Asian Journal of Conservation Biology, July 2022. Vol. 11 No. 1, pp. 41–48 AJCB: FP0181/69836
ISSN 2278-7666 ©TCRP Foundation 2022 https://doi.org/10.53562/ajcb.69836

Research Article

Predicting the effects of climate change on tadpole stage fitness in the Korean brown frog *Rana uenoi* Matsui, 2014 (Amphibia: Ranidae)

Uhram Song[a,1,*], Eunjeong Yang [b,1], Min Woo Kim[c], Byoengwoo Kim[a], Sangeun Kwak[a], Seokhyeon Oh[a], Myeongjin Hong[a], Jiman Heo[a], Seungyeop Lee[a], Seorin Jeong[a], Hyeonsoo Kim[a], Hyunju Oh[a], Hun Park[d] ,Hojun Rim[e]

[a]*Department of Biology, Jeju National University, Jeju, 690-756, Korea*
[b]*Department of biological sciences, Seoul National University, Seoul, 08826, Korea*
[c]*Operational System Development Department, National Institute of Meteorological Sciences, Seguipo 63568, Korea*
[d]*Institute for Sustainable Development, Seoul National University, Seoul, 08826, Korea*
[e]*Center for Ecological Research, Kyoto University, Otsu, Shiga, 520-2113, Japan*

(Received: June 09, 2021; Revised: February 22, 2022; Accepted: April 11, 2022)

ABSTRACT

Jeju Island, South Korea is a biodiversity hotspot, but the vulnerability of Jeju Island fauna to climate change has not been thoroughly investigated. In this paper, we tested the adaptability of the Korean large brown frog *Rana uenoi* to future climate change. While increased temperatures did not affect the hatchability of frog eggs, hatched tadpoles in greenhouse treatment with less exposure to sunlight (less maximum temperature) were significantly (14%) shorter in length after three weeks of growth, indicating increased temperature of early season helps growth of tadpoles. However, right after three weeks, all tadpoles in a higher-temperature treatment (outside) died after the experiment logged a maximum temperature of 34.3ºC. In the field, the average temperature of puddles without tadpoles (puddles in the middle of the rivulet with all day exposure to sunlight) was 33.2ºC. During an indoor temperature elevation experiment, tadpoles suddenly died at 32.4ºC. Therefore, the expected maximum survivable temperature for *R. uenoi* is around 32ºC. Our results therefore suggest that *R. uenoi* tadpoles are vulnerable to elevated temperatures and could suffer mortality from climate change. Climate change will continue to cause extreme weather events like this (sudden elevation of temperature in spring), and frogs, especially tadpoles with restricted movement, will be particularly vulnerable to these events. Identifying how adaptable these amphibian species are to the weather events predicted by future climate change scenarios will be key for their conservation on Jeju Island.

Key words: Brown frog, global warming, hatchability, Jeju island, Climate data

INTRODUCTION

Biodiversity conservation is one of the most important challenges humans are facing. The projected loss of biodiversity in the 21st century has generated concern for several reasons, ranging from aesthetic and spiritual to purely commercial (Duffy, 2009), and biodiversity change is now considered an important global change in its own right (Sala *et al*., 2000) and many related studies are reported (Gallardo *et al*., 2018; Habibullah, Din, Tan, & Zahid, 2022; Sage, 2020). Understanding how the looming extinctions of plant and animal species will affect human welfare is undoubtedly the most important issue of all. However, even though changes in biodiversity are as important for ecosystem health as they are for human welfare, there was limited research examining how climate change will alter biodiversity (Sala *et al*., 2000) and research on how climate change affects local biodiversity is still lacking for many ecosystems (Nunez *et al*., 2019). Especially, islands are often reported to be vulnerable to climate change (Veron *et al*., 2019) and especially islands in pacific region adaptation to climate change by ecological resilience is very important study cases for humans to prepare galobal changes (Mcleod *et al*., 2019).

Jeju Island, South Korea is a biological hotspot for species diversity and has been designated as a 'Biosphere Reserve' by the United Nations Educational, Scientific and Cultural Organization (UNESCO) (Jo *et al*., 2012). However, limited research related to biodiversity (Cho *et al*., 2014; Kim, 2009) or species conservation (Lim, 2012; Song, Jang, & Kang, 2012) has been conducted on Jeju Island, and the effects of climate change have only been addressed in relation to crop pests (Kim, Jang, & Song, 2009) and fish communities (Jung, Ha, & Na, 2013). To our knowledge, no study has examined the relationships between climate change and biodiversity or species conservation for any terrestrial species on Jeju Island. As Jeju Island is at either the northern or southern geographic limit of many species' ranges (Banjade *et al*., 2019), the effects of future climate change may be seen more immediately in populations living on the island. Knowing whether species on Jeju Island will adapt to climate change or be threatened by extinction is important for preparing conservation measures tailored towards species with low adaptability. As changes of dynamics, population and diversity of herpetofauna represent habitat alteration and climate change (Priambodo *et al*. 2019), we have used a frog species for testing vulnerability of biodiversity and

**Corresponding Author's E-mail: uhrami@hanmail.net*

amphibian adaptation to climate change. In this study, we therefore tested the vulnerability of the Korean brown frog *Rana uenoi* to future climate change.

