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부화 온도가 맹꽁이(*Kaloula borealis*)알의 부화에 미치는 영향[1]

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The Effect of Incubation Temperature at Egg Hatching of the Boreal Digging Frog, *Kaloula borealis*[1]

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요 약

본 연구는 부화 온도가 맹꽁이(*Kaloula borealis*) 알의 부화 기간에 영향을 미치는 여부를 알아보기 위해 수행되었다. 본 연구에서 맹꽁이 알에서 올챙이가 생긴 것을 기준으로 알 부화 과정을 기록했다. 연구 결과 맹꽁이의 모든 알은 산란 후 48시간 이내에 부화하였으며, 28.1%(±10.8, n=52)가 24시간 이내에, 99.9%(±0.23, n=49)가 산란 후 48시간 이내에 부화했다. 수온의 차이에 따라 맹꽁이 알의 평균 부화율은 유의미한 차이를 나타냈다. 산란 후 15~24시간 사이의 평균 부화율은 24.1(±0.2)°C보다 21.1(±0.2)°C의 수온에서 더 높았다. 본 연구 결과는 비교적 낮은 수온에서 빠른 부화가 되는데 이는 비가 오는 계절에 일시적인 연못이나 웅덩이에 알을 낳는 번식 습성으로 웅덩이가 마르기 전에 빠른 부화가 필요하기 때문으로 추측한다. 본 연구 결과는 멸종위기종인 맹꽁이 알의 최적 부화온도를 이해하는데 도움이 된다.

주요어: 맹꽁이, 부화 온도, 부화 기간, 초기 성장

ABSTRACT

This study aimed to determine the egg-hatching period of boreal digging frogs, *Kaloula borealis*, and investigate whether the incubation temperature affects the hatching period. In this study, the egg hatching was recorded based on the appearance of the tadpole. The results of this study showed that all the eggs hatched within 48 hours after spawning, with 28.1% (±10.8, n=52) hatching within 24 hours and 99.9% (±0.23, n=49) within 48 hours after spawning. The mean hatching rate of tadpoles showed significant differences depending on the difference in water temperature. The mean hatching rate between 15 and 24 hours after spawning was higher at a water temperature of 21.1 (±0.2) °C than at 24.1 (±0.2) °C. The results suggest rapid hatching occurs at relatively low water temperatures because the spawning habits that spawn eggs in temporary ponds or puddles in the rainy season require rapid hatching before the puddles dry out. The results of this study are helpful for understanding the most suitable temperature conditions for the incubation of eggs of the endangered species, boreal digging frog.

KEY WORDS: KALOULA BOREALIS, INCUBATION TEMPERATURE, HATCHING DURATION, EARLY DEVELOPMENT

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Introduction

Optimal environmental conditions are extremely important for improving the reproductive success and development rate of anurans (Han & Lu 2001, Feng *et al*. 2004). The incubation temperature is a major environmental condition that directly influences the rate of embryonic development in different amphibian species (Pollister & John 1937, Moore 1939, Stewart 1956, Herreid & Kinney 1967, Bradford 1990). For example, the optimal incubation temperature improves the early embryonic development rate and survival of eggs of the Black-spotted frog (*Rana nigromaculata*) (Yu *et al*. 2013).

The boreal digging frog, *Kaloula borealis*, is commonly found in central to northeast China and Korea including Jeju-do Island (Yang & Yu 1978). In Korea, *K. borealis* is classified as an endangered category II species (Park & Kaplan 2013, The ministry of environment, 2004). *K. borealis* breeds during the annual rainy season, and the breeding habitat is generally shallow ponds, ditches, or stagnant rainwater pools that form during the rainy season and do not exceed 50 cm in depth (Han 1992). Previous studies have shown that the drying up of the temporary aquatic habitats of *K. borealis* could result in a shortening of the early egg development period as well as the egg survival rates. These studies have also shown that the mean proportion of tadpole appearance from the eggs was higher at high than at low temperatures because of increased evaporation rates. These studies conducted an experiment to test the effects of temperature on the early development of *K. borealis* eggs.

Methods

For this study, previous field surveys were conducted at a farm pond nearby Gwacheon City, Gyeonggi-do, Korea from 20015 to 2016. This pond was used as a breeding site for boreal digging frogs(*K. borealis*) for several years and was shallow, not exceeding 10 to 40 cm in depth, during the rainy seasons around June and July. Although after the rainy season, the pond dried up, the soil remained wet. Adult frogs dug burrows underground and emerged sometimes only at night to feed. The mean water temperature was 24.5 (±0.5, n=30) ℃ of the day during previous surveying period. In a related study, Han (1992) reported that *K. borealis* inhabited waters with temperatures of 21−23.7 °C in other ponds and rice paddies in Korea. This study was conducted based on previous observations and related research.

