AN ABSTRACT OF THE THESIS OF

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Abstract approved : _____

Andrew Blaustein

Massive die-offs of *Bufo bufo gargarizans* were observed in May 2008 in Mangwal Gee, Korea. To find out the causes for this disaster several different elements were examined: pH, total nitrogen, dissolved oxygen (DO), and dissolved heavy metal, suspension, introduced species, habitat change, disease, temperature changes, and precipitation and humidity. After checking pH, total nitrogen, dissolved oxygen, and heavy metal, the quality of water appeared to be acceptable for toads. No introduced species were found which could kill or compete with toads. Even though there were environment changes surrounding Mangwal Gee, thanks to government efforts the condition of the pond has not changed for years. The pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* responsible for amphibian population declines worldwide was not found. The average temperature was little higher, and precipitation was lower compared to last 30 years, however there was no evidence found to indicate as a cause of mass die-off.

Key Words : Korean toads, mass die-off, different elements

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Toad Populations Decreasing in South Korea.

by

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Introduction

As a part of a ‘biodiversity crisis’, many amphibian populations are in decline throughout the world. There are several factors that may contribute to declining amphibian populations. These include habitat destruction, pathogens, increasing ultraviolet radiation, introduced non-native species, global environmental change, diseases, and contaminants. There does not seem to be a single reason for these declines. (Blaustein and Kiesecker, 2002).

In March 2007, millions of juvenile *Bufo bufo gargarizans* died at Daegu, South Korea. These toads were migrating to the mountains and were just beginning to breathe through their lungs after completing metamorphosis. A year later in May 2008, another mass die-off was observed. This time only about a thousand toads were migrating and those who did not migrated died in the pond. The causes of mass die-off of toads in Daegu, remain a mystery. For this reason, the focus of this project was to investigate the potential causes for mortality of *Bufo bufo gargarizans* in Daegu.

Factors contributing to the die-offs may affect each species differently. As mentioned before, there are several possibilities affecting toad populations and that could cause mortality. To consider the possibilities, several types of data need to be collected. The nitrogen, oxygen level, and pH of the pond are critical to toads and can affect toad respiration. Contamination caused by pesticides, heavy metals, may affect toads. Since amphibians have moist, permeable skin and unshelled eggs, they are directly exposed to soil, water, and sunlight (Blaustein et al., 2003). For this reason, contamination of water can cause toad mortality. Introduced new species to the pond, habitat changes, and diseases caused by viruses, bacteria, parasites, protozoans, oomycetes, and fungi may also play a role in causing toad mortality

Thesis Statement

Amphibian populations are declining throughout the world, and according to several researchers there are several possible factors causing this phenomenon. The declining populations of toads in South Korea and the specific mortality event at Daegu may be explained through one or more of several factors.

Methodology

There could be several different factors that can affect the mass die-off of toads. It could be low quality of water, contaminants in the water, introduced species, habitat changes, temperature changes or diseases caused by pathogens.

To check the quality of water, total amount of nitrogen, dissolved oxygen, pH of the pond should be analyzed. To test whether the pond was contaminated, data about the amount of heavy metal, suspension of particulates in the pond was needed. Introduced species had to be examined and toad skin had to be examined to see whether they had disease caused by pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*. For all of these comparisons, I used data collected by the Daegu Ministry of Environment.

Temperature is another important factor needing to be compared. I used the temperature data collected by the Korean Meteorological Administration during last 30 years.

Based on the data gathered, I attempted to provide several possibilities that could cause the mass die-off of Korean toads.

Results

1) Species

The most abundant species which was found in my focal pond, Mangwal Gee in Daegu is *Bufo bufo gargarizans.*

The length of *Bufo* is from 56 to 102 mm. It has similar characteristics of common toads (*Bufo bufo*), but has spines on the dorsal skin tubercles and a black band which extends from the outer surface of the parotoid gland. Dorsal skin tubercles are large and tympanic membranes are either very small or covered with skin. There are several different dorsal colors: dark-gray, olive-gray, or olive-brown, with three wide longitudinal bands. (Sergius L., 2009)

A wide dark band extends from the inner surface of the parotoid onto the body flank. This dark band is interrupted posteriorly into large spots. They have a gray or yellow belly. Females are larger than males, and females have longer hindlegs and wider heads than males. (Sergius L., 2009)

Bufo gargarizans lives in China, Korea, Japan, and in the Russian Far East. The population density could reach 3600 individuals per square kilometer, but this depends on the habitat varies by years. (Sergius L., 2009)