MATERIALS AND METHODS

Research site

Jeju Island is one of the most-visited tourist islands in the world and boasts the world's busiest flight route, with 180 daily flights (Asquith, 2020). Tourists are primarily drawn to Jeju Island for its remarkable biodiversity. The main reason the island became famous is because of its preserved nature. The island is biological hotspot for species diversity that designated as 'Biosphere Reserve' by UNESCO. Jeju Island is situated at the northern or southern geographic limit of the range of several endangered species (Banjade *et al*., 2019), and, like other islands, is particularly vulnerable to biodiversity loss due to climate change (Harter *et al*., 2015). It therefore poses a major challenge for biodiversity conservation.

Species selection

The brown frog *Rana uenoi* (Dybowski's frog) is found in northeast Asian countries including China, Japan, Korea, Mongolia and Russia (Feng *et al*., 1999). This species is categorized as "Least Concern" (LC) by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species, but in some parts of the world, such as Korea and China, this species is threatened by mass harvesting for use in traditional Chinese medicine. Because *R. uenoi* is not endangered and can therefore be used for scientific experiments, we chose this species as a representative for testing the effects of climate change on frog species of Jeju Island. Jeju Island is also the southern geographic limit of *R. uenoi* (Feng *et al*., 1999), as the southernmost range of this species in other countries (Liaoning Province in China and Tsushima Island in Japan) is at higher latitudes than the southern part of Jeju Island (33°06′37" N, 126°16′03" E, Altitude: 281m). Although *R. uenoi* is more closely related to boreal species and would presumably be more vulnerable to elevated temperatures, previous studies on this species have only focused on the effects of freezing temperatures (Xiao *et al*., 2008).

Species collection

In the spring of 2020, we collected *R. uenoi* eggs from puddles in an otherwise dry rivulet bed located at 33°27′ 37" N, 126°33′57" E on Jeju Island. The tadpole species were identified in 2019 by growing the tadpole to froglets in previous research (Baek *et al*., 2021). The rivulet is about 15 m wide and has a full forest canopy at its edges. The bed of the rivulet is bumpy volcanic rock with little soil or vegetation and is usually dry except for small puddles that form after precipitation. The rivulet flows only when it rains more than about 100 mm.

Hatchability and growth experiments

To determine egg hatchability under different conditions, we placed 20 eggs each in bowls (30 cm upper diameter, 20 cm lower diameter) filled with underground water. Bowls were then stored indoors, outdoors, or in a greenhouse, with five replicates performed for each treatment. We checked for hatchability every four days. The temperature of the water and air during the experiment were logged using an RC-5 data logger (Elitech, London, UK). This experiment was repeated twice, from March 27 to April 12, 2020 and again from April 14 to April 23, 2020.

We monitored bowls for approximately one week after the second hatchability experiment to see if there was another spawning event, in which case we would have conducted a third hatchability experiment, but there was no further large-scale spawning, and the hatched tadpoles were therefore used for a growth experiment in April 29th. Tadpoles remained housed in the experimental treatment (indoors, outdoors, or greenhouse) where they were hatched. Additional tadpoles, hatched separately from the hatchability experiments, were added to bowls with low hatchability to ensure that each bowl contained 20 tadpoles. The water and air temperatures were logged, as before, and the daily high temperature of the water was measured at 1500 (3:00pm) using an Orion Star A221 Portable pH Meter (ThermoFisher Scientific, Massachusetts, United States). Because tadpoles at our field site appeared to be eating leaf litter, we supplied leaf litter collected at our field site as a food source (4 leaves from field site per bowls). We then measured the body length (head to end of tail) of five random tadpoles per bowls three weeks after hatching.

Temperature elevation experiment

We conducted an additional temperature elevation experiment to test how tadpoles respond to warmer temperatures. For this experiment, four tadpoles were placed in a beaker (30 cm tall, 8.1 cm wide) filled with water at 20ºC. The water temperature was then elevated slowly to 30ºC within four hours using an SH-75 aquarium heater (Zhongshan Canhu Electric, Guangdong, China). Due to discrepancies between the temperature displayed on the aquarium heater and the exact temperature of the water, the exact temperature was also measured with an Orion Star A221 Portable pH Meter. After the water had reached the target temperature, we added a small amount of goldfish food to the beaker. The following day, we observed if the food had been eaten by the tadpoles. We additionally monitored for any behavior indicating stress, such as rapid movement or continued proximity to the surface of the water. The target temperature of the first experiment was 30 ºC and aimed to increase 1 ºC by each sequel experiment, with new tadpoles used for each experiment. After each experiment, tested tadpoles were grown indoors for a few weeks and monitored for any delayed responses such as irregular eating caused due to the elevated temperature experiment.

