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Prevalence of malformed frogs in Kaoping and Tungkang river basins of southern Taiwan

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Abstract: *In this study, we found many amphibians with bizarre appearances, known as malformations in Pingtung County, southern Taiwan. For this investigation we collected frogs inhabiting the Kaoping and Tungkang river watersheds between February 2006 and June 2007. Among the total number of 10,909 normal frogs (i.e., anurans) collected during the investigation period, the Indian rice frogs (Rana limnocharis) account for the greatest number; next is the Chinese bullfrog (Rana rugulosa). Of all the 244 captured malformed frogs, the Indian rice frog account for the greatest proportion. These malformed frogs have their main distribution in upstream areas of these two rivers. Our result indicates that the appearance rate of malformed frogs is 1.8% in the upstream reaches of the Kaoping River and 2.6%, and 0.8%, respectively in the upstream and midstream reaches of the Tungkang river. The most-commonly-found malformation is the lack of palms, followed by the lack of appendages, exostosis, and a malformed appendicular. It is, therefore, reasonable to speculate that the causes for the malformation may be related to the increased organic pollutants and agricultural chemicals used in the upstream reaches of these two rivers.*

Key words: *Anurans, Malformed frog, Southern Taiwan, Indian rice frog (Rana limnocharis)*
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Introduction

Anurans reproduce and lay eggs mostly in water, and the tadpoles grow up in the water and become amphibious in their adulthood (McDiarmid and Altig, 1999). Their complicated life cycle and extreme sensitivity to environmental changes make amphibians a good index for environment monitoring (Venturino *et al.*, 2003). In the past two decades, frog populations have rapidly declined worldwide due to global climate changes, increased pollutants, and decreases and degradation of habitats (Rodriguez-Prieto and Fernandez-Juricic, 2005). In addition to rapid declines in frog populations, malformed frogs are also an important issue. Mass population decreases and strange phenomena like malformations indicate that potential crises exist in our environment (Loman and Andersson, 2007).

Currently, there are three hypotheses proposed about frog malformation: ultraviolet radiation, water pollution and parasites. The ozonosphere is becoming thinner, allowing stronger ultraviolet radiation to reach the surface of the earth; thus, the radiant energy destroys genes that control growth and results in frog malformation (Ankley *et al.*, 2002; 2004; Bruner *et al.*, 2002). Moreover, the various pollutants, such as pesticides-methoxychlor, xenoestrogen-diethylene glycol, and heavy metal- copper etc. in water may cause the formation of malformed frogs. (Fort *et al.*, 2004a; Fort and Paul, 2002; Fort *et al.*, 2004b). In addition to the influence of increased ultraviolet radiation and water pollution, frog malformation may also result from parasitic (*Ribeiroia ondatrae*) infection (Johnson *et al.*, 2001). However, none of the above three hypotheses can be conclusively verified with existing evidence (Burkhart *et al.*, 2000; Cohen Jr, 2001). Reports have it that many malformed frogs have been discovered in the US and Europe in recent years (Geer and Krest, 2000; Meteyer, 2000; Vandenlangenberg *et al.*, 2003). So far, no reports have documented the appearance of malformed frogs in Taiwan. In this study, we have collected anurans in the drainage basins of the Kaoping and Tungkang rivers, located in Pingtung County, southern Taiwan, to analyze the possible causes, to explore the present existence and prevalence of malformed frogs.

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Materials and Methods

This study mainly investigated the quantity and distribution of malformed anurans in the drainage basins of the Kaoping and Tungkang rivers, Taiwan. Each river was divided into upstream (Kaoping upstream, KPU and Tungkang upstream, TU), midstream (Kaoping midstream, KPM and Tungkang midstream, TM), and downstream (Kaoping downstream, KPD and Tungkang downstream, TD) sites (Fig. 1).

