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Quantitative analysis of courtship and mating behaviours in the big-headed turtle, *Platysternon megacephalum*

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Turtles are an excellent group for understanding the theory of sexual selection, sexual dimorphism and the evolution of courtship behaviour. Asia has a rich diversity of turtle species, but quantitative analysis of courtship behaviour has only been conducted on a single species. This study quantitatively analysed courtship and mating behaviours of captive *Platysternon megacephalum* to serve as a basis for future comparisons with other freshwater turtles. A total of 259 courtship behaviour sequences stemming from 66 pairings between 12 males and 24 females were analysed. Seven (approaching, sniffing, chasing, resting, mounting, subduing female, copulating) and three mutually exclusive motor patterns (fleeing, mating resistance, mating acceptance) were performed by males and females, respectively. The temporal sequences of courtship and mating behaviours were analysed using Chi-square tests and Kappa analyses, from which a flow diagram was constructed. Male courtship display patterns involved tactile, visual and olfactory cues for conspecific and sexual recognition. In response, females may have emitted olfactory cues regarding their sex and reproductive status. Male *P. megacephalum* exhibited biting, but no head movement or foreclaw display in courtship, which differs from other freshwater turtles. This study provides the first record of male biting during courtship behaviour in an Asian turtle species. Recommendations for captive breeding of the endangered species *P. megacephalum* are presented.

Key words: Asia, behavioural sequences, biting, cinematographic techniques, kappa analysis, Platysternidae

INTRODUCTION

Animal courtship is a process that results in two mature members of a species becoming a couple, usually with the intent to mate and produce offspring (West, 2009). Courtship behaviour influences the evolution of mate choice and sexual dimorphism (Liu et al., 2013; Setoguchi et al., 2014) and differences in courtship behaviour function as isolating mechanisms (Fritz, 1999). Turtles are an excellent group for understanding the underpinning of sexual selection, sexual dimorphism and the evolution of courtship behaviour, because they inhabit a diversity of habitats and exhibit a range of mating strategies (Berry & Shine, 1980; Liu et al., 2013). Although studies on the courtship and mating behaviour of turtles have been carried out for over a century (Liu et al., 2013), most of the earlier reports are anecdotal descriptions (Baker & Gillingham, 1983). The first quantitative study of courtship in turtles was conducted by Jackson & Davis (1972), who documented the courtship motor patterns and temporal sequences for red-eared sliders (*Trachemys scripta*).

To date, only a few studies have attempted a quantitative analysis of courtship and mating behaviour in turtles (Bels & Crama, 1994; Liu et al., 2008). Most studies have focused on species in the Emydidae, Kinosternidae, and Pleurodira in America, Australia and Europe; few studies have focused on the Geoemydidae, Trionychidae and Chelydridae (Liu et al., 2013). Asia has a rich diversity of turtle species, but quantitative analysis of courtship behaviour has only been conducted on a single species (the four-eyed spotted turtle *Sacalia quadriocellata*; Liu et al., 2008).

The big-headed turtle *Platysternon megacephalum*, the sole member of the monotypic family Platysternidae, inhabits rocky mountain streams throughout China, Vietnam, Thailand, Myanmar, Laos and Cambodia (van Dijk et al., 2011). IUCN has listed *P. megacephalum* as Endangered (IUCN, 2012) due to commercial harvesting and habitat destruction. One approach in turtle conservation is captive breeding, as it decreases the risk of extinction by establishing captive populations. However, *P. megacephalum* has rarely been bred in captivity, usually due to failures in courtship as a result of fighting or mating resistance (Gong et al., 2013a, b; Wei et al., 2014). In this study, we present a detailed quantitative analysis of courtship and mating behaviours in captive *P. megacephalum*, to serve as a basis for future

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Table 1. Definition and description of courtship and mating motor patterns in *Platysternon megacephalum.*

