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Adaptive plasticity in anuran metamorphosis: response of tadpoles of *Polypedates maculatus* (Anura: Rhacophoridae) to pond drying

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Abstract

Adaptive plasticity in anuran metamorphosis: response of tadpoles of *Polypedates maculatus* (Anura: Rhacophoridae) to pond drying. The influence of desiccation on metamorphic traits (larval duration and size at emergence) was studied in *Polypedates maculatus* under laboratory conditions. Gosner Stage 23 tadpoles were exposed to decreasing water levels (gradual or rapid) until the beginning of metamorphic climax (Stage 42). A control group was reared in constant water levels. Tadpoles reared in decreasing water levels reached the metamorphic climax earlier and metamorphosed at a smaller size than those reared in constant water levels. Further, tadpoles experiencing rapid depletion of water reached the metamorphic climax earlier and metamorphosed at a smaller size than those experiencing gradual depletion of water levels. Tadpoles of *P. maculatus* showed adaptive plasticity in metamorphosis to pond drying. Survival of tadpoles in treatments and the control was 100%. The study revealed that tadpoles of *P. maculatus* have plastic development in response to water levels; the trade-off between growth and development favors development, which results in early metamorphosis at a small size.

Keywords: Desiccation, Metamorphic traits, Phenotypic plasticity, Tadpoles, Tree frog.

Resumo

Plasticidade adaptativa na metamorfose de anuros: resposta dos girinos de *Polypedates maculatus* (Anura: Rhacophoridae) ao dessecamento do tanque. A influência do dessecamento nas características metamórficas (duração do estágio larval e tamanho na emergência) foi estudada em *Polypedates maculatus* sob condições de laboratório. Girinos no Estágio 23 de Gosner foram expostos a níveis de água decrescentes (graduais ou rápidos) até o início do clímax metamórfico (Estágio 42). Um grupo-controle foi criado em níveis de água constantes. Os girinos criados em níveis de água decrescentes atingiram o clímax metamórfico mais cedo e se metamorfosearam em um tamanho menor do que aqueles criados em níveis de água constantes. Além disso, os girinos que sofreram um rápido esgotamento da água atingiram o clímax metamórfico mais cedo e se metamorfosearam em um tamanho menor do que aqueles que sofreram um esgotamento gradual dos níveis de água. Os girinos de *P. maculatus* mostraram plasticidade adaptativa na metamorfose em

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relação ao dessecamento do tanque. A sobrevivência dos girinos nos tratamentos e no grupo-controle foi de 100%. O estudo revelou que os girinos de *P. maculatus* têm um desenvolvimento plástico em resposta aos níveis d'água; a compensação entre crescimento e desenvolvimento favorece o desenvolvimento, o que resulta em metamorfose precoce em um tamanho pequeno.

Palavras-chave: Características metamórficas, Dessecamento, Girinos, Pererecas, Plasticidade fenotípica.

Introduction

Phenotypic plasticity is widespread in nature and especially in organisms with complex life histories. Anuran amphibians are the best example of phenotypic plasticity (Wilbur 1980, Newman 1992, Pigliucci 2005, Miner *et al.* 2005). Phenotypic plasticity affects many life-history traits in anurans, especially those related to reproduction and larval development (Wells 2010, Urban *et al.* 2014). Timing of metamorphosis is a trade-off between opportunities for growth and risk of mortality in aquatic and terrestrial habitats (Wilbur and Collins 1973, Werner 1986, Rudolf and Rödel 2007, Mogali *et al.* 2011a, 2017). In most anurans, the timing of and size at metamorphosis are highly plastic, and both depend on multiple factors such as water level (Loman 1999, Mogali *et al.* 2016, 2017), larval density (Newman 1998, Mogali *et al.* 2016), predators (Mogali *et al.* 2011b, Orizaola *et al.* 2013), temperature (Gómez-Mestre and Buchholz 2006, Maciel and Juncá 2009), food availability (Laurila and Kujasalo 1999, Enriquez-Urzelai *et al.* 2013), and the complex interaction between these variables (Grözinger *et al.* 2014, Mogali *et al.* 2016).

