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Role of photoperiod, temperature and food on development of *Polypedates teraiensis* (Dubois, 1987) tadpoles

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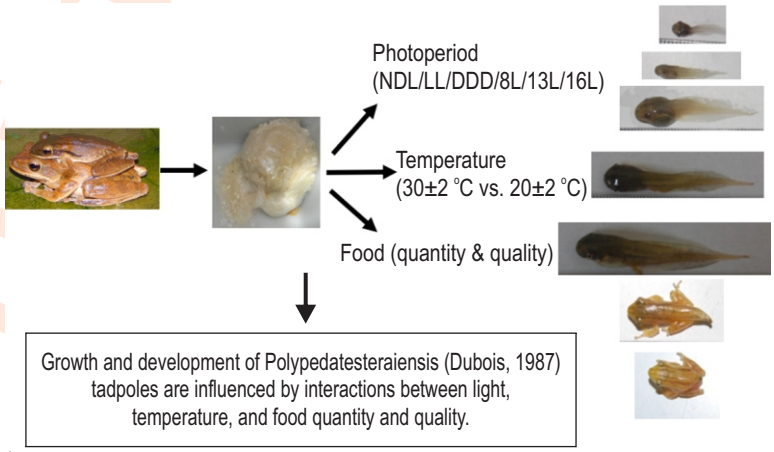
Abstract

Aim: To determine the effect of environmental factors on the growth and development of *Polypedates teraiensis* (Dubois, 1987) tadpoles.

Methodology: The study was conducted in two phases. In the first study effect of light on tadpole growth and development was conducted. Experiment No. 1 examined the effects of natural light (NDL), continuous light (LL), and constant dark (DD). Experiment No. 2 investigated the role of day length by exposing three groupsof tadpoles to either a short photoperiod (8L:16D) or a long photoperiod (13L:11D and 16L:8D). Experiment No. 3 examined the effects of light quality on tadpoles by exposing them to either short wave length (450 nm; blue light), long wavelength (650 nm; red light), or white light at equal intensity (0.45 W m^2) under an equinox photoperiod (12L:12D). In the second study, the effects of food quantity and quality, as well as temperature (30±2°C vs. 20±2°C) was calculated on tadpole growth and development.



Results: DD photoperiod produced the fastest growth,but long photoperiods (13L:11D and 16L:8D) delayed the growth when compared to short photoperiods (8L:16D). Furthermore, although long wavelength (650 nm; red light) delayed growth, short wave length (450 nm; blue light) speed it up. Food restriction delayed the growth and development, with 4hr group growing at a slower rate than the 12hr group. A protein-rich food combined with high temperature (30±2°C) accelerated growth. Taken together, these findings suggest that interactions among environmental factors may affect anuran development and metamorphosis.

Interpretation: These findings can potentially improve amphibians' captive breeding programs and aid tools for amphibian conservation.

Key words: Food, Photoperiod, *Polypedates teraiensis*, Tadpoles, Temperature

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Introduction

The frog's life cycle is complex in comparison to other vertebrates, with an intermediate larval stage called a 'tadpole,' which feeds and metamorphoses. The timing of metamorphosis is a critical fitness component that can be influenced by a wide range of environmental factors. Seasonal variations in photoperiod, temperature, humidity, rainfall, food availability, altitudinal and latitudinal gradients, and other environmental and geographical factors often leads to genetic clines in growth and development rates (Conover and Schultz, 1995; Reed, 2005; Bekhet *et al.,* 2014; Strong *et al.,* 2017, Weerathunga and Rajapaksa, 2020; Kikuyama *et al.,* 2021). The photoperiod, wavelength, and intensity of light all have an effect on species' behavior and physiology (Kumar *et al.,* 2000). For example, when various mammals are deprived of light or exposed to a particular visual field during an early and critical developmental period, their growth is abnormal (Grobstein and Chow, 1975). Similarly, occlusion of one eye reduces precision in *Xenopus* tadpoles near metamorphosis, while rotation of one eye changes the initial development of connections between that eye and ipsilateral visual tectum (Keating, 1977). When tadpoles are deprived of light for the first 10 days of development, their light preference decrease; however, when tadpoles are exposed to light throughout their first 10 days of development, their light preferences increase (Copp and McKenzie, 1984).

Amphibian life-history traits are greatly influenced by ecological variables such as temperature and hydric conditions, which have a significant impact on their energy consumption, activity patterns, and the onset of critical life-history states such as hibernation, breeding, and development (Gao *et al.,* 2015). Because anurans lack an effective physiological thermo regulation mechanism, temperature may be the most important proximal driver of size and age variations during metamorphosis. Low temperatures cause differentiation to be slowed down, resulting in an increase in stage-specific sizes (Smith-Gill and Berven, 1979). Also, the quality and quantity of food accessible during the larval stage can affect the timing and rate of metamorphosis (Leips and Travis, 1994). Here, we investigated the effect of environmental factors (light, food, and temperature) on the growth, development, and metamorphosis of tadpoles of *P. teraiensis* (Dubois, 1987) under laboratory conditions due to a dearth of study on the subject.

Materials and Methods

The Institutional Animal Ethics Committee approved all experimental protocols for this study. A newly laid foam nest recovered from a natural pond within the Mizoram University Campus in Aizawl, Mizoram (23^0N, 92^0E), was allowed to hatch in the laboratory. All experiments were performed on day one hatched tadpoles. Tadpoles were kept in a clean and easy-to-manage polypropylene cage (size 28.5 x 22.1 x 14 cm) and fed boiled cabbage (*Brassica oleracea*) *ad libitum* unless otherwise mentioned. The temperature of the room was maintained at 24±2 $^{\circ}$C unless otherwise mentioned. The water was changed every day. An individual tadpole's body mass was measured with a top pan balance to an accuracy of 0.01g, while body length, tail length, interorbital distance (IOD), internarial distance (IND), and maximum tail height (MTH) were measured with a Vernier Calliper to an accuracy of 0.01mm at ten-day interval.