Sex	Motor pattern (Abbreviation)	Definition and description
Male	Approaching (*AP*)	This behaviour occurs at the beginning of every courtship interaction. When a male encounters a female, the male gazes at and moves slowly (swimming or walking) toward the female.
Male	Sniffing (*SN)*	Once close to the female, the male extends his neck and sniffs, touching the head, neck, bridge, and cloacal region of the female.
Male	Chasing (*CH*)	This is an initiative motor pattern, which occurs when the female flees or moves. The male pursues the fleeing female (quickly swimming or walking on the bottom of the aquarium).
Male	Resting (*RE*)	Sometimes, males stops chasing and do not move for a while. Stopping for more than 10s is considered resting behaviour.
Male	Mounting (*MO*)	This behaviour follows *SN* or *CH*. The male climbs onto the female's back from the front, rear or side and grasps the female's marginal scutes with the claws on his forelimbs. At this time, the turtles' bodies are parallel. For some head-to-tail mounts, the male adjusts to a head-to-head orientation and keeps his body parallel with the female.
Male	Subduing female (*SF*)	After *MO*, the male frequently extends his neck to gently bite the head and neck of the female and his tail clings to the female's cloacal region.
Male	Copulating (*CO*)	After *SF*, the male stops biting the female's head and supports the front part of his body on his fore limbs. In this position, their bodies form an angle of approximately 30–50 degrees. Simultaneously, the male moves his hind limbs to clasp the female's rear marginal plastron and grasps the female's tail with his tail. While the female's hind limbs are controlled by the male's hind limbs, he then protrudes and inserts his penis into the female's cloaca.
Female	Fleeing (*FL*)	This is the pattern exhibited in response to *SN* or unsuccessful *MO*. The female moves away.
Female	Mating acceptance (*MA*)	This is the pattern exhibited in response to *SF*. The female remains subdued (or stationary), and extends her tail so the male can copulate with her.
Female	Mating resistance (*MR*)	This is the pattern exhibited in response to *SF*. The female bites the male, struggles, and escapes, so the male cannot copulate with her.

comparisons with other freshwater turtles, and to aid in the captive breeding of this endangered species.

MATERIALS AND METHODS

Experiments were undertaken in the Guangdong Xiangtoushan National Nature Reserve (114°22′ E，23°15′ N), Huizhou, Guangdong Province, China, where there is a naturally occurring population of *P. megacephalum*. A total of 50 sexually mature (estimated to be over six years old based on growth rings on scutes; Bonin et al., 2006) *P. megacephalum* (25 males and 25 females) were selected from the Turtle Conservation Center in Guangdong Xiangtoushan National Nature Reserve (an animal research station for the South China Institute of Endangered Animals). All turtles had been illegally collected from the mountainous area of eastern Guangdong Province by local villagers between 2009 and 2012, and were later rescued and kept at the Turtle Conservation Center. To house these turtles, an environment mimicking their natural habitat (resembling natural ponds) was created along a natural mountain stream. Before the mating experiments, all turtles were measured (Table 2) and marked for individual recognition with a number code painted on the carapace.

Mating experiments were conducted between 0830 and 1730 hours from March to June 2014 (during the breeding season of this species; Gong et al., 2013b). In each experiment, one male and one female were selected randomly as a mating pair. The pairs were placed in an indoor, rectangular, glass aquarium (100×50×50 cm) with a water depth of 20–25 cm. Based on the natural habitat characteristics of *P. megacephalum*, we designed a simple environment with shallow water and stones to form a terrestrial area. Water plants (*Acorus calamus*, a common plant in the habitat of *P. megacephalum*) were arranged around the aquarium to provide shelter for the turtles (Gong et al., 2013b). The range of indoor

Table 2. Measurements of the 50 *Platysternon megacephalum* individuals used in this study.