During the early breeding season (early to middle June), 108 adults were collected from ponds, of which 54 each were males and females with snout-length (SVL) between 52 and 53 mm. The SVL was measured to obtain females of similar size.

In this study, 54 plastic tubs filled with 3 L of water were used and kept in a room with an ambient temperature of 19-20 ℃. One pair of *K. borealis* was added to each tub, and 27 tubs were maintained at 21.1 (±0.2) °C and the remaining at 24.1(±0.2) °C. The water temperatures were automatically maintained at a constant rate. After spawning under the above conditions, the eggs were checked at 15, 24, and 48 h, to confirm hatching. Water was added if necessary to maintain a constant water level of 3 L at each observation. After hatching, the tadpoles were transferred to another plastic tub with 10 L of water to maintain the tadpoles before releasing them into the pond from which the adult frogs were collected.

Seven of the 54 tubes were infected. Some of the eggs between 15:01 and 24:00 h (n=2) and between 24:01 and 48:00 h (n=4) at 24.1 °C and between 24:01 and 48:00 h (n=1) at 21.1 °C were infected with mold resulting in tadpole mortality. The plastic tube with the infected eggs was excluded from the analysis.

Results

The egg hatching was recorded based on the appearance of the tadpole. Figure 1 shows the duration of embryonic development of the eggs. Most of the eggs hatched within 48 h after spawning. The mean rate of tadpole appearance from the eggs between 15:01 and 24:00 h and between 24:01 and 48:00 h after spawning was 28.1% (±10.8, n=52) and 99.9% (±0.23, n=49), respectively. A significant difference was noted in the mean rate of tadpole appearance between water temperatures of 21.1 (±0.2) ℃ and 24.1 (±0.2) ℃ at 15:01 to 24:00 h after spawning (ANOVA, F=1755.9, *df*=1, *P*<0.0001), suggesting that the hatching rate was high at a suitable temperature in the early stages of egg development; no significant difference was noted between 24:01 and 48:00 h at both temperatures (Fig. 2).

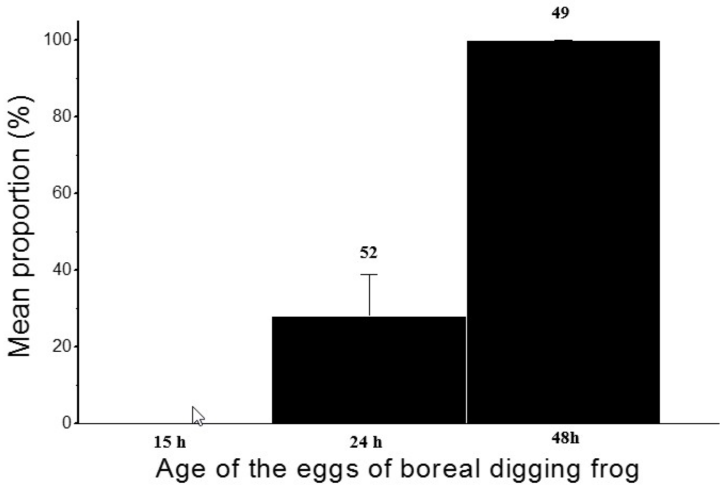

Figure 1. The mean proportion of hatching from eggs at 15, 24, 48 hours after spawning in boreal digging frog, *Kalula borealis*. Mean S.D and the sample size is given above each bar.

