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Metamorphic strategies of the Indian rice frog, *Fejervarya limnocharis*, in response to irrigation regimes

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ABSTRACT: Organisms gain benefits from phenotypic plasticity by possessing traits better cope with environmental variations. Although cohort-dependent life-history strategy may be ubiquitous in amphibians, it is rarely studied. I investigated whether Indian rice frog, *Fejervarya limnocharis*, populations from cultivated fields with different irrigation regimes have differential cohort-dependent metamorphic strategies. I tested the hypothesis that populations inhabiting temporally disrupted breeding habitats would, while populations inhabiting temporally constant breeding habitats would not show cohort-dependent metamorphic strategies in response to seasonal temperature variation. I assessed cohort-dependent strategies by comparing metamorphic weight, age, and growth rate between spring and summer cohorts in response to low and high temperatures in a factorial common garden experiment. The results showed that the plasticity of metamorphic weight and age were both very extensive in the Indian rice frog. Tadpoles from disrupted irrigation (rice paddy) populations had cohort-dependent metamorphic strategies. In contrast, tadpoles from constant irrigation (water bamboo field) populations did not show cohort-dependent metamorphic strategies. More research on cohort-dependent life-history traits is badly needed to further our understanding the evolution of life history strategies.

KEY WORDS: Irrigation, Life-history strategy, Metamorphosis, Plasticity, Rice paddy, Water bamboo field.

INTRODUCTION

Phenotypic plasticity is the capacity of a certain genotype to produce multiple phenotypes in response to variable environments. Organisms gain benefits from plasticity by possessing traits better cope with environmental challenges (Schlichting and Pigliucci, 1998; Pigliucci, 2001; Miner *et al.*, 2005). Amphibians have a complicated life-history that often include an aquatic larval stage and a semi-terrestrial adult stage. The size at and timing of metamorphosis from larvae to adults affect the fitness of both stages. Plasticity in one or both traits is commonly seen in larval amphibians. "Bigger is better" is a robust rule in ectotherms including amphibians (Kingsolver and Huey, 2008). Larger metamorphs not only perform better after metamorphosis in many physiological and reproductive aspects (Pough and Kamel, 1984; Hetherington, 1988; Newman and Dunham, 1994; Wells, 2007), but also have higher survival and growth rate on land (Morey and Reznick, 2001; Altwegg and Reyer, 2003; Cabrera-Guzman *et al.*, 2013). On the other hand, early metamorphosis is generally advantageous for anuran larvae. It allows larvae escape from risky aquatic habitats, for instance, predation pressure or pond desiccation (Newman, 1988; Relyea, 2007; Mogali *et al.*, 2011).

Temperature is the most powerful environmental cues for inducing phenotypic plasticity in larval anurans due to its direct influence on the metabolic rates that drive growth and development (Atkinson, 1996). Because developmental rate is accelerated more than growth, high temperature often causes larvae to undergo metamorphosis earlier (Harkey and Semlitsch, 1988; Beck and Congdon, 2000; Alvarez and Nicieza, 2002; Castañeda *et al.*, 2006; Kuan and Lin, 2011). Several studies have observed spatial divergence in larval growth and/or development within species along geographic temperature gradients, including altitudinal (Berven *et al.*, 1979; Berven and Gill, 1983; Lai *et al.*, 2002) and latitudinal (Blouin and Brown, 2000; Stahlberg *et al.*, 2001; Laugen *et al.*, 2003; Merila *et al.*, 2004; Liess *et al.*, 2013) clines. For example, tadpoles from northern populations (cold environments) had faster development rate and higher growth rate than southern ones under the same high temperature treatment (Stahlberg *et al.*, 2001). Overall, these studies suggested that geographic populations varied in responses to the same temperatures. In contrast, few studies on metamorphosis have examined responses within populations along temporal frames, such as seasons. Those few studies focused on over-wintering tadpoles (Chuang, 2006; Hsu *et al.*, 2012), however. Temporal variation in environment is a fact of life faced by many amphibians, particularly prolonged breeders, such as *Bufo calamita* (Sinsch, 1988) and *Fejervarya limnocharis* (Kuan and Lin 2011). One would expect tadpoles respond with phenotypic plasticity to those temporal variations. Indeed, Kuan and Lin (2011) found an intriguing seasonal cohort-dependent response. They demonstrated that spring and summer cohorts of Indian rice frogs, *F. limnocharis*, used different metamorphic strategies in response to temperature

variation. They proposed that the discontinuous breeding environment due to the interruption of irrigation between the spring and summer rice crops had contributed to the cohort effect. Such a proposition could be tested by comparing the metamorphic traits of populations inhabiting temporally continuous versus discontinuous breeding habitats.

