

Thermal preference of the yellow-margined box turtle (*Cuora flavomarginata*) (Testudines: Geoemydidae) inhabiting a mesic lowland forest, northern Taiwan

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Abstract. Thermal preference of free-living yellow-margined box turtle, *Cuora flavomarginata*, was studied in a mesic forested habitat using a combination of radiotransmitters and temperature dataloggers attached to individual turtles. Patterns of mean weekly shell surface temperature variation of selected individuals were highly related to seasonal change of environmental temperature, with a peak temperature of 24.5-25.1°C in males and 26.0-27.0°C in females during June and July. Minimum shell surface temperature occurred in late January (4.5-7.6°C). Despite a small sample size, there were significantly intersexual differences in mean weekly shell surface temperature during nesting season. Individual females of *C. flavomarginata* tended to maintain higher shell surface temperature during nesting season (May-July). This difference was mostly pronounced during the day. There were no obviously intersexual differences prior to and after nesting season. Our results do not support the previous predictions that gravid *C. flavomarginata* may select higher environmental temperatures prior to nesting season.

Keywords: *Cuora flavomarginata*, Reptilia, Taiwan, thermal ecology.

Introduction

The yellow-margined box turtle (*Cuora flavomarginata*) is an endangered species, inhabiting mesic forested habitats in low elevation of east Asia, including south-eastern China, Taiwan and southern Ryukyu Archipelagos of Japan (Ernst and Barbour, 1989; Zhao, 1998; Lue and Chen, 1999). It is threatened by overexploitation for pet and food, habitat loss and fragmentation (Zhao, 1998; Chen, Lin and Chang, 2000). For endangered species, better understanding of their ecology is urgently needed and will provide the basic information for further conservation.

Both body temperature and environmental temperature play important roles in many aspects of the ecology of reptiles, affecting activity, metabolism, reproduction and growth (Huey, 1982; Congdon, 1989). As ectotherms, turtles regulate their body temperature within

preferred ranges by using physiological and behavioural regulations to cope with environmental temperature fluctuation, such as shuttling among different thermal microclimate (Avery, 1982; Huey, 1982). Turtles, like many other ectotherms, can take advantage of the thermal environment to regulate their body temperatures through microclimate selection. Thus, habitat temperature is an important resource for turtles in their thermoregulation. Terrestrial turtles and tortoises inhabiting in shady habitats thermoregulate by exploring the thermal gradient in the habitat, such as exposure to solar radiation in forest gap or forest edge (Donaldson and Echternacht, 2005; Luiselli, 2005). Most available information relevant to the thermal preference of *C. flavomarginata* is anecdotal or based on observation of captive individuals (Zhang, 1986; Huang, Zhao and Wang, 2007). In a previous study (Lue and Chen, 1999), *C. flavomarginata* was found to have seasonal and sexual-specific patterns in its habitat preference; females were captured more frequently than males and usually stayed near forest border during gravid season. For ectotherms, differences in habitat selection for turtles often

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imply differences in thermoregulatory preference (do Amaral, Marvin and Hutchison, 2002). Thus, we hypothesize that female *C. flavomarginata* will select higher environmental temperature during gravid season.

In this study, we investigate the thermal preference, daily and seasonal variation of shell surface temperature of free-living *C. flavomarginata* in a northern Taiwan population, with emphasis on intersexual differences in terms of thermal environment preference.

Materials and methods

Fieldwork was conducted from September 2002 to August 2003 in a mountain basin in the Feitsui Reservoir Protected Area of northern Taiwan. The study site was a mesic forest located 17 km SE from Taipei City. Since this reservoir is the main municipal water supply for northern Taiwan, the study site has been blocked from public access after flooding in 1984. The landscape of the study site is hilly, with elevation ranging from 150-300 metres above the sea level. The mountain slopes were mainly covered with primary or secondary evergreen forest consisting of tall broad-leaf tree, lower shrubs, and herbaceous undergrowth. There were some patchy abandoned agricultural land, mainly tea plantations and bamboo shrubs. The abandoned farmland was gradually replaced by dense herbaceous shrubs (mainly *Miscanthus floridulus*).

