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Evaluation of the effect of ecological factors on cannibalism in *Pelophylax bedriagae* (Pallas, 1771) (Amphibia: Anura)

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Abstract

Marsh frog, *Pelophylax bedriagae* tadpoles habituate in temporary wetlands where intraspecific aggression has been observed. In this study, we examined the independent and interactive effects of predator cues, water level and density on head width and head, tail and whole-body cannibalism of larval *P. bedriagae*. We designed a 2x2x3 factorial experiment, by crossing two levels of predatory cues (presence/absence), two levels of density (low/ high) and three levels of water (low/ high/ decreasing) that were carried out within 29 weeks. Despite using conditions that might facilitate cannibalism (i.e., the presence of predator cues, low water level, and high density), a low rate of cannibalism was observed in all treatments. Independently, the highest percentage of total cannibalism was indicated in the presence of predator cues (2.26 %), high water level (2.04 %) and high density (2.24 %). Interactively, the highest rate of total cannibalism was recorded for larvae reared in the high density/absence of predator cues/low water level treatment (3.55%). In contrast, the lowest rate of total cannibalism was observed in the both the low density/presence of predator cues/decreasing water level and low-density/presence of predator cues/high water level treatments (0.44%). The results of the MANOVA indicated that predator cues, water level and density independently and interactively had not significant effect on rate of total cannibalism. At the end of experiment, the maximum size of head width was measured in low-density/absence of predator cues/high water level treatment (9.60 ± 0.39 mm), and there was no significant relationship between the maximum head width and the highest rate of cannibalism. According to the results of this experiment, it seems that *P. bedriagae* larvae may function to avoid the risk of cannibalism by conspecific.

Key words: Amphibia, Intraspecific predation, Predatory cues, Density, Tadpole, Water level, Marsh frog

INTRODUCTION

Cannibalism or intraspecific predation represents the interactions of animals with individuals of the same species in response to environmental conditions (Polis, 1981; Wakano et al. 2002). This phenomenon is observed in diverse animal taxa including amphibians and is suggested to be a predictable evolutionary consequence of crowding, low availability of food resources, and starvation (Nishimura & Isoda, 2004). In amphibian species that reproduce a large number of offspring (Relyea, 2004; Richter-Boix et al. 2007),



cannibalism is common during the hatching to metamorphosis stages (larval-larval cannibalism) (Wakano et al. 2002; Gibbons et al. 2003; Zhang et al. 2003). Similar to interspecific predator-prey interactions, cannibalistic interactions contribute to the regulation of population structure and to the evolution of antipredator behavioral responses (Dong & Polis, 1992).

Climate change influence on amphibian community and their habitats (Walther et al. 2002; Brooks, 2004; Blaustein et al. 2010). These changes can lead to alterations in precipitation and temperature regimes, particularly in aquatic environments. Agonistic behaviors in amphibians can be influenced by a variety of factors including desiccation, population density, competition for food, temperature, and the presence of predator (Blaustein et al. 2010). In particular, pond desiccation and high temperature can increase cannibalism rates and have negative impacts on survival and reproduction in populations (Crumrine, 2010). As temporary wetlands gradually disappear, larval population density can increase (Wildy, 2001). Jefferson et al. (2014) have suggested that climate changes could alter population density for larval salamanders, leading to increased cannibalism rates. Maneti et al. (2015) found that aggression among larval *Salamandra salamandra* increased with density. Under such conditions, limitations to food resources may restrict their ability to metamorphosis (Enriquez-Urzelai et al. 2013) and intraspecific predation may avoid (Claessen & De Roos, 2003; Persson et al. 2004). Food limitations can also play a significant role in size variations of individuals which can facilitate cannibalism, although typically the cannibals are the largest individuals in the population (Fox, 1975; Polis, 1981; Crump, 1992). Pizzatto and Shine (2008), have demonstrated *Bufo marinus* tadpoles eat eggs, and large adults consume smaller conspecifics.