Body measurements	Male		Female	
	Sample size	Mean ±SD (Range)	Sample size	Mean ±SD (Range)
Carapace length (mm)	25	127.07±13.34 (108.77–156.50)	25	113.06±6.94 (99.88–124.90)
Body mass (g)	25	380.04±113.51 (251.50–626.70)	25	262.28±34.88 (182.90–316.70)

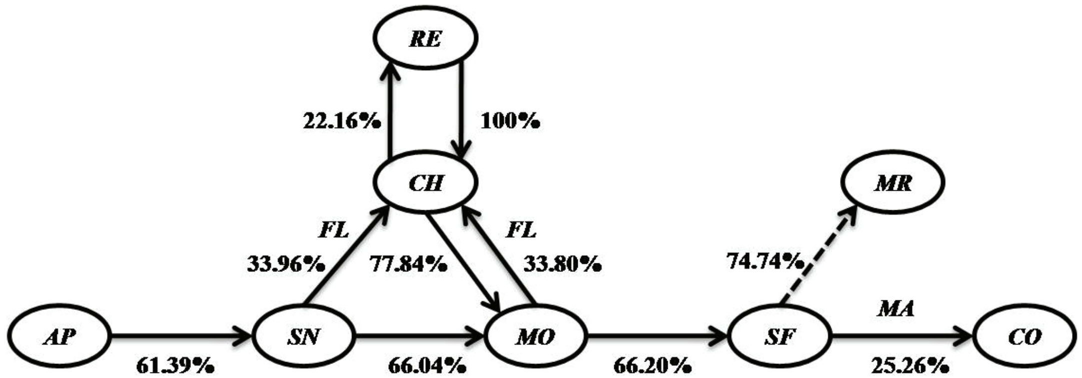

Fig. 1. Flow diagram of the courtship and mating motor patterns in *Platysternon megacephalum* (see Table 1 for abbreviations). The percentages represent the probabilities of transitions between preceding and following behaviours.

light intensity was 8–15 Lux, water temperature ranged from 22–27°C, air temperature was 22–28°C and relative humidity ranged from 70–95%. A digital surveillance system using an infrared video camera (HZH-38603K, Guangzhou, China) was fixed above the aquarium; the video was monitored in real time in another room to minimise disturbance. Based on our pre-experimental observations, if a pairing displayed mating behaviour, it usually occurred within one hour of being placed in an aquarium; thus, each pairing was observed for a minimum of one hour and the experiment was terminated if there was no courtship behaviour. When males initiated courtship behaviours (approaching and sniffing), the entire courtship and mating process was recorded using a video camera. When one pairing resulted in two matings, both courtship and mating behaviour sequences were recorded in one experiment.

Cinematographic analysis was used to describe the motor pattern components of courtship and mating behaviour. Following the method of Whitehurst et al. (1986), an intra-individual dyadic transition matrix was used to identify motor-pattern dependencies. In this matrix, all of the motor patterns were rearranged and combined into a single form that included all pattern sequences and their frequency of occurrence. We calculated the probabilities of all permutations and combinations, and the highest probabilities were used to construct the behavioural sequence. Significant transitions were identified using Chi-square tests and Kappa analyses (Whitehurst et al., 1986). Chi-square tests were used to evaluate whether or not the frequencies of all permutations and combinations were distributed randomly. If one behaviour randomly followed another, it meant that the behaviour sequence was invalid. If a behaviour was non-random, a Kappa analysis was used to determine whether the next pattern reliably followed the previous one; and a courtship and mating flow diagram was constructed from this analysis. The statistical analysis software package SPSS v.18.0 was used to analyse data.

RESULTS

A total of 185 pairings were conducted. Of these, 22 pairings (involving 9 males and 13 females) exhibited courtship and mating behaviour, 44 pairings (11 males, 21 females) showed only courtship behaviour, while the remaining 119 pairings displayed neither courtship nor mating behaviour. No courtship or mating behaviour was observed in pairings with males smaller than females. Among the 50 selected turtles, 43 individuals (19 males, 24 females) were used more than once for experimentation. A total of 259 courtship behaviour sequences were recorded. Following Liu et al (2008) with modifications, seven mutually exclusive motor patterns in males (Approaching, *AP*; Sniffing, *SN*; Chasing, *CH*; Resting, *RE*; Mounting, *MO*; Subduing female, *SF*; Copulating, *CO*) and three motor patterns in females (Fleeing, *FL*; Mating acceptance, *MA*; Mating resistance, *MR*) were defined (see Table 1 for detailed definitions and descriptions of each motor pattern).