In temporary water bodies, desiccation is a threat, and completion of metamorphosis before drying of aquatic habitats is necessary. Slow growth rates and/or prolonged larval periods in unpredictable hydroperiods decrease the chances of tadpoles completing metamorphosis before habitats dry (Altwegg and Reyer 2003). In contrast, faster larval development can lower larval mortality, but it is invariably at the cost of growth, resulting in a smaller size at metamorphosis that may have consequences in later survival and reproductive success (Reques and Tejedo 1997, Morey and Reznick 2000, Altwegg and Reyer 2003). When larval mortality risk increases because of pond desiccation, early metamorphosis may be favored despite the costs associated with smaller size. Phenotypic plasticity involving a trade-off among certain life history traits (e.g., larval growth, length of the larval period, size at transformation) is a useful strategy. The original Wilbur and Collins' model of amphibian metamorphosis predicts that in an aquatic environment when conditions are favorable for larval growth (i.e., in permanent or slowly desiccating ponds), tadpoles should delay metamorphosis and transform at a larger size. But when conditions of temporary ponds become precarious, a strategy to adjust developmental processes to favor early metamorphosis and emergence from the aquatic habitat is useful (Wilbur and Collins 1973). A developmental strategy of phenotypic plasticity can decrease exposure to risky conditions and thereby increase survival rate.

In the city of Dharwad in Southern India, many anuran species reproduce in rain-filled ephemeral habitats formed during the southwestern monsoons. Tadpoles living in such ponds face a perennial threat of desiccation because of intermittent rains (Mogali *et al.* 2011a, 2017). *Polypedates maculatus* (Gray, 1830) is widely distributed in India. In Southern India, populations breed only during the rainy season. Females deposit eggs in foam nests. Nests are attached to vegetation, underneath stones above a water body, in bushes over rain-filled puddles, or to walls of cement cisterns

(Girish and Saidapur 1999, Mogali *et al.* 2022). Early embryonic development (up to Stage 23 of Gosner 1960) occurs inside the foam nests, after which tadpoles drop into the water where they undergo further development and metamorphosis. Tadpoles of *P. maculatus* occur in both ephemeral and permanent ponds, thus providing an excellent model to study developmental plasticity in response to varying degrees of evaporation of pond water. The present study was designed to determine, in a laboratory, the influence of gradual or rapid water depletion on the two major metamorphic traits, the larval period and size at emergence. We hypothesized that tadpoles with depleted water levels (either low or high rates of desiccation) would metamorphose earlier and at a smaller size than those developing in constant water levels. We also hypothesized that tadpoles facing rapidly depleting water would metamorphose earlier and at a smaller size than those developing in gradually declining water levels. The experimental design permitted us to exclude the influence of confounding factors such as food scarcity and predator pressure that generally interfere with growth and development of tadpoles in nature.

Materials and Methods

Three foam nests of *P. maculatus* were collected in July 2015 from temporary ponds on the Karnatak University Campus (15.440407° N, 74.985246° E) Dharwad, Karnataka State, India. They were transported to the laboratory and each nest was placed in a separate plastic tub (32 cm diameter and 14 cm deep) with 1 L of water and with substratum collected from the same pond. Tadpoles stages are according to Gosner (1960). The tadpoles hatched from the foam nests almost synchronously after five days at Stage 23. Tadpoles from all three nests were then mixed to normalize genetic difference among the groups. Tadpoles (Stage 23) were selected randomly and were reared in the plastic tubs (32 cm diameter and 14 cm deep) with 3–0.6 L of aged tap water until the onset of metamorphic climax stage (Stage 42). Fifteen tubs with 10 tadpoles each were maintained (in total 150 tadpoles). The experimental groups of 50 tadpoles were as follows:

Group I. Constant water: Tadpoles were reared in constant water levels (3 L).

Group II. Gradual desiccation: Tadpoles were reared in 3 L of water for the first 4 days and then subjected to 0.2 L decrease in water at 4 day intervals.

