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Nocturnal Activity Rhythm of Chinese giant Salamander (*Andrias davidianus*) during the Reproductive Period

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Abstract

Nocturnal activity rhythm of Chinese giant salamander during the reproductive period was monitored by recording the time when it exited and entered the den using a digital monitoring system. Chinese giant salamanders exited the dens during 17:22~19:03, and mostly during 18:15~18:35 for female and during 18:00~18:15 for male, respectively. Water temperature (WT) ranged from 16.1℃ to 23.0 ℃ when the animal exited the dens. Movement of the animals outside of the dens peaked during 21:00~23:00 for female and during 21:00~01:00 for male, respectively. Female entered the dens during 23:01~01:02, while male during 06:19~08:38. WT ranged from 15.5℃ to 22.8 ℃ for females, and from 15.0℃ to 22.3 ℃ for males, when entering the dens. The time when the animals existed the dens was significantly correlated with WT for both females and males. However, the time when they entered the dens was not significantly correlated with WT.

Key words

Activity rhythm, Water temperature, Chinese giant salamander (*Andrias davidianus*).

1. Introduction

Chinese giant salamander (*Andrias davidianus*) is the largest amphibian in the world, which originated from the middle Jurassic [1] or early Cretaceous [2], and thus being considered as "living fossil" [3]. Because of overfishing and habitat destruction, the number of wild *A. davidianus* becomes scarce, it was listed as a Grade Two Protected Species in China since 1988 [4]. To protect this species, the Chinese government established 25 nature reserves in the mountains of Wuling, Qinling, Minshan, etc [5]. The National Nature Reserve was established in Hunan Zhangjiajie City in November, 1996.

Diurnal activity rhythm is a relatively stable process in which animals perform various activities obliged to daily life, and it is a comprehensive adaptation to diurnal changes in environmental conditions such as light, temperature, humidity, food, and natural enemies [6]. Besides, it is affected by many factors, such as physiology, digestion, morphological characteristics, habitat area, food distribution and so on [7-8]. In brief, animal activity rhythm is an important part of behavioral ecology. Research on animal behavior can help to quantify animal behavior and to further explore its function, mutual conversion and mutual relations, which is essential for the protection and artificial breeding of animals.

The daily movement of Hellbender (*Cryptobranchus alleghaniensis*) peak during about 2 h after dark [9-10]. The activity rhythm of Hellbender is influenced by gender, temperature, illumination and season. The depth of stream is positively related to nocturnal activity, while there is not significant correlation between water temperature (WT) and activity [11]. The daily activity for *A. davidianus* breeded in pool stayed in the den to rest in day and exited den in night [12]. *A. davidianus* under imitated-natural ecological conditions moved out at 20:30, their activity peaked in 21:30 and reminded den at 01:00 (next day) [13]. Illumination and WT affected the nocturnal activity rhythm of the *A. davidianus* during the earlier reproductive period. *A. davidianus* began to leave or return when the illumination was 685~1 431 lx or the relative illumination was 3%. The WT of their activities peak ranged from 17.1 to 18.4 ℃ [14]. The above report on *A. davidianus* behavior were mainly conducted through interviews or occasional observations and lack comprehensive and systematic records. Most reports were descriptive, and lack the quantitative analysis.

Wild populations of the *A. davidianus* inhabit underground rivers and are very difficult to access. In this paper, their breeding behaviors were monitored with a digital monitoring system in a simulated natural habitat. We aimed to ascertain the time and temperature when *A. davidianus*

exited and entered den which can elucidate its behavioral pattern. It provides some basis for the ways to optimize the breeding technology and the conservation of the wild population. We hypothesized that (1) there is a regular time scope when *A. davidianus* exit and enter den and there is a time difference between the female and the male, and (2) the time when the animals exist and enter the dens is affected by WT.

2. Materials and Methods

2.1 Study Area and Time

We chose two natural-like breeding streams at the Zhangjiajie Zhuyuan Biological Technology of Chinese Giant Salamander Co. Ltd which located on 29°28′N 110°22′E. Ten dens were built on both sides of each stream, with plants growing on top of the dens. Past records showed that the reproductive period of *A. davidianus* is from Jul. to Sept. The time of observation is from 27 Jul to 1 Oct, 2016.

2.2 Trial Materials

(1) *A. davidianus*. their weight and age of each one were 5-8 kg, and 8-9 years, respectively.