The species, *F. limnocharis*, studied by Kuan and Lin (2011) distributes widely in lowland Taiwan. It breeds from February to October primarily in rice paddies. Its breeding activities are deeply affected by rice field irrigation (Alexander *et al.*, 1979). The juvenile (tadpole) stage typically lasts 3-4 weeks in the field. The life events during the adult stage of the species remain unclear (Alexander *et al.*, 1979). The rice crops in northern Taiwan are generally planted in spring (March - June) and summer (August - October) with a complete drainage of the paddies between crops (July). The start of irrigation at the beginning of each crop triggers the breeding of the species. The species also breeds in other aquatic habitats, such as water bamboo fields, that differ in irrigation regimes from that of rice paddies. Since few studies (Sinsch, 1988; Kuan and Lin, 2011) have investigated cohort-dependent life history in amphibian. To further the understanding of such a peculiar phenomenon, in this study, I investigated the metamorphic traits of *F. limnocharis* tadpoles inhabiting temporally continuous versus discontinuous breeding habitats due to irrigation patterns in northern Taiwan. I hypothesized that populations inhabiting temporally disrupted breeding habitats would, while populations inhabiting temporally constant breeding habitats would not show cohort-dependent metamorphic strategies in response to seasonal temperature variation.

MATERIALS AND METHODS

Study sites

I collected *F. limnocharis* tadpoles from two sites, Sanzhi (25°15'0.5"N, 121°30'16"E) and Daxi (24°54.5'53.6"N, 121°15'51.1"E) in northern Taiwan that differ in irrigation regimes. Both sites have expansive rice fields historically, and most extant rice paddies have been cultivated for over 50 years. However, the majority of rice paddies at Sanzhi have been transformed into water bamboo (*Zizania latifolia*) fields in the last three decades. Water bamboos at Sanzi are cultivated annually from March to October. The fields hold a constant water level at 5–20 cm throughout the year. Two crops of rice are grown at Daxi in spring (March–June) and summer (August–October), respectively. The rice fields are irrigated from March to October except for July, when the fields are drained, and remain dry for 2–3 weeks between the two crops. Therefore, the water bamboo fields at Sanzi and rice paddies at Daxi have distinctly different irrigation regimes. The former provide constant and the latter disrupted breeding habitats for the *F. limnocharis*, respectively. I obtained ambient temperature regime records of the two sites from the Environmental Protection Administration of Taiwan. (http://dacet.ntu.edu.tw/ch/data/epa/download.php?species=14).

Experimental design

The experimental procedures followed Kuan and Lin (2011) closely. I randomly collected a total of 40 *F. limnocharis* tadpoles at Gosner 25 stage (Gosner, 1960) from four water bamboo fields at Sanzhi in April (spring cohort, hereafter) and August (summer cohort, hereafter), 2011, each. The fields were on average 400 m apart. Similarly, I randomly collected 40 *F. limnocharis* tadpoles at Gosner 25 stage from three rice paddies at Daxi in April and August, 2011, each. The paddies were on average 750 m apart. All 80 tadpoles were transported to the laboratory immediately, and remained at room temperature before the experiments started within 24 hours of collection.

For each location (irrigation regime), I used a 2×2 factorial design in a common garden experiment to examine the effects of temperature (low vs. high) and cohort (spring vs. summer) on larval growth and development. I set two growth chambers with 12:12-hr light/dark cycle (lights on at 0600 am) at 22°C (LT, hereafter) and 29°C (HT, hereafter) to represent the spring and summer temperature, respectively. Both temperatures were within the natural temperature ranges at the two sites. I randomly assigned 10–16 healthy tadpoles to each temperature treatment for each cohort. I raised tadpoles individually in opaque plastic containers containing 250 ml tap water, and provided boiled spinach *ad libitum*. I checked food supply daily and changed water weekly.

