

# Density But Not Kinship Regulates the Growth and Developmental Traits of Chinese Tiger Frog (*Hoplobatrachus chinensis*) Tadpoles

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**Abstract** Kinship and density are believed to affect important ecological processes such as intraspecific competition, predation, growth, development, cannibalism, habitat selection and mate choice. In this work, we used Chinese tiger frog *Hoplobatrachus chinensis* tadpoles as an experimental model to investigate the effects of kinship and density on growth and development of this species over a 73 day period. The results showed that density can affect the growth and developmental traits (survival rate, larval period, size at the limb bud protrusion/metamorphic climax and body mass at different life stages) of *H. chinensis* tadpoles, while kinship does not. Tadpoles took longer to develop and potential metamorphosis was greater in high density groups of both sibling and non-siblings. The interaction of kinship and density did not significantly influenced growth traits of *H. chinensis* tadpoles during the experimental period. For coefficient variations of each growth trait, no differences were detected between sibling and non-sibling groups. These findings provide valuable information on the basic ecology of *H. chinensis* which will be helpful in future studies of other anuran species.

**Keywords** Kinship, density, intraspecific competition, growth, metamorphic climax, *Hoplobatrachus chinensis*

## 1. Introduction

How relatedness affects intraspecific competition has been discussed for a long time (Orizaola and Laurila, 2008). The intensity of intraspecific competition can affect the evolution of social behavior, reproductive ecology (including growth, survival and life history), and habitat selection in many organisms (Twomey *et al.*, 2008). However, the effect of intraspecific competition can be modified through the interaction with genetic relatedness among individuals (Orizaola and Laurila, 2008). Currently, there are two theories that predict the role of relatedness in intraspecific competition: kinship theory and heterogeneous advantage theory (Hamilton, 1964, Twomey *et al.*, 2008). According to kinship theory, we speculate that groups of closely related individuals should compete less intensely than unrelated

individuals. Behaviors that reduce aggression and interference between kins may increase an individual's inclusive fitness and therefore be favored by natural selection. For instance, an increase in density leads to an increase in competitive interactions between organisms, and changes in density are frequently associated with modifications of growth, survival, and life history, such as *Crinia georgiana* (Dziminski, 2009), *Rana arvalis* (Loman, 2001). Meanwhile, the resultant competitive interactions can be altered by environmental factors such as abiotic conditions, predation risk, or relatedness among competitors (Orizaola and Laurila, 2008). On the other hand, heterogeneous advantage predicts that the potential for exploitative competition is higher because individuals in a group will overlap more in their resource use (Sammata and Levins, 1970). Consequently, intraspecific competition is predicted to be stronger between siblings than unrelated individuals (Twomey *et al.*, 2008).

Larval amphibians have been widely used as an idea model system for studying the effects of relatedness and density on individual fitness since they often form aggregations that may be composed of kins or non-

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kins, and larval success is often dependent on the density (Waldman, 1991; Wilbur, 1977). Although larval amphibians may preferentially link to kinship, the adaptive significance of this behavior varies dramatically depending on the species or experimental conditions (Halverson *et al.*, 2006). There is strong evidence that some species showed their individuals reared in sibling groups grew faster and have a larger mass at metamorphosis than those reared in mixed groups. Previous studies supported kin selection theory in *Bufo melanostictus* (Saidapur and Girish, 2001), *R. catesbeiana* (Smith, 1999), and *Scaphiopus couchii* (Newman, 1988). These findings also discovered higher variations of growth in sibling groups than in mixed groups. However, some studies have found opposite results showing certain species grow better in mixed groups, such as *R. cascadae* (Hokit and Blaustein, 1994; Hokit and Blaustein, 1997), *R. temporalis* (Girish and Saidapur, 2003) and *B. melanostictus* (Saidapur and Girish, 2001). These studies thus supported the heterogeneous advantage hypothesis, while other studies have found no significant differences in growth between sibling and mixed groups such as *Ambystoma opacum* (Walls and Blaustein, 1995), *Tomopterna breviceps* (Gramapurohit *et al.*, 2004) and *Ameerega bassleri* (Twomey *et al.*, 2008).