Turtles were captured with wire mesh rodent traps (29 × 17 × 14 cm) baited with banana. Captured turtles were measured (straight carapace length in mm), sexed and uniquely marked by filing notches or drilling holes in the marginal scutes. After necessary handling and measurements, turtles were released at the point of capture. Selected adults (four males and five females) were fixed with radio transmitters (13 g, model P2RLM-G3, AVM Instrument Co., Livermore, CA, USA) and temperature dataloggers (15 g, -4-38°C, StowAway TidBit, Onset Computer Corporation, Pocasset, MA, USA) on their front carapaces. The two units were attached to the front lateral scutes of turtles with epoxy cement. A rubber cap was also fastened to each datalogger to avoid erosion of the optical data readout point. Fasten devices weighted 32-35 g, approximately 5-7% of the body weight of the turtles, within a maximum of 10% for reptiles (Beaupre et al., 2004). Dataloggers were programmed to record ambient temperature at intervals of 30 min. To compare the reliability of body temperature estimation with external dataloggers attached on turtle carapace (T_s), we obtained 20 measurements of cloacal temperatures from seven radio-tagged turtles in September-October 2002 and late March 2003, and the ambient air temperatures from 10 cm above ground surface at the exact location where turtles were observed with concurrent of data logger records. Because most *C. flavomarginata* closed their shells tightly when

handled, we have not taken the cloacal temperatures for all the turtles radio-tracked to avoid causing severe disturbance to their normal activities. We located radio-tagged turtles by using an AVM receiver (model LA 12Q) and a hand-held H-shaped antenna (150-154 MHz, Telonics Inc., Mesa, AZ, USA), and the temperature records were downloaded at intervals of one to two months by using an Onset optic data shuttle (Onset Computer Corporation). We have not downloaded the records in January and February to avoid inducing accident arousal of turtles from wintering. We also selected three temperature stations for the representative habitat types (open, edge and forested) where turtles were usually found and the air temperatures (T_e) at each site were monitored with HOBO data loggers (H8 Pro, Onset Computer Corporation) for the duration of the study. Each datalogger was fixed under a plastic shelter set 30 cm above the ground level. Datalogger station was located along an open trail in open habitat, under a cultivated bamboo bush two metres away from the trail in edged habitat, and 30 metres into the broad-leaf forest in forested habitat.

Series of T_s values obtained from a single turtle are not independent and may be misleading (Blouin-Demers and Weatherhead, 2001; do Amaral, Marvin and Hutchison, 2002; Luiselli, 2005). Therefore, we used the averaged data for each individual in the analyses. To examine temperature trends among seasons, we compared the mean T_s and T_e by week. The mean weekly temperatures for each group were determined by averaging datalogger records for each day, and then averaged these daily means by week. Since the sample size was small in September 2002 and August 2003 because of limited numbers of turtles radio-tagged and battery failure of radiotransmitters, we exclude the data from these two months. Because *C. flavomarginata* used the forested habitat exclusively during non-active season (Lue and Chen, 1999), we used the environmental temperature data from forested habitat in the analyses from December-March, and averaged the data from three representation sites in the other seasons.

Results

Results suggest that the datalogger records on shell surface can be used as reliable estimation of body temperature in the field. Among the temperature measurements from datalogger, cloaca, ground surface and ambient environment, there were no significantly differences among data logger record, cloacal temperature and air temperature (pair-*t* test: datalogger vs. cloacal, $t = 1.3449$, $P = 0.1945$; air vs. cloacal, $t = 0.2536$, $P = 0.8025$; datalogger vs. air, $t = 1.5348$, $P = 0.1413$) (table 1).

In the environmental stations, the mean environmental temperature and variation was the

Table 1. Measurements of cloacal and air temperatures at turtle locations taken concurrently with datalogger from seven radio-tagged *Cuora flavomarginata* ($n = 20$) in a northern Taiwan population.

Temperature measured	Measurements (°C)	
	mean \pm s.d.	range
Data logger temperature	21.9 \pm 2.5	17.5-27.9
Cloacal temperature	22.2 \pm 2.6	19.2-27.5
Air temperature	22.2 \pm 2.2	19.8-27.0

highest in open area, then followed by edge and forested habitat (fig. 1). Patterns of mean weekly T_s variation of selected individuals of *Cuora flavomarginata* were correlated with the seasonal change in environmental temperature (fig. 2). Mean weekly T_s steadily decreased through October and November, and the differences between T_s and T_e in both sexes also decreased greatly (fig. 3). During early winter (December), both T_s and T_e dropped to approximate 15°C (figs 2 and 4), suggesting the inactivity of *C. flavomarginata*, although some occasional increases of T_s corresponding to T_e fluctuations were observed. However, mean weekly T_s were 0.5-1.0°C higher or lower than T_e in non-active period (December-March), indicating that *C. flavomarginata* may be able to select more stale microhabitats to cope with low and varying temperature during winter. There were no sudden increases of T_s or significant differences from T_e as in active period (fig. 4), indicating that *C. flavomarginata* were thermoconforming in winter. In this study, minimum T_s occurred in late January (4.5-7.6°C). From early April, mean weekly T_s and T_e increased gradually to >20°C in mid-April (figs 2 and 5), indicating arousal from wintering. Maximal mean weekly T_s was found in late June and July (males: 24.5-25.1°C; females: 26.0-27.0°C).