Local climate analysis

We obtained long-term climate data for our study site by using the average temperature data from the Sancheondan area (33° 26′ 49.6" N 126° 33′ 54.8" E) automated weather station, which is the closest weather station (1.5 km distance) to our egg collection site. The data, which is recorded in an hourly format, were downloaded from the Korea Meteorological Administration (KMA) for the period 2002-2020.

Statistical analysis

Differences in water temperature, hatchability, and tadpole length between two groups were evaluated using the Wilcoxon two-sample test when normality assumptions were violated. Differences in non-normally distributed variables among three or more groups were analyzed using the Kruskal-Wallis test. All statistical

Table 1. Logged temperature (ºC) and egg hatchability rate (%) by treatment.

Trial	Temperature	Outside ambient	Greenhouse ambient	Indoor water bowl	Outside water bowl	Greenhouse water bowl
1st	Maximum	14.0	17.2	21.4	23.8	21.6
	Minimum	4.7	5.2	15.3	2.6	3.3
	Average	9.7	10.7	18.8	10.9	10.4
	Hatchability			95.0 ± 2.2	98.0 ± 1.2	98.0 ± 2.0
2nd	Maximum	16.1	29.4	20.9	34.6	36.0
	Minimum	7.7	10.6	15.4	4.2	8.2
	Average	11.0	16.7	18.8	13.5	18.0
	Hatchability			99.0 ± 1.0	100 ± 0.0	97.0 ± 2.0

Hatchability values represent the mean ± SE of five replicates.
There were no statistically significant differences in hatchability (Kruskal-Wallis test).
The first experiment was conducted from March 27 to April 12, 2020, and the second experiment from April 14 to April 23, 2020.
The logged temperatures of the outdoor and greenhouse water bowls during the second experiment are erroneously high (see Results).

Table 2. Tadpole body length three weeks after hatching.

	Indoor	Outside	Greenhouse	*p* value
Length	2.60 ± 0.07	2.62 ± 0.03	2.28 ± 0.06	0.0002

Values represent the mean ± SE of 25 replicates

tests were conducted with SAS 9.1 (SAS Institute Inc., North Carolina, USA), and statistical significance was inferred when $p<0.05$.

RESULTS

Hatchability rate experiment

Frog eggs showed over 95% hatchability regardless of treatment (Table 1). In the first hatchability experiment, the water temperature of bowls placed indoors was stable near an average of 18.8ºC, whereas the water temperature of bowls placed outside or in a greenhouse fluctuated more widely around an average of less than 11ºC.

There was an error in the second experiment that affected the temperature data. For all replicates, the temperature logger was placed in a plastic bag and then secured in the water bowl using stones. In the second experiment, the temperature loggers for the outdoor and greenhouse treatments were untied from the stones and were left floating in the water. The loggers therefore recorded falsely high temperatures because they were exposed to direct sunlight and the temperature inside the plastic bags increased. We did not discover the floating temperature loggers for three days, which is why the maximum recorded temperature was artificially high. To resolve this problem in subsequent experiments, we measured the water temperature in each bowl at 3:00pm on each sunny day using a portable pH meter. The average temperature of outdoor water bowls was 30.1±0.2ºC (mean ± SE of five replicates), significantly higher (Wilcoxon test) than the greenhouse water bowls at 26.7±0.5ºC.

Growth experiment and field temperature

Three weeks after hatching, at first measurement, tadpoles grown in the greenhouse were significantly shorter in length than tadpoles grown indoors or outdoors (Table 2). The maximum daily temperature of the greenhouse treatment was significantly lower than outside while the daily temperature change was very severe (Table 3). However, every tadpole in the outdoor treatment died two days after the first body length measurement (May 13), preventing us from taking further measurements. The logged maximum water temperatures of the greenhouse and indoor treatments during the growth experiment were 31.8ºC and 20.9ºC, respectively, whereas the maximum water temperature of the outdoor treatment was 34.3ºC (Table 3). The average water temperature in the greenhouse treatment, measured daily at 3:00pm using a portable meter, was 25.1ºC (Table 4), whereas the average water temperature of bowls kept outside was significantly higher at 33.2ºC. At the field site, puddles without tadpoles were significantly warmer (33.2ºC) than puddles with tadpoles (29.9ºC) (Table 4).

Temperature elevation experiment

Tadpoles that were held at temperatures up to 31.2ºC readily consumed goldfish food, showed no signs of stress, and grew well after the experiment, suggesting that they were not affected by the warmer temperatures. However, at the next sequel experiment, all tadpoles held at 32.4ºC died (Table 5).

Local climate analysis

It is important to know the temperature of the study region because research conducted outdoors is invariably affected by it. Temperatures at the Sancheondan weather station in 2020 were above average from January to March and unusually low in April (Figure 1). According to climate analysis by the National Institute of Meteorological Sciences (provided by author Min Woo Kim), the overall average temperature from January to July 2020 was the third-highest on record, but there were large fluctuations in monthly temperatures. January, February, March, and June all had average temperatures that were among the warmest three on

Table 3. Logged temperature (ºC) during the tadpole growth period.