I weighed and recorded Gosner stage for each tadpole weekly until they reached metamorphosis (Gosner 42). As one of the forelimbs emerged (Gosner 42), I recorded body weight (metamorphic weight, hereafter), and calculated the number of days elapsed between Gosner 25 and Gosner 42 (metamorphic age, hereafter). Each tadpole was removed from its residing container with a cloth dip-net, placed on a paper tower, and blotted dry delicately. Then, it was placed in a small pre-weighed petri-dish with 5 ml water and weighed with an electronic balance (accuracy: 0.1 mg, Sartorius, Germany). I then calculated growth rates as the difference in body weights between Gosner 25 and 42 divided by the metamorphic age.

Data analyses

I calculated two temperature parameters from the hourly air temperature of the two sites from 1997 to 2010: (1) mean daily temperature (the average of hourly air temperature in a day) and (2) coefficient of variation (the coefficient of variation of hourly air temperature in a day). The daily values were first averaged by season: spring (March–June) and summer

(August–October). I then regarded average values from different years as replicates (N=14), and used t-tests to compare between seasons and between two sites.

Although some tadpoles came from the same fields (or paddies) at the same time, I treated individual tadpoles as replicates. I used logistic regression (R software version 2.15.2 by R Development Core Team, www.r-project.org) to analyze the effects of temperature, cohort and irrigation regime (site) on whether or not tadpoles reached metamorphosis. I performed three-way ANOVAs with temperature, cohort and irrigation regime as fix factors to examine their effects on metamorphic weight, and growth rate. In order to specifically examine cohort-dependent metamorphic strategies under each irrigation regime, I followed up with two-way ANOVAs with temperature and cohort as fix factors for constant (Sanzhi) and disrupted (Daxi) irrigation regimes, respectively. Type III sums of squares were used to account for the unequal sample sizes among treatments. Since data sets of metamorphic age and growth rate did not conform to normal distribution (Kolmogorov-Smirnov tests for normality, P <0.01 in both cases). The growth rate was square root transformed, while metamorphic age was square-root-and-reciprocal transformed before statistical analysis. All analyses were conducted using SYSTAT 12 (Systat Software Inc., CA, USA).

RESULTS

Temperature regimes of study sites

Daily temperature was significantly cooler (comparing means) and more variable (comparing CVs) in spring than summer at both sites (t-tests, P < 0.001 for both parameters at both sites, Table 1). Thus, generally speaking, spring tadpoles experienced not only cooler but also more variable temperature than summer ones at both sites (Table 1). Comparisons between two sites showed that summer temperature of Sanzhi was significantly warmer, though only by 0.8ºC, than that of Daxi (t-tests, P < 0.001, Table 1). Water temperature in the rice paddies follows the air temperature closely (Kuan and Lin 2011). In summary, there was only a slight difference in temperature regime between the two sites.

Table 1. Mean and coefficient of variation of daily temperature during the spring (March–June) and summer (August–October) at two study sites: Sanzhi and Daxi from 1997 to 2010.

		Sanzhi	Daxi	*P* value
Mean daily temperature (ºC)	Spring	^{A}22.51±3.72	^{A}22.20±4.03	0.13
	Summer	^{B}26.54±2.20	^{B}25.72±2.45	<0.001
Coefficient of variation (%)	Spring	^{A}16.52±2.05	^{A}18.17±2.38	0.06
	Summer	^{B}8.30±2.07	^{B}9.52±1.90	0.12

[A] For each variable, significant differences between seasons at a site are indicated by different capital letters, while *P* values show the differences between sites for a season.

Percentage of tadpoles reached metamorphosis

Not all tadpoles made it to Gosner 42. Some died during the experiment; some had extended larval periods and did not enter G42 at the end of the experiment (week 14). There was no main effect or interaction of site, cohort or temperature treatment on the percentage of tadpoles reached metamorphosis (logistic regression, P > 0.05 in all cases, Table 2). In short, similar percentages of tadpoles reached metamorphosis regardless of site, cohort, or temperature treatment. The mortality of tadpoles should not affect our comparisons between sites, cohorts, or temperature treatments.

Table 2. The number and percentage of *F. limnocharis* tadpoles that reached G42 at the end of experiment (week 14) in spring and summer raised under two temperature treatments.