Table 3. Duration of courtship and mating motor patterns in *Platysternon megacephalum*. Abbreviations are defined in Table 1.

Motor pattern	Sample size	Duration of motor pattern		Proportion of total time (%)
		Mean±SD (s)	Range (s)	
AP	259	11.49±10.80	2–86	14.54
SN	159	11.82±13.45	1 –83	9.19
CH	194	13.31±20.88	2 –204	12.62
RE	43	29.63±26.77	10 –120	6.23
MO	356	4.41±5.86	1 –73	7.68
SF	190	18.55±12.80	3 –64	17.22
CO	48	138.65±112.03	24 –667	32.52

Table 4. Intra-individual (male) dyadic transition matrix of courtship and mating motor patterns for *Platysternon megacephalum*. Numbers above are observed frequencies and values in parentheses are expected frequencies. Abbreviations are defined in Table 1.

Preceding behaviour	Following behaviour						Total
	SN	*CH*	*RE*	*MO*	*SF*	*CO*	
AP	159	0	0	100	0	0	259
	(41.6)	(50.8)	(11.2)	(93.1)	(49.7)	(12.6)	
SN	-	54	0	105	0	0	159
	-	(31.2)	(6.9)	(57.2)	(30.5)	(7.7)	
CH	0	-	43	151	0	0	194
	(31.2)	-	(8.4)	(69.8)	(37.2)	(9.4)	
RE	0	43	-	0	0	0	43
	(6.9)	(8.4)	-	(15.5)	(8.3)	(2.1)	
MO	0	97	0	-	190	0	287
	(46.1)	(56.2)	(12.5)	-	(55.1)	(13.9)	
SF	0	0	0	0	-	48	48
	(7.7)	(9.4)	(2.1)	(17.3)	-	(2.3)	
Total	159	194	43	356	190	48	990

All courtship and mating behaviours occurred underwater, and males always initiated courtship (motor pattern *AP*; approximately 11s). *AP* occurred via walking (204 cases, 78.76%) or swimming (55 cases, 21.24%). When a male approached a stationary female, he usually extended his neck and sniffed (*SN*; approximately 12s), contacting the female's cloacal region, marginal plastron, or neck. After *SN,* the male mounted (*MO*; approximately 4s) the female. However, usually, the female fled (*FL*) during *SN* or *MO*, initiating chasing (*CH*; approximately 13s) by the male. During chasing, the male often rested (*RE*; approximately 30s). If *CH* was successful, the male mounted the female.

A mount was considered successful when the male was parallel to the female and was in a head-head orientation, tightly clasping her marginal plastrons. Some head-to-tail mounts were made (10 cases), but these were adjusted to head-to-head orientation within 1–3s. Following a successful mounting, the male began to subdue the female (*SF*; approximately 18s). Sometimes, when the male grasped the female, their bodies turned 90 degrees (7 cases) or 180 degrees (5 cases) in the water. During *SF*, females exhibited two motor patterns, *MA* or *MR*. If *SF* was successful, it resulted in the female's *MA* behaviour. However, if a male met a female with strong *MR* behaviour, *SF* was unsuccessful, the female escaped and they maintained separation until the end of the trial.

Following a successful *SF*, the male began the copulation motor pattern (*CO*; approximately 139s). During copulation, the male often expanded and contracted his neck slowly (2–3 times per min). At the end of copulation, the male released the female, and the female escaped. Table 3 presents the durations of each male motor pattern.

Based on 990 motor pattern transitions (Table 4), the intra-individual dyadic transition matrix was constructed. The results of the Chi-square tests showed that the sequence of motor patterns occurred in a nonrandom pattern (χ^2=2589.95, p<0.001, df=25, n=990). Kappa and Z values were calculated for all dyadic pairs (Table 5) and transitions demonstrating p<0.001 significance or better (i.e., Z value>4.90) were used to construct the flow diagram for courtship and mating motor patterns in *P. megacephalum* (Fig. 1).