The Chinese tiger frog, *Hoplobatrachus chinensis*, is usually found in farmland across south and southeast Asia. It has been recorded in most Chinese provinces (Fei *et al.*, 2009). The breeding season lasts from April to September. The egg clutches of *H. chinensis* are laid scattered granular ranging from 300 to 4000 at the edges of shallow, slow-flowing streams, or farmland. In our laboratory, we observed that *H. chinensis* tadpoles showed loosely structured aggregations often consisting of fewer number than the clutch size, and cannibalism was found within sibling groups and mixed groups. Hence, *H. chinensis* is an ideal organism to investigate the possible significance of social interaction on growth and metamorphosis of its tadpoles. In the present study, we examined the effects of sibling versus non-sibling interactions and the density of rearing on growth and metamorphosis in *H. chinensis* tadpoles.

## 2. Materials and Methods

We captured five pairs of amplexing adults to collect eggs from artificial pond at the herpetological laboratory of Lishui University at night 25 May 2012. We carefully placed them into opaque plastic cages (60 cm length  $\times$  40 cm width  $\times$  30 cm height) individually, with 15 cm–20

cm depth of dechlorinated tap water, respectively. The following morning, we took the parents from the cages and hatched the eggs following outdoor temperatures, while adding water to a level of 30 cm in depth. Small tadpoles from each clutch hatched out in two days. All the tadpoles were 25 stages temporality and their body sizes were similar (Gosner, 1960).

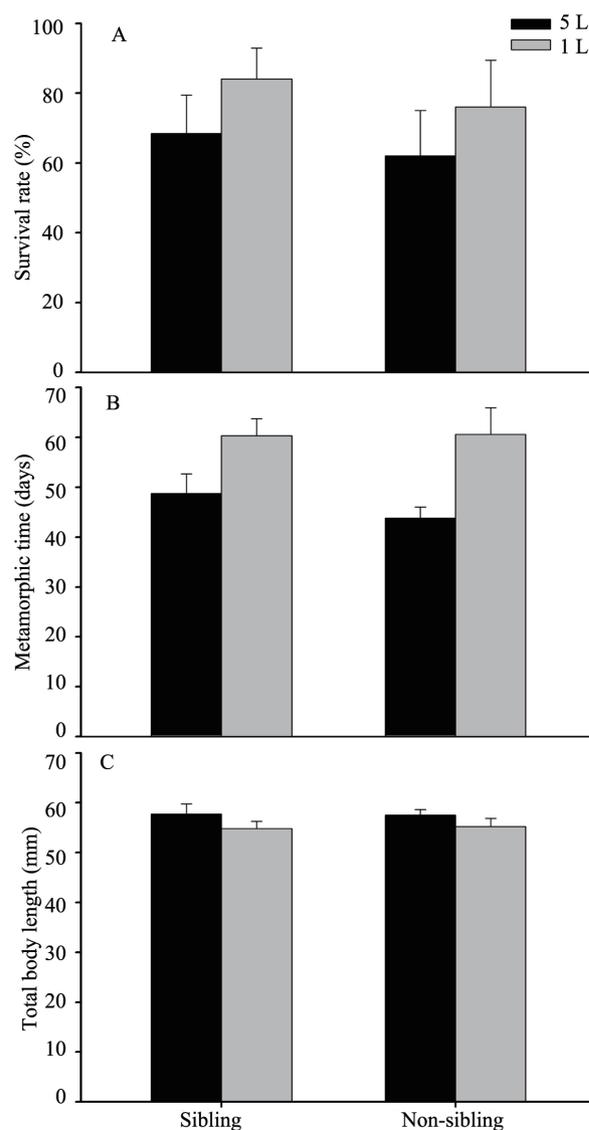
To investigate the effects of kinship and density on the mean and variation in growth and development traits of larval *H. chinensis*, we conducted 2 (kinships)  $\times$  2 (densities) factorial design experimental treatments. Kinships were comprised of sibling groups (high-relatedness) and non-sibling groups (low-relatedness), whereas density included low density and high density following the methods described by Hokit and Blaustein (1994), Girish and Saidapur (1999), Orizaola and Laurila (2008). Therefore, four experimental treatments were conducted in this study: (1) low density sibling group; (2) high density sibling group; (3) low density non-sibling group; (4) high density non-sibling group. Sibling groups were comprised of 10 tadpoles pooled from one egg clutch/parent line, while non-sibling groups were comprised of 10 tadpoles that mixed two tadpoles from the five different clutches, respectively. Thus any tadpole had only one sibling present in each treatment and it was presumed to be unrelated to the other eight tadpoles in non-sibling group. Subsequently, we assigned 10 tadpoles to each corresponding experimental treatment. All tadpoles were raised in small opaque plastic boxes (30 cm length  $\times$  20 cm width  $\times$  10 cm height) individually, with 5 liters and 1 liter of dechlorinated tap water for low density (10 tadpoles/5L) and high density (10 tadpoles/1L), respectively. The sibling groups and non-sibling groups were replicated five times respectively. We positioned all the boxes on the outdoor ground under natural conditions. We randomly assigned box positions and made no marking to indicate kinship composition to remove directional effect. The tadpoles in each box were reared with commercial fish meal (Shanghai Tech-bank feed industry Co. LTD). Each box was checked every day, with the dead being removed. The water was changed every 2 days. Because it was easy to cause mortality for new hatchling tadpoles, we did not directly measure body mass. As a result, the first time of measuring body mass was at day 10 after the experiment was initiated, while day 20, day 30 and day 40 were for the second, third and fourth time, respectively. When a tadpole's one or two forelimbs (metamorphic climax point, Gosner 42) begin to emerge, it was considered to have reached a critical step in metamorphosis and its body mass, total body