	22ºC		29ºC	
	Spring	Summer	Spring	Summer
Daxi (disrupted irrigation)				
Initial tadpole number	12	16	14	16
Tadpoles completed metamorphosis	10	9	9	13
Percentage of metamorphosis (%)	83.3	56.3	64.3	81.3
Sanzhi (constant irrigation)				
Initial tadpole number	11	12	11	14
Tadpoles completed metamorphosis	5	6	7	8
Percentage of metamorphosis (%)	45.5	50.0	63.6	57.1

Cohort-dependent metamorphic strategies

Tadpoles collected from disrupted irrigation regime (Daxi) showed cohort-dependent metamorphic strategies in all three metamorphic traits (Two-way ANOVAs, cohort effect, P < 0.01; Table 3). On the other hand, tadpoles collected from constant irrigation regime (Sanzhi) did not show any significant effect of cohort in all metamorphic traits measured (Two-way ANOVA, P> 0.05 in both cohort effect and cohort x temperature

Table 3. Two-way ANOVAs that examine the effects of cohort and temperature on **(A)** metamorphic weight, **(B)** metamorphic age and **(C)** growth rate of *F. limnocharis* tadpoles at Daxi (disrupted irrigation).

Source	Type III SS	df.	Mean square	F ratio	*P* value
A. Metamorphic weight					
Cohort	137185	1	137185	29.33	<0.001
Temperature	4031	1	4031	0.86	0.36
Cohort × Temperature	23553	1	23553	5.04	0.03
Error	173056	37	4677		
B. Metamorphic age[a]					
Cohort	0.02	1	0.015	48.38	<0.001
Temperature	0.07	1	0.071	226	<0.001
Cohort × Temperature	<0.001	1	<0.001	0.49	0.49
Error	0.01	37	<0.001		
C. Growth rate					
Cohort	424.4	1	424.4	37.39	<0.001
Temperature	533.8	1	533.8	47.03	<0.001
Cohort × Temperature	3.6	1	3.6	0.32	0.58
Error	420.0	37	11.4		

[a] Data of metamorphic age was transformed by square root and reciprocal transformation.

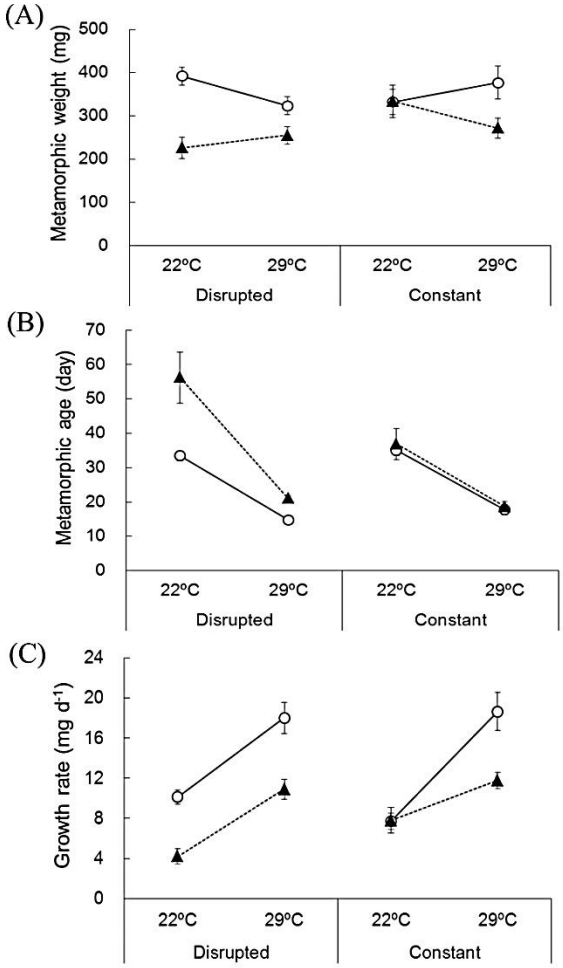

Fig. 1. Life history parameters: **(A)** metamorphic weight, **(B)** metamorphic age and **(C)** growth rate of spring (blank) and summer (filled) *F. limnocharis* tadpoles raised under two temperatures from two sites with disrupted (Daxi) versus constant (Sanzhi) irrigation regimes. Values are given in MEAN±1SE. Sample sizes are given in Table 2.

interaction; Table 4). Three-way ANOVAs (irrigation regime x cohort x temperature, Table 5) disclosed the effects of irrigation regime with further details as follows.