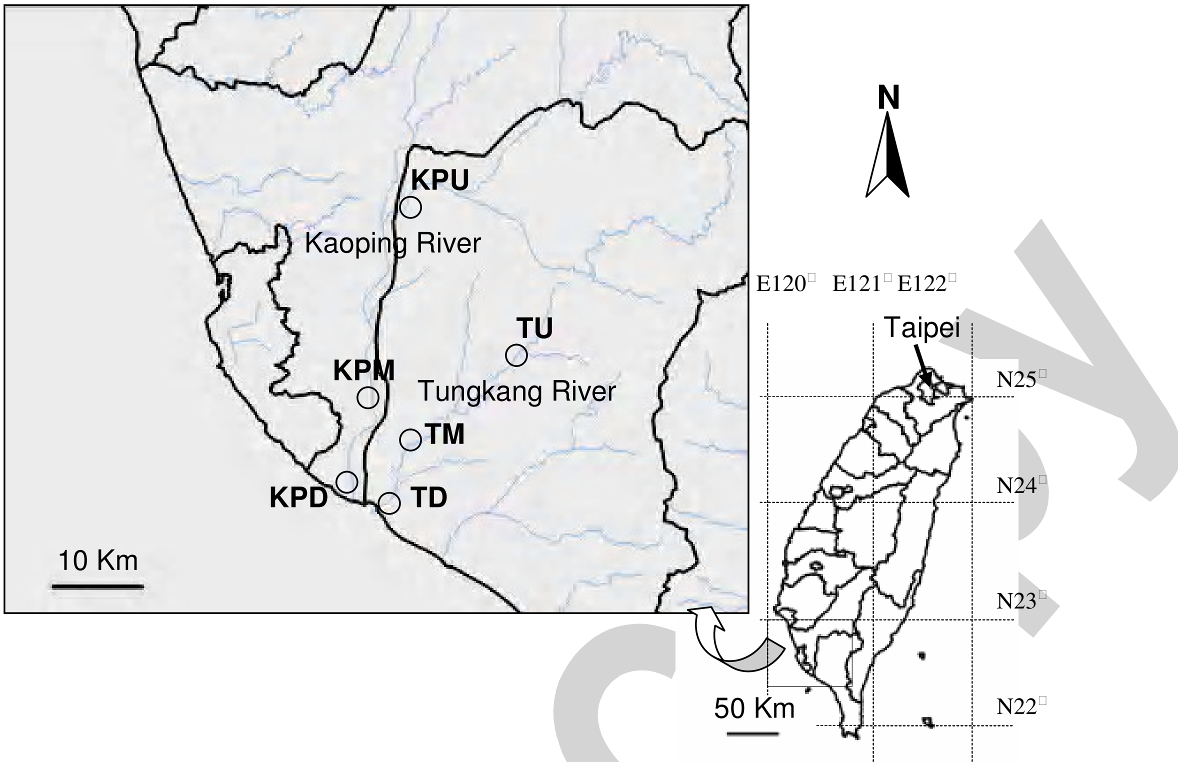

Fig. 1: Location of study sites in the Kaoping and Tungkang river basins. Kaoping upstream (KPU), Kaoping midstream (KPM) and Kaoping downstream (KPD); Tungkang upstream (TU), Tungkang midstream (TM) and Tungkang downstream (TD)

A visual encounter method was used for amphibian collection (Lin and Lue, 2004). On each sampling day, 2 hr of sampling was conducted from 19:00 to 21:00 using a strong light and collecting net around anuran habitats, such as rice fields, drainage areas, and banana farms in the study area. The amphibian densities were calculated as numbers per 100 m^2.

Amphibians were collected from study sites and identified to species level (Yang, 1998). The sex was determined and recorded, and the snout-vent length (SVL) was also measured. In addition, any abnormal morphology was identified in the frogs. Abnormal frogs were brought back to the laboratory for further examination (Meteyer, 2000).

SPSS vers. 12.0 (SPSS, 2003) was used for the statistical analysis. The snout-vent lengths between normal and abnormal frogs were compared, using Student's *t*-test (paired assay, $p<0.05$). Comparisons of malformation frequencies between males and females were examined by Chi-squared analysis in this study.