length and larval period were measured and recorded. At the day that all the surviving tadpoles emerged their one or two forelimbs, we terminated the experiment. Subsequently, we counted the numbers of metamorphic tadpoles in each cage.

Prior to using the growth data, all data were tested for normality and homogeneity of variances using the methods of Kolmogorov-Smirnov test and Bartlett test. We first conducted a MANOVA for all growth indices at kinship, density and their interactions, respectively. Then for each index and its CV values comparison, we used Two-way ANOVA. All statistics were performed in SPSS 16.0 for Windows Software. Descriptive data were expressed as Mean  $\pm$  SD, and the significance level ( $\alpha$ ) was set at 0.05.

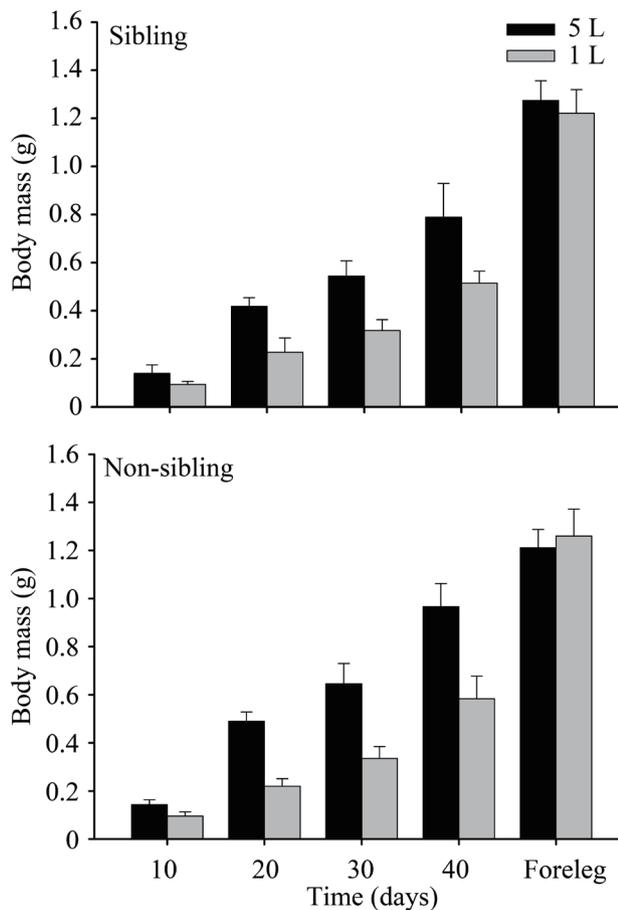
### 3. Results

The whole experimental period lasted for 73 days. For the five parent lines reared in isolation, we found metamorphic traits including body mass, body length and larval period showed no significant variation in both high-density and low-density treatments (all  $P > 0.05$ ). Density but not kinship can affect the growth and developmental traits of tiger frog tadpoles (MANOVA, Table 1). Density levels significantly influenced survival rate, duration of larval period, total body length at forelimb emerged (metamorphic climax, Gosner 42) and body mass from day 10 to day 40, but did not significantly influenced the body mass at forelimb emerged (ANOVA, Table 1). Tadpoles took longer to develop and proportionally more potential metamorphs were produced at higher densities in both sibling and non-sibling groups (Figure 1). Total body length of the potential metamorphs (Gosner 42) in low density treatments were larger than those in high density treatments (ANOVA, table 1). Body mass at all measuring points of tadpoles produced in low density treatments were heavier than those in high density treatments during the experimental period, except with the metamorphic body mass (mass at foreleg emergence) produced from non-sibling group (Figure 2). No significant differences were found between sibling and non-sibling groups with respect to survival rate, duration of larvae period, total body length and body mass (ANOVA, Table 1). Likewise, the interaction between kinship and density did not significantly influenced survival rate, duration of larval period, body length and body mass of tiger frog tadpoles during the experimental period (MANOVA, ANOVA, Table 1).