Intersexual differences in thermal preference were found during nesting season (May-July); mean weekly T_s of females were higher than those of males (paired- t test, $t = 4.0190$, $P < 0.01$). Daily thermal profiles obtained from April to July also showed that mean T_s of females were higher than those of males from

May through July (fig. 5). Females selected the thermal environment close to mean T_e , however, the mean T_s of males were 1-4°C lower than the mean T_e during the day. The difference was pronounced during the day and hotter days.

There were no obvious differences between sexes in mean weekly T_s during non-nesting periods (paired- t test: April, $t = 1.2388$, $P = 0.3035$; October-November, $t = -2.3208$, $P = 0.0533$), although some T_s increases of males were observed (fig. 3). The preferred T_s ranges of male and female *C. flavomarginata* were not significantly different during cold months from December to March (paired- t test: $t = 0.2524$, $P = 0.8040$).

Discussion

Numerous studies have shown that the temperature measurements of dataloggers attached on turtle carapace can provide good estimates of body temperature under most circumstances (Peterson, 1987; Nussear, Esque and Tracy, 2002; Grayson and Dorcas, 2004). The differences between internal temperature and those recorded by externally attached dataloggers may be substantial during periods of large environmental fluctuation, such as warming or basking (Zimmermann et al., 1994; Sajwaj and Lang, 2000; Nussear, Esque and Tracy, 2002; Grayson and Dorcas, 2004). Although we have not compared the internal core temperature of *C. flavomarginata*, the measurements of dataloggers attached on turtle carapace can still be used as a reliable estimate of turtle body temperature, as it is at least a labor-efficient tool for thermal preference study (Nussear, Esque and Tracy, 2002).

Climate factors and female breeding condition may play important roles in the seasonal variation in activity of *C. flavomarginata*. In the preliminary observations, *C. flavomarginata* will enter hibernation when environmental temperature drops to lower than 12-15°C (Chen and Xie, 1988; Huang, Zhao and Wang, 2007).