Our analysis identified *AP* as the key motor pattern initiating the courtship sequence. From *SN* to *MO*, there were various linear sequences and the female's *FL* was the key incentive for pattern-shifting in the male. The transfer rates of motor patterns were relatively high during *AP*-*SN*-*MO*-*SF*, and each of the transfer rates was over 60% (Fig. 1). Although the male's *SF* was a key prerequisite for *CO*, the female's *MA* was necessary for a successful *SF* and *CO*. The transfer rate was rather low (only 25.26%) from *SF* to *CO* and the female *MR* rate was 74.74% (Fig. 1). From the total of 259 courtship behaviour sequences, 48 reached *CO* (Table 3). Consequently, the probability of a completed sequence from *AP* to *CO* was 18.53%.

DISCUSSION

The courtship behavioural sequences of *P. megacephalum* include five major phases (*AP*, *SN*, *MO*, *SF* and *CO*; Fig. 1). Like most other species (Bels & Crama, 1994; Hidalgo, 1982), male *AP* is the key motor pattern to initiate the courtship sequence in *P. megacephalum*. In species using open aquatic environments (i.e., Chelids), this approach consists mainly of a swimming motor pattern (Murphy & Lamoreaux, 1978); in Geoemydids, Chelydrids and the majority of Kinosternids, the approach activity is a walking motor pattern (Bels & Crama, 1994; Liu et al., 2008). In *P. megacephalum*, the approach consists mainly of a walking motor pattern. In the initial phase, visual cues including body shape may be used to recognise potential mates. Body shape can be an important visual cue in the initial phase of turtle courtship (Hidalgo, 1982; Baker & Gillingham, 1983; Liu

Table 5. Kappa and *Z* values for the sequence of courtship and mating motor patterns for *Platysternon megacephalum*. Numbers above are kappa values and Z values are given in parentheses. Abbreviations are defined in Table 1. Notes: *Kappa value>0 and Z value>3.30 (*p*<0.001).

Preceding behaviour	Following behaviour					
	SN	*CH*	*RE*	*MO*	*SF*	*CO*
AP	1.00*	-0.35	-0.35	0.04	-0.35	-0.35
	(23.12)	(-9.25)	(-3.99)	(1.03)	(-9.13)	(-4.23)
SN	-	0.18*	-0.19	0.47*	-0.24	-0.19
	-	(4.98)	(-2.93)	(8.63)	(-6.71)	(-3.11)
CH	-0.24	-	1.00*	0.65*	-0.24	-0.24
	(-6.79)	-	(13.58)	(13.55)	(-7.57)	(-3.51)
RE	-0.19	1.00*	-	-0.56	-0.24	-0.05
	(-2.93)	(13.58)	-	(-5.02)	(-3.27)	(-1.51)
MO	-0.41	0.30*	-0.41	-	1.00*	-0.41
	(-8.79)	(7.19)	(-4.28)	-	(24.00)	(-4.54)
SF	-0.19	-0.24	-0.05	-0.56	-	1.00*
	(-3.11)	(-3.51)	(-1.51)	(-5.32)	-	(31.46)

et al., 2008). For example, Hidalgo (1982) found that male *Rhinoclemmys pulcherrima incisa* show a positive response to moving objects that resemble turtles. Some studies have indicated that colour cues have important effects on the recognition of potential mates (Mansfield et al., 1998; Rovero et al., 1999). Liu et al. (2008) suggested that head bobbing in male *S. quadriocellata* functions to display the bright red stripes on the ventral part of the neck to females. In this study, visual cues including body shape were used by *P. megacephalum* to recognize potential mates because the male gazed at the female before *AP*. If the male was smaller than the female, the male always moved away and did not exhibit *AP.* No obvious colour difference between male and female *P. megacephalum* was found.