(2) Streams and dens. Two artificial streams were selected as test points, and there are 10 dens beside each one. The width and depth of the natural-like stream were 1.10 m and 0.40 m, respectively, with pebble and soil on the bottom. Each den was cuboid-shaped and approximately 1.44 m^2, with an entrance of 0.25 m and 0.3 m in height and width, respectively, with a water depth of 0.2 m and sand and pebble on the bottom.

(3) Digital monitoring system. Three dens in each steam randomly selected to place digital monitoring cameras (HIKvision, Hangzhou hikivision digital technology Co, Ltd.) on top of the dens. In addition, one camera was installed above each stream to observe behavior outside the dens. There were 8 video screens altogether.

2.3 Observation Index

Nocturnal activity rhythm of the *A. davidianus* during the reproductive period, were observed using the digital monitoring system, which included times when *A. davidianus* leave and enter den very day. WT was monitored with a temperature recorder (SIN-RC-4, Hanghzou Sinomeasure automation Co. Ltd).

2.4 Data Processing

The data were statistically analyzed using the SPSS 20.0 package. One-way ANOVA was performed to study differences in the time and WT of entering and exiting between the male and female. A correlation analysis was made with WT and time when *A. davidianus* enter and exit den.

3. Results Analysis

3.1 Time and WT When *A. davidianus* Exited den

(1) Time of exiting den. During the *A. davidianus'* reproductive period, the times of their exiting den were shown in Fig. 1. The *A. davidianus* exited den during 17:22~19:03, and were 18:10±0:27 for femal and 17:54±0:20 for male, respectively. The times when the male exited den was earlier in Aug 26 to Sept 15, 2016, they were between 17:25 and 17:50. The else times of the male exiting den were later in the early and late observation stage, and their peaks were 18:15~18:35 and 18:00~18:15 respectively. The times when the female exited den were similar with the male in Aug 26 to Sept. 19, which range from 17:35 to 17:50. But, in early and late stage, the times were 18:25~18:45 and 18:15~18:30, respectively, which were later than the times for the male. The results from One-way ANOVA showed that there was extremely significant difference in time of exiting den between the female and the male during the early, middle and late stages (Tab. 1). As *A. davidianus* exited den, it put the head to the entrance of the den slowly at the first, then observe around surrounding for 10 min. After *A. davidianus* confirmed the security, it exited den completely.

Tab.1. One-way ANOVA of Exiting Den Time Between the Female and Male

	F/P Value		
	Early Stage	Medium Stage	Late Stage
Female-Male	10.773/0.002**	6.511/0.014**	7.800/0.008**

Note: **P<0.01

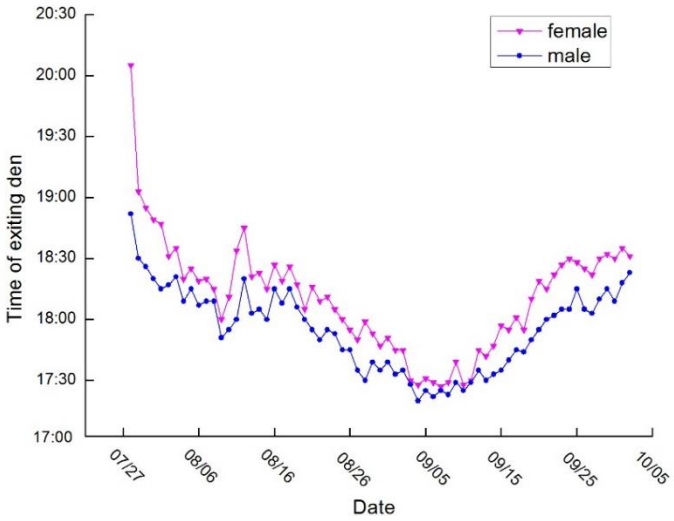

Fig.1. The Time When *A. davidianus* Exited Den

(2) WT of exiting den. The WT when *A. davidianus* exited den is shown in Fig. 2. It is found that the WT when the female exited den was between 16.50℃ and 22.80℃, and the mean temperature was (18.86±2.02) ℃. It is shown a downward trend from early to late stage. They were (20.92±1.15) ℃, (18.87±1.74) ℃ and (16.97±0.47) ℃ during the early, medium and late stage, respectively (Fig. 2a). The WT when the male exited den range from 16.10℃ to 23.00℃, and the mean temperature was (18.89±2.03) ℃. They were (20.94±1.19) ℃, (18.94±1.73) ℃ and (16.97±0.48) ℃ during the early, medium and late stage, respectively (Fig. 2b). On the Jul. 30, water height in breeding pool was reduced from lack of water so that the WT risen to 27℃ when *A. davidianus* exited den. It has been excluded from the data statistics for the rare. The results of One-way ANOVA showed that the difference of WT of exiting den between the male and female was not significant (F=0.006，P=0.936). Correlation analysis showed that the time and WT exiting den were significant correlation for the male and female, respectively (Tab. 2).