The effects of predator cues on animal behavior and ecology are well documented, both in amphibian and non-amphibian taxa (Kats & Dill 1998; Fraker, 2009; Kerby et al. 2011). For example, Sih (1986) found that mosquito larvae (*Culex pipiens*) reduce their activity when exposed to *Notonecta undulata* predators. Cannibals often exhibit faster growth, larger body size and broader head morphology compared to non- Cannibals (Fox, 1975; Polis, 1981). Cannibal larvae also tend to attack and consume the tails and developing limbs of non-cannibal larvae (Crump, 1986). Kishida et al. (2011) examined how the presence or absence of predation risk from larval dragonflies (*Aeshna nigroflava*) affected cannibalism dynamics in their prey, larval salamanders (*Hynobius retardatus*). They suggested that the positive feedback dynamics between size structure and cannibalism, and their modification by predation risk may also operate in other systems to shape the population dynamics of cannibalistic prey species, as well as overall community dynamics.

Amphibian tadpoles have been observed to exhibit anti-predator behavior in response to natural predators (Mogali et al. 2011, 2012). Such behaviors include a reduction in movement, avoidance of the predator compartment and an increase in shelter use (Lima & Dill, 1990; Mogali et al. 2015, 2019, 2020). Invasive predators, such as mosquitofish, have been introduced into various freshwater habitats, often resulting in declines in native amphibian populations due to predation on eggs, embryos, and tadpoles (Pyke, 2008). The mosquitofish, *G. holbrooki*, is a small viviparous fish that was introduced to Iran in the 20th century (1922–1930) from Italy and Azerbaijan as part of a mosquito control program (Patimar et al. 2011).

The Middle East is one of the most vulnerable regions to the impacts of climate change, largely due to water scarcity (Elasha, 2010). Iran located in an arid and semi-arid region is particularly susceptible to the effects of global climate change on its ecosystems (Amiri & Eslamian, 2010). The IPCC estimates an increase of 1.5-4 °C in mean temperatures by the year 2100, and predicts a steady decline in annual rainfall (~30%) in Iran (Nazaripour & Daneshvar, 2014). Subtropical species may be more sensitive to temperature increases than temperate species (Deutsch et al. 2008; Dillon et al. 2010; Duarte et al. 2012). The Marsh frog *P. bedriagae* (Pallas, 1771) is the most common amphibian species in Iran, often found in aquatic environments such as ponds, streams, and wetlands, and rarely straying far from water bodies (Baloutch & Kami, 1995). In nature, Marsh frog tadpoles are dependent on aquatic environments and frequently face ecological challenges, including desiccation threats and crowding. The

aim of this study is to evaluate the independent and interactive effects of three ecological factors- predator cues, water level and density- on cannibalism of *Pelophylax bedriagae* larvae.

MATERIAL AND METHODS

Marsh frog (*Pelophylax bedriagae*) eggs were collected from Sarab-e-Barnaj, a freshwater wetland, Kermanshah Province in Iran (N 34° 28' E 47° 22'). Egg masses were transported to Razi University, and maintained in glass aquarium filled with de-chlorinated water. From this stock, when the eggs reached to Gosner 26 stage (Gosner, 1960), start of the larval stage, individuals (N=540) of similar size and developmental stage were randomly chosen for the experimental containers. *Gambusia holbrooki* specimens were collected from Sarab-e-Youan (N 34° 38' E 46° 35'), 35 km to northwest Kermanshah city, Kermanshah Province in Iran.

The Experimental Design

The experiment consisted of 2×2×3 factorial design incorporating two levels of density (low, n =5 and high, n =25), two levels of predatory (present of predatory cues and without of predatory cues), and three levels of water (low: 300 cc, high 1500 cc, and decreasing 150 cc of water, 10 days once) including: 1) high density/ high water level/ no predator (HD/HW/NP); 2) high density/ low water level/ no predator (HD/LW/NP); 3) high density/ decreasing water level/ no predator (HD/DW/NP); 4) low density/ high water level/ no predator (LD/HW/NP); 5) low density/ low water level/ no predator (LD/LW/NP); 6) low density/ decreasing water level/ no predator (LD/DW/ NP); 7) high density/ high water level/ predator (HD/HW/P); 8) high density/ low water level/ predator (HD/LW/P); 9) high density/ decreasing water level/ predator (HD/DW/P); 10) low density/ high water level/ predator (LD/HW/P); 11) low density/ low water level/ predator (LD/LW/P); low density/ decreasing water level/ predator (LD/DW/P).