Group III. Rapid desiccation: Tadpoles were reared in 3 L of water for a day and from the second day onward 0.1 L of water was reduced each day.

In the two groups with receding water, when the water reached 0.6 L (day 49 in Group II; day 25 in Group III) no further reductions were made. Groups II and III thus provided low and high desiccation risks. All tadpoles were fed boiled spinach ad libitum. Water was changed on alternate days and fresh food was provided. The rearing tubs were placed on a flat surface in a room with natural photoperiod and temperature. The positions of tubs were randomized on alternate days to avoid possible effects of position. Water temperature (°C) in tubs was recorded twice daily at 10:00 and 15:00 h. Following the onset of metamorphic climax (MC, emergence of forelimbs, Stage 42), subjects were transferred to small plastic tubs (19 cm diameter and 7 cm deep) with a small amount of water, covered with fine nylon mesh, and placed on an incline to provide a semi-terrestrial environment to facilitate emergence. The days to reach MC were noted for each individual. After completion of metamorphosis (Stage 46), snout–vent length (SVL in mm; measured using a digital caliper, accuracy 0.01 mm) and body mass (in mg; measured using an electronic balance, accuracy 0.001 g) were recorded. No tadpoles died during the course of the experiment. After completion of the experiments, the froglets were released near natural water bodies. We used the mean values of each variable (days to reach MC, SVL, body mass of froglets, and water temperature) within each tub for analysis in order to avoid pseudo replication. Data were analyzed by one-way ANOVA using mean tub days to reach MC, SVL, and body size at metamorphosis as the response variables in separate tests, and treatment (water level: constant, gradual desiccation, rapid desiccation) as the effects, followed by Tukey's post-hoc tests. Data for each parameter were organized into frequency distributions to determine the percentage of individuals falling within a particular dataset.

Results

Time taken to reach MC and size at metamorphosis (SVL and body mass) differed significantly between treatment groups ($p < 0.001$, Table 1). Tadpoles reared in declining water levels (Groups II and III) reached MC earlier ($p < 0.001$) and metamorphosed at a smaller size ($p < 0.001$) than those reared in constant water levels (Group I). Tadpoles experiencing rapid depletion of water (Group III) reached MC earlier ($p < 0.001$) and metamorphosed at smaller sizes ($p < 0.001$) than those experiencing gradual depletion in water levels (Group II).

The daily water temperature of various tubs fluctuated between 22–23°C and did not differ significantly throughout the course of the experiments (morning hours: $F = 6.125$, $df = 2, 12$, $p = 0.224$; afternoon hours: $F = 0.452$, $df = 2, 12$, $p = 0.647$). The effects of water temperature, if any, were uniform across the control and experimental groups.

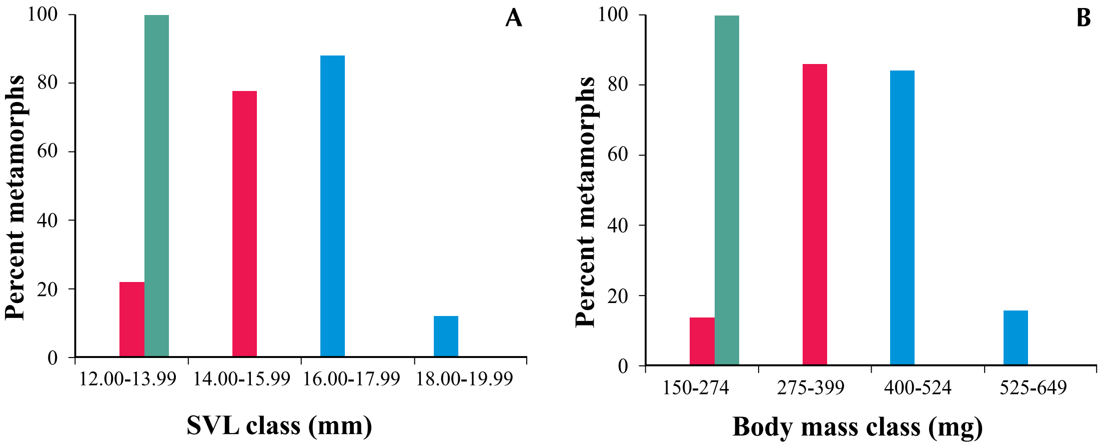
The frequency distribution data showed that all individuals (100%) from the rapid desiccation group and 22% of individuals from the gradual desiccation group metamorphosed (Stage 46) at < 14.00 mm SVL, but none of the individuals subjected to constant water levels metamorphosed at comparable SVLs (Figure 1A). All individuals (100%) from the rapid desiccation group and only 14% of individuals in the gradual desiccation group metamorphosed at a smaller body mass (< 275 mg), but none of the individuals subjected to constant water levels metamorphosed at a comparable body mass (Figure 1B). The data on days to onset of MC showed that all individuals (100%) reared in the rapid desiccation group and 12% of individuals from the gradual desiccation group took < 63 days, but no individuals subjected to constant water levels initiated MC by this same time (Figure 1C).