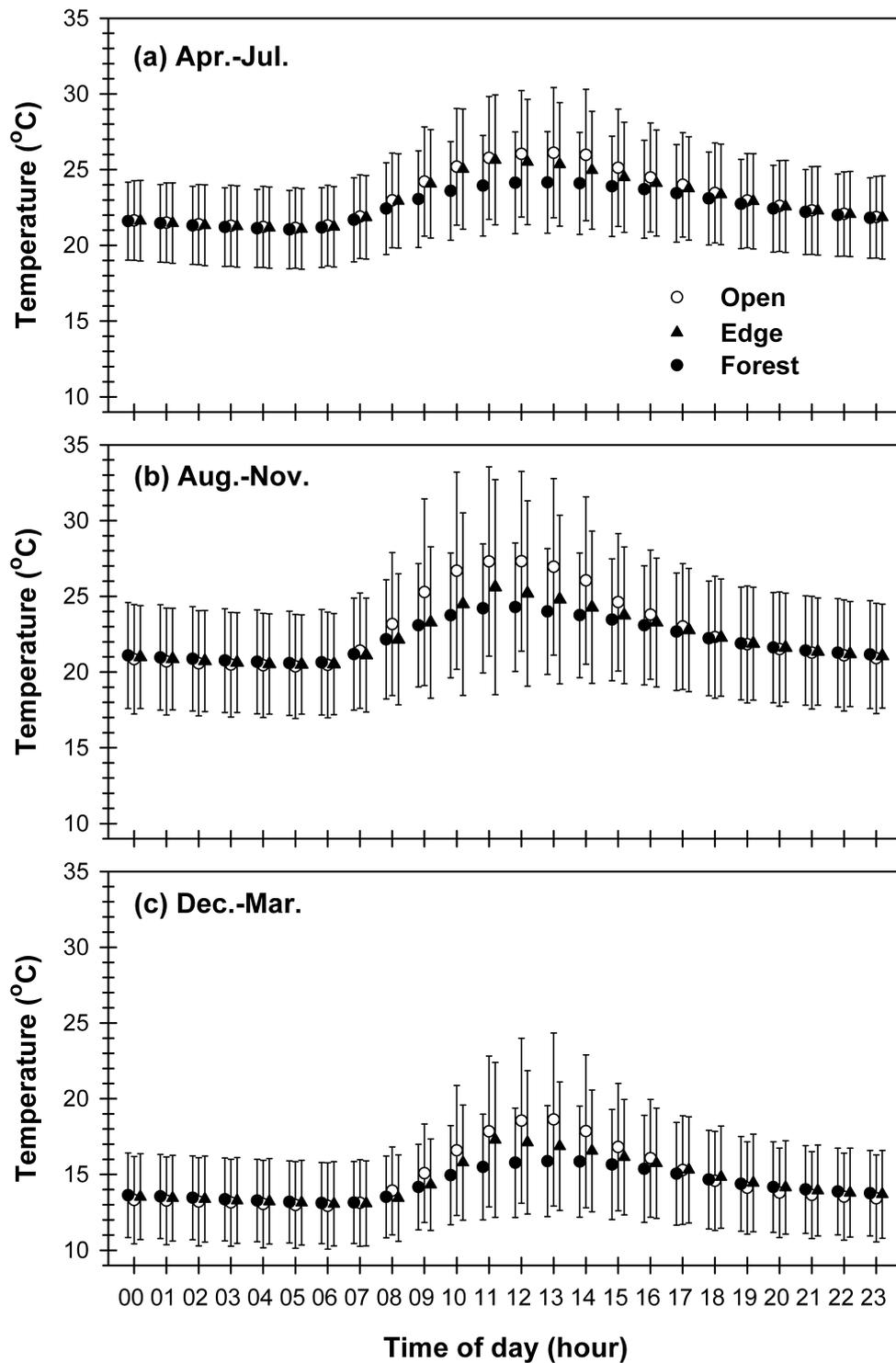


Figure 1. Hourly mean and standard deviation environmental temperature during (a) gravid season (April-July), (b) non-gravid season (August-November) and (c) non-active season (December-March) at three temperature stations of the representative habitat type in the Feitsui Reservoir Protected Area, northern Taiwan.

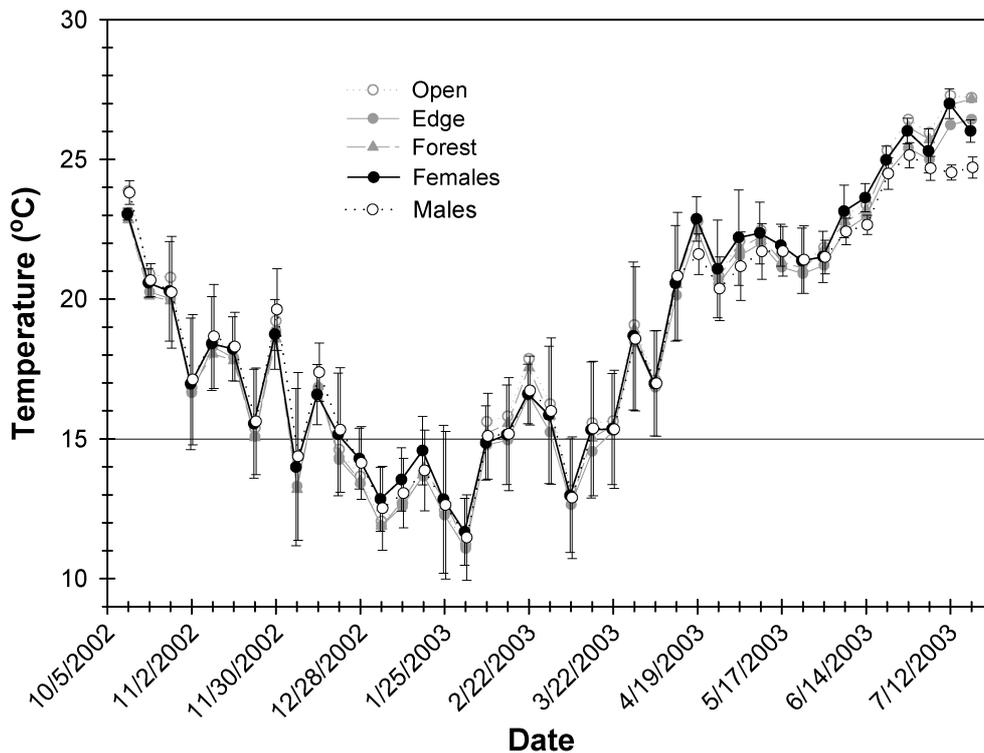


Figure 2. Mean weekly carapace surface temperature (T_s) for *Cuora flavomarginata* during the study period. The line for 15°C indicates that turtles may enter wintering (see text for explanation).