Tab.2. Correlation Coefficient Between WT, Daily Mean WT and Time of Exiting Den

Index	Correlation coefficient	
	Time when the female exited den	Time when the male exited den
WT of exiting den	0.355*	0.404*
Daily mean WT	0.488**	0.536**

Note: *P<0.05, **P<0.01 (the same as below)

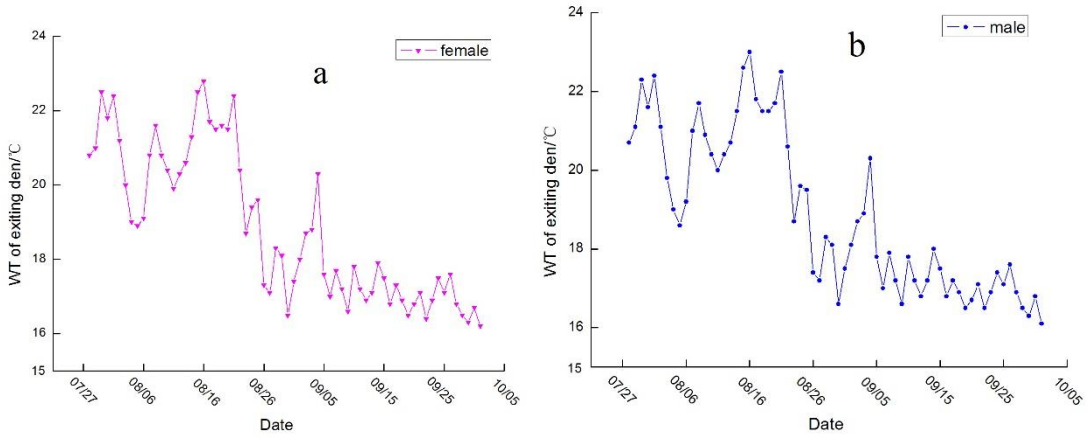

Fig.2. WT When *A. davidianus* Exited Den

The daily mean WT. The daily mean WT when the female and male exited den is shown in Fig. 3. They were between 16.10 ℃ and 22.35 ℃, and mean value was (18.32±1.86) ℃. They were (20.29±0.96) ℃, (18.08±1.76) ℃ and (16.75±0.27) ℃ in the early, medium and late stage, respectively, which shown a downward trend from early to late stage. Correlation analysis showed that the time and daily mean WT of exiting den were significant positive correlation for the female and male, respectively (Tab. 2). The higher of the WT, the later of time of exiting den.

A correlation analysis was performed on the WT of exiting den and daily mean WT. The results showed that they were a highly significant correlation (Tab. 3). In other words, the higher of the daily WT, the higher of the WT of exiting den.

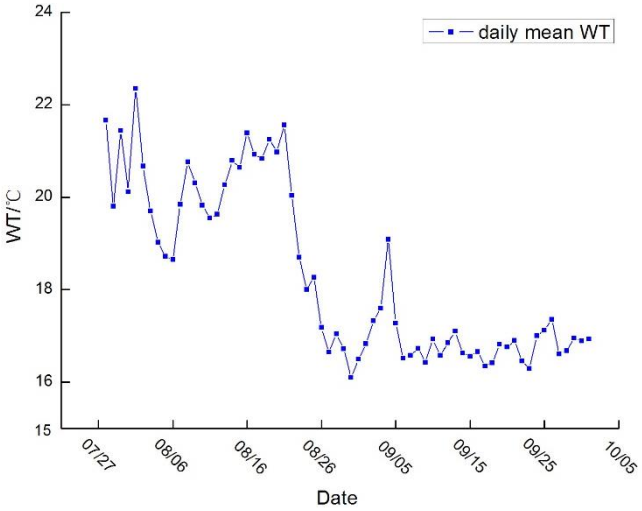

Fig.3. Daily Mean WT When *A. davidianus* Exited Den

Tab.3. Correlation Coefficient Between WT and Daily Mean WT When *A. davidianus* Exited Den

Index	Correlation coefficient	
	WT when the female exited den	WT when the male exited den
Daily mean WT	0.960**	0.956**

3.2 Activity out of Den

After *A. davidianus* exited den, it slowly creeped or quietly lay in the artificial stream. Once there was slight movement around environment, it quickly reacted and returned to den. A few *A. davidianus'* climbed up bank on the sunny day while many ones on the rainy day. Besides, *A. davidianus* is a little anxious in the rainy day, they entered and exited den frequently in the day and night. *A. davidianus* preyed, paired, bathed and acted others outside den. The activity for the female and the male peaked during 21:00~23:00 and 21:00~01:00, respectively.