Reproduction occurs in lakes, ponds, swamps, puddles, old riverbeds, ditches and rivulets with stagnant or slow-flowing water. Males enter breeding areas before females. Females lay two strings of eggs, 1.5-2.3m in length, and containing 1200-7400 eggs. Usually metamorphosis occurs in summer. Mass

mortality of *B. gargarizans* larvae occurs in pools with high tadpole density of the brown frog *Rana dybowskii*. (Sergius L., 2009)

Sexual maturity of *B. gargarizans* occurs during the 3rd-4th year of life, so the majority of adults are 5-6 years old. Adult *B. gargarizans* consume insects, and other invertebrates including Mollusca, Myriapoda and Arachnoidea. (Sergius L., 2009)

Most of the time, hibernation occurs from September - October to April – May on land and also occurs in rivers and lakes at depths of more than one meter. (Sergius L., 2009)

2) Examine the Water

Table 1. Water quality standard

Level		Standard				
		pH	Biochemical oxygen demand (BOD) (㎎/L)	Suspension (㎎/L)	Dissolved Oxygen (DO) (㎎/L)	Colon *Bacillus* (numbers/100mL)
Excellent	Ia	6.5 ~ 8.5	Less than 1	Less than 25	Over 7.5	Less than 50
Very Good	Ib	6.5 ~ 8.5	Less than 2	Less than 25	Over 5.0	Less than 500
Good	II	6.5 ~ 8.5	Less than 3	Less than 25	Over 5.0	Less than 1,000
Okay	III	6.5 ~ 8.5	Less than 5	Less than 25	Over 5.0	Less than 5,000
Slightly bad	IV	6.0 ~ 8.5	Less than 8	Less than 100	Over 2.0	-
Bad	V	6.0 ~ 8.5	Less than 10		Over 2.0	-
Very Bad	VI	-	Over 10	-	Less than 2.0	-

(Ministry of Environment)

Seven samples of water quality were evaluated through March, 2008 to June, 2008, from three different locations: the water entrance of the pond where the water is introduced, the center of the pond, and the opposite side of the entrance.

Table 2. Water quality of the pond in three different locations

2.1) pH

	2008 3.20	2008 4.2	2008 4.16	2008 4.30	2008 5.14	2008 5.27	2008 6.2
Entrance	7.6	7.7	7.1	7.4	6.8	8.6	9.8
Middle	7.6	7.7	7.1	7.4	6.8	8.6	9.8
Opposite	7.6	7.6	7.2	7.3	7.1	9.2	9.9

(Daegu Ministry of Environment)

The pH differed from month to month, but from March, 2008 to May, 2008 it was about pH 7.3 and after that it was between 8 and 9. The pH did not show much difference with location, since the entrance, the middle of the pond, and the opposite side of the entrance had similar pH.

2.2) DO (mg/L)

	2008 3.20	2008 4.2	2008 4.16	2008 4.30	2008 5.14	2008 5.27	2008 6.2
Entrance	11.3	10.6	9.6	10.1	8.1	11.1	13.3
Middle	10.9	9.5	9.6	10.5	9.2	13.1	13.9
Opposite	10.4	9.6	10.1	10.2	9.1	11.3	13.6

(Daegu Ministry of Environment)

According to the Daegu Ministry of Environment, good quality water has to contain over 5.0 mg/L of dissolved oxygen(DO). Gathered data showed around 10mg/L which means this pond had sufficient dissolved oxygen. Like the pH of the pond, dissolved oxygen (DO) levels did not change a lot with the location of the pond. On March and April of 2008, DOs were around ten at all three locations: entrance of the pond, middle of the pond, and the opposite side of the pond entrance. It was lower number in April (9.6). DO was higher in May and June. DO went up to 13.9mg/L on June.

2.3) Total Nitrogen (mg/L)

	2008 3.20	2008 4.2	2008 4.16	2008 4.30	2008 5.14	2008 5.27	2008 6.2
Entrance	1.690	0.874	1.210	0.974	1.094	0.058	0.058
Middle	0.826	0.806	1.186	0.965	0.888	0.730	0.595
Opposite	0.662	0.730	1.248	0.926	0.864	0.754	0.648

(Daegu Ministry of Environment)

Total amount of nitrogen was also measured and most of the time it showed less than 1mg/L. However, on March, 2008 at the entrance of the pond showed 1.69mg/L, which means much higher than other areas and other time periods. Other than that, the amount of nitrogen was really similar no matter where it was measured.