Based on radio-tracking results, the activity of *C. flavomarginata* decreased after late November, although occasionally short-distance movements could be detected (Lue and Chen, 1999). Our temperature data from shell surface of *C. flavomarginata* also suggest that they may have decreased activity level as the shell surface temperature decreases sharply in November. As in many reptiles, the immediate stimulus to enter hibernation for *C. flavomarginata* is likely a combination of temperature, photoperiod and endogenous rhythms (Gregory, 1982; Ultsch, 1989). During cold months, the behaviour of terrestrial box turtles is affected by ambient air and soil temperature (Congdon, Gatten and Morreale, 1989). In cooler winter, the fluctuation of shell surface temperature was coincident with the variation of environmental temperature; this pattern suggests that *C. flavomarginata* were thermoconforming during cold season.

Several factors have been suggested to be responsible for arousal of terrestrial turtles from hibernation, including warming of soil temperature, precipitation and ground moisture (Ultsch, 1989; Grobman, 1990). In previous study, radio-tracked *C. flavomarginata* started to emerge from over-wintering sites in mid-March (Lue and Chen, 1999). In this study, most of tracked individuals have left wintering sites in late March (unpubl. data). However, we have not found apparent increases of environmental and shell surface temperatures in March. Environmental temperature may not be the crucial cue for their emergence from wintering. Some other factors, such as endogenous rhythm or photoperiod, may have involved in emergence from wintering like other reptiles (Gregory, 1982).

The gravid season of *C. flavomarginata* extends from April to mid-July, and it nests mainly in May and June (Chen and Lue, 1999). In this study, we have not found apparently intersexual