Results and Discussion

In this study, 10,909 frogs were collected from six study sites of KPU, KPM, KPD, TU, TM, and TD during February 2006 to June 2007. The Indian rice frog (*Rana limnocharis*) was the most dominant (accounting for a capture rate of over 95%), followed by the Chinese bullfrog (*R. rugulosa*) and Asiatic painted frog (*Kaloula pulchra pulchra*). The average densities (D) were 9.4 (KPU), 0.1 (KPM), 4.8 (TU) and 0.8 (TM) individuals (ind.) m^{-2} respectively (Table 1). In the six survey sites, the greatest numbers of individuals were in the KPU and TU in order; in both the KPM and TM sites the numbers were on the low end; and no individuals were captured in the KPD or TD sites (Table 1). These data reveal an obvious fact that in the downstream of both rivers the habitat alterations and pollutants had greatly affected both local amphibian populations (Green, 2003). Much environmental monitoring research has evaluated environmental conditions mainly based on changes in amphibian populations (Ankley *et al.*, 2004; Burkhart *et al.*, 2000; Klaassen, 2001; Liao *et al.*, 2006; Ouellet *et al.*, 1997; Venturino *et al.*, 2003). A report issued by the Pingtung County Environmental Protection Bureau confirms that the water quality of the Kaoping and Tungkang Rivers has been deteriorating from upstream to downstream (EPB/Pingtung-County, 2006). The worsening situation may be most likely caused by the following reasons. At the KPU and TU sites, livestock wastewater and agricultural chemicals are mostly responsible for the severe pollution. At the KPM and TM sites, aside from livestock wastewater and agricultural chemicals, the gradually growing human population, plus the increasing amounts of discharged domestic wastewater have further worsened the quality of both rivers. And at the KPD site, more wastewater from factories on both banks flows into the river while at the TD site, agricultural and livestock wastewater is gradually increased (Jen *et al.*, 2004). Our results indicate that the KPU and TU sites are better habitats for amphibians, followed by the KPM and TM sites, while the KPD and TD sites are the worst.