Temperature	Outside ambient	Greenhouse ambient	Indoor water bowl	Outside water bowl	Greenhouse water bowl
Maximum	21.3	27.4	20.9	34.3	31.8
Minimum	12.2	10.2	16.1	7.5	7.5
Average	17.6	18.4	19.9	18.8	19.0

Temperatures were recorded from April 29 to May 13, 2020.

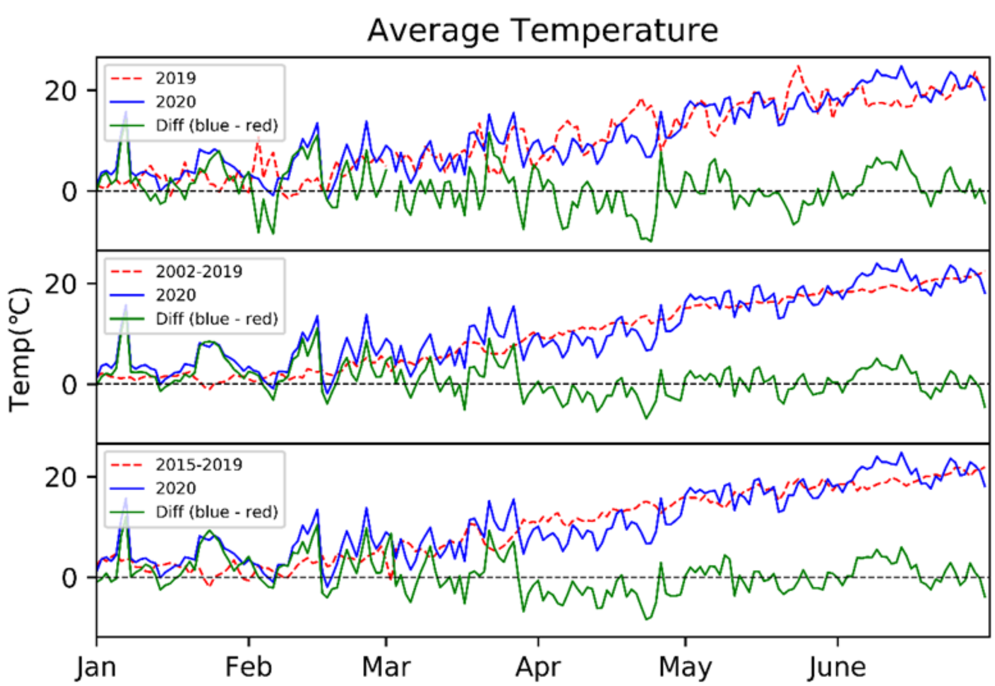
Table 4. Measured water temperature (ºC) at the time of the death of the experimental animal

Greenhouse water bowl	Outside water bowl	*p* value
25.08 ± 0.26	**33.22 ± 0.29**	**0.01141**
Field puddle with tadpoles	Field puddle without tadpoles	*p* value
29.94 ± 0.17	33.22 ± 0.75	0.01193

Values represent the mean ± SE of five replicates.
*Only *p*-values < 0.05 (significantly different) are presented.

Table 5. Tadpole responses to different temperatures during the temperature elevation experiment.

Temperature (ºC)	Eating
30.0	O
31.2	O
32.4	X (Dead)



*Diff: Temperature differences.

Figure 1. Average temperatures based on automated weather station data from the Sancheondan area during different time periods. (a) shows the average temperatures in 2019 and 2020, (b) shows the average temperatures from 2002-2019 and in 2020, and (c) shows the average temperatures from 2015-2019 and in 2020. All graphs additionally show the difference between the 2020 temperatures and the average for the previous time period.

record for each month, but April and July were each only the 44th-warmest on record, with relatively low temperatures occurring for new millennium not only on Jeju Island but also across South Korea. This unusually cold April delayed tadpole growth, which negatively affected the tadpoles as the weather warmed up in May. For example, many tadpoles in fields did not have their forelimbs by the first weeks of May, even 6 weeks after hatching while our previous monitoring of the study site (Baek *et al*., 2021) in 2019 showed presence of forelimbs for most individuals of *Rana uenoi*.

DISCUSSION

Hatchability rate experiment

The hatchability experiment was designed to see if elevated temperatures affect the hatchability of frog eggs. Hatchability rates were over 95% in all treatments, indicating that the observed temperature differences among treatments did not affect hatchability (Table 1). However, the hatchability differences among treatments were not statistically significant, despite large differences in maximum, minimum and average temperatures (Table 1). Hatching occurred within the first four days in all treatments, even though much lower minimum water temperatures (approximately 3ºC) were recorded outdoors and in the greenhouse.