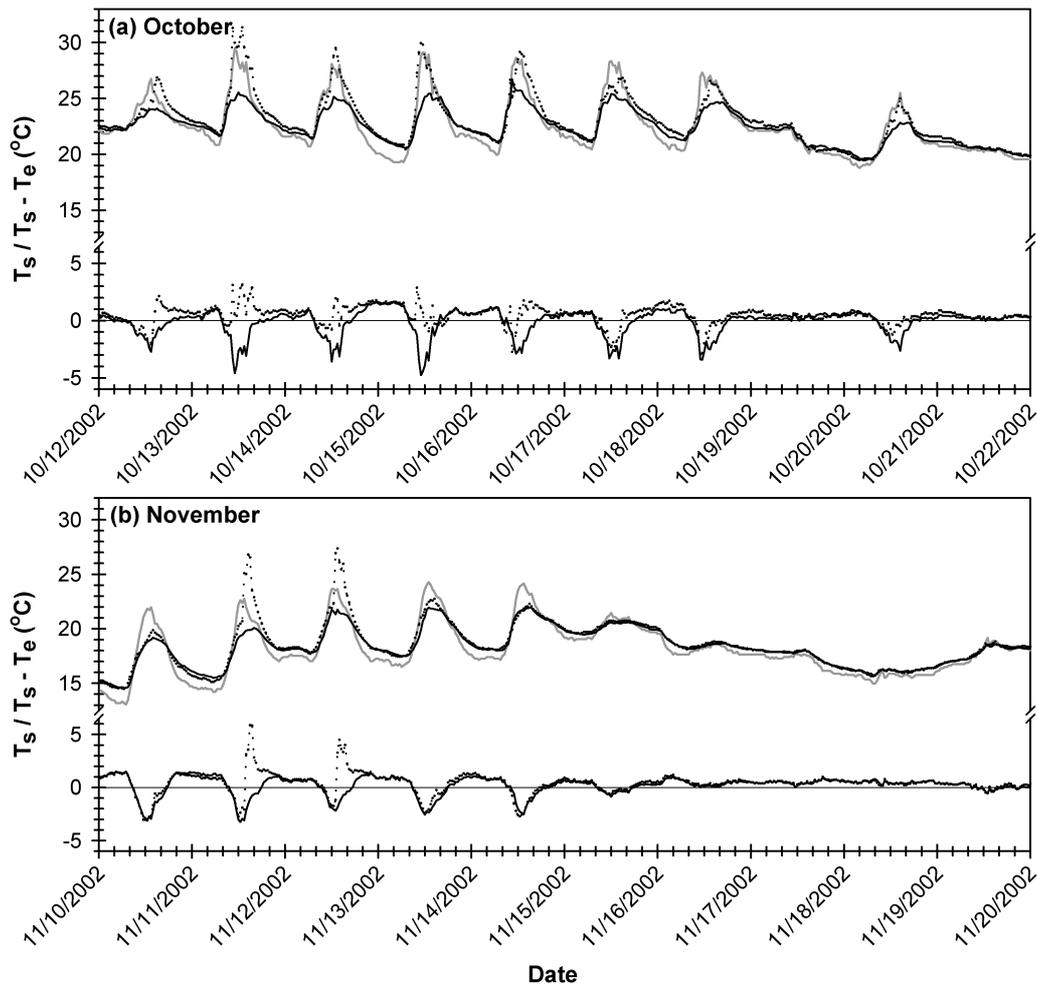


Figure 3. Representative time series of mean carapace surface temperature (\bar{T}_s) and differences between carapace surface temperature and ambient air temperature ($T_s - T_e$) between sexes of *Cuora flavomarginata* after gravid season (October–November) in the Feitsui Reservoir Protected Area, northern Taiwan (males: dot lines, females: solid lines, ambient air temperature: grey lines).

difference in thermal preference prior to nesting season (April). Although we have not investigated the reproductive condition for each female in this study, most of the radio-tagged females were believed to have nested because of the high nesting frequency in this turtle (Chen and Lue, 1999). Despite a limited sample size, our results provide evidence of intersexual thermal preference for individual *C. flavomarginata* in nesting season and also indicate the relatively broad range of shell surface temperature these turtles experienced. Based on trapping and radio-tracking results, the capture frequency for fe-

male *C. flavomarginata* was higher than that for males in April–August, and the gravid females preferred the forest edge in April–July (Lue and Chen, 1999). The seasonal shifts in habitat use and activity patterns could be in response to thermophilic requirements (nesting and hibernation) (Litzgus and Brooks, 2000). Obtaining higher body temperature can have many benefits for turtles, such as enhanced digestive rates, increased fat mobilization and rates of follicle development (Avery, 1982; Congdon, 1989). Numerous authors have suggested that gravid female turtles are likely to maintain higher body

