology of Fishes, 96: 67–75.

Choi, Y. U., Rho, S., Park, H. S., and Kang, D. H. 2012. Population characteristics of two seahorses, *Hippocampus coronatus* and *Hippocampus mohnikei*, around seagrass beds in the southern coastal waters of Korea. Ichthyological Research, 59: 235–241.

Clutton-Brock, T., and Vincent, A. 1991. Sexual selection and the potential reproductive rates of males and females. Nature, 351: 58–60.

Conover, D. O., and Murawski, S. A. 1982. Offshore winter migration of the Atlantic silverside, *Menidia menidia*. Fishery Bulletin, 80: 145–150.

Correia, M. 2015. Trends in seahorse abundance in the Ria Formosa, South Portugal: recent scenario and future prospects. PhD thesis, Universidade do Algarve, Faro, 128 pp.

Curtis, J. M. R., and 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.

Eggert, L. S., Buij, R., Lee, M. E., Campbell, P., Dallmeier, F., Fleischer, R. C., Alonso, A. *et al.* 2014. Using genetic profiles of African forest elephants to infer population structure, movements, and habitat use in a conservation and development landscape in Gabon. Conservation Biology, 28: 107–118.

Excoffier, L., and Lischer, H. E. L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10: 564–567.

Faleiro, F., Narciso, L., and Vicente, L. 2008. Seahorse behaviour and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? Aquaculture, 282: 33–40.

Faleiro, F., Baptista, M., Santos, C., Aurelio, M. L., Pimentel, M., Pegado, M. R., Paula, J. R. *et al.* 2015. Seahorses under a changing ocean: the impact of warming and acidification on the behaviour and physiology of a poor-swimming bony-armoured fish. Conservation Physiology, 3: cov009-cov009.

Foster, S. J., and Vincent, A. C. J. 2004. Life history and ecology of seahorses: implications for conservation and management. Journal of Fish Biology, 65: 1–61.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics, 147: 915–925.

Han, Z. Q., Gao, T. X., Yanagimoto, T., and Sakurai, Y. 2008. Deep phylogeographic break among white croaker *Pennahia argentata* (Sciaenidae, Perciformes) populations in North-western Pacific. Fisheries Science, 74: 770–780.

Hellberg, M. E. 2009. Gene flow and isolation among populations of marine animals. Annual Review of Ecology Evolution and Systematics, 40: 291–310.

The IUCN Red List of Threatened Species. Version 2016-2. www.iucnredlist.org (last accessed 7 September 2016).

Jin, X., Shan, X., Li, X., Wang, J., Cui, Y., and Zuo, T. 2013. Long-term changes in the fishery ecosystem structure of Laizhou Bay, China. Science China-Earth Sciences, 56: 366–374.

Kanou, K., and Kohno, H. 2001. Early life history of a seahorse, *Hippocampus mohnikei*, in Tokyo Bay, Japan. Ichthyological Research, 48: 361–368.

Koldewey, H. J., and Martin-Smith, K. M. 2010. A global review of seahorse aquaculture. Aquaculture, 302: 131–152.

Kwak, S. N., Huh, S. H., and Klumpp, D. W. 2004. Partitioning of food resources among *Sillago japonica, Ditremma temmincki, Tridentiger trigonocephalus, Hippocampus japonicus* and *Petroscirtes breviceps* in an eelgrass, *Zostera marina*, bed. Environmental Biology of Fishes, 71: 353–364.

Lazzari, M. A., and Able, K. W. 1990. Northern pipefish, *syngnathus fuscus*, occurrences over the mid-atlantic bight continental-shelf - evidence of seasonal migration. Environmental Biology of Fishes, 27: 177–185.

Librado, P., and Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451–1452.

Lin, Q., Gao, Y. L., Sheng, J. Q., Chen, Q. X., Zhang, B., and Lu, J. 2007. The effects of food and the sum of effective temperature on the embryonic development of the seahorse, *Hippocampus kuda* Bleeker. Aquaculture, 262: 481–492.

Lin, Q., Li, G., Qin, G., Lin, J., Huang, L., Sun, H., and Feng, P. 2012. The dynamics of reproductive rate, offspring survivorship and growth in the lined seahorse, *Hippocampus erectus* Perry, 1810. Biology Open, 1: 391–396.

Lin, Q., Lin, J., and Zhang, D. 2008. Breeding and juvenile culture of the lined seahorse, *Hippocampus erectus* Perry, 1810. Aquaculture, 277: 287–292.

Lin, Q., Lin, J. D., Zhang, D., and Wang, Y. B. 2009. Weaning of juvenile seahorses *Hippocampus erectus* Perry, 1810 from live to frozen food. Aquaculture, 291: 224–229.

Lourie, S. A., Green, D. M., and Vincent, A. C. J. 2005. Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). Molecular Ecology, 14: 1073–1094.