**Figure 1** Survival rate (A), metamorphic time (B) and total body length (C) of metamorphs produced per box for sibling group and non-sibling group at both low (black bars) and high density (grey bars).

Coefficient variations ( $CVs$ ) of metamorphic time, total body length at metamorphic climax (Gosner 42) and body mass on different life stages (d10, d20, d30, d40 and days of foreleg emergence) were lower in high-density of sibling group than the remaining three groups. No differences between sibling and non-sibling groups were identified for all  $CV$  values (MANOVA, ANOVA, Table 1). However, there were strong effects of density on  $CVs$  of body mass at metamorphic time, the interactions of kinship and density on  $CVs$  of metamorphic time and total body length at metamorphic climax, respectively (ANOVA, Table 1).



**Figure 2** The body mass on day 10, day 20, day 30, day 40 and metamorphic climax point (fore limb emergence) measured from sibling (upper) and non-sibling (lower) group at both low (black bars) and high density (grey bars), respectively.

#### 4. Discussion

Studies on the effects of intraspecific competition (relatedness or unrelatedness) on metamorphic traits (such as survival rate, duration of larval period, body length and mass) can provide insights into the evolution and maintenance of kin selection in anurans (Orizaola and Laurila, 2008). In the present study, no significant variation in growth of the five parental lines was found whether tested in high-density (1L) or low-density (5L). Thus, we predict that *H. chinensis* tadpoles reared in isolation revealed no genetic effect on larval growth traits. If it exists, genetic effect may be expressed in tadpoles when they are exposed to different social situations such as association exclusively with siblings or with non-kins (Gramapurohit *et al.*, 2004). These results were in agreement with previous studies on *Rana temporalis* (Girish and Saidapur, 1999) and *Bufo melanostictus* (Saidapur and Girish, 2001). In other species, such as *R. arvalis*, *R. catesbeiana* and

*Scaphiopus couchii*, significant variations were found in the growth, development and survival rates of tadpoles of different sibships when reared in groups under different conditions (Newman, 1988; Smith, 1999). Thus, variation in the metamorphic traits of tadpoles among sibships may depend upon species (Gramapurohit *et al.*, 2008).

Effects of kinship on larval development may vary depending upon the species as well as in the context or environmental conditions (Pakkasmaa and Laurila, 2004). Previous studies on *Rana temporalis* (Girish and Saidapur, 1999), *B. melanostictus* (Saidapur and Girish, 2001) and *R. catesbeiana* (Smith, 1999) reared in sibling groups showed uniform growth and bigger size at metamorphosis. Furthermore, size of the individual tadpoles for the three species in sibling groups were less variable than those of mixed groups, indicating a positive influence of kinship on larval growth and metamorphic traits. In contrast, tadpoles of *Rana cascadae* (Hokit and Blaustein, 1997), *R. temporalis* (Girish and Saidapur, 2003), and *R. arvalis* (Shvarts and Pyastolova, 1970) reared in non-sibling treatments grew better than sibling groups. Even in some other species such as *Tomopterna breviceps* (Gramapurohit *et al.*, 2004), *Rana lessonae* (Orizaola and Laurila, 2008) and *Bufo scaber* (Twomey *et al.*, 2008), there were no significant effects of kinship on growth and metamorphosis. In our study, the effects of kinship on growth and development traits were not significant at both low-density and high-density. *H. chinensis* grew in low density conditions were larger than those grew in high density during the experiment period except for body mass at limb bud protrusion (metamorphic climax) produced from high-density mixed groups. Thus, low density conditions may lead to reduced competition resulting in faster development.

Larval period and size at metamorphosis were two main metamorphic traits for amphibian species. Natural selection may favor either of the two traits depending upon the ecological context (Twomey *et al.*, 2008). In general, species inhabiting semipermanent or permanent habitats may attain larger body size at metamorphosis and take longer time for larval period than those grow in ephemeral conditions, but they also undergo many pressures from a large number of predators in complex environments. For individuals growing in ephemeral conditions, water resources are the primary ecological factor. Because larvae should be completely metamorphosed in short time so that they can reduce mortality prior to the pond desiccation. As a result, they can metamorphose earlier at the cost of relative smaller size (Gramapurohit *et al.*, 2008; Tejedo

**Table 1** Results of MANOVA for overall effects of kinship, density, and kinship  $\times$  density interactions and of ANOVAs for each response variable and its coefficient variation (*CV*) within each of the main effects. Response variables are proportions of survival numbers on day 40 (survival rate), total body length and mean duration of up to metamorphosis (larval period/metamorphic time), and mass at different time recorded (day 10, day 20, day 30, day 40 and forelimb emergence). Note: \*indicated significant differences at  $P < 0.05$ .