3) Contamination

Table 3. Contamination of the pond

3.1) Suspension (mg/L)

	2008 3.20	2008 4.2	2008 4.16	2008 4.30	2008 5.14	2008 5.27	2008 6.2
Entrance	5.9	5.9	3.6	9.7	4.6	11.3	19.4
Middle	4.4	2.9	2.3	8.4	2.2	5	14.6
Opposite	4.1	3	2	7.9	9.1	11.3	13.6

(Daegu Ministry of Environment)

Suspension of particulate matter in the pond is shown in the Table 3. Most of the time it was below 5 mg/L, but on April 30th it increased to 9mg/L. The level of suspension was lower at the beginning of May in all three different locations of the ponds. However, it increased again at the end of May. The entrance of the pond showed 11.3 mg/L which was almost double compared to the beginning of March. On June second, the amount of suspension increased to 15mg/L at the middle and the opposite side of the entrance of the pond.

3.2) Chlorophyl-a (Chl-a) (mg/ m^3*)*

	2008 3.20	2008 4.2	2008 4.16	2008 4.30	2008 5.14	2008 5.27	2008 6.2
Entrance	13.8	17.3	13.4	16	14	33	39
Middle	15	11	17	16	9	29	44
Opposite	10	10	17	17	9	26	44

(Daegu Ministry of Environment)

The amount of chlorophyll-a is shown in the Table 3. From March to May 14th, it was less than 20mg/m^3. However, it increased after at the end May 27th, when it

was 33mg/m^3 at the entrance of the pond, and 39 mg/m^3 on Jun 2nd. The amount of chlorophyll-a increased rapidly between at the end of the May and at the beginning of June.

3.3) Colon Bacilus (mg/m^3*)*

	2008 3.20	2008 4.2	2008 4.16	2008 4.30	2008 5.14	2008 5.27	2008 6.2
Entrance	5000000	5000	160000	900	330	900	170
Middle	500000	900	900	23	170	130	50
Opposite	80000	500	3000	50	110	50	80

(Daegu Ministry of Environment)

The number of colon *Bacillus* was also checked to examine the quality of water. It showed high number on March at all three locations. All three locations showed over 80000 mg/m^3. From the end of the April the number was reduced to 1000 mg/m^3. It was 900 mg/m^3 at the entrance of the pond, and 23 mg/m^3 at the center of the pond. The number decreased temporally and finally in June, it showed 170 mg/m^3 at the entrance of the pond, 50 mg/m^3 at the middle of the pond, and 80 mg/m^3 at the opposite side of the entrance.

Heavy metals are other types of contamination sources. Thus, heavy metals were checked on June 2nd, 2008.

Table 4. Heavy Metals and organics in the Water

	Unit	Standard	Entrance of the pond	Middle of the pond	Opposite side of the pond
Cadmium (Cd)	mg/L	Less than 0.005	0.000	0.000	0.000
Arsenic (As)	mg/L	Less than 0.05	0.000	0.005	0.008
Cyanogen (CN)	mg/L	0	0.00	0.00	0.00
Mercury (Hg)	mg/L	0	0.0000	0.0000	0.0000
Polychlorinated biphenyl (PCB)	mg/L	0	0.0000	0.0000	0.0000
Lead (Pb)	mg/L	Less than 0.05	0.00	0.00	0.00
Cr^{6+}	mg/L	Less than 0.05	0.00	0.00	0.00
CCl_4	mg/L	Less than 0.004	0.000	0.000	0.000
1,2-CH_2Cl_2	mg/L	Less than 0.03	0.000	0.000	0.000
Tetrachloroethylene (PCE)	mg/L	Less than 0.04	0.000	0.000	0.000
Dichloromethane	mg/L	Less than 0.02	0.000	0.000	0.000
chloroform	mg/L	Less than 0.08	0.000	0.000	0.000

(Daegu Ministry of Environment)

Based on the data collected by the Daegu Ministry of Environment, the pond had 0.005 mg/L of arsenic at the middle and at the opposite side of the pond. However, less than 0.05 mg/L of arsenic is an acceptable quantity for animals. Other than arsenic, no other heavy metals were found.

4) Introduced species

Table 5. Found species in the Pond

		Number of individuals found
Amphibians	*Bufo bufo gargarizans*	More than 30
	Rana nigromaculata	Less than 5
	Rana rugosa	Less than 10
Reptiles	*Trachemys scripta elegans*	2
	Rhabdophis tigrinus tigrinus	1
	Elaphe dione	1
Others	*Mollusca (Gastropoda)*	4
	Annelida (Hirudinea)	2
	Arthropoda (Crustaceas)	1
	Arthropoda (Insecta)	13

(Daegu Ministry of Environment)

Data shown in Table 5 was gathered by the Daegu ministry of Environment and it was collected after examining three different places near where the water sample was collected. There are not many different types of species in the pond.

Bufo bufo gargarizans was the only toad found in the pond. Two different types of frogs were found: *Rana rugosa* and *Rana nigromaculata*. Two different types of snake (*Elaphe dione and Rhabdophis tigrinus tigrinus)* were found near the pond, and several insect species were found in the pond.