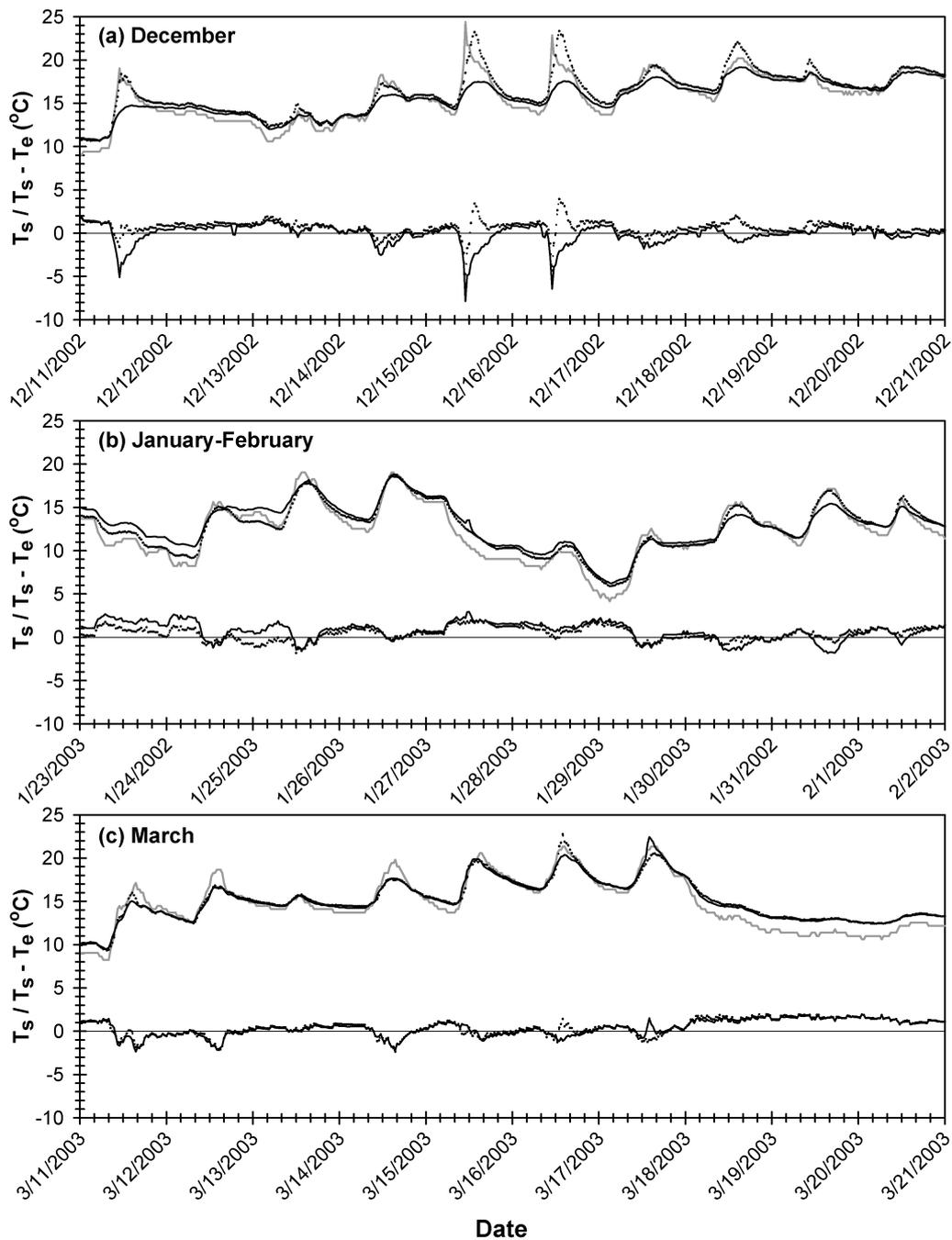


Figure 4. Representative time series of mean carapace surface temperature (\bar{T}_s) and differences between carapace surface temperature and ambient air temperature ($\bar{T}_s - \bar{T}_e$) between sexes of *Cuora flavomarginata* during non-active season (December-March) in the Feitsui Reservoir Protected Area, northern Taiwan (males: dot lines, females: solid lines, ambient air temperature: grey lines).

temperature to accelerate egg development (Obbard and Brooks, 1979; Vogt, 1980; Lue and

Chen, 1999). However, the higher basking frequency was observed only during nesting sea-