The experiment was continued past the initial hatching event, for two weeks in the first experiment and ten days in the second experiment, to see if any eggs would hatch late. However, eggs that did not hatch in the first four days of the experiment did not hatch during this extended observation period. The maximum water temperatures in the outdoor treatment were 23.8ºC during the first experiment and 30.1ºC (measured by a portable meter because of the floating logger error) during the second experiment, which was evidently warm enough to hatch the eggs without being too warm(hot) that hatchability was affected. We noted that the second sampling period and hatchability experiment also seemed to be the climax of *R. uenoi* spawning activity in the field, as spawning activity rapidly decreased since then.

Growth experiment and field temperature

After the second hatchability experiment, we monitored puddles in field for approximately one week to see if there was another spawning event. Occasional spawning was observed, but there were no further mass spawning events even though it rained on the third day of the observation period. Hatched tadpoles were therefore used for a growth experiment. Because most eggs hatched within four days of each other, we only replaced dead or less-developed tadpoles with tadpoles of equal age that had been hatched in spare bowls. Fallen leaf litter appeared to be the primary source of food for tadpoles at our field site, so we supplied litter collected from our field site as a food source during this experiment. Most eggs hatched before April 20 during the second hatchability experiment, so we considered May 11 to be three weeks after hatching for all tadpoles. After three weeks, tadpoles housed in the greenhouse were significantly shorter (14% shorter) than tadpoles housed indoors or outdoors (Table 2). Compared to the other treatments, indoor water had a higher, more stable average temperature at approximately 20ºC (Table 1 and Table 3), which was closest to the optimal temperature of related brown frog species (23ºC), *Rana chensinensis* (Yu, Pang, & Chen, 2015). We therefore expected tadpoles housed indoors to show the most growth in our three-week study. However, the fact that tadpoles in the greenhouse showed significantly less growth, despite the relatively stable temperature of the greenhouse treatment compared to bowls kept outdoors, raises the possibility that rise of puddle temperatures due to climate change in future could have negative effects on tadpole growth.

Because water temperature did not have a strong effect on egg hatchability, but warmer temperatures significantly affected tadpole growth, the adaptability of *R. uenoi* to temperature increase may vary among growth stages. Our results suggest that the temperatures during tadpole growth, as well as during the spring spawning season, are important measures for assessing the species' vulnerability to climate change. In our experiment, outdoor water temperatures were higher than greenhouse water temperatures because the greenhouse had a lower light intensity. The light intensity outdoors was 2276 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (mean of three replicates, measured using an LI-250 Light Meter with Quantum Sensor (Li-Cor, Nebraska, USA), whereas the light intensity inside the greenhouse was 1164 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (mean of three replicates). Bowls in the greenhouse therefore received less heat from the sun than bowls kept outdoors. This was unexpected, as we thought the greenhouse treatment would result in higher water temperatures than the outdoor treatment. Although we originally intended to continue the experiment into the summer because the water temperatures might change, all tadpoles in the outdoor treatment died two days after body length measurement, ending the growth experiment. Since the frog did not die by small numbers for several days, but died all at once within two days, it is unlikely to be stress caused by an environment other than temperature. Factors other than temperature are unlikely to have changed rapidly within two days. When all the tadpoles in the outdoor treatment died, the maximum recorded water temperature of outdoor bowls was 34.3ºC, whereas the maximum recorded water temperature for the greenhouse treatment was 31.8ºC (Table 3). In the indoor treatment, the temperature recorded by the logger was within ±0.1ºC of the temperature measured by a portable meter, but in the outdoor treatment, the temperature recorded by the logger was artificially elevated because some parts of the plastic bag that held the logger were warmed by the sunlight. We therefore used a portable meter to measure the daily hottest water temperature at the time of death of each tadpole. Based on measurements taken with a portable meter at 3:00pm each day, the average temperature of the outdoor bowls 33.2ºC, significantly warmer than the greenhouse water at 25.1ºC (Table 4). It can therefore be assumed that the temperature in the outdoor treatment exceeded the maximum survivable temperature of *R. uenoi*.

To verify the maximum survivable temperature of *R. uenoi* and to determine if similar temperatures are being reached in the field, we measured the water temperature of puddles at our field collection site. The average water temperature of puddles that contained tadpoles was 29.9ºC, whereas puddles without tadpoles were significantly warmer at 33.2ºC (Table 4). In general, puddles in the middle of the rivulet were warmer, were exposed to direct sunlight because they had no canopy cover, and harbored only unhatched eggs and small, dead, rotting tadpoles. On the other hand, tadpoles were able to survive in pools on the edge of the rivulet where sunlight was obscured by the canopy.

Of course, puddles in both areas receive direct sunlight in the middle of the day, but the total duration and intensity of sunlight exposure throughout the day would be lower in shaded puddles on the edge of the rivulet. These field observations confirm our experimental result that 33ºC exceeds the maximum survivable temperature of *R. uenoi*.