Study on the effect of photoperiod on growth and development of tadpoles

Effect of natural light, continuous light, and continuous dark: Day one old tadpoles were divided into three groups (N=30 each group). Group 1 was exposed to NDL (natural day length conditions receiving sunlight), Group 2 to continuous light LL (LL-bright light; light intensity 300 ± 10 lux was delivered using compact fluorescent tubesfrom Phillips, India), and Group 3 to constant dark DD.

Effect of light duration: Day one old tadpoles were divided into three groups (N=30 each group). The first group was exposed to a short photoperiod (8L:16D; SD), the second to a long photoperiod (13L:11D; LD), and the third to an even longer photoperiod (16L:8D; LD). All groups received light intensity of 300 ± 10 lux at the level of the bottom of a cage.

Effect of light spectrum: Day one old tadpoles were divided into three groups (N=30 each group). All groups hadthe same photoperiod (12L:12D) and intensity (0.45 W m^2 at floor level of the cage), but different wavelengths of light. The first group was exposed to a short-wavelength (blue light; 450 nm), the second to a long-wavelength (red light; 640 nm), and the third to normal light as a control. A Q203 quantum radiometer (Macam Photomatrix Ltd., Scotland, U.K.) was used to measure the intensity of light. The desired wavelength was achieved by using monochromatic filters (Supergel Rosco, Germany) and covering black pastel sheet papers (Borah *et al.,* 2018).

Study on the effect of non-photic cues on the development of tadpoles

Effect of timed food availability: Day one old tadpoles were divided into nine groups (N=20 each group). All groups were exposed to 12L:12D photoperiod and were fed freshly chopped boiled cabbage. Group 1 was fed ad libitum, Group 2 was fed for 12hr during the light phase, Group 3 was fed for the first 4hr of light phase, Group 4 was fed for 4hr during the middle of the day, Group 5 was fed for last 4hr of the day, Group 6 was fed for 12hr during dark hours, Group 7 was fed for first 4hr of dark phase, Group 8 was fed for 4hr during the middle of dark phase, and Group 9 was fed for last 4hr of dark phase.

Effect of food quality: Day one old tadpoles were divided into two groups (N=20 each group) and exposed to a 12L:12D photoperiod. The first group was fed freshly chopped boiled cabbage on daily basis, whereas the second group was fed protein-rich food consisting of 35% crude protein, 3% crude fiber, and 4% of crude fat (Hello pets, Maharashtra aquarium, Mumbai, India).

Effect of temperature: Day one old tadpoles were divided into two groups (N= 30 each) and exposed to a 12L:12D photoperiod. Group 1 was exposed to a higher temperature (30±2°C) whereas Group 2 was exposed to a lower temperature (20±2°C).

Data analysis: Data are represented as mean and standard error (Mean± SE). One-way ANOVA followed by Newman–Keuls test was used to find out significant differences in the body weight, body length, tail length, IOD, IND, and MTH within the group. Two-way ANOVA followed by Bonferroni post hoc test was employed to compare two factors: the effects of photoperiodic conditions (factor 1) and time of day (factor 2) on different parameters studied. The significance level was set at $P < 0.05$.

Results and Discussion

The light conditions influenced the growth of tadpoles, with DD group developing fastest, followed by NDL, and the LL group developing slowest (Fig. 1a-f). Under DD, by week six, froglet formation had began, and 11% of tadpoles had metamorphosed,81% by week seven and 100% by week nine. In NDL, 19% of froglets metamorphosed by week six, 50% by week seven, and 100% by week 10 (Fig. 1g). Under LL, the metamorphosis was delayed and took 13 weeks to complete (Fig. 1g). Tadpoles grew and developed in both short (8L:16D) and long photoperiods (13L:11D and 16L:8D) ($P < 0.0001$; One-way ANOVA; Fig. 2a-e). The fastest development was observed undershort photoperiod (8L:16D), while the slowest development was observed under long photoperiod (16L:8D). Under 8L:16D, just 8% of tadpoles had forelimbs by week five, and it took eight weeks for all tadpolesto develop forelimbs (Fig. 2f).

Under 13L:11D,only 20% of the tadpoles had forelimbs by week six, and all tadpoles had forelimbs by week 12. In 16L:8D,only 21% of tadpoles had forelimbs by week six, while it took 13 weeks for all tadpoles to develop forelimbs (Fig. 2f). Under 8L:16D,15% of tadpoles had metamorphosed into froglets by week six, and by week nine, 100% of tadpoles had metamorphosed into froglets. Under 13L:11D, only 3% of froglets had metamorphosed, and it took 13 weeks to complete the metamorphosis. Under 16L:8D, development was significantly slower, with just 21% of froglets developing by week seven, and completing growth by 14 weeks (Fig. 2g). Tadpoles exposed to blue, red, and white light had a significant change in growth over time ($P < 0.0001$; One-way ANOVA; Fig. 3a-e). However, light quality (light wave length) affected the growth of tadpoles ($P < 0.0001$; Two-way ANOVA; Fig. 3a-e). The fastest development was observed in blue light group, followed by the red light group, while the slowest development was recorded in the white light group. Development of forelimbs was fastest in the blue light group, with forelimbs emerging in 48% of the population by six weeks, and in 100% of tadpoles by nine weeks (Fig. 3f). However, growth was delayed under red light,and by six weeks, only 42% of the population had forelimbs, while it took ten weeks for all tadpoles to develop the forelimbs (Fig. 3f). White light produced the slowest growth, withonly 32% of tadpoles developing forelimbs in six weeks, and 100% developing forelimbs in twelve weeks (Fig. 3f). Froglet formation was fastest in the blue light group, starting at six weeks and completing at ninth week, followed by the red light group, where it began at seven weeks and completed at twelve weeks (Fig. 3g). Both the quantity and quality of light affected the growth and development of *P. teraiensis* tadpoles. Development and metamorphosis were faster under DD conditions whereas they were slowest under LL conditions. A delay in the growth under continuous light conditions could result from the loss of melatonin rhythm.