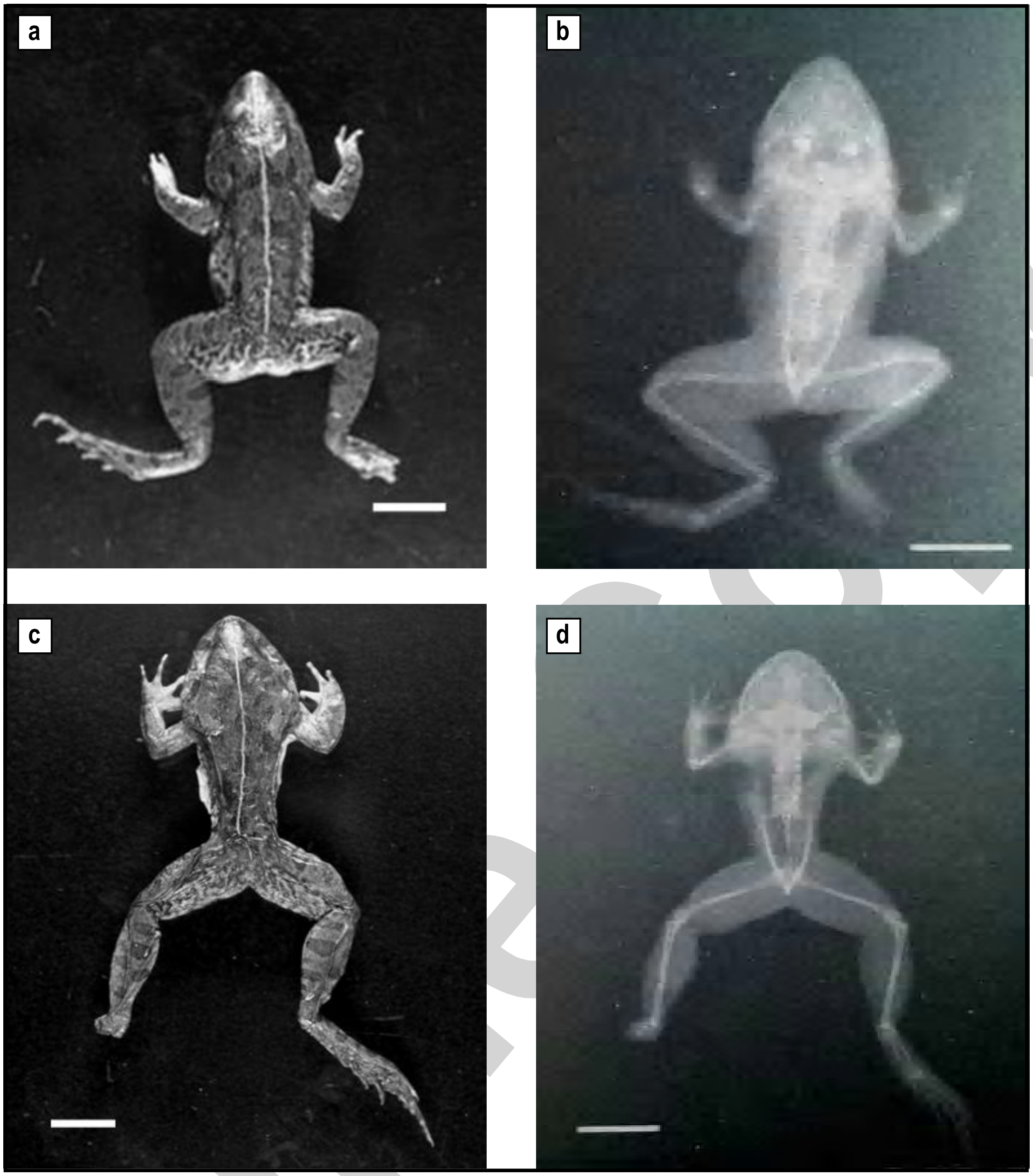

Fig. 2: No foot/partial foot. The tibiofibular bone was present but the tibiale and fibulare were shorter than normal bones in the right limb (a). The absence of the tibiale and fibulare in the left limb (c). b and d are x-ray pictures corresponding to a and c, respectively. (Bar = 1 cm)

This investigation revealed that most frogs were found in the reaches of the KPU and TU. In the Kaoping River, the frog population was higher in summer (May to July) 2006 and 2007, and lower in autumn (August to October) and spring (February to April). In the Tungkang River, frog population numbers, from high to low, were in the order of spring 2006, summer 2007 and autumn 2006 (Table 1). Wu *et al.* (2006) reported that the population of Indian rice frog increased after the breeding season (March to October). The same pattern was found in our observations at the KPU site but not at the TU site. It is probably a key factor that the higher level of human activities in the upstream areas of the Tungkang River has interfered with the growth of frog populations. In comparison with the density data of the Lanyang River of northeastern Taiwan (3-13 ind. 100 m^{-2}), the central mountainous area of Taiwan, southern Taiwan (2.5-18.0 ind. 100 m^{-2}), and the Zhoushan Archipelago of China (Huang and Hou, 2004; Lin and Lue, 2004; Wu and Liu, 2004; Wu *et al.*, 2006), we have found out that the amphibian populations at the KUP and TU sites are slightly lower than those of the Zhoushan Archipelago, and are comparatively higher than Lanyang River of northeastern Taiwan.

In this study, 244 (2%) malformed frogs in total were collected (malformed/normal, 244/10,909), among which 161 were collected at the KPU site, 59 at the TU site, and 24 at the TM site

Table - 1: Seasonal variation in density and percentage of malformed frogs

Season	Kaoping river						Tungkang river					
	Upstream		Midstream		Downstream		Upstream		Midstream		Downstream	
	D	m%	D	m%	D	m%	D	m%	D	m%	D	m%
Spring/2006	8.0	1.87	0.3	0	0	0	9.1	1.56	—	—	—	—
Summer/2006	13.9	3.15	0	0	0	0	0.9	7.50	2.9	4	0	0
Autumn/2006	8.7	0.97	0.1	0	—	—	5.9	2.67	1.3	0	—	—
Winter/2006	0.9	1.27	0	0	—	—	4.2	0.77	0	0	—	—
Spring/2007	6.1	2.93	0	0	0	0	2.2	0	0	0	0	0
Summer/2007	18.7	0.67	0	0	0	0	6.6	2.93	0	0	0	0
Mean	9.4	1.8	0.1	0	0	0	4.8	2.6	0.8	0.8	0	0
SD	5.7	0.9	0.1	0	0	0	2.7	2.4	1.1	1.6	0	0