Although the temperature logger in the outdoor treatment recorded a maximum temperature of 33.8ºC on April 30, the actual temperature of the water was likely less than 33ºC because, as mentioned earlier, sunlight passing through the water would increase the temperature of the plastic bag holding the logger. In addition, the temperatures recorded one hour before and after the 33.8ºC maximum were less than 33ºC, indicating that the temperature only briefly went up and then dropped. However, our results still suggest that tadpoles spawned and hatched at the end of April, which is the peak spawning season for *R. uenoi*, would have difficulties surviving in May when puddle temperatures rise above 33ºC. Climate change will invariably increase the frequency of warmer puddles, which could greatly affect the survival of *R. uenoi*, so we conducted an additional temperature elevation experiment to further verify the maximum survivable temperature of tadpoles.

Temperature elevation experiment

Our original plan for the temperature elevation experiment was to determine the maximum temperature at which tadpoles could survive, with four tadpoles for each replicates and four replicates tested at each temperature. The design was altered following the recommendations of the Animal Care and Use Committee, which suggested that we minimize animal suffering by not using replicates and by determining the temperature at which tadpoles no longer eat rather than the temperature at which they die. Despite these precautions, tadpoles still died unexpectedly during the temperature elevation experiment. The target temperature of the first experiment was 30 ºC and aimed to increase 1 ºC by each sequel experiment, but the measured temperature was a little different, perhaps because the area (or level) we have measured temperature and thermal sensor of the heater might be slightly different. Tadpoles in water raised to 31.2ºC ate foods well, showed no sign of stress activity, and survived and grew well after the experiment (Table 5), suggesting that they were not affected much at 31.2ºC. However, all tadpoles raised to 32.4ºC suddenly died, leaving no time to monitor for any stressed behavior.

Based on this experiment and the measured water temperatures of the indoor beaker, the field puddles, and the outdoor water bowls where tadpoles died, the maximum survivable temperature for tadpoles is somewhere just above 32ºC. The fact that there were no surviving tadpoles in sun-exposed puddles located in the middle of the rivulet at our field site, whereas tadpoles could be found in canopy-shaded puddles at the edge of the rivulet, suggests that spring water temperatures have already reached the temperature limit (just above 32ºC) of *R. uenoi*. Related brown frog species have optimal temperatures around 23ºC, and a previous study has concluded that temperatures over 30ºC could have negative effects on frogs *Rana chensinensis* (Yu *et al*., 2015). Increasing climate instability could therefore affect the long-term survival of *R. uenoi*.

This study is not making conclusion that *R. uenoi* is endangered by climate change. However, certainly such case (death by extreme weather) has occurred in field and indoor experiment. The results of our study carefully suggest that *R. uenoi* tadpoles are vulnerable to elevated temperatures and are therefore could be threatened by climate change. Our sampling period (spring of 2020) was unusual in that record lows for April precipitation and mid-April temperatures on Jeju Island were set in the spring of 2020 (Lee, 2020) and April and early May temperatures were generally colder than average, making for a relatively cold spring (Fig. 1). This unusually cold weather, not only in April (the beginning of the experiment) but also in May (just before when the tadpoles died), may have delayed tadpole growth and development and could explain why we observed many tadpoles with no fore limbs. Temperatures then rose considerably after mid-May, increasing the water temperature of puddles in the rivulet bed. However, because the tadpoles were not developed enough (legs development) to move outside the water, some of the tadpoles in sun-exposed puddles died when the water became too hot, over 32ºC.

The case of *R. uenoi* is a clear example of how vulnerable frogs are to the extreme weather events caused by climate change, and as climate change continues, these extreme weather events will occur more often. In 2020, at least six new climatic records were set on Jeju Island, including hottest night (Ko, 2020), warmest January (Park, 2020), longest monsoon season (Lee, 2020), highest average temperature in the first half of the year (Han, 2020), lowest precipitation in April, and lowest temperature in mid-April (Lee, 2020). Within the past five years, Jeju Island has recorded its hottest summer (Jeon, 2017), strongest typhoon ever (KMA, 2018), lowest temperature (Guardian, 2016), and largest snowfall (Guardian, 2016). Climate change will continue to cause extreme weather events like this, and frogs, especially tadpoles with restricted movement, will be particularly vulnerable to these events. It is therefore necessary to identify how adaptable these species are to the weather predicted by future climate change scenarios. This information will be essential for the conservation of amphibians on isolated islands like Jeju Island.

Our target species, *R. uenoi*, is recently identified as a new species (Suk *et al*., 2021) and is a close sister species to *R. dybowskii*, which has a relatively wide range of habitats and is found in many countries (Feng *et al*., 1999). Although this paper is before identification (2014) and used '*R. dybowskii*', it is clearly about *R. uenoi* as most of the field site is Korea and China where *R. dybowskii* is now identified as *R. uenoi*. The southern (Jeju Island, Korea) and northern (Khabarovsky, Russia) limits of its range are separated by almost 15º in latitude (Feng *et al*., 1999) and have very different climates, indicating that *R. uenoi* has a broad habitat tolerance. Nevertheless, our study suggests that *R. uenoi* can be greatly affected by increasing temperatures. Other frog species with smaller habitat ranges are likely to be more susceptible to climate change than generalists like *R. uenoi*. For example, Jeju Island is habitat to frog species like *Pelophylax chosenicus, Hyla japonica* and *Bombina orientalis*, which have a narrower habitat range than *R. uenoi* and are also listed on the IUCN Red List (Shi *et al*., 2018; Yang & Koo, 2016). *Hyla japonica* and *Bombina orientalis* are also found in this rivulet (Baek *et al*., 2021). These specialist species may be more vulnerable to climate change than *R. uenoi*. Our work on a generalist species that is available for scientific research therefore suggests the potential threats climate change poses for specialist species.