Metamorphic weight

There was a strong cohort effect ($F_{1, 65}$ = 20.11, P < 0.001) and a three-way interaction among irrigation regime, cohort and temperature (Three-way ANOVA, $F_{1, 65}$ = 7.34, P = 0.01; Table 5A). Generally, spring cohort at both sites had larger metamorphic weights (359.4±13.7 mg; Fig. 1A) than summer cohort (265.1±13.2 mg). However, in disrupted irrigation (Daxi) populations only, there was an interaction effect between cohort and temperature (Two-way ANOVA, $F_{1, 39}$ = 5.04, P = 0.03; Table 3A). Spring cohort had significantly larger metamorphic weight than summer cohort at 22ºC, but not 29ºC (Fig. 1A). Such strong cohort effect and cohort x temperature interaction did not occur in constant irrigation (Sanzhi) populations (Two-way ANOVA, P > 0.05 in both cases; Table 4A and Fig. 1A).

Table 4. Two-way ANOVAs that examine the effects of cohort and temperature on **(A)** metamorphic weight, **(B)** metamorphic age and **(C)** growth rate of *F. limnocharis* tadpoles at Sanzhi (constant irrigation).

Source	Type III SS	df.	Mean square	F ratio	*P* value
A. Metamorphic weight					
Cohort	16816	1	16816	2.47	0.13
Temperature	495	1	495	0.07	0.79
Cohort × Temperature	17952	1	17952	2.64	0.12
Error	149804	22	6809		
B. Metamorphic age					
Cohort	11.9	1	11.9	0.26	0.61
Temperature	1985.5	1	1985.5	44.03	<0.001
Cohort × Temperature	1.3	1	1.3	0.03	0.87
Error	992.1	22	45.1		
C. Growth rate					
Cohort	1.15	1	1.15	3.91	0.06
Temperature	7.53	1	7.53	25.54	<0.001
Cohort × Temperature	1.12	1	1.12	3.78	0.07
Error	6.49	22	0.30		

[a] Data of growth rate was transformed by square root transformation.

Table 5. Three-way ANOVAs that examine the effects of irrigation regime (constant vs. disrupted), cohort (spring vs. summer) and temperature (low vs. high) on **(A)** metamorphic weight, **(B)** metamorphic age and **(C)** growth rate of *F. limnocharis* tadpoles.

Source	Type III SS	df	Mean squares	F ratio	*P* value
A. Metamorphic weight					
Irrigation	13301	1	13301	2.43	0.12
Cohort	110063	1	110063	20.11	<0.001
Temp.[a]	3236	1	3236	0.59	0.45
Irrigation × Cohort	16527	1	16527	3.02	0.09
Irrigation × Temp.	484	1	484	0.09	0.77
Cohort × Temp.	92	1	92	0.02	0.90
Irrigation × Cohort × Temp.	40137	1	40137	7.34	0.01
Error	322860	59	5472		
B. Metamorphic age					
Irrigation	0.001	1	0.001	1.25	0.27
Cohort	0.007	1	0.007	13.60	<0.001
Temp.	0.093	1	0.093	184.2	<0.001
Irrigation × Cohort	0.005	1	0.005	9.68	0.003
Irrigation × Temp.	0.001	1	0.001	1.29	0.26
Cohort × Temp.	<0.001	1	<0.001	0.36	0.55
Irrigation × Cohort × Temp.	<0.001	1	<0.001	0.007	0.93
Error	0.030	59	0.001		
C. Growth rate					
Irrigation	0.33	1	0.33	1.12	0.30
Cohort	8.72	1	8.72	29.24	<0.001
Temp.	19.81	1	19.81	66.43	<0.001
Irrigation × Cohort	1.62	1	1.62	5.42	0.02
Irrigation × Temp.	0.02	1	0.02	0.08	0.79
Cohort × Temp.	0.34	1	0.34	1.15	0.29
Irrigation × Cohort × Temp.	1.14	1	1.14	3.83	0.06
Error	17.59	59	0.30		

[a] Temp. was the abbreviation of temperature.

Metamorphic age

There was a strong cohort effect ($F_{1, 65}$ = 13.60, P < 0.001) and a two-way interaction between irrigation regime and cohort (Three-way ANOVA, $F_{1, 65}$ = 9.68, P

= 0.003; Table 5B). Although, overall, spring cohort had shorter metamorphic age (larval days, 24.7±1.8 days) than summer one (32.0±3.2 days), the pattern was significant in disrupted irrigation (Daxi) populations (Two-way ANOVA, $P < 0.001$; Table 3B and Fig. 1B), but not in constant irrigation (Sanzhi) populations (Two-way ANOVA, $P = 0.61$; Table 4B and Fig. 1B).