— = Not sampled, SD = Standard deviation, D = Individuals 100 m^{-2}, m% = Percentage of malformed frogs

Table- 2: Frequency of symptoms in abnormal frogs

	Kaoping river		Tungkang river		Total
	Upstream	Midstream	Upstream	Midstream	
Amelia	12	0	7	2	21
Ectromeila	39	0	14	4	57
No foot/ Partial foot	67	0	22	12	101
Entire toe missing	11	0	0	0	11
Exostosis	15	0	6	0	21
Multiple toes	10	0	5	3	18
Short toes	1	0	2	0	3
Bone bridge/ Bone Rotation / Proportionately short limb	3	0	2	1	6
Other	3	0	1	2	65

Table - 3: Frequency of symptoms in abnormal male and female frogs

	Kaoping river		Tungkang river		Total	
	Male	Female	Male	Female	Male	Female
Amelia	8	4	4	5	12	9
Ectromeila	22	18	10	7	32	25
No foot/ Partial foot	45	22	18	16	63	38
Entire toe missing	8	3	0	0	8	3
Exostosis	7	7	4	3	11	10
Multiple toes	5	4	6	3	11	7
Short toes Bone bridge/	0	1	1	1	1	2
Bone Rotation / Proportionately short limb	2	1	0	2	2	3
Other	1	3	1	2	2	5

(Table 2). In species, of all the malformed frogs, 240 were Indian rice frogs, and only 4 were Chinese bullfrogs. The fact that more numbers of malformed frogs were found at the KPU and TU sites may be associated with the higher populations of amphibians. However, very few numbers of malformed frogs were found in the midstream and downstream reaches, the environment of which is thought to be more appropriate for amphibians to live in (Feidieker *et al.*, 1995; Loeffler *et al.*, 2001; Rodriguez-Prieto and Fernandez-Juricic, 2005). The malformation rate of frogs generally falls between 2 and 3% (Stocum, 2000). Our results indicated the frog malformation percentages in all seasons at the KPU and TU sites during 2006 to 2007 were around 0.67-3.15% and 0-7.5%, respectively. Comparatively, though these data were lower than those reported in northern California (0.6-9%; Bettaso, 2004) and Minnesota (0.3-22.8%; Canfield *et al.*, 2000), still the percentages at the KPU and TU sites are quite high, which implies there must be some potential factors causing these abnormalities in amphibians.

Among the 244 frogs with a peculiar appearance, there are 171 with one malformation symptom, 64 with two, 7 with three, and 2 with four. For the malformation symptoms, foot/partial foot