3.3 Time and WT of Entering Den

Time of entering den. The times when *A. davidianus* entered den during the reproductive period were shown in Fig. 4. The times when the female and the male entered den during 23:01~01:02 and 06:19~08:38, respectively, and the male' s was about 7 hours later than the female's. During the whole observation period, the female and the male had a similar time of enter den.

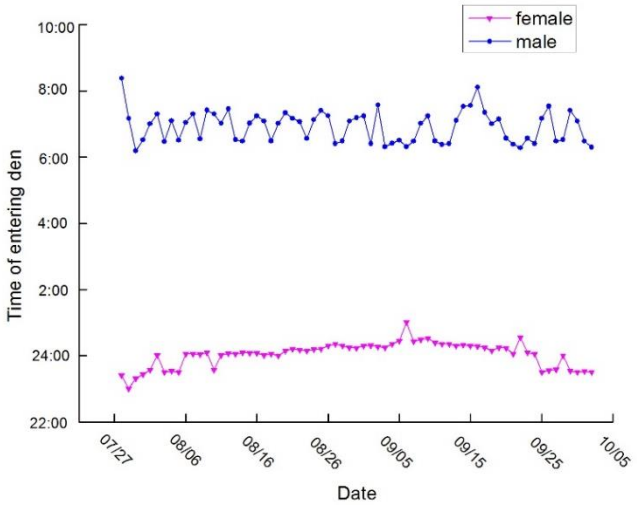

Fig.4. The Time When *A. davidianus* Entered Den

WT of entering den. The WT when *A. davidianus* entered den is shown in Fig. 5. It is found

that the WT of female entering den range from 15.50 ℃to 22.80 ℃. In the early, medium and late stage, they were (20.61±0.97) ℃, (17.88±2.02) ℃and (16.12±0.35) ℃, respectively (Fig. 5a). The WT when the male entered den range from 15.00 ℃to 22.30 ℃. In the early, medium and late stage, they were (19.35±1.01) ℃, (17.09±1.57) ℃ and (15.64±0.34) ℃, respectively (Fig. 5b). One-way ANOVA showed that the WT of entering den differed between the male and the female significantly (F=4.713, P =0.032<0.05), the male' s was significantly lower than the female's. Correlation analysis showed that the time and the WT of entering den were not correlative significantly for the male and the female, respectively.

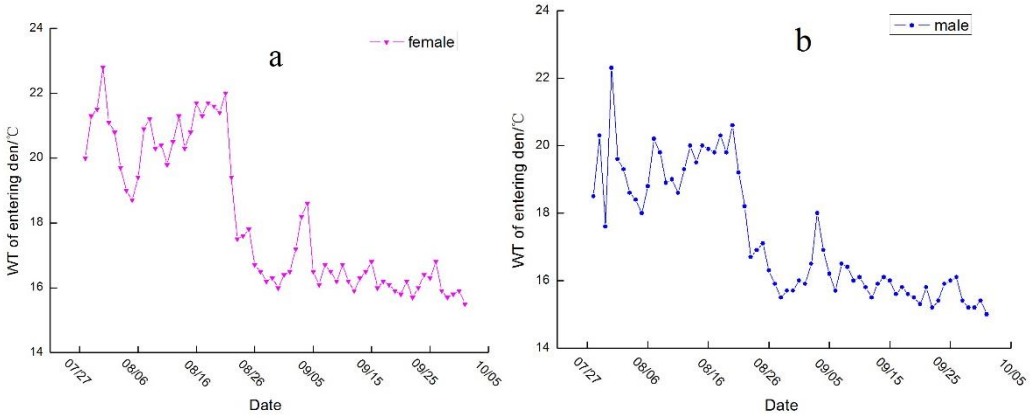

Fig.5. The WT When *A. davidianus* Entered Den

4. Discussion and Conclusion

4.1 Nocturnal Rhythm During the Reproductive Period

The *A. davidianus* left den during 17:22~19:03, and the fastigias of the female and the male were 18:15~18:35 and 18:00~18:15, respectively. The male' s was significantly earlier than the female's. The female entered den during 23:01~01:02, while during 06:19~08:38 for the male. The times of activity out of den were 3~5 h and 12~14 h for the female and male, respectively, and the male' s were about 8~9 h longer than female's. Their fastigia were 21:00~23:00 and 21:00~01:00 for the female and male, respectively. There was a significantly difference in the nocturnal rhythm between the female and the male.