MANOVA(Wilks $\lambda$ )		Response variables			<i>CV</i> of response variables		
		<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
	Kinship	8, 9	1.26	0.365	7,10	2.29	0.114
	Density	8, 9	28.73	< 0.001*	7,10	2.64	0.080
	Kinship $\times$ Density	8, 9	1.91	0.178	7,10	1.45	0.288
ANOVAS							
Survival rate	Kinship	1, 16	1.88	0.190			
	Density	1, 16	7.93	0.012*, L < H			
	Kinship $\times$ Density	1, 16	0.02	0.881			
Larval period	Kinship	1, 16	1.75	0.205	1, 16	0.01	0.921
	Density	1, 16	65.67	< 0.001*, L > H	1, 16	1.23	0.283
	Kinship $\times$ Density	1, 16	2.16	0.161	1, 16	4.93	0.041*
Total body length	Kinship	1, 16	0.01	0.904	1, 16	4.27	0.055
	Density	1, 16	13.09	0.002*, L > H	1, 16	2.40	0.141
	Kinship $\times$ Density	1, 16	0.21	0.650	1, 16	8.07	0.012*
Body mass on d10	Kinship	1, 16	0.049	0.827	1, 16	2.22	0.156
	Density	1, 16	20.82	< 0.001*, L > H	1, 16	1.63	0.220
	Kinship $\times$ Density	1, 16	0.0020	0.965	1, 16	0.23	0.640
Body mass on d20	Kinship	1, 16	2.771	0.115	1, 16	0.25	0.627
	Density	1, 16	144.94	< 0.001*, L > H	1, 16	0.90	0.356
	Kinship $\times$ Density	1, 16	4.36	0.053	1, 16	0.09	0.767
Body mass on d30	Kinship	1, 16	4.27	0.055	1, 16	0.76	0.395
	Density	1, 16	91.89	< 0.001*, L > H	1, 16	0.83	0.377
	Kinship $\times$ Density	1, 16	2.15	0.162	1, 16	0.15	0.706
Body mass on d40	Kinship	1, 16	7.47	0.015*, L < H	1, 16	0.56	0.465
	Density	1, 16	53.58	< 0.001*, L > H	1, 16	0.25	0.625
	Kinship $\times$ Density	1, 16	1.47	0.242507	1, 16	0.20	0.664
Body mass at metamorphosis (foreleg)	Kinship	1, 16	0.08	0.777	1, 16	0.01	0.927
	Density	1, 16	0.003	0.957	1, 16	5.40	0.034*, L < H
	Kinship $\times$ Density	1, 16	1.51	0.237	1, 16	1.31	0.269

*et al.*, 2010). In this study, we found that larval period, body mass and size at metamorphic climax (Gosner 42) of *H. chinensis* tadpoles were shorter, heavier and larger in low-density groups of both sibling and mixed groups than those reared in high-density groups. Interestingly, we found *CVs* produced from high-density treatments were lower than low-density treatments. This may be caused by cannibalism in *H. chinensis* during premetamorphic stages. Thus, in *H. chinensis* low density conditions may lead to reduced competition resulting in faster development. By contrast, in high-density treatments, individuals from different parent lines may mutually suppress and grow at uniform size under higher competition condition, and more individuals survived and metamorphosed eventually. Thus, the effects of kinship,

group composition and density on larval history traits may vary with ecological context (Sadeh, 2012).

In summary, our present study indicated that the density strongly influenced the growth and development traits of *H. chinensis*. Resources at different densities and species life habits should more or less affect growth of some individuals. Uniform growth and later metamorphosis seen in high-density groups of *H. chinensis* may be attributed to intraspecific competition. Although gregarious behaviours were observed in different life stages of larvae, subadult or adult period, kinship of *H. chinensis* is not believed to play an important role in the regulation of growth, development, and survival of individuals by equally sharing the resources like food and space. Because *H. chinensis* is

carnivorous, and cannibalism is often found within this species including sibling and/or nonsibling groups at different life stages. Therefore, for economic breeding of *H. chinensis*, we should control individuals at optimal breeding density.

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