Although melatonin levels were not messured in this study, Trivedi and Kumar (2014) suggested that extended exposure to bright light inhibits melatonin production Several other studies have also suggested a functional link between melatonin and offspring growth. For example, tadpoles of *Xenopus laevis* raised in constant light were significantly smaller than those raised in constant darkness or 12L:12D, whilst those raised in melatonin water showed delayed growth (Delgado *et al.,* 1987). Similarly, in *Carassius auratus*, pinealectomy decreased the growth rate, while melatonin treatment accelerated growth and weight (De Vlaming, 1980). Male offspring's physical features, neuro development, and cognition were all delayed in rats deprived of maternal melatonin during gestation and lactation (Motta-Teixeira *et al.,* 2018). Our findings support the idea that the quality of light (light spectrum) has an effect on growth and development rates, with blue light promoting the fastest growth, followed by red and white light, which is consistent with the findings in *Rhacophorus maximus* (Borah *et al.,* 2018). Joshi and Mohinuddin (2003) demonstrated the effect of light spectrum on the development of *R. cyanophlycti*s, with white light being more effective than red light. However, the above-cited study examined development solely in the presence of red and white light conditions.

The anuran amphibians, *Xenopus laevis*, *Rana catesbeiana, Rana nigromaculata*, and *Bufojaponica* have all been found to have opsin-like immunore activities (Okano *et al.,* 2000). Currie *et al.* (2016) also demonstrated that *Xenopus laevis* larvae deep brain photoreceptors are sensitive to short wavelengths. It has been established that endocrine mechanisms play a role in mediating evolutionary changes in metamorphic life-history strategies. Variation in light quality may alter the thyroid axis, primary endocrine regulator of metamorphosis, mechanistically (Buchholz *et al.,* 2011). Thyroid axis consists of a series of central regulators that mediate the production and release of thyroid hormone from thyroid gland, as well as peripheral regulators that mediate tissue-specific responses to circulating hormone (Buchholz *et al.,* 2011). Changes that affect metamorphic timing can occur at any level of thyroid axis, and evolutionary changes at one level will often have impact on other levels. Tadpoles grew over time under all food conditions ($P < 0.0001$; One-way ANOVA; Figs. 4, 5). However, the timing of food availability had an effect on tadpole growth and development ($P < 0.0001$; Two-way ANOVA). Growth was faster in the *ad libitum* group compared to 12hr food ($P<0.05$; Bonferroni test; Figs. 4 and 5). The response to 12hr food

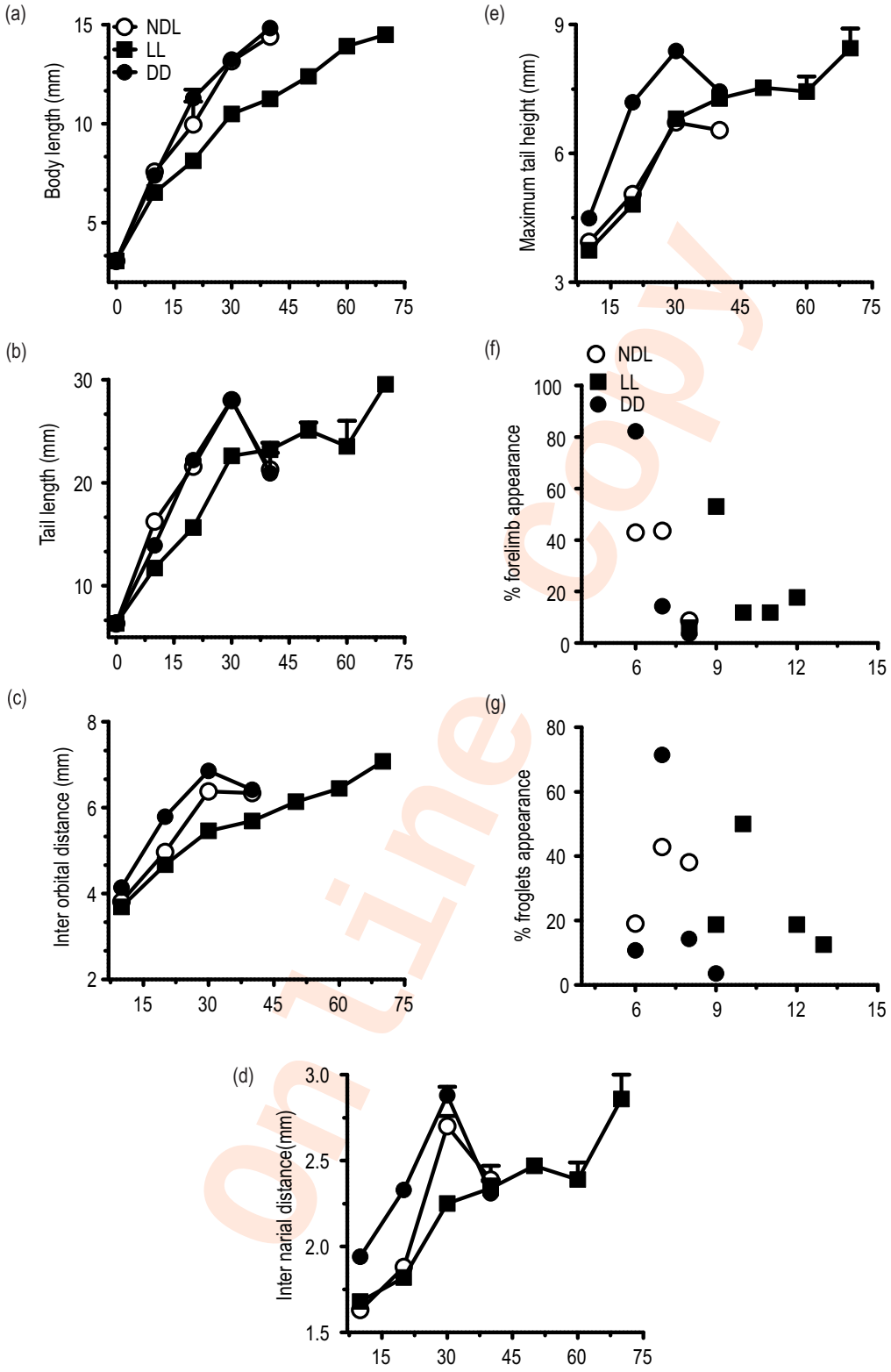

Fig. 1: Development and metamorphosis of *Polypedates teraiensis* tadpoles under NDL (natural day length but captive conditions, hallow circle), DD (continuous dark, filled circle) and LL (continuous light, filled rectangle) light conditions. Fig. 1f and g represents percent forelimb and froglet metamorphosed.