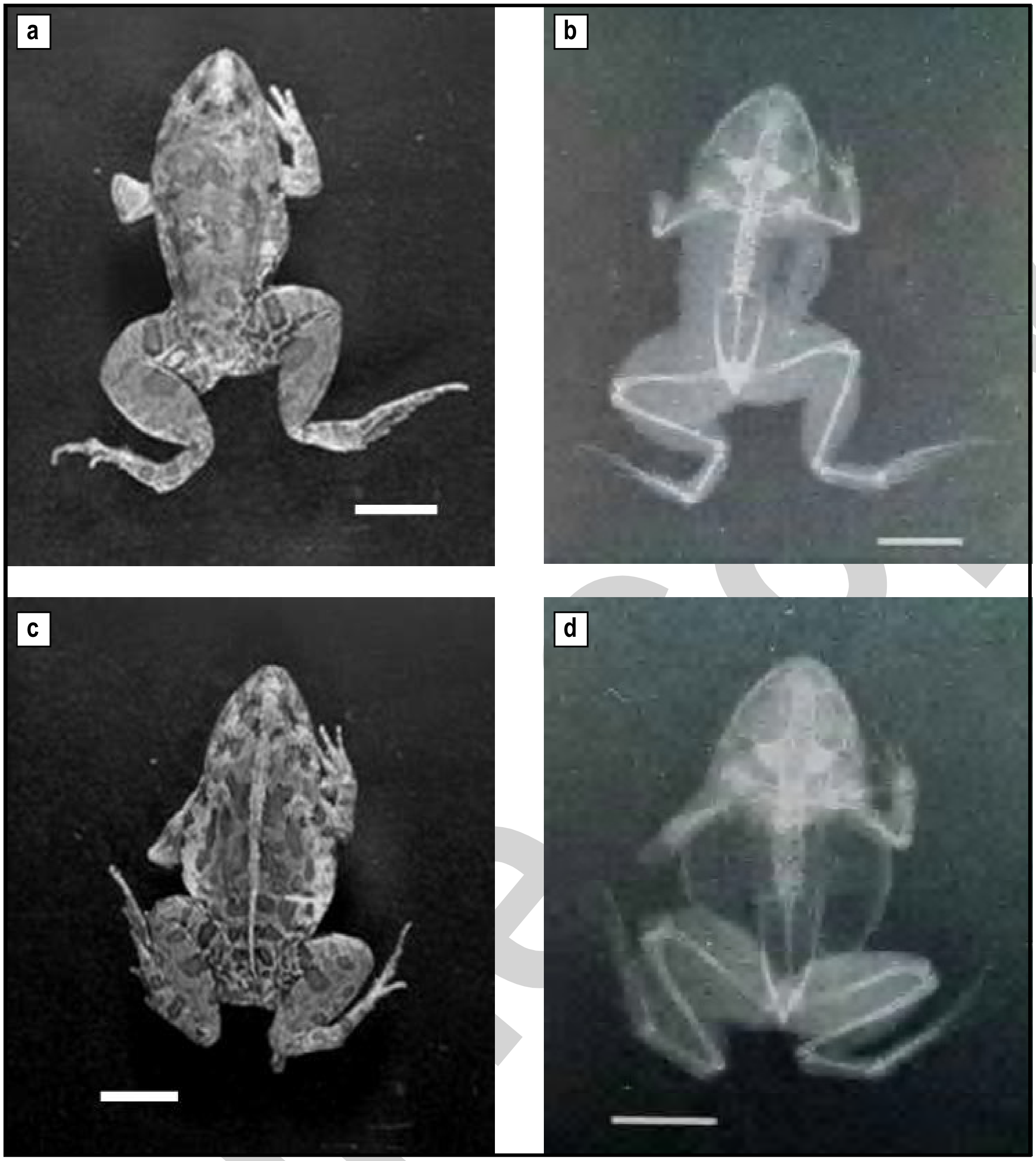

Fig. 3: Ectromeila. The stifle (knee) is present, and a portion of the tibiofibular bone is also present (a). The absence of the stifle (knee) and femur also occurred as exostosis in the femur bone (c). b and d are x-ray pictures corresponding to a and c, respectively. (Bar = 1 cm)

(Fig. 2) is the most prevalent; ectromeila (Fig. 3) the second, and amelia the third respectively. Except for the no foot/partial foot symptoms, other symptoms do not significantly differ between the KPU and TU sites (Table 2). In our investigation, the number of those malformed frogs that have more than one malformation symptom is 64, among which exostosis most often occurs, accompanied by other symptoms. Exostosis in malformed frogs was mostly accompanied by ectromeila and no foot/partial foot. This phenomenon may be caused by trying to act with incomplete limbs which might result in limb wearing or injury, causing a chronic inflammatory reaction and bone tissue proliferation (Smithuis, 1964).

There were more males than females in number in the collected malformed frogs. A Chi-squared analysis of the frequencies of symptoms in abnormal frogs shows no significant difference between males and females in either the Kaoping or Tungkang Rivers (Table 3).

The average snout-vent length (SVL, 42.62 mm) of abnormal individuals is shorter than that of normal frogs (45.16 mm, $p<0.01$). In the Kaoping River basin, the same pattern is observed in female frogs; the average SVL shows no difference between normal and abnormal males in spring and summer; however, it is shorter for abnormal individuals than for normal frogs (Fig. 4a).