Discussion

Environmental heterogeneity plays a key role in the evolution of biological phenotypic plasticity (Sultan and Spencer 2002). Natural selection favors organisms with the most suitable phenotype for interacting with their environment, and variations in phenotype and the corresponding genotype exist to adapt to a specific environment

Table 1. Snout–vent length (SVL), body mass of metamorphs, and days required for the onset of metamorphic climax (MC, Gosner Stage 42) in *Polypedates maculatus* reared in containers with different levels of desiccation. Data represent mean ± SE; $N = 50$ tadpoles for each group (150 tadpoles in total); superscripts (a, b, c) indicate significant differences between the groups in the same column; significance level was set to 0.05.

Rearing groups	SVL (mm)	Body mass (mg)	Onset of MC (days)
I. Constant water	17.34 ± 0.10^{a}	466.76 ± 7.00^{a}	74.84 ± 0.73^{a}
II. Gradual desiccation	14.60 ± 0.09^{b}	315.30 ± 4.85^{b}	64.54 ± 0.23^{b}
III. Rapid desiccation	13.44 ± 0.06^{c}	229.04 ± 2.93^{c}	60.16 ± 0.21^{c}
$F_{2, 12}$	1120.0	817.863	1411.0
p value	< 0.001	< 0.001	< 0.001



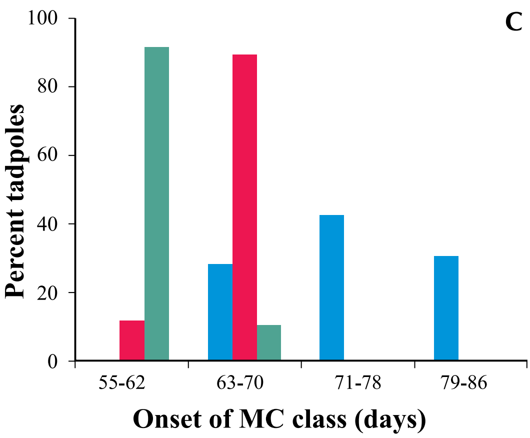


Figure 1. Percent metamorphs of *Polypedates maculatus* (**A**) per snout–vent length class (mm) and (**B**) per body mass class (mg) in different groups. (**C**) Percent tadpoles of *P. maculatus* per class (days) to reach metamorphic climax (MC, Gosner Stage 42) in different groups. Rearing groups: constant water (blue bars), gradual desiccation (red bars), rapid desiccation (green bars).

(Whitlock 1996). Indeed, the ephemeral ponds many anuran amphibians use for egg deposition and those with growing tadpoles are typical examples of environmental heterogeneity.

Anuran amphibians are characterized by phenotypic plasticity both in time to metamorphosis and in size at metamorphosis, especially for species that breed and develop in temporary ponds or highly unpredictable environments (Crump 1989, Newman 1992, 1994, Denver *et al.* 1998, Loman 1999, Mogali *et al.* 2011a, Richter-Boix *et al.* 2011).