5) Habitat Change

This pond has existed for at least 50 years. During this period the surroundings were developed with schools, restaurants and a temple built near the pond. While developing the area the city tried not to ruin the pond.

Since some surrounding restaurants were throwing out water without purification, the government introduced water purification construction on April 2008. To purify water, the environmental organization called ‘greendaegu’ placed water hyacinth (*Eichhomia crassipes*) into the pond, on April 18th, 2008, and May 22nd, 2008. The city government also put some water into the pond on April, 2008 since they thought the pond did not have enough water. The water was from another pond located above my focal pond, Mangwal Gee. Before taking the water from the other pond, the quality of water was checked and no huge differences in water quality was found.

To help the migration of toads the government built a path to make toads easier to move up to the mountain and to reduce the mortality rate of toads caused by cars, or people while migration.

6) Disease caused by pathogenic fungus

Two different facilities the Korean Research Institute of Bioscience and Biotechnology, and the Seoul National University research team tested for *Batrachochytrium dendrobatidis* on May 30th, and July 8th of 2008 by using polymerase chain reaction (PCR). The data they gathered are not accessible. However, both facilities reported that there was not any pathogenic chytrid fungus: *Batrachochytrium dendrobatidis.*

7) Temperature

Table 6. Temperature

6.1) Annual Temperature at Daegue on 2008

Month	1	2	3	4	5	6	7	8	9	10	11	12
Average(℃)	1.5	1.5	9.1	14.8	19.7	21.1	28.4	25.8	23.0	17.8	9.5	3.3
Mean Maximum (℃)	5.6	7.0	14.8	21.0	25.7	25.8	33.1	30.7	28.0	23.6	14.7	8.7
Mean Minimum(℃)	-2.0	-3.2	3.9	9.3	14.2	17.2	24.8	22.2	18.9	12.6	4.9	-1.2
Highest(℃)	11.2	14.6	19.5	28.9	33.6	33.4	36.2	35.2	33.1	28.8	22.9	15.9
Lowest(℃)	-8.2	-8.5	-2.0	3.4	8.0	13.2	19.8	17.5	9.6	7.3	-3.8	-8.2

(Korean Meteorological Administration)

6.2) Average monthly temperature at Daegue between 1971 and 2000

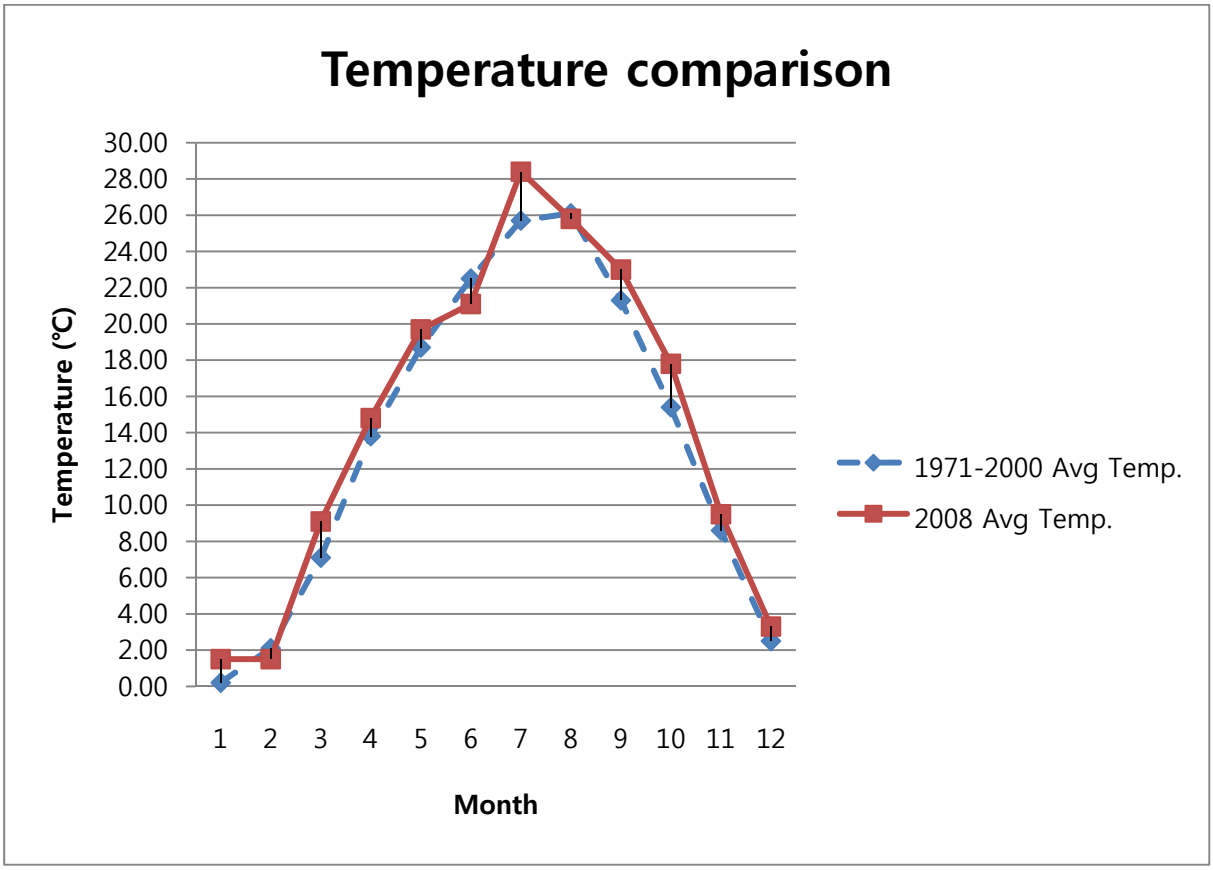
Month	1	2	3	4	5	6	7	8	9	10	11	12
Average Temp. (℃)	0.2	2.1	7.1	13.8	18.7	22.5	25.7	26.1	21.3	15.4	8.6	2.5
Highest Temp. (℃)	5.3	7.5	12.9	20.1	25.0	28.0	30.3	30.9	26.6	21.7	14.4	8.0
Lowest Temp.(℃)	-4.1	-2.4	2.1	7.9	12.8	17.8	22.1	22.4	16.9	10.1	3.7	-2.0