Noeske and Coatne found that the hellbenders less act during the day and frequently act during 2 h after dark[9-10]. It was in line with the fastigia of movement of *A. davidianus* in this study. Liang G found that *A. davidianus* moved out of den at 20:30, their activity peaked during 21:00~01:00 (next day)[13]. Yu reported that *A. davidianus* moved out of den at 19:30, their activities peaked during 21:30~01:30 (next day)[14]. After 06:30, all *A. davidianus* returned to den, which were similar to the activity peak in this study and different to the time of exiting den. The

times from above reports were later 1.5~2.5 h and 0.5~1.5 h than this study, respectively. They maybe result from the different climate conditions of trial sites due to difference region. Chen found that *A. davidianus* breeding in the pool left den to ingestion and crawled around the pool in 19:00~06:00[12]. There were three activities peaks, which were 20:00~21:30, 23:00~0:00 and 01:00~02:00, respectively. It was different from this paper. It may be result from the different ecological factors such as light and WT and so on existing the pools inside and outside of room.

During reproductive period, the male act outside longer than the female. Maybe the male need eat more food to store more abundant nutrients and energy preparing for parental care. In addition, their activity outside den for a long time benefit to look for spouse. However, the female was weight because of a large of eggs in body, so they less act outside.

4.2 The Effect of WT on the Nocturnal Activity Rhythm of *A. davidianus*

During observation period, the WT when the female exited den was 16.50~22.80 ℃, and 16.10~23.00℃ for the male. The appropriate WT for *A. davidianus'* growth was 16~23 ℃, and the WT when the female and male exited den on this study was accorded with it. The time when *A. davidianus* exited den positively correlated with the WT same time, the higher the WT, the later the time exiting den. *A. davidianus* is a poikilotherm, and the temperature is the main factor that affects its physiology and behavior. In addition, the observation was performed in hot season, the outside temperature was higher than it in den. The later time when *A. davidianus* exit den, the lower of WT, which were suitable for the *A. davidianus* more. In winter, the WT outside is lower than the den, maybe the higher of WT, the earlier time when *A. davidianus* exited den. The timeof exiting den significantly positive correlated with WT and mean daily WT. Thus, the time when *A. davidianus* exit den maybe seasonal.

During observation period, the WT when the female entered den was 15.50~22.80 ℃, while the male' s was 15.00~22.30 ℃. They were slightly below the WT of exiting den, and they approach 16~23℃ which is the appropriate WT for *A. davidianus'* growth, There was no correlation between the time and WT of entering den. During observation period, the time of *A. davidianus* entering den was stable, maybe in the proper WT range, the time when *A. davidianus* enter den might be mainly affected by the biologic clock.

Yu (2013) found that the number of *A. davidianus* exiting den was significantly correlated with WT, and the temperature was the main factor to activity peaks [14]. When WT was 17.1~18.4℃, their activity reached peak. They returned to den when WT was 16.7~16.8℃. The temperatures were similar to this study. Chen (2006) reported that the nocturnal active rhythm was

changed with WT [12]. In this study, we found the time when *A. davidianus* exited den was affect by WT, while the time when it entered den was not. The *A. davidianus* is poikilotherm animal, its physiological function is affected by temperature. So temperature can affect its active rhythm. The temperature has a greater effect on the circadian rhythm of poikilotherm animal. The daily activity rhythm of the plateau and minshan toad, which was located in the norge wetland, was significantly correlated with temperature. But, Humphries (2000) reported there was not significant correlation between WT and activity for Hellbender [11]. In this study, only the time of exiting den was significant correlate with WT. Maybe, WT isn't the only factor affecting the activity. Therefore, in the case of artificial breeding for *A. davidianus*, especially in its ecological reproduction, the WT still required to be kept within the proper range in order to satisfy it normal growth and breeding.

Conclusion

This paper systematically recorded the time and WT when *A. davidianus* enter and exit den, found its nocturnal active rhythm during reproduction period, and analyzed the effect of WT on its nocturnal active rhythm. In addition, the relevant image date was first filmed and stored. Light intensity is the key factor which affect the outside activity of the *A. davidianus* [14], but it was not measured in the study. Thus, further study on it and other ecological factors were required.

Acknowledgments

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