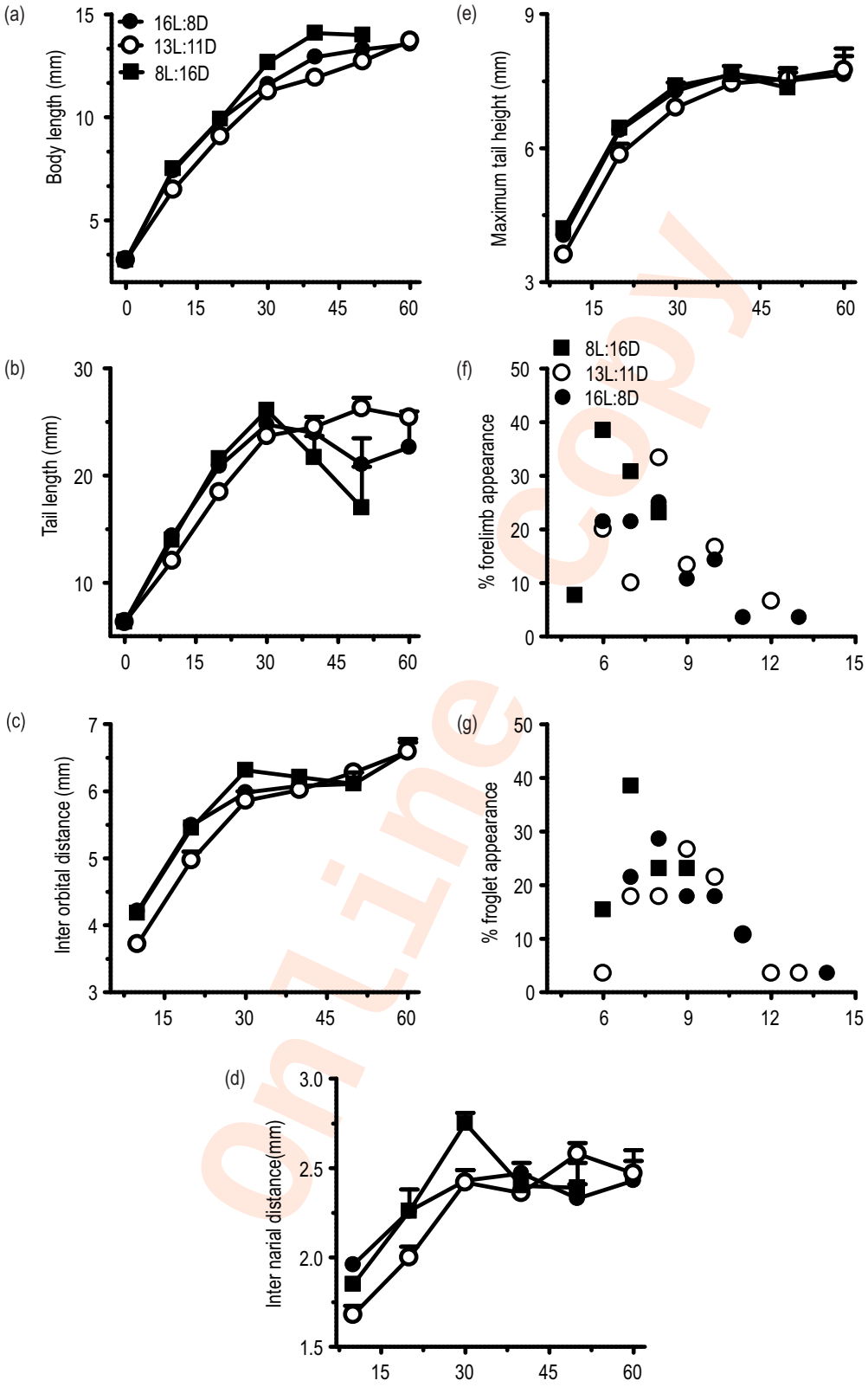

Fig. 2: Development and metamorphosis of *Polypedates teraiensis* tadpoles under 8L:16D, 13L:11D, and 16L:8D light conditions. Fig. 2f and g represents percent forelimb and froglet metamorphosed.