In the second phase (*SN*) the male *P. megacephalum* extends his neck to sniff and contact the head, neck, bridge and cloacal region of the female. *SN* mainly involves olfactory cues, and appears to be involved in recognition of species, gender, and sexual maturity (Bels & Crama, 1994; Liu et al., 2008). The cloacal scent produced by a receptive female *R. p. incisa* can elicit trailing in males (Hidalgo, 1982). In other turtles, the cloacal scent serves a discriminatory function (*Geochelone carbonaria* and *G. denticulate*, Auffenberg, 1965; *Gopherus polyphemus*, Auffenberg, 1966). During *SN*, the male's nose touches the female's cloacal area or bridge. Therefore, tactile and olfactory cues could simultaneously be involved. In *Elseya latisternum* and *Emydura macquarii*, the touching of a female's vent will result in her turning 180° to face the male (Murphy & Lamoreaux, 1978), a similar phenomenon was observed in *S. quadriocellata* (Liu et al., 2008). However, in *P. megacephalum*, male touching may stimulate the female's *FL*, following which the male exhibits the *CH* motor pattern as a response.

The third phase, *MO,* is an important "contact" motor pattern. In *P. megacephalum*, males can mount females from any direction, and males typically adjust their position on the female's back (as in some other turtle species, Davis & Jackson, 1973; Murphy & Lamoreaux, 1978; Hidalgo, 1982; Bels & Crama, 1994; Liu et al., 2008). If *MO* is unsuccessful, the male will drop from the female's back and resume *CH* and *MO* behaviours. Auffenberg (1977) hypothesised that repetition of mountings could increase female receptivity in tortoises. Studies on *S. minor* supported this hypothesis (Bels & Crama, 1994). In *P. megacephalum*, repetition of mountings may similarly increase the rate of successful *MO* and repetition of mountings occurred in 9 pairings with female receptivity.

The fourth phase, *SF*, is a key prerequisite for *CO*. In other studies, this motor pattern has not been specifically defined, but similar motor patterns are included in the *MO*. For example in *S. quadriocellata*, the male's neck fully extends and his head points at the female's head; sometimes the male's chin contacts the female's neck during *MO*, and these actions seem to calm the females (Liu et al., 2008). Similar motor patterns, such as chin-rubbing, also occur in some emydine turtles (Mahmoud, 1967; Baker & Gillingham, 1983). Biting is common in the social interactions of turtles (Liu et al., 2013). However, biting in courtship differs from aggressive male–male biting; it most likely functions to subdue females and force their compliance, which is essential for copulation (Hidalgo, 1982; Harrel et al., 1996; Liu et al., 2008). Male biting in courtship includes both visual and tactile cues; it may be feigned and end with a touch or light hit, or males may bite with sufficient vigor to leave scars on the females (Liu et al., 2013). Female biting seems to be a response to male *SF*. A strong bite often means female *MR*. A female *MA* is essential for male *MO*, *SF*, and final *CO* (Gibbons & Lovich, 1990). *MO* is often not followed by copulation because of female refusal (Murphy & Lamoreaux, 1978; Baker & Gillingham, 1983; Liu et al., 2008). In *P. megacephalum*, the female *MA* rate (or male *SF* success rate) was only 25.26% (Fig. 1). The female's *MR* rate was very high, which is also normal in other turtle species (Mahmoud, 1967; Hidalgo, 1982; Liu et al., 2008). The high female *MR* rate could, however, be caused by the circumstances of captivity where encounters are frequent (Liu et al., 2008).