Growth rate

There was a strong cohort effect ($F_{1, 65} = 29.24$, $P < 0.001$) and a two-way interaction between irrigation regime and cohort (Three-way ANOVA, $F_{1, 65} = 5.42$, $P = 0.02$; Table 5C). Generally, spring cohort had 1.5 times higher growth rate (14.0±1.1 mg/day) than summer one (8.9±0.7 mg/day). However, the pattern was significant in disrupted irrigation (Daxi) populations (Two-way ANOVA, $P < 0.001$; Table 3C and Fig. 1C), but not in constant irrigation (Sanzhi) populations (Two-way ANOVA, $P = 0.06$; Table 4C and Fig. 1C).

Range of plasticity of key metamorphic traits

Metamorphic weight (size) and metamorphic age are two of the most important components of amphibian fitness (see introduction). To demonstrate the ranges of plasticity in these two traits, I used 95% confidence intervals (Fig. 2). In general, tadpoles from a disrupted irrigation regime (rice paddies at Daxi, Fig. 2A) had more extreme metamorphic traits than those from a constant irrigation regime (water bamboo fields at Sanzhi, Fig. 2B). The metamorphic weights of the former ranged between 226.9–392.4 mg, while those of the latter ranged between 272.0–377.0 mg (Table 6A and Fig. 2). The metamorphic age of rice paddy tadpoles ranged between 14.8–56.2 days, while those of water bamboo field tadpoles ranged between 17.7–36.8 days (Table 6B and Fig. 2).

DISCUSSION

Phenotypic plasticity in metamorphic traits of amphibians such as weight and age at metamorphosis and growth rate is regularly reported (Alvarez and Nicieza, 2002; Lind, 2009; Liess *et al.*, 2013). As demonstrated by Kuan and Lin (2011), my study species, the Indian rice frog, *F. limnocharis*, exhibits extensive plasticity (Fig. 2). Either spring or summer cohort (a surrogate of genotype) showed significant differences in one or more metamorphic traits when raised under different temperatures (Table 6) and/or food availability (Kuan, 2016). In addition, Kuan and Lin (2011) found that tadpoles born in different seasons exhibit different metamorphic plasticity in response to the same set of environmental gradients. For example, spring tadpoles had greater body growth rates when raised under spring (low) temperature; whereas summer tadpoles had greater body growth rates when raised under summer (high) temperature. That is, the patterns

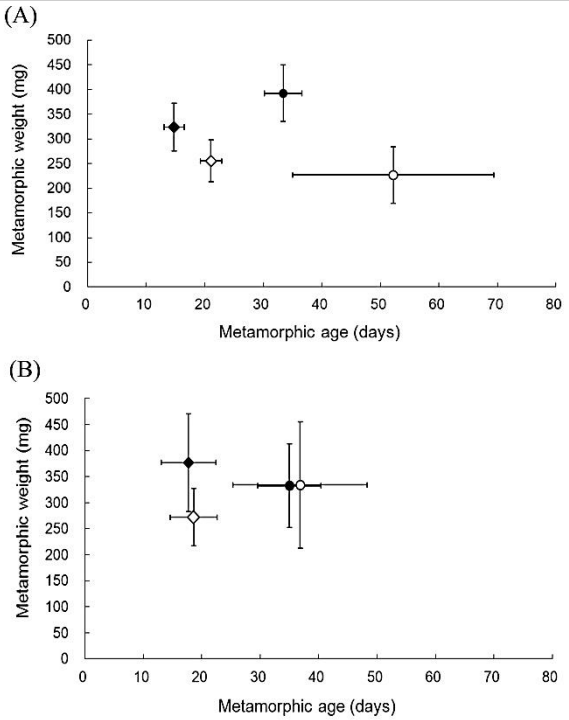

Fig. 2. Mean metamorphic age and weight of spring (filled) and summer (blank) *F. limnocharis* cohorts under **(A)** a disrupted and **(B)** a constant irrigation regime, raised under 22ºC (circle) and 29ºC (diamond), respectively. The horizontal and vertical error bars show 95% confidence intervals.