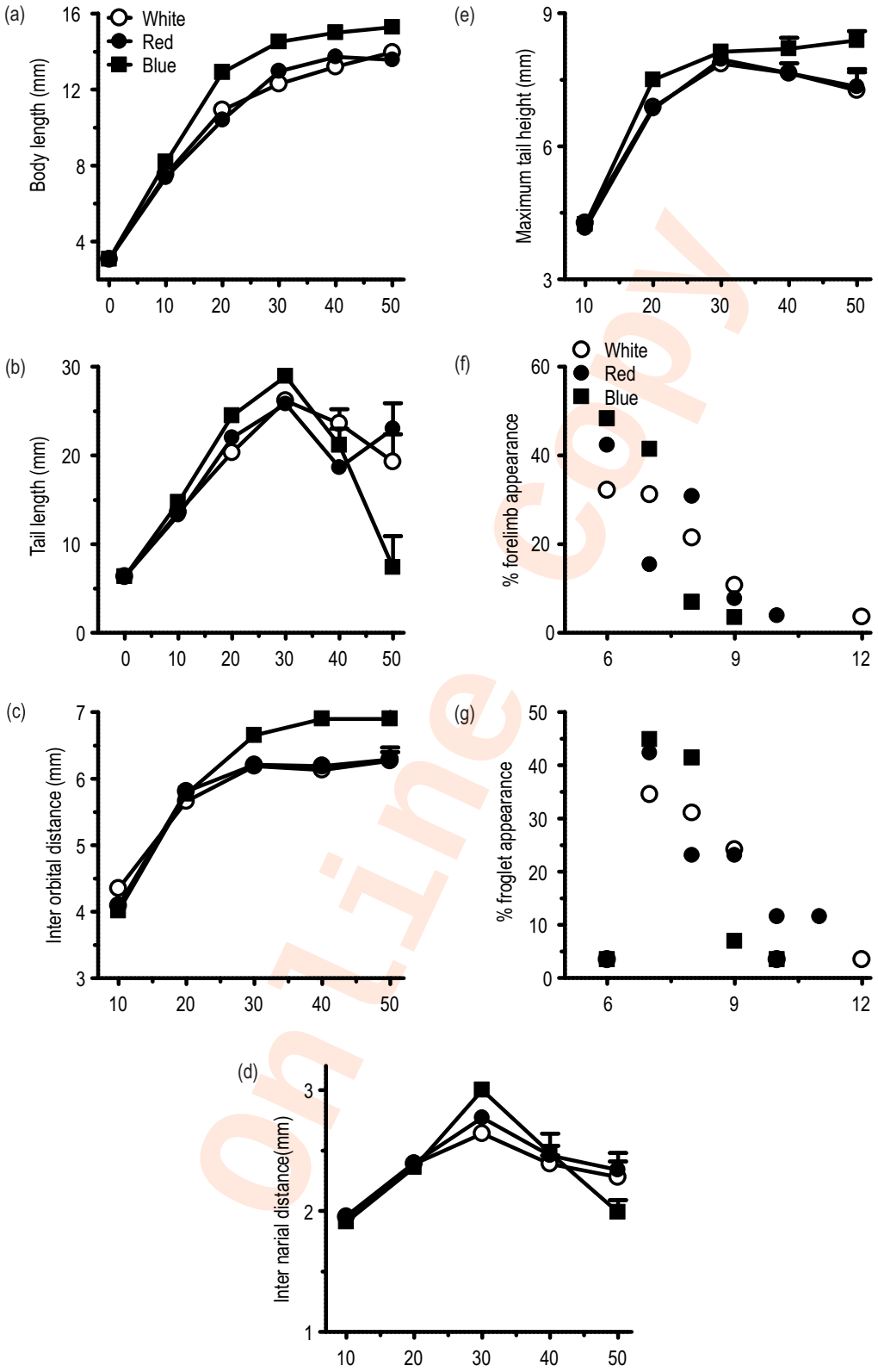

Fig. 3: Development and metamorphosis of *Polypedates teraiensis* tadpoles under white light (hallow circle), red light (filled circle) and blue light (filled rectangle) conditions. Fig. 3f and 3g represents percent forelimb and froglet metamorphosed.

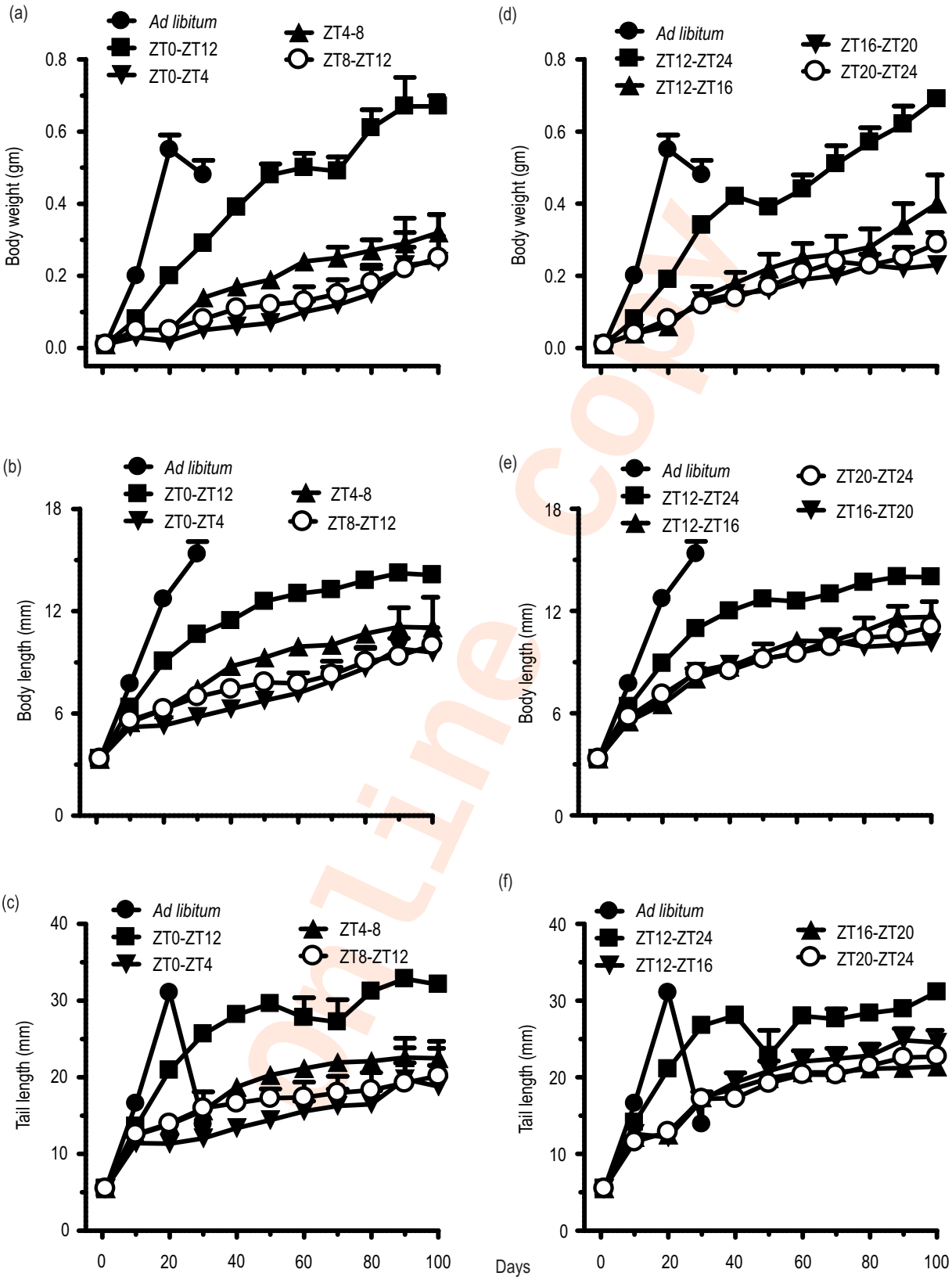

Fig. 4: Changes in body weight (a,d), body length (b, e), tail length (c, f), of *Polypedates teraiensis* tadpoles during different food cycles.