(Korean Meteorological Administration)

6.3) Temperature comparison between 1971-2000 and 2008

Month	1	2	3	4	5	6	7	8	9	10	11	12
2008 Average(℃)	1.5	1.5	9.1	14.8	19.7	21.1	28.4	25.8	23	17.8	9.5	3.3
1971-2000 Average(℃)	0.2	2.1	7.1	13.8	18.7	22.5	25.7	26.1	21.3	15.4	8.6	2.5

(Korean Meteorological Administration)



Graph 1. Temperature comparison between 2008 and 1971-2000

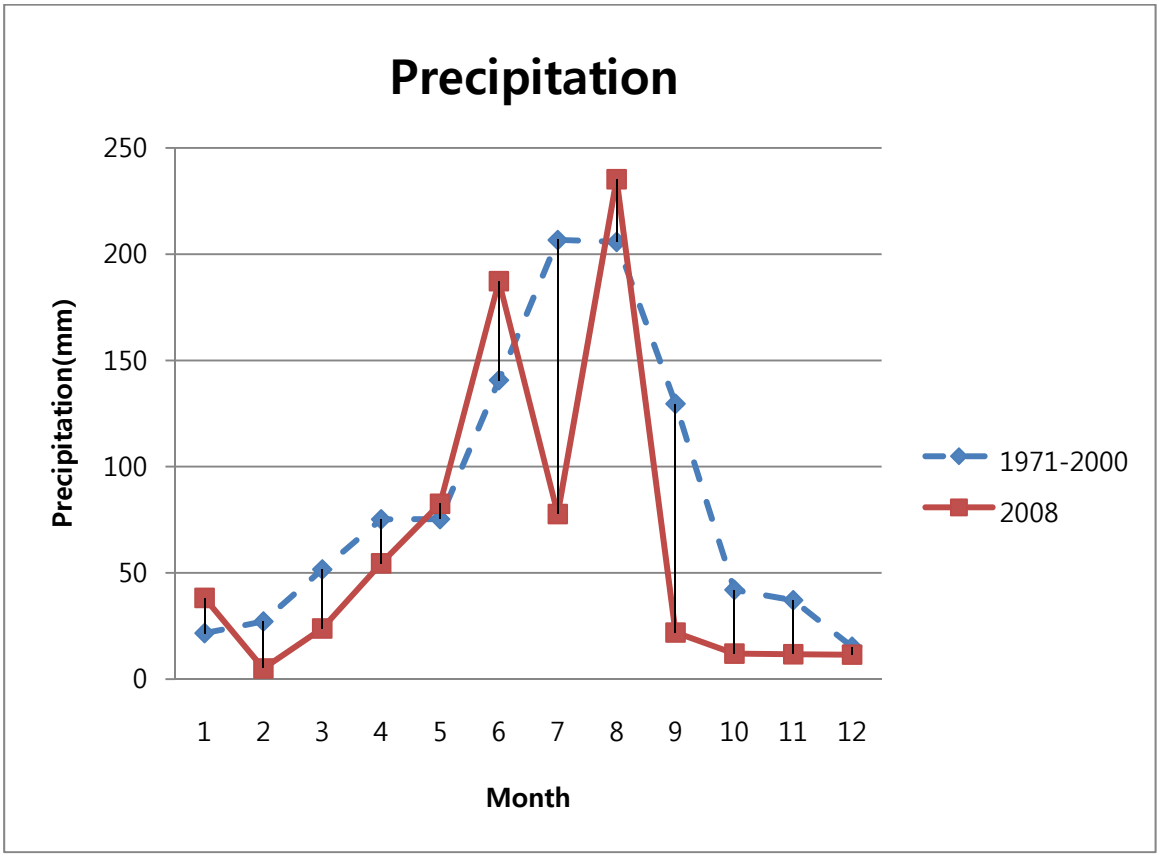
Temperature data were collected by the Korean Meteorological Administration. Even though the time of interest is 2008, to check whether the range of temperatures were normal, data were gathered from past years. The monthly average temperatures of 2008 were about 1℃ higher from March to June and November to December compared to past 30 year's average, and about 2℃ higher between July and October. On the other hand, during January, February and June the data collected on 2008 were 1℃ lower than the past 30 year's average.