Lourie, S. A., Pollom, R. A., and Foster, S. J. 2016. A global revision of the Seahorses *Hippocampus* Rafinesque 1810 (Actinopterygii: Syngnathiformes): taxonomy and biogeography with recommendations for further research. Zootaxa, 4146: 1–66.

Lourie, S. A., and Vincent, A. C. J. 2004. A marine fish follows Wallace's Line: the phylogeography of the three-spot seahorse (*Hippocampus trimaculatus*, Syngnathidae, Teleostei) in Southeast Asia. Journal of Biogeography, 31: 1975–1985.

Lowe, W. H., and Allendorf, F. W. 2010. What can genetics tell us about population connectivity? Molecular Ecology, 19: 3038–3051.

Mascaro, M., Amaral-Ruiz, M., Huipe-Zamora, I., Martinez-Moreno, G., Simoes, N., and Rosas, C. 2016. Thermal tolerance and phenotypic plasticity in juvenile *Hippocampus erectus* Perry, 1810: Effect of acute and chronic exposure to contrasting temperatures. Journal of Experimental Marine Biology and Ecology, 483: 112–119.

Olden, J. D., Hogan, Z. S., and Vander Zanden, M. J. 2007. Small fish, big fish, red fish, blue fish: size-biased extinction risk of the world's freshwater and marine fishes. Global Ecology and Biogeography, 16: 694–701.

Otsuka, Y., Suzuki, H., and Akagawa, I. 2009. Occurrence, gonad morphology and maturity of Japanese seahorse *Hippocampus mohnikei* in Matsushima Bay, Japan. Journal of the School of Marine Science and Technology Tokai University, 7: 11–22.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945–959.

Russell, I. A. 1994. Mass mortality of marine and estuarine fish in the Swartvlei and Wilderness Lake Systems, Southern Cape. South African Journal of Aquatic Science, 20: 93–96.

Shokri, M. R., Gladstone, W., and Jelbart, J. 2009. The effectiveness of seahorses and pipefish (Pisces: Syngnathidae) as a flagship group to evaluate the conservation value of estuarine seagrass beds. Aquatic Conservation-Marine and Freshwater Ecosystems, 19: 588–595.

Song, W. P. 2009. The analysis of the structure of T-S and the current characteristics in Baohai Sea during winter and summer. Ocean University of China, China.

Song, Z. B., Song, J., and Yue, B. S. 2008. Population genetic diversity of Prenant's schizothoracin, *Schizothorax prenanti*, inferred from the mitochondrial DNA control region. Environmental Biology of Fishes, 81: 247–252.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123: 585–595.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30: 2725–2729.

Teske, P. R., Cherry, M. I., and Matthee, C. A. 2004. The evolutionary history of seahorses (Syngnathidae: *Hippocampus*): molecular data suggest a West Pacific origin and two invasions of the Atlantic Ocean. Molecular Phylogenetics and Evolution, 30: 273–286.

Teske, P. R., Hamilton, H., Matthee, C. A., and Barker, N. P. 2007. Signatures of seaway closures and founder dispersal in the phylogeny of a circumglobally distributed seahorse lineage. BMC Evolutionary Biology, 7: 1–19.

Thangaraj, M., and Lipton, A. P. 2007. Occurrence of the Japanese seahorse *Hippocampus mohnikei* Bleeker 1854 from the Palk Bay coast of south-eastern India. Journal of Fish Biology, 70: 310–312.

Tinti, F., Di Nunno, C., Guarniero, I., Talenti, M., Tommasini, S., Fabbri, E., and Piccinetti, C. 2002. Mitochondrial DNA sequence variation suggests the lack of genetic heterogeneity in the Adriatic and Ionian stocks of *Sardina pilchardus*. Marine Biotechnology, 4: 163–172.

Vincent, A. C. J. 1994. Seahorses exhibit conventional sex roles in mating competition, despite male pregnancy. Behaviour, 128: 135–151.

Vincent, A. C. J., Foster, S. J., and Koldewey, H. J. 2011. Conservation and management of seahorses and other Syngnathidae. Journal of Fish Biology, 78: 1681–1724.

Wen, L. K., Li, J. D., Wan, D. G., Ren, Y., and Guo, J. L. 2013. Market survey and identification of hippocampus (Haima). China Journal of Chinese Materia Medica, 7: 969–972.

Whitfield, A. K. 1995. Threatened fishes of the world: *Hippocampus capensis* Boulenger, 1900 (Syngnathidae). Environmental Biology of Fishes, 44: 362–362.

Zhang, Y., Pham, N. K., Zhang, H., Lin, J., and Lin, Q. 2014. Genetic variations in two Seahorse Species (*Hippocampus mohnikei* and *Hippocampus trimaculatus*): evidence for middle Pleistocene population expansion. Plos One, 9: e105494.

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