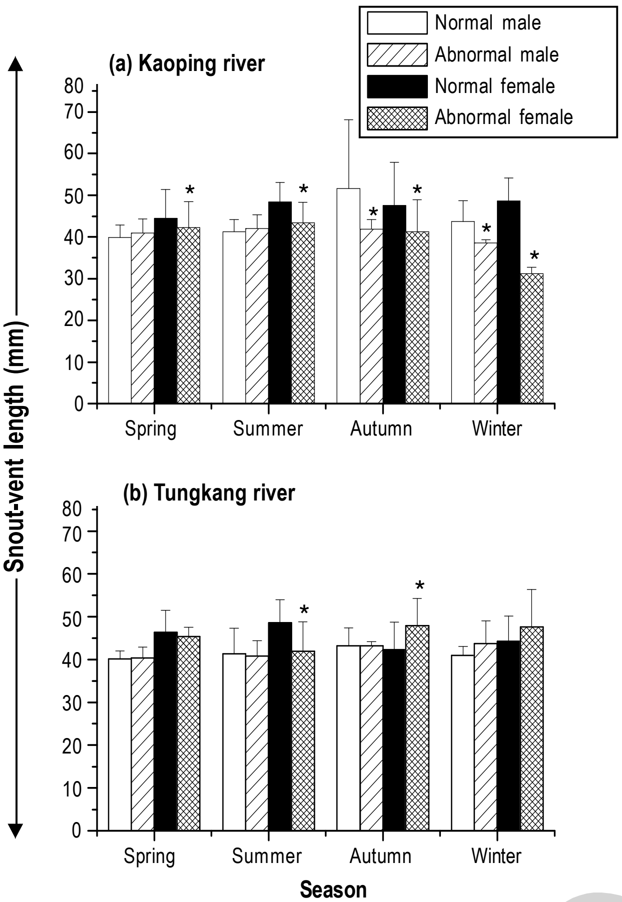

Fig. 4: Snout-vent length of normal and abnormal frogs sampled during February 2006 to June 2007. Asterisk (*) indicates significant at $p<0.05$

Abnormal frogs with a shorter SVL are thought to be less active, resulting in malnutrition due to the difficulty in capturing prey (Ouellet *et al.*, 1997). However, a shorter SVL in abnormal frogs was not observed in the Tungkang River basin (Fig. 4b).

Ouellet et al. (1997) found higher malformation rates in agricultural fields. Agricultural chemicals such as pesticides and herbicides are the most often mentioned materials among various contaminants that can lead to creature malformation (Clements *et al.*, 1997; El-Merhibi *et al.*, 2004; Gillilland *et al.*, 2001; Lu, 2001). Furthermore, some agricultural chemicals such as pesticides are found to posses estrogen-like activity that can affect the normal development of bones (Pickford and Morris, 2003). In addition, our results reveal that numerous malformed frogs were found during the 1-2 months after high agricultural activities in summer. Therefore, agricultural chemicals are very likely the factor that affects frog malformation in the Kaoping and Tungkang River basins. Kiesecker (2002) indicated that, when parasites exist, the amphibian malformation rates that occurred in water bodies containing pesticides are higher than those without pesticides. As a result, a combination of parasites and pesticides (Johnson *et al.*, 2001) may contribute to the appearance of malformed frogs at the KPU and TU sites.

In addition to ultraviolet radiation, parasites and pollutants,, rising temperatures can also cause malformations in amphibians (Lu, 2001). The Central Weather Bureau/ROC (2006) showed that the temperature in Pingtung went up from 25.1 to 25.9°C during 2001 to 2006. Rising temperatures and organic material contamination can expand the microbiological populations such as of *R. ondatrae* and cyanobacteria (Hynes, 1974).

This study suggest that, as the result of human activities, the pollution from upstream to downstream has been increasingly worsening and thus causes the decreasing number of frog populations. Potential sources that possibly lead to the malformation are in the upstream areas, where frog malformation may be closely related to organic wastewater and chemicals released through agricultural activities.

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