8) Humidity and precipitation

Table 7. Annual precipitation at Daegue

Precipitation (mm)	1	2	3	4	5	6	7	8	9	10	11	12
1971-2000	21.6	27.1	51.6	75.2	75.3	140.7	206.7	205.8	129.6	42	37.1	15.2
2008	38.2	5.0	23.8	54.5	82.5	187.3	77.7	235.3	21.9	12.0	11.7	11.5

(Korean Meteorological Administration)

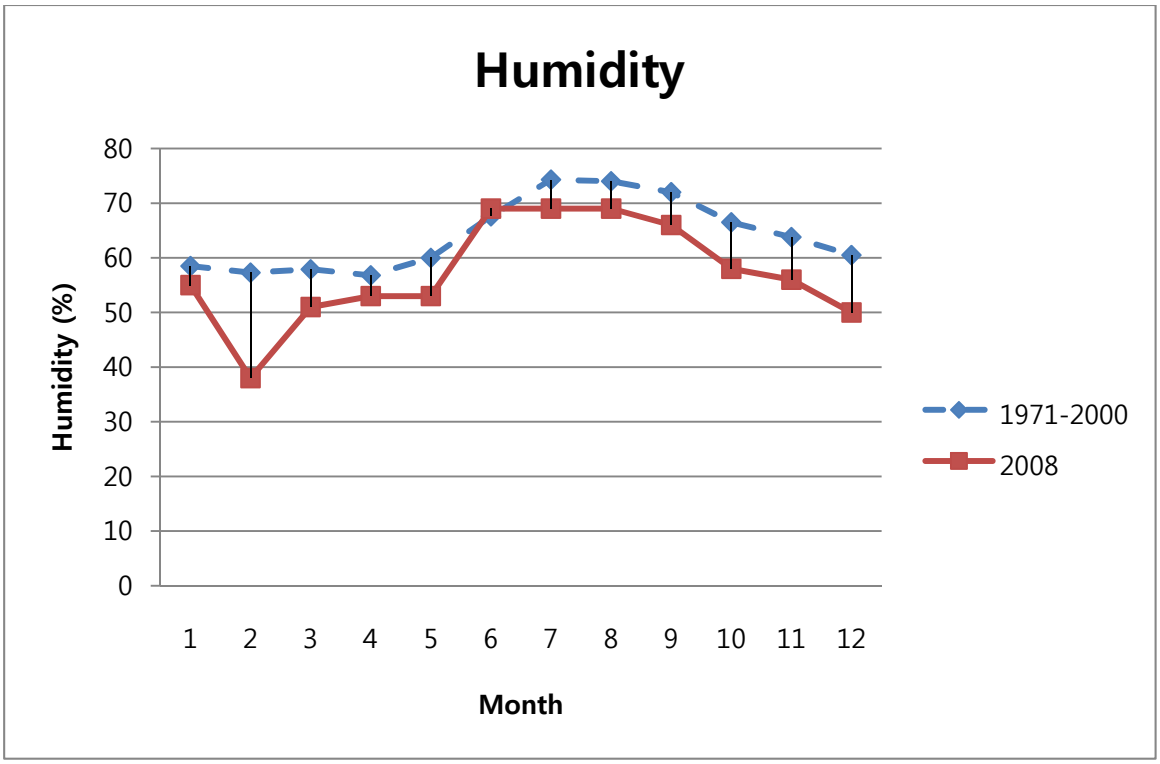


Graph 2. Precipitation comparison

Table 8. Annual Humidity at Daegue

Humidity (%)	1	2	3	4	5	6	7	8	9	10	11	12
1971-2000	58.5	57.3	57.9	56.8	60	67.6	74.3	74	72	66.5	63.8	60.5
2008	55	38	51	53	53	69	69	69	66	58	56	50

(Korean Meteorological Administration)



Graph 3. Humidity comparison

February to April on 2008 had less rain than before, and humidity was also lower than in the past. Especially the average humidity of February during last 30 years was 57.3% but on 2008 it was only about 38%. In July, there were average 206.7 mm rain falls during last 30 years, but on 2008 it was only about 77.7 mm. Overall, 2008 was drier compared to last 30 years.