Our study demonstrated that tadpoles of *P. maculatus* have phenotypic plasticity and that timing of metamorphosis responds to the availability of water. Tadpoles raised in constant water levels metamorphosed later and at a larger size than tadpoles from decreasing water levels (gradual or rapid). Tadpoles raised in gradually depleted water levels metamorphosed later and at a larger size than tadpoles from rapidly depleting water levels. Tadpoles that experienced a severe desiccation threat (rapidly depleting water levels) metamorphosed earlier and at smaller sizes. Hence, tadpoles of *P. maculatus* showed adaptive plasticity in metamorphosis to pond drying. Our results are in accordance with Wilbur and Collins' model (1973) for amphibian metamorphosis, which assumes that tadpoles encountering favorable conditions postpone metamorphosis, capitalizing on the opportunity for additional growth, while tadpoles exposed to hostile conditions (rapid desiccation threat) develop quicker and leave the aquatic habitat earlier. Our results support previous research (Newman 1989, Denver *et al.* 1998, Loman 1999, Mogali *et al.* 2011a, 2017, Székely *et al.* 2017).

The mechanisms proposed to explain the acceleration of metamorphosis in anuran tadpoles facing the risk of desiccation differ. Elevated temperature (Newman 1992, Tejedo and Reques 1994) or a decrease in food (Alford and Harris 1988, Newman 1994) has been attributed to lowered growth rate with accelerated developmental rate. Other studies indicated that temperature has no influence on developmental rate (Loman 1999, Laurila and Kujasalo 1999, Márquez-García *et al.* 2009, Mogali *et al.* 2017). In the present study, water temperature of the rearing containers did not vary, and excess food was provided to all groups. The main factor influencing accelerated metamorphosis of tadpoles of *P. maculatus* in this study was the desiccation threat rather than temperature or shortage of food availability.

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References

Alford, R. A. and R. N. Harris. 1988. Effects of larval growth history on anuran metamorphosis. *American Naturalist 131:* 91–106.

Altwegg, R. and H. U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution 57:* 872–992.

Crump, M. L. 1989. Effect of habitat drying on developmental time and size at metamorphosis in *Hyla pseudopuma*. *Copeia 1989:* 794–797.

Denver, R. J., N. Mirhadi, and M. Phillips. 1998. An experimental analysis of adaptive plasticity in amphibian metamorphosis: developmental response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology 79:* 1859–1872.

Enriquez-Urzelai, U., O. San Sebastián, N. Garriaga, and L. A. Llorente. 2013. Food availability determines the response to pond desiccation in anuran tadpoles. *Oecologia 173:* 117–127.

Girish, S. and S. K. Saidapur. 1999. Mating and nesting behavior, and early development in the tree frog *Polypedates maculatus*. *Current Science 76:* 91–92.

Gómez-Mestre, I. and D. R. Buchholz. 2006. Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proceedings of National Academy of Sciences USA 103:*19021–19026.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica 16:* 183–190.

Grözinger, F., J. Thein, H. Feldhaar, and M. O. Rödel. 2014. Giants, dwarfs and the environment-metamorphic trait plasticity in the common frog. *PLoS ONE 9:* e89982.

Laurila, A. and J. Kujasalo. 1999. Habitat duration, predation and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *Journal of Animal Ecology 68:* 1123–1132.

Loman, J. 1999. Early metamorphosis in common frog *Rana temporaria* tadpoles at risk of drying: an experimental demonstration. *Amphibia-Reptilia 20:* 421–430.

Maciel, T. A. and F. A. Juncá. 2009. Effects of temperature and volume of water on the growth and development of tadpoles of *Pleurodema diplolister* and *Rhinella granulosa* (Amphibia-Anura). *Zoologia 26:* 413–418.

Márquez-García, M., M. Correa-Solis, M. Sallaberry, and M. A. Méndez. 2009. Effects of pond drying on morphological and life history traits in the anuran *Rhinella spinuosa* (Anura: Bufonidae). *Evolutionary Ecology Research 11:* 803–815.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution 20:* 685–692.