Table 6. Life history parameters: **(A)** metamorphic weight, **(B)** metamorphic age and **(C)** growth rate of spring and summer *F. limnocharis* tadpoles raised under two temperatures from two sites with different irrigation regimes (constant vs. disrupted). Values are given in MEAN±1SE.

	22ºC		29ºC	
	Spring	Summer	Spring	Summer
(A) Metamorphic weight (mg)				
Disrupted	$^{A}392.4\pm20.5^{a}$	$^{A}226.9\pm24.8^{b}$	$^{A}323.8\pm20.8^{ac}$	$^{A}255.3\pm19.6^{bc}$
Constant	$^{A}332.5\pm28.9^{a}$	$^{B}334.2\pm37.1^{a}$	$^{B}377.0\pm38.3^{a}$	$^{A}272.0\pm23.2^{a}$
(B) Metamorphic age (day)				
Disrupted	$^{A}33.4\pm1.4^{a}$	$^{A}56.2\pm7.4^{b}$	$^{A}14.8\pm0.7^{c}$	$^{A}21.1\pm0.8^{d}$
Constant	$^{A}35.0\pm1.5^{a}$	$^{B}36.8\pm4.5^{a}$	$^{A}17.7\pm1.5^{b}$	$^{A}18.6\pm1.5^{b}$
(C) Growth rate (mg/day)				
Disrupted	$^{A}10.1\pm0.7^{a}$	$^{A}4.2\pm0.8^{b}$	$^{A}18.0\pm1.6^{c}$	$^{A}10.9\pm1.1^{a}$
Constant	$^{A}7.7\pm0.8^{a}$	$^{B}7.8\pm1.3^{a}$	$^{A}18.7\pm1.9^{b}$	$^{A}11.8\pm0.8^{ab}$

[A] Significant differences between two irrigation regimes based on t-test are indicated by different capital letters along a column. Significant differences among the four temperature x cohort treatments based on Scheffé's test are indicated by different lower case letters along a row.

of plasticity were cohort-dependent. This is one of the rare studies of amphibian cohort-dependent metamorphic strategy (also see Sinsch, 1988). Kuan and Lin (2011) further proposed that the disrupted irrigation regime of rice fields had caused *F. limnocharis* seasonal cohorts to diverge in metamorphic strategies.

Because Indian rice frog populations also inhabit other habitats than rice paddies, I took a step further and asked: do frog populations in cultivated fields with

diverse irrigation regimes have different cohort-dependent metamorphic strategies? To answer this question, I tested the hypothesis that populations inhabiting temporally disrupted breeding habitats (rice paddies) would, while populations inhabiting temporally constant breeding habitats (water bamboo fields) would not show cohort-dependent metamorphic strategies in response to seasonal temperature variation. The results from the three traits I measured: metamorphic weight, metamorphic age and growth rate together strongly support the hypothesis.

In metamorphic weight, a strong cohort effect occurs in populations inhabiting temporally disrupted breeding habitats (Table 3A). Spring cohort (359.4±13.7 mg) weights 35% more than the summer cohort (265.1±13.2 mg, Table 6A). Furthermore, a significant interaction between cohort and temperature indicates that the difference in weight is much greater at 22ºC than 29ºC (Fig. 1A left panel). Populations inhabiting temporally constant breeding habitats show neither cohort effect nor interaction (Table 4A, Fig. 1A right panel). In metamorphic age (larval period), a strong cohort effect occurs in populations inhabiting temporally disrupted breeding habitats (Table 3B, Fig. 1B left panel). Spring cohort (24.7±1.8 day) has a 22% shorter larval period than the summer cohort (32.0±3.2 day, Table 6B). The cohort effect does not occur in populations inhabiting temporally constant breeding habitats (Table 4B, Fig. 1B right panel). Finally, in growth rate, a strong cohort effect occurs in populations inhabiting temporally disrupted breeding habitats (Table 3C, Fig. 1C left panel). Spring cohort (14.0±1.1 mg/day) has a 57% higher growth rate than the summer cohort (8.9±0.7 mg/day, Table 6C). Populations inhabiting temporally constant breeding habitats do not have significant cohort effect or cohort x temperature interaction (Table 4C, Fig. 1C right panel).