Discussion

1) Examine the Water

To check the quality of water, total amount of nitrogen, pH, dissolved oxygen (DO) and other characteristics were measured.

More than 1 mg/L of nitrogen in the water could be harmful for amphibians. Lethal and sub-lethal effects were shown from 3 mg/L of nitrogen in the water (Jeremy et al., 1999). According to the data, the average amount of nitrogen was 0.847 mg/L. Thus, nitrogen amount is not the reason for mass die-off.

There have been many studies related to low pH and toad populations, and low pH results in high death rates or developmental abnormalities in amphibians (Freda, 1986). Since the average pH in the pond was 7.9, low pH cannot be the reason of mass die-off of toads. Leslie et al (1995) examined the effect of pH/ UV-B in amphibians. There is no evidence to indicate pH caused mass die-off.

Even though amount of nitrogen and pH of the water were not in the optimal range, there were sufficient amount of dissolved oxygen (DO). According to the Ministry of Environment, high quality water has over 5mg/L of oxygen. Since, the pond had DO of 10mg/L on average, it is difficult to suggest that insufficient of oxygen is the reason of mass die-off.

2) Contamination

In this research I treated suspension, amount of chlorophyll-a in the water, and dissolved heavy metal as contaminants.

Suspensions are particles that cannot be dissolved in water and often float in or above the water. According to the Ministry of Environment having less than 25mg/L of suspension is acceptable. The maximum suspension was 19.4mg/L on 2nd June. Thus, suspension cannot be the reason causing mass die-off of toads.

The acceptable amount of colon *Bacillus* is less than 5000/100ml. Average data showed 270000/100ml of Colon *Bacillus* in the pond. The high average was from March to middle of the April. During this time period, water contained abnormal amount of colon *Bacillus*. However, it declined to 170/100ml at the beginning of the June. Traditionally, lots of Korean farmers add manure as a fertilizer frequently at the beginning of March to middle of the June. Since there is a small farm above the Mangwal Gee, fertilizer run off was the reason why the pond contained high amount of Colon *Bacillus*. The city did water purification construction in April and poured water into the pond in April. So this might be why the amount of Colon *Bacillus* decreased dramatically from the middle of the April. If Colon *Bacillus* is coming from fertilizer, this might be not the cause of mass die-off since this technique was used for more than 100 years.

There is a study showing that nitrate and nitrite from fertilizers causes high mortality rates within 15 days of applying, even if sensitivity is varies among species (Aldolfo et al., 1999). Only the concentration of nitrogen was gathered from the pond of interest, thus it is hard to say nitrate or nitrite was problematic

resources from fertilizer. However, since this fertilizer was from human sources rather than chemically produced, I will say nitrate and nitrite are not main causes of mass die-off.

Arsenic was the only heavy metal found in the pond. However, the amount measured was not over 0.05mg/L which thought to be a lethal amount based on Korean Ministry of Environment. For this reason, contamination caused by heavy metals is not likely to be a cause of toad mortality in the pond.

There was huge increase of chlorophyll-a between at the end of the May and beginning of the June. Before this time, it measured about 17mg/m^3 but it increased to 35mg/m^3 on average, while the highest was 44.2mg/m^3. Increased chlorophyll-a was due to increased green algae: *Microcystis aeruginosa*. Previous studies showed that microcystis had no effect on neuromuscular systems. However, it caused liver damage in mammals, and it is only toxic in endothermic vertebrates (John and Ikawa, 1980).

Further research was not done with *Microcystis aeruginosa* and *Bufo bufo gargarizans.* Thus, it is not clear that previous research could apply to *Bufo bufo gargarizans* and say it was affected by green algae and this is one of the reason caused mass die-off.

3) Introduced species

According to Sergius (2007), mass mortality of *Bufo bufo gargarizans* larvae occurs in pools with high tadpole density of the brown frog *Rana dybowskii*. *Rana dybowskii* was not found in the pond, but *Rana nigromaculata, and Rana rugosa* were found in the pond. Feeding behavior of *Rana nigromaculata* is not known, but *Rana rugosa* eats variety of insects, and consuming arachnids, crustaceans, chilopods, mollusks, and small frogs are possible (Okada; Maeda and Matsui; Hirai and Matsui, 2000,2001). I do not know if the sympatric frog species are eating or competing with toads in the focal pond.