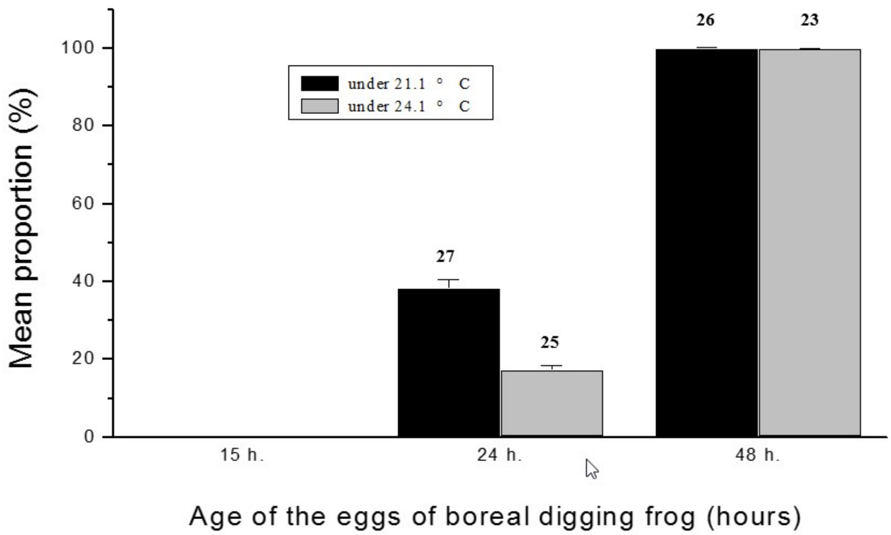

Figure 2. Comparison of the mean proportion of hatching from eggs at 15, 24 and 48 hours after spawning at different water temperature in boreal digging frog, *Kalula borealis*. Mean S.D and the sample size is given above each bar.

Discussion

In this study, we showed that the development from eggs to tadpoles of *K. borealis* is faster than that of most other frogs in the study area. It was also clear that temperature affected the early stages of the egg development of *K. borealis*. These results could be used to develop strategies to increase the survival rates of eggs in temporary stagnant rainwater pools.

For some frogs, temperature reduced the total incubation period during the early (Zou *et al*. 2001) and late (Zhang 1990) stages of embryo development. Contrary to the results of the above studies, our observations showed that the mean proportion of tadpoles emerging from eggs 15:01 to 24:00 h after spawning at of 21.1 ℃ was higher than at 24.1 ℃. Thus, *K. borealis* could lower the total incubation period during their early embryo development stages. Similarly, in another species of frog, Yu *et al*. (2013) reported that the total incubation period at 20.87 °C was lower than that at 18.20 °C during the early embryonic development of *Pelophylax nigromaculata*.

Although all organisms try to maximize their reproductive success, tadpoles of amphibians in temporary aquatic habitats are likely to experience the stress of pond desiccation, which adversely affects the survival of the tadpoles. Hence, pond desiccation can be a major factor resulting in reproductive failure as faster pond drying will result in higher mortality rates (Semlitsch & Wilbur 1988, Semlitsch *et al*. 1996, Gamble *et al*. 2006). Thus, tadpoles have to devise a strategy to maximize survival rates in a rapidly drying pond. One such strategy that has evolved in tadpoles is to accelerate embryo developmental rates (Newman 1988, Tejedo and Reques 1994, Bridges 2002). In this study, all of the eggs hatched into tadpoles within 48 h; this finding is consistent with that of Hwang (2000) who reported that *K. borealis* eggs hatched within 36h after spawning.

Global warming has been a growing concern since the 20th century. Darrow *et al*. (2004) reported a drastic increase in the average global temperature over the last 100 years. Increased environmental temperatures may seriously threaten the survival of many species, especially amphibians and insects, and could have drastic effects on their development. For example, the hatching duration of eggs in the wood frog, *Rana sylvatica* decreased as the water temperature increased from 9 to 26 °C (Darrow *et al.* 2004). In a detailed study, Censky *et al.* (2001) found that the optimal growth temperature for the wood frog was 22 °C. Similarly, our study demonstrated that the percentage of infected and dead eggs was greater at a water temperature of 24.1 °C (n = 6) than at 21.1 °C (n=1). Hence, we can presume that the survival rates of the *K. borealis* eggs will gradually decrease if there is an increase in global temperature.

It is important for the timing of metamorphosis to coincide with the drying of pools during raining season to minimize the mortality risk of tadpoles. For example, Semlitsch & Skelly (2007) reported that some species of anuran tadpoles, including the American toad, *Bufo americanus,* receive environmental cues such as the increase in temperature or changes in water level and could accelerate larval growth and development in rapidly drying ponds. Similarly, the Red-Spotted newt (*Notophthalmus viridescens*) has been shown to decrease larval developmental rates during periods of long inundation (Healy 1973). These studies demonstrate that some amphibians can adjust the timing of metamorphosis to ensure reproductive success in transient aquatic environments.

In conclusion, this study demonstrated that environmental conditions such as temperature is an important determinant in influencing the developmental rate of *K. borealis.* However, the findings of this study are limited because tadpoles can actively thermoregulate by moving between shallow and deep water, a fact not considered in this study. Additionally, this study has no data on other factors influencing developmental rate such as duration of metamorphosis according to water level, and crowding at various temperatures, which requires further investigations. Nevertheless, the results suggest that metamorphosis underwater was faster at 21.1 °C than at 24.1 °C within 24 h after spawning. We, therefore, concluded that the optimal water temperature for increasing the survival rates of *K. borealis* eggs is about 21 °C.

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