Mogali, S. M., S. K. Saidapur, and B. A. Shanbhag. 2011a. Receding water levels hasten metamorphosis in the frog, *Sphaerotheca breviceps* (Schneider, 1799): a laboratory study. *Current Science 101:* 1219–1222.

Mogali, S. M., S. K. Saidapur, and B. A. Shanbhag. 2011b. Levels of predation modulate antipredator defense behavior and metamorphic traits in the toad *Bufo melanostictus*. *Journal of Herpetology 45:* 428–431.

Mogali, S., S. Saidapur, and B. Shanbhag. 2016. Influence of desiccation, predatory cues, and density on metamorphic traits of the bronze frog *Hylarana temporalis*. *Amphibia-Reptilia 37:* 199–205.

Mogali, S., S. Saidapur, and B. Shanbhag. 2017. Influence of desiccation threat on the metamorphic traits of the Asian common toad, *Duttaphrynus melanostictus* (Anura). *Acta Herpetologica 12:* 175–180.

Mogali, S. M., B. A. Shanbhag, and S. K. Saidapur. 2022. Sensory basis of food detection in tadpoles of *Polypedates maculatus* (Anura: Rhacophoridae): an experimental approach. *Phyllomedusa 21:* 59–65.

Morey, S. and D. Reznick. 2000. A comparative analysis of plasticity in larval development in three species of spadefoot toads. *Ecology 81:* 1736–1749.

Newman, R. A. 1989. Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. *Ecology 70:* 1775–1787.

Newman, R. A. 1992. Adaptive plasticity in amphibian metamorphosis. *Bioscience 42:* 671–678.

Newman, R. A. 1994. Effects of changing density and food level on metamorphosis of a desert amphibian, *Scaphiopus couchii*. *Ecology 75:* 1085–1096.

Newman, R. A. 1998. Ecological constraints on amphibian metamorphosis: interactions of temperature and larval density with responses to changing food level. *Oecologia 115:* 9–16.

Orizaola, G., E. Dahl, A. G. Nicieza, and A. Laurila. 2013. Larval life history and anti-predator strategies are affected by breeding phenology in an amphibian. *Oecologia 171:* 873–881.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology and Evolution 20:* 481–486.

Reques, R. and M. Tejedo. 1997. Reaction norms for metamorphic traits in natterjack toads to larval density and pond duration. *Journal of Evolutionary Biology 10:* 829–851.

Richter-Boix, A., M. Tejedo, and E. I. Rezende. 2011. Evolution and plasticity of anuran larval development in response to desiccation: a comparative analysis. *Ecology and Evolution 1:* 15–25.

Rudolf, V. H. W. and M. O. Rödel. 2007. Phenotypic plasticity and optimal timing of metamorphosis under uncertain time constraints. *Evolutionary Ecology 21:* 121–142.

Sultan, S. E. and H. G. Spencer. 2002. Metapopulation structure favors plasticity over local adaptation. *American Naturalist 160:* 271–283.

Székely, D., M. Denoël, P. Székely, and D. Cogălniceanu. 2017. Pond drying cues and their effects on growth and metamorphosis in a fast developing amphibian. *Journal of Zoology 303:* 129–135.

Tejedo, M. and R. Reques. 1994. Plasticity in metamorphic traits of natterjack tadpoles: the interactive effects of density and pond duration. *Oikos 71:* 295–304.

Urban, M. C., J. L. Richardson, and N. A. Freidenfelds. 2014. Plasticity and genetic adaptation mediate amphibian and reptile responses to climate change. *Evolutionary Applications 7:* 88–103.

Wells, K. D. 2010. *The Ecology and Behavior of Amphibians.* Chicago. University of Chicago Press. 1148 pp.

Werner, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *American Naturalist 128:* 319–341.

Whitlock, M. C. 1996. The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. *American Naturalist 148:* 65–77.

Wilbur, H. M. 1980. Complex life cycles. *Annual Review of Ecology and Systematics 11:* 67–93.

Wilbur, H. M. and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. *Science 182:* 1305–1314.

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