Rhabdophis tigrinus tigrinus eats mice, frogs, and toads (Species Restoration Center, 2005). The other snake, *Elaphe dione*, also eats shrews, mice, moles, birds and their eggs, frogs, lizards, snakes and toads (Gidi & Wim Van De Belt, 2005).However, toads were dead in or near the water, not eaten by other animals. Thus, existing *Rhabdophis tigrinus tigrinus, and Elaphe dione* cannot be the cause of mass die-off in Daegu.

4) Habitat Change

The Daegu government put lots of effort to improve the environment around the pond and inside the pond. To prevent pollution the government did water purification construction, and put some water hyacinth (*Eichhomia crassipes*) into the pond. Most of these operations are done in April but according to water data there was no huge improvement in water quality. The only difference was the number of Colon *Bacillus* in the pond. Until the end of the April, number of Colon *Bacillus* was 1291475/100ml at the entrance of the pond, 125455.8/100ml at the middle of the pond, and 20887.5/100ml at the opposite side of the pond on average. After all the purification efforts, the number of Colon *Bacillus* decreased to 466.67/100ml, 116.67/100ml, and 80/100ml.

There were not any noticeable habitat changes that could cause the mass die-off of toads.

5) Disease caused by pathogenic fungus

Even though the data were not included in this paper, PCR was done to check whether the increased death rate was due to pathogenic chytrid fungus (Sample size : 10). Since, *Batrachochytrium dendrobatidis* is a well known pathogen that kills amphibians (Blaustein et al., 2006), two research facilities examined toad's skin for *Batrachochytrium dendrobatidis* and none was found.

However, there are several other pathogens that could cause diseases. For example, 96% of mortality in Wyoming toad were caused by microscopic fungus: *Basidiobolus ranarum* (Taylor et al., 1999). Another study about population declines of Yosemite Toads in the Eastern Sierra found that *Aeromonas hydrophila* and *enterobacter* species could cause diseases on toads (Kagarise Sherman, 1993).

It is unknown if other diseases other than *Batrachochytrium* are contributing to the massive die-offs at Daegu.

6) Temperature and Precipitation

The average temperature between March and May in 2008 was about 1℃ higher than in the past. Desiccation caused by increased temperature could be one of the reasons for mortality. Timing of emigration is related to temperature (Sung et al., 2007). At the low temperature the physical activity of toads reduced and delaying migration time (Sung et al., 2007). On 2008, from March to May the average temperature was about 1℃ higher than the past 30 years. This caused the earlier migration time, which was middle of the May.

During May the average precipitation was 82.5mm which was higher than the past: 75.3mm. However, the humidity decreased from 60% to 53%. These data indicates that it rained during specific time and the rest of days were without precipitation. However, desiccation is not the case of Asian toads *Bufo gargarizans.* The reason is that Asian toads live in several different places during the non-breeding period and thus this toad increased tolerance to dry situations compared with anuran species (Hillman 1980, Burggren and Vitals 2005). For this reason, desiccation cannot be the reason of mass die-off of toads.

Subject for Continued Study

I could not find ultraviolet radiation (UV) data for the last 30 years and 2008. For this reason, it was impossible to investigate the UV effect on toads. Since, toads are sensitive to ultraviolet radiation (UV) (Blaustein et al., 2003), this should be checked to see whether it is one of the reason of mass die-off.

As I mentioned above only one type of pathogenic disease was checked, even though there are several other possibilities. Since no significant evidence was found other possible infections have to be considered.

After comparing different possibilities, the differences caused by all other elements except increased chlorophyll-a and introduced species were explained why that could not be one of the reason causing mass die-off. Thus, increased chlorophyll-a and introduced species have higher possibilities that resulted in mass die-off. For this reason, further study related to *Microcystis aeruginosa* and *Bufo bufo gargarizans* has to be done. Looking for other species living in the pond, and the feeding behavior of those species has to be studied.

However again, probably is not one reason causing mass die-offs. Temperature, precipitation, and arsenic cannot be ruled out.

Acknowledgment

I first heard about the mass die-off of toads in South Korea in March, 2008. Decreasing toads occurred during last two years, and last year (2009) there was no toad migration. Since there was serious drought and continued abnormal high temperature during March and April the situation got worse. I wanted to find out the cause of this disaster by doing this research and prevent further seriousness. However, it was hard to find one specific cause of the mass die-off Korean toad in Daegu.

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