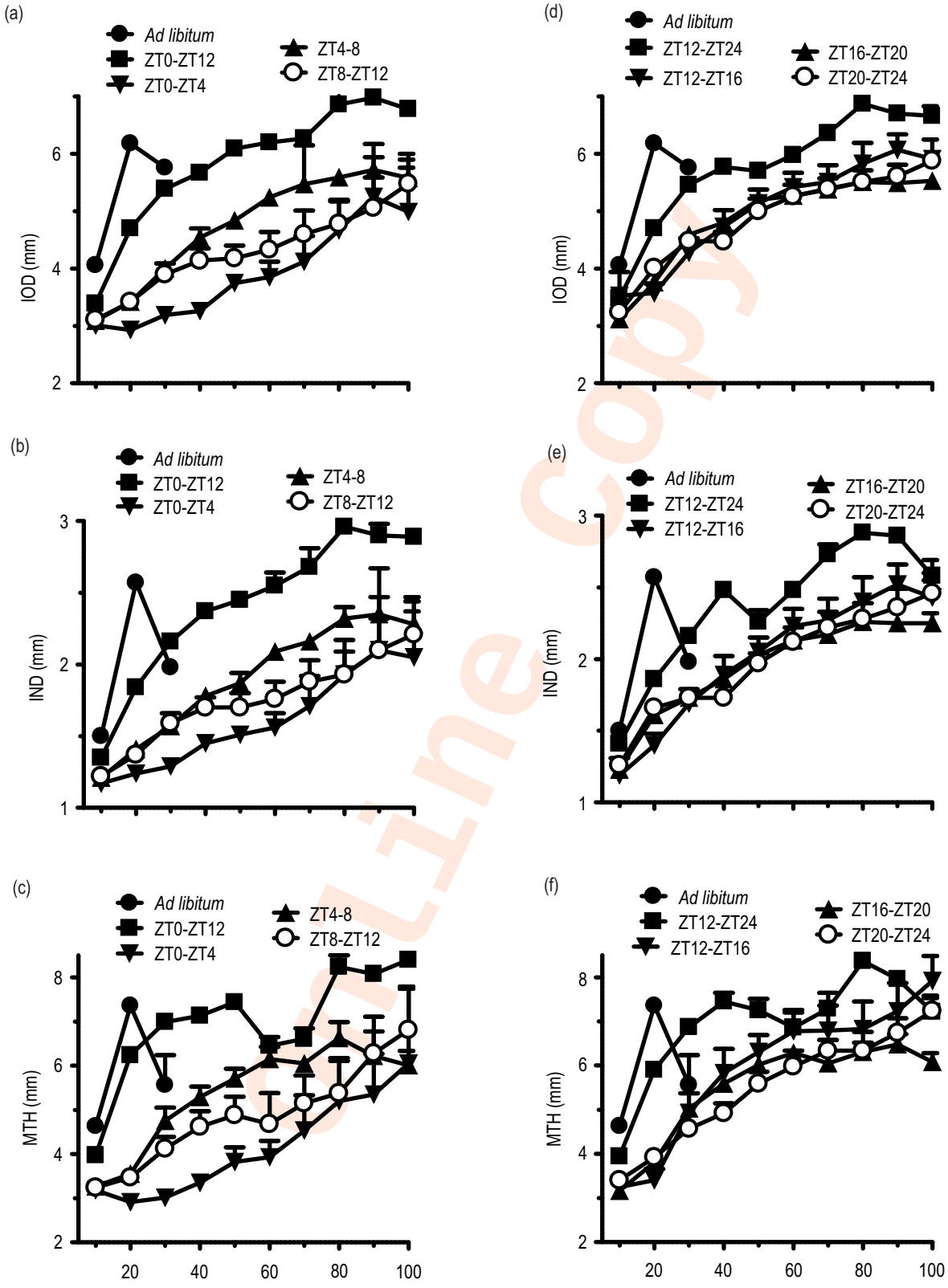

Fig. 5: Changes in interorbital distance (a,d), internarial distance (b,e) and maximum tail height (c,f) of *Polypedates teraiensis* tadpoles during different food cycles.