Even for frog species that are not specialists, frog populations on Jeju Island are more vulnerable to climate change because they are unable to move north to cooler environments. Experiments to determine how adaptable each species is to rising temperatures are therefore especially important in isolated areas with high biodiversity, such as Jeju Island. Knowing the temperature limitations of each species will help determine the urgency of conservation activity and the appropriate conservation actions for each species. In particular, we found that susceptibility to elevated temperatures varies between stages of development, which should be an important reference for future research. In conclusion, *R. uenoi* may be vulnerable to climate change and will need to be continuously studied as climate change progresses. This study is not making conclusion that *R. uenoi* is endangered by climate change, but suggesting that there are possibilities. Our study shows the need for comprehensive research on how frogs respond to climate change and the need for detailed experimental designs that test each stage of growth with all native frog species found in each areas.

CONCLUSION

As climate change will continue to cause extreme weather events, frogs, especially tadpoles with restricted movement, will be more vulnerable to events like elevated temperature in spring. Therefore, in isolated areas with high biodiversity such as Jeju Island, it is important to identify how adaptable amphibian species are to the weather events predicted by future climate change scenarios .

ACKNOWLEDGEMENT

The experiment is approved by the Institutional Animal Care and Use Committee of Jeju National University (No. 2020-0032).

ABBREVIATIONS

UNESCO: United Nations Educational, Scientific and Cultural Organization, LC: Least Concern, IUCN: International Union for Conservation of Nature and Natural Resources, KMA: Korea Meteorological Administration.

REFERENCE

AmphibiaWeb. 2020. *AmphibiaWeb,* University of California, Berkeley, California, USA**.** http://amphibiaweb.org. Cited 31 Oct 2020.

Asquith, J (2020) Do You Know What The World's Busiest Flight Route Is With 180 Daily Flights? Forbes**.**https://www.forbes.com/sites/jamesasquith/2020/01/21/do-you-know-what-the-worlds-busiest-flight-route-is-with-180-daily-flights/#60857f2d702e. Cited 31 Otc 2020.

Baek, S.Y., M.H. Lee, Y.S. Kim, S.R. Bae, U.R. Song and C.K. Kang. 2021. Selective oviposition by Oriental fire-Bellied Toads in temporally fluctuating environments. Current Herpetology*,* 40**,** 120-128.

Banjade, M., Han, S.H., Jeong, Y.H., Jun-Won, L. and Oh, H.S. 2019. Long-term trends of bird community at Dongbaekdongsan and 1100-Highland Wetland of Jeju Island, South Korea. The Korean Society of Phycology 26**:** 54-61.

Cho, I.Y., Kang, D.W., Kang, J., Hwang, H., Won, J.H., Paek, W. K. and Seo, S.Y. 2014. A study on the biodiversity of benthic invertebrates in the waters of Seogwipo, Jeju Island, Korea. Journal of Asia-Pacific Biodiversit*,* 7**:** e11-e18.

Duffy, J. E. 2009. Why biodiversity is important to the functioning of real-world ecosystems. Frontiers in Ecology and the Environment 7**:** 437-444.

Feng, X., Changyuan, Y., Liang, F., Jianping, J., Xiaomao, Z. and MatsuI, M. 1999. Taxonomical studies on brown frogs (Rana) from Northeastean China (Amphibia: Ranidae). Dong wu fen lei xue bao= Acta Zootaxonomica Sinica 24**:** 224-231.

Gallardo, B., Bogan, A. E., Harun, S., Jainih, L., Lopez-Lima, M., Pizzarro, M., Rahim, K. A., Sousa, R., Virdis, S. G., and Zieritz, A. 2018. Current and future effects of global change on a hotspot's freshwater diversity. Science of the Total Environment, 635: 750-760.

Guardian, T. 2016. Jeju hits record for lowest temperature and snowfall. https://www.theguardian.com/world/2016/jan/25/south-korean-snow-strands-86000-on-holiday-island-of-jeju. Cited 28 Otc 2020.

Habibullah, M. S., Din, B. H., Tan, S.H.,and Zahid, H. 2022. Impact of climate change on biodiversity loss: Global evidence. Environmental Science and Pollution Research, 29(1): 1073-1086.

Harter, D. E., Irl, S. D., Seo, B., Steinbauer, M. J., Gillespie, R., Triantis, K. A., Fernãndez-Palacios, J.M. and Beierkuhnlein, C. 2015. Impacts of global climate change on the floras of oceanic islands–Projections, implications and current knowledge. Perspectives in Plant Ecology, Evolution and Systematics 17**:** 160-183.