The last phase, *CO*, is the most important motor pattern of the whole courtship and mating sequence because it integrates all of the previous efforts. Generally, copulation in turtles involves two major male postures: i) the male grasps the female's carapace either with all four limbs, as occurs in the Chelidae, Emydidae, and Kinosternidae; or ii) the male grasps the female's carapace with his forelimbs and supports his body with his hind limbs planted on the substrate (Liu et al., 2013). In *P. megacephalum*, the male copulation posture seems to be distinct – hind limbs clasp the female's rear marginal plastron (not carapace) and control her hind limbs. For aquatic turtles to copulate, the male's plastron usually contacts the female's carapace (Liu et al., 2013), but plastron-to-plastron mating is also observed (Tronzo, 1993; Mitchell & Mueller, 1996). We did not observe plastron-to-plastron mating in *P. megacephalum.*

Platysternon megacephalum exhibited biting (in male *SF* and female *MR*), an aggressive form of courtship behaviour, as seen in Chelydridae, Kinosternidae, Emydinae and South American Pleurodira (Liu et al., 2013). *Platysternon megacephalum* exhibited no head movement or foreclaw display, which are apomorphic and evolved independently in Geoemydinae, Deirochelyinae and Australian Pleurodira (Liu et al., 2013). In the Asian *S. quadriocellata*, only the female exhibits biting when the male exhibits head-bobbing or sniffing patterns (Liu et al., 2008). Based on the phylogenomic analysis by Crawford et al. (2015), Platysternidae have closer relations with Emydidae and Geoemydidae than with Chelydridae, Kinosternidae and Pleurodira. This study found the first evidence of male biting in courtship behaviour in Asian turtle species. It seems that there is no clear phylogenetic relationship with regard to biting in courtship behaviour.

Head movement, foreclaw display and biting appear to calm females and facilitate mounting (Murphy & Lamoreaux, 1978; Hidalgo, 1982; Baker & Gillingham 1983; Liu et al., 2008). Berry & Shine (1980) suggested that less courtship and more coercion or forceful insemination of females occur in species with larger males, whereas smaller males display more elaborate courtship behaviours. In this study, the body sizes of males were always larger than those of females for pairings that exhibited courtship behaviour. When the males were smaller than females, males always tried to flee and showed no courtship behaviour. Therefore, our study seems to support the view of Berry & Shine (1980). However, Gibbons & Lovich (1990) did not completely agree with this view and suggested that it is difficult for a male turtle to achieve forcible intromission with an unreceptive female. Based on a meta-analysis, Liu et al. (2013) found that there was a highly significant positive correlation between larger females (relative to male body size) and a less aggressive courtship strategy; males, in species with larger females, tend to adopt a less aggressive mating strategy involving foreclaw displays and head movements instead of biting. This result is consistent with Berry & Shine's (1980) view of mating strategy and sexual dimorphism. Further, Liu et al. (2013) suggested that the evolution of courtship behaviour in male freshwater turtles might accompany the evolution of sexual dimorphism, which is directly subject to natural selection. In *P. megacephalum*, some field studies have indicated that the body sizes (i.e. the maximum and mean of carapace length) of males are usually larger than females (Kendrick et al., 2011; Sung et al., 2013). It seems that there is sexual size dimorphism in *P. megacephalum* but further study is needed.

Female courtship behaviour in turtles is less obvious than that of males (Liu et al. 2013). Only a few female courtship behaviours have been reported. For example, Hidalgo (1982) reported active nose-to-nose contact and biting by female *R. p. incise*. In *P. megacephalum*, the male seems to play a positive role and initiate the courtship, while the female seems passive and exhibits only a few motor patterns in response to the male's pattern. Some studies suggested that females may play passive roles during courtship (Jackson & Davis, 1972). However, some other studies, disputing this suggestion, indicated that the success or failure of an attempted mating ultimately depends on the female's acceptance or rejection (Harless, 1979). For example, although male *Emydoidea blandingi* initiate courtship, ultimately females decide to accept or reject the male's copulation (Baker & Gillingham, 1983). No obvious positive courtship behaviour was observed in female *P. megacephalum*. For a female showing *MA*, no or less mating resistance, and extending her tail, may be the positive response to male courtship. It is difficult to test whether female acceptance is positive or passive, as that may lead to a biased interpretation of observed courtship behaviour.

Based on our results, we present some recommendations for captive breeding of *P. megacephalum*: (i) when selecting mating pairs, the male should be larger than the female; and (ii) a suitable water pool is necessary for courtship and mating activity because courtship and mating were never observed on land.

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