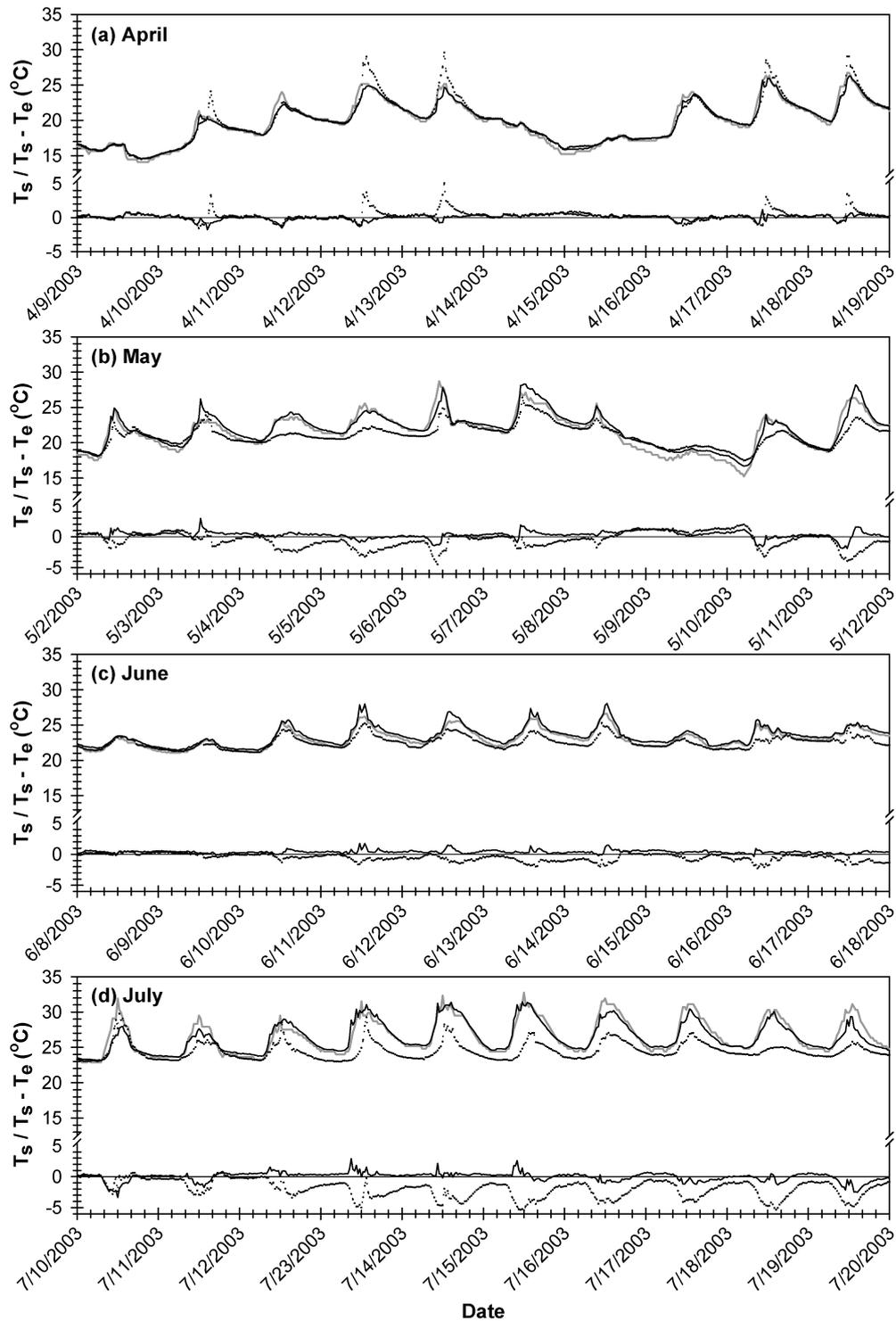


Figure 5. Representative time series of mean carapace surface temperature (\bar{T}_s) and differences between carapace surface temperature and ambient air temperature ($\bar{T}_s - \bar{T}_e$) between sexes of *Cuora flavomarginata* during gravid season (April-July) in the Feitsui Reservoir Protected Area, northern Taiwan (males: dot lines, females: solid lines, ambient air temperature: grey lines).

son in aquatic turtles, and has not supported the correlation between basking behavior and egg development (Krawchuk and Brooks, 1998). In the preliminary observations with limited sample size, the follicles of female *C. flavomarginata* may have matured during hibernation (Chen and Li, 1979; Chen, 1991). Previous studies have also indicated that most of egg development in turtles is complete before winter of previous season (Moll, 1979; Hsieh, 2005; Cheng, 2007). The preference for higher environmental temperatures for female *C. flavomarginata* during nesting season may not have been explained by accelerating egg development. Gravid females of *C. flavomarginata* usually use the edged habitat and nest in more cleared area with more exposure to solar radiation in the forest edge or forest gap (Lue and Chen, 1999). The thermal environment of seasonal habitat utilization may enhance the higher shell surface temperature directly.

In captivity, *Cuora flavomarginata* were active with an optimal environmental temperature, ranging from 26 to 31°C in terms of feeding efficiency and growth rate (Huang, Zhao and Wang, 2007). In our results from the field study, the T_s of *C. flavomarginata* only reached the lowest limit of optimal thermal range. Like other ectotherms, the physiological processes and activity were temperature-dependent (Hutchison, 1979; Huey, 1982). The increase of body temperature can enhance digestive efficiency and activity level. However, this indicates that turtles have to expend more energy to maintain high metabolic rate and the trade-off of growth rate (Steyermark, 2002; Litzgus and Hopkins, 2003). The lower optimal thermal range of free-living *C. flavomarginata* may be the results of balance between benefit of growth and cost of energy consumption. Female *C. flavomarginata* may stay in the forest edge during nesting season to conserve energy from shuttling among different habitats for nest site searching.

Our results of the intersexual difference in thermal preference indicated that female *C. flavomarginata* favor the forest edge or forest

gap during nesting season. They are more likely to be encountered and collected illegally near open habitat. This may lead to sex-biased population structure and detrimental effects on population sustainability; it is especially true for the removal of gravid females with high reproductive potential. In Taiwan, *C. flavomarginata* are exploited extensively to feed the food market in China or collected by pet hobbyists during accidental encounters. Legal enforcement and public education for illegal collection of *C. flavomarginata* are needed.

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