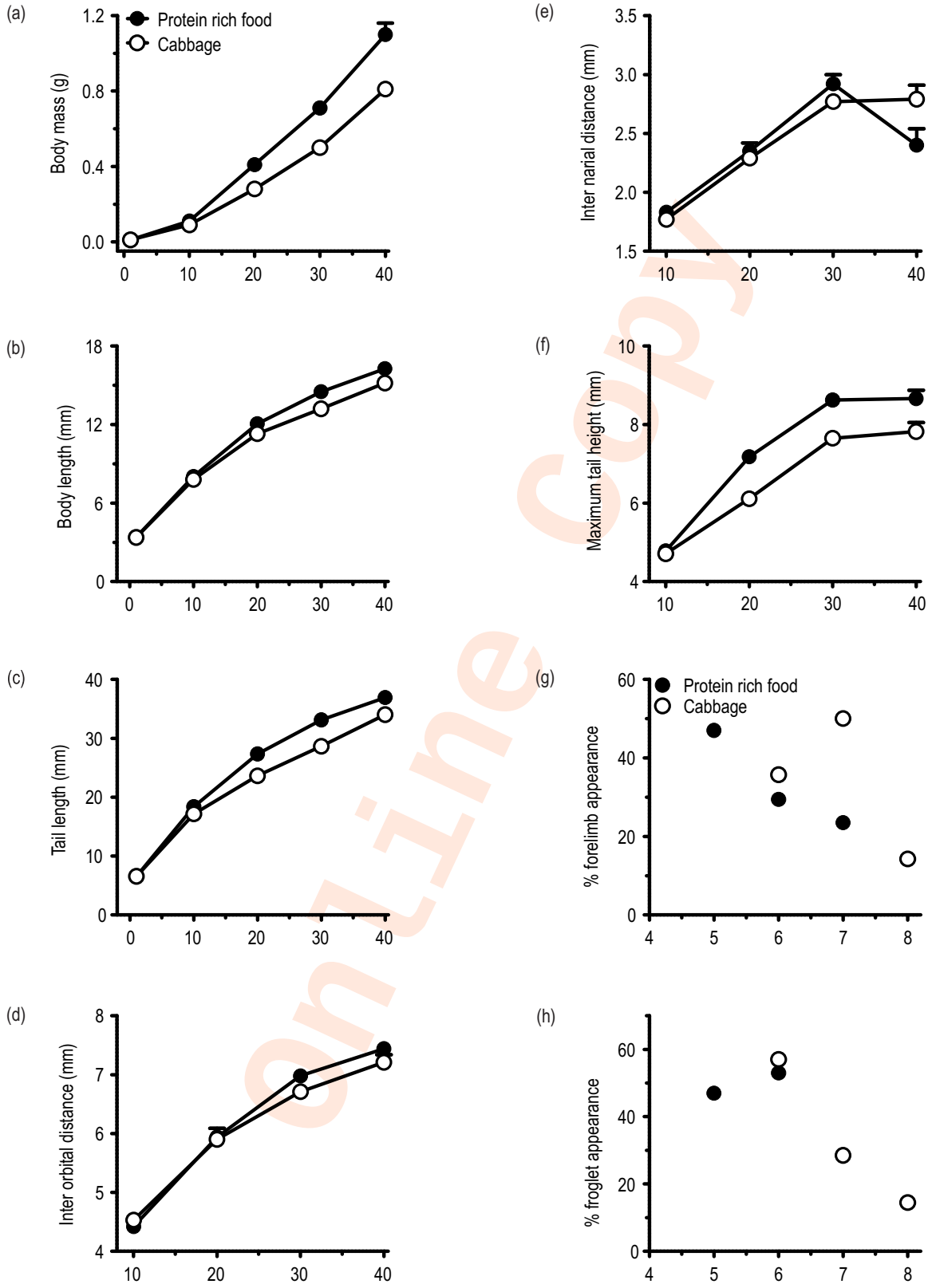

Fig. 6: Development and metamorphosis of *Polypedates teraiensis* tadpoles in cabbage (hallow circle) and protein-rich food (filled circle). Fig. 6g and h represents percent forelimb and froglet metamorphosed.

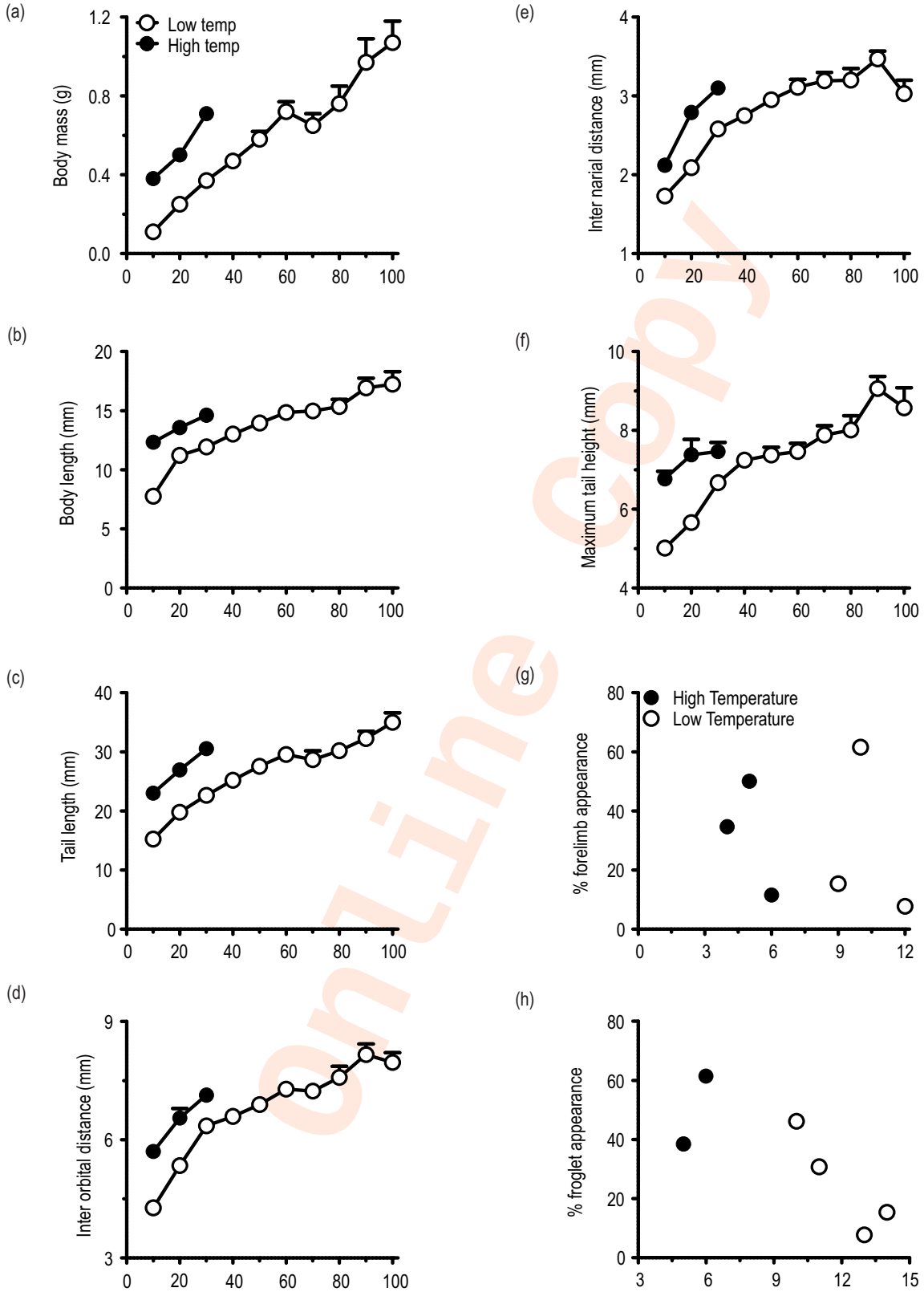

Fig. 7: Development and metamorphosis of *Polypedates teraiensis* tadpoles at low temperature (20±2°C; hallow circle) and high temperature ((30±2°C; filled circle) conditions. Fig. 7g and h represents percent forelimb and froglet metamorphosed, respectively.