Han, K. 2020. The temperature in the first half of the year was the highest on record. JEMINNEWS, Jeju, South korea. http://www.jemin.com/news/articleView.html?idxno=663776 (Presented in Korean). Cited 31 Otc 2020.

Jo, Y.S., Kim, T.W., Choi, B.J. and Oh, H.S. 2012. Current status of terrestrial mammals on Jeju Island. Journal of Species Research 1**:** 249-256.

Jung, S., Ha, S. and Na, H. 2013. Multi-decadal changes in fish communities Jeju Island in relation to climate change. Korean Journal of Fisheries and Aquatic Sciences 46**:** 186-194.

Kim, C.-S. 2009. Vascular plant diversity of Jeju island, Korea. Korean Journal of Plant Resource 22**:** 558-570.

Kim, D.S., Jang, Y. S. and Song, J. H. (2009). The effects of elevated temperatures on the population phenology and abundance of citrus pests in Jeju, Korea. International Symposium on Climate Change and Insect Pest 96-98.

KMA (Korea Meteorological Administration) 2018. Strongest typhoon to ever hit Jeju. https://www.kma.go.kr/download_01/Annual_Report_2018.pdf. Cited 28 Otc 2020.

Ko, J.T. 2020. Jeju sets record for hottest night. Korea Herald, Seoul, South Korea. http://www.koreaherald.com/view.php?ud=20200806000613. Cited 31 Otc 2020.

Lim, D.O. 2012. Plant diversity and conservation in Oruem of Jeju City. Korean Journal of Environment and Ecology 26**:** 635-653.

Lee, J.M. 2020. Jeju sets record for lowest precipitation of April and lowest temperature of mid-April. MEDIAJEJU, Jeju, South Korea. http://www.mediajeju.com/news/articleView.html?idxno=323178 (Presented in Korean). Cited 31 Otc 2020.

McLeod, E., M. Bruton-Adams, J. Forster, C. Franco, G. Gaines, B. Gorong, R. James, G. Posing-kulwaum, M. tara and E. Terk. 2019. Lessons from the Pacific Islands–adapting to climate change by supporting social and ecological resilience. Frontiers in Marine Science, 6**,** 289.

Nunez, S., Arets, E., Alkemade, R., Verwer, C. and LeemanS, R. 2019. Assessing the impacts of climate change on biodiversity: is below 2° C enough? Climatic Change 154: 351-365.

Priambodo, B., H. Permana, F. Akhsani, S. E. Indriwati, S. Wangkulangkul, S. R. Lesrati and Rohman F. (2019) Characteristic of water sources in malang, based on the diversity, community structure, and the role of herpetofauna as bioindicator. Eurasian Journal of BioSciences, 13**,** 2279-2283.

Sage, R. F. 2020. Global change biology: A primer. Global Change Biology, 26(1): 3-30.

Sala, O. E., Stuart Chapin , F., III, Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-sanwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D. M., Mooney, H. A., Oesterheld, M. N, Poff, N. L., Sykes, M. T., Walker, B. H., Walker, M. and Wall, D. H. 2000. Global Biodiversity Scenarios for the Year 2100. Science 287: 1770-1774.

Shi, Y., Yu, L., Han, X., Zhao, S., Niu, T. and XU, C. 2018. Development of 12 microsatellite markers for Bombina orientails based on RNA-Seq and their usefulness in population genetic diversity. Molecular biology reports 45: 2811-2814.

Song, G.P., Jang, C.G. and Kang, S.H. 2012. Conservation and vegetation structure of Euchresta japonica (Leguminosae) in Jeju Island. Korean Journal of Plant Resources 25: 89-95.

Xiao, X., Zheng, D., Yang, C. and Chai, L. 2008. Survival and metabolic responses to freezing temperature in the northeast forest frog Rana dybowskii. Asiatic Herpetol Research 11**:** 147-152.

Yang, D.S. and Koo, B. H. 2016. A Study on the Improvement Plan for a Habitat of'Gold-spotted Pond Frog (Pelophylax chosenicus)'in Danger of Regional Extinction in the Urban Area-Case on the Abandoned Railroad Site on Su-in Line. Journal of the Korea Society of Environmental Restoration Technology 19**:** 95-107.

Jeon, J.H. 2017. hottest summer of Jeju on record. Yonhap News Agency, Seoul, South Korea. https://www.yna.co.kr/view/AKR20170901123500056 (Presented in Korean). Cited 31 Otc 2020.

Park, B.R. 2020. Jeju sets record for warmest January. Yonhap News Agency, Seoul, South Korea. https://en.yna.co.kr/view/AEN20200204005900315. Cited 31 Otc 2020.

Vulnerability to climate change of islands worldwide and its impact on the tree of life. Scientific Reports*,* 9**,** 1-14.

Yu, T. L, Pang, R. H. and Chen, K. 2015. Plasticity in metamorphic traits of Chinese brown frog (*Rana chensinensis*) tadpoles: the interactive effects of food level and rearing temperature. Animal Biology 65**:** 233-240.