The pattern of cohort-dependent metamorphic strategy I observed at Daxi (disrupted irrigation) is generally consistent with the findings by Kuan and Lin (2011), in which the experiment was performed in rice paddies as well at Ankang. Rice paddies at both Ankang and Daxi have a disrupted irrigation regime. Both studies found temporal divergence in metamorphic strategies between spring and summer cohorts. The spring cohort responds to low temperature with high body weight at metamorphosis, while the summer cohort does not have such a response. On the other hand, while both spring and summer cohorts respond to low temperature with retarded developmental rates (long larval periods), summer cohort has a significantly greater magnitude of retardation than the spring one (Fig. 2, Table 6). In comparison, summer tadpoles generally face a shorter growing season (3 months, August – October) than spring one (4 months, March – June) in rice fields because of the cultivation practice and weather change. Therefore, summer tadpoles need to accelerate development rate to reach the metamorphosis as soon as possible. Otherwise, they will need to postpone metamorphosis until the next breeding season (Stahlberg *et al.*, 2001; Laugen *et al.*, 2003; Lind and Johansson, 2007). Indeed, in both studies, several individuals of summer cohorts underwent arrested development and did not enter metamorphosis by the end of experiments. The arrested development may allow tadpoles overwinter as larvae, and contribute to the high plasticity of metamorphic age for summer cohorts I observed (Fig. 2). Overall, the spring and summer cohorts in rice paddies employ distinct life-history strategies to increase their fitness: the former used a 'size' strategy, the latter a 'rate' strategy (Kuan and Lin 2011). The similarity of metamorphic trait responses at both rice paddy sites suggested that cohort-dependent metamorphic strategies is a consistent pattern occur across multiple sites, and not a result of site-specific factors.

My study used one location for disrupted irrigation regime and the other location for constant irrigation regime. Thus, the experimental design likely confounded the effects of location (Sanzhi vs. Daxi) and irrigation regimes (constant vs. disrupted). However, as I mentioned in the last paragraph that the responses of tadpoles from two rice paddy locations (Daxi & Ankang) were virtually the same. Thus, cohort-dependent metamorphic strategies are likely a strong pattern across various habitats. In addition, the weather patterns of the Daxi and Sanzhi are nearly the same. Summer temperature of Sanzhi is warmer than that of Daxi only by 0.8ºC (or 3%), a minimal difference. The location effect probably does not affect the life-history of tadpoles too much, compared to the effects of irrigation regime. I believe the differential cohort-dependent metamorphic strategies between Daxi (disrupted irrigation regime) and Sanzhi (constant irrigation regime) sites were due to the difference in irrigation regimes rather than other site-specific factors. Tadpoles live in constant irrigation regime (water bamboo fields) not only have different cohort-dependent traits from those in disrupted irrigation regime (rice paddies), they do not show significant cohort-dependent metamorphic traits at all. A caveat of my study was that although tadpoles were collected from multiple fields, some tadpoles came from the same fields (or paddies) at the same time. By treating individual tadpoles as replicates I inflated sample sizes. Although the irrigation effects were strong (large effect sizes, > 20% in all cases), the results need to be interpreted with cautions.

Were the differential cohort-dependent traits from constant vs. disrupted irrigation regime habitats genetically fixed or environmentally triggered? I could

not be certain. Since I used a common garden experiment, the results seem to indicate the critical role of genetic differentiation on the differential responses. However, the tadpoles entered the experiment at the Gosner 25 stage. They had already experienced the natal habitat during pre-Gosner 25 stages, which could be enough stimuli for triggering differential responses (Kaplan and Phillips, 2006; Watkins and Vraspir, 2006). To resolve the relative importance of genetic vs. environmental influences on cohort-dependent life history traits require further studies on the genetics of life history traits.

In conclusion, the plasticity of both metamorphic weight and age are very extensive in the Indian rice frog. The highly plastic life-history traits may contribute to the wide geographic distribution of the *F. limnocharis*. My study indicates that irrigation regimes have significant effects on cohort-dependent metamorphic traits. Tadpoles from disrupted irrigation populations show, while tadpoles from constant irrigation populations do not show cohort-dependent metamorphic strategies. Many amphibians are prolonged breeders (Sinsch, 1988), different breeding cohorts would face drastically different environmental factors, such as temperature, food and predators that vary seasonally. Thus, cohort-dependent metamorphic strategy is an important topic in amphibian life history research. The current study is only one of the handful of studies that deal with this topic. More research is badly needed to further our understanding of life history strategies.

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