at either time of the day (12hr day time food vs. 12hr night time food) was comparable (Figs. 4 and 5). Similarly, restricting food to 4hr delayed the development of tadpoles ($P < 0.0001$; Two-way ANOVA; Figs. 4 and 5). However, when food was available during dark hours, relative growth was faster in the 4hr group (day 4hr food vs. night 4hr food availability). Tadpoles grew and developed in both cabbage and protein-rich food groups ($P<0.0001$; One-way ANOVA; Fig. 6a-f). However, food quality affected the growth of tadpoles ($P < 0.0001$; Two-way ANOVA; Fig. 6a-f) and it was faster in protein-rich food group than in the cabbage group ($P<0.05$; Bonferroni test; Fig.6). Forelimb appearance is directly related tothe quality of food provided (Fig. 6 g). In protein-rich food, 47% of tadpoles had forelimbs by fifth week, and by seventh week they had developed in 100% tadpoles (Fig. 6 g).

In the cabbage group, forelimbs had started to developing by sixth week, and it took eight weeks for all tadpoles to develop forelimbs (Fig. 6 g). Metamorphosis followed a similar pattern. In protein-rich food, froglets were formed by the fifth week, and all the tadpoles were metamorphosed by seventh week (Fig. 6 h). In the cabbage group, metamorphosis started at sixth week and completed in eight weeks (Fig. 6 h). Tadpoles have developmental plasticity and can thus adapt to changes in their environment to maximize their survival chances (Doughty and Roberts, 2003). Food availability for 12hr led a significant reduction in growth when compared to the *ad libitum* group (Fig. 4 and 5). Similarly, availablity of food for 4hr further delayed the developmental processes. Furthermore, 4hr food restriction during daytime has a more severe effect than night time,as evidenced by delayed growth in 4hr daytime group. Vaissi and Sharifi (2012) demonstrated that when yellow-spotted mountain newts *Neurergus microspilotus* larvae were grown at a high food level and high temperature, metamorphosis occurred earlier as compared to individuals raised at a low food level and low temperature. In principle, if feeding rates are unchanged, a more nutritious and energetic food should result in faster growth and development, allowing for either shorter larval periods and the increased metamorphic size or longer larval periods to capitalize on rapid-growth opportunity (Wilbur and Collins, 1973).

In this study, tadpoles fed on protein-rich food had faster rate of development in comparison to tadpoles fed on cabbage. Until now, only few studies have demonstrated that dietary protein accelerates metamorphosis in tadpoles by influencing thyroid hormone production (Alvarez and Nicieza, 2002). Temperature played a significant role in tadpole growth, with different effects observed under high (30±2°C) and low temperature (30±2°C) treatments. In general, temperature conditions had a significant effect ($P < 0.0001$; Two-way ANOVA; Fig. 7a-f) and growth was faster under high temperature ($P < 0.05$, Bonferroni test; Fig. 7). Forelimbs development was much faster in high-temperature group. By fourth week of treatment, 35% of tadpoles had developed fore limbs, and by week five 85% of the tadpoles had developed forelimbs, with the remaining tadpoles developing forelimbs by 6th week (Fig. 7g). However, in low-temperature group, only 15% of tadpoles had forelimbs by week nine, and it took 12 weeks for all tadpoles to have forelimbs (Fig. 7g). Similarly, froglet formation was significantly faster in the high-temperature group, with 39% of tadpoles metamorphosing into froglets by week five and all tadpoles metamorphosing into froglets by week six (Fig. 7h). The rate of metamorphosis retarded at low-temperatures, and by week 10, 46% of tadpoles had metamorphosed into froglets whereas it took 14 weeks to complete the metamorphosis (Fig. 7h).

Lower temperature conditions during winter season are unfavorable for growth and reproduction in ectotherms. Therefore, most of these ectotherms must reach a certain developmental stage or body size before hibernating as winter approaches (Bradshaw and Holzapfel, 2007). Our results also suggest that temperature has a significant impact on the growth and metamorphosis of *P. teraiensis* tadpoles. During the experiment, higher temperatures (30±2°C) resulted in faster tadpole growth. Similar findings were made with the yellow-spotted mountain newts *Neurergus microspilotus*, where tadpoles raised at lower temperatures took longer to metamorphos than those raised at higher temperatures. High temperature (28°C) favored growth and development in endangered green and golden bell frog *Litoria aurea* (Browne and Edwards, 2003). The role of temperature in the feeding behavior of tadpoles has also been studied, with high temperatures (26°C) favoring high ingestion in *Rana clamitans* (Warkentin, 1992). As summer season is marked by higher temperatures, it appears to favour faster growth and development than winter season, which is marked by lower temperatures. This could explain why growth is slowed in a low-temperature environment.

In conclusion, our study suggests that photoperiod, temperature, and time restricted food availability all influence the rate of growth, development, and metamorphosis of *P. teraiensis* tadpoles. These findings do imply that anuran development and metamorphosis are influenced by interactions among environmental factors. These findings may aid in the improvement of captive frog breeding programmes and amphibian conservation.

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Add-on Information

Authors' contribution: B.K. Borah: Execution of experiment and analysis of data; **Z. Renthlei:** Execution of experiment and analysis of data; **A. Tripathi:** Manuscript editing and mentoring; **A.K. Trivedi:** Supervisor and manuscript and writing.

Research content: The research content of manuscript is

original and has not been published elsewhere.

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