In order to prevent the direct contact of the *G. holbrooki* with larvae of *P. bedriagae*, the meshed cages (11.5 cm diameter × 6 cm depth) were designed, and were placed inside glass containers (12.5 cm diameter and 14 cm depth) during experiment. Within each meshed cage, five females *G. holbrooki* were placed at an average total length and head width 31.29 mm ± 8.33 and 5.12mm ± 1.43, respectively.

Monitoring

The experiment was performed under natural photoperiod at air and water temperature of approximately 24°C ± 1.40 and 21°C ± 1.60, respectively. Water in rearing containers was renewed each week. Larvae were fed daily with boiled spinach ad libitum (0.1 g per larva in the first month and 0.2 g per larva in the next months). Predators were fed daily with *P. bedriagae* tadpoles (each with one larva). The experiment was terminated when all tadpoles of all groups metamorphosed (Gosner stage 46).

Measurement

Photos of consumed larvae were taken with a digital camera (SONY, DSC-HX9V, 3.6V) on a tripod at a fixed height (30 cm) (Figs. 1-2). All larvae were measured for pre-ocular head width (width across the head through bisecting line through the external nares) by Digimizer version 4.6.0. Also, the morphological variables of larvae were surveyed daily including head (biting or eating all or part of head), tail (tail shortening, tail narrowing and eating all part of tail), whole body (larva disappearance in container) and total (head, tail and whole body) cannibalism. In *G. holbrooki* total length was calculated by drawing a line from the tip of snout to the end of caudal fin and head width were calculated by drawing a line between left and right jaw angles. Measurements were performed by Digimizer version 4.6.0.

Statistical Analysis

We calculated the average values of each variable for statistical analyses. All data are expressed as mean ± SD. After checking for normality, data were analyzed using multivariate analysis of variance (MANOVA) for testing independently and interactively effects of predatory cues, water level and density on tail, head, whole and total cannibalism and head width. All statistical tests were performed using SPSS software (ver. 22.0) and Stata (ver. 16.0).



FIGURE 1. Cannibalistic larval *P. bedriagae* consume smaller conspecifics in the 17th week of experiment.



FIGURE 2. An example of larval *P. bedriagae* cannibalism in the 28th week of experiment.

RESULTS

The results of the experiment indicate that cannibalism rates were generally very low across all treatments. However, the highest percentage of cannibalism was observed in the presence of predator cues ($2.26 \pm 0.89\%$), high water levels ($2.04 \pm 1.10\%$), and high density ($2.24 \pm 0.72\%$). In contrast, the lowest

percentage of cannibalism was recorded in the absence of predators ($1.16 \pm 0.66\%$), decreasing water levels ($1.26 \pm 0.77\%$), and low density ($1.18 \pm 0.87\%$) (Fig. 3 A-C).

The independent and interactive effects of three factors (predator cues, water level and density) on head cannibalism surveyed in experimental treatments. The percentage of head cannibalism demonstrated from high to low in HD.NP.LW ($0.17 \pm 0.46\%$), HD.P. HW ($0.17 \pm 0.68\%$), HD.NP.DW ($0.08 \pm 0.34\%$) and HD.P. DW ($0.08 \pm 0.34\%$) treatments, respectively. No head cannibalism was observed in the other treatments (Fig. 4). The results of MANOVA indicated that the factors of predatory cues, water level and density, independently and interactively had not significant effect on percentage of head cannibalism (Table 1).

The percentage of tail cannibalism recorded from high to low in LD.NP.LW ($1.33 \pm 2.16\%$), HD.NP.LW ($0.44 \pm 0.96\%$), LD.NP.DW ($0.44 \pm 1.71\%$), HD.NP.HW ($0.35 \pm 0.60\%$), HD.P. LW ($0.35 \pm 0.60\%$), HD.NP.DW ($0.17 \pm 0.68\%$), HD.P. DW ($0.17 \pm 0.68\%$) and HD.P. HW ($0.17 \pm 0.46\%$) treatments, respectively. In other treatments was not observed tail cannibalism (Fig. 4). The results of MANOVA indicated that predator cues, water level and density factors independently and interactively had not significant effect on total rate of tail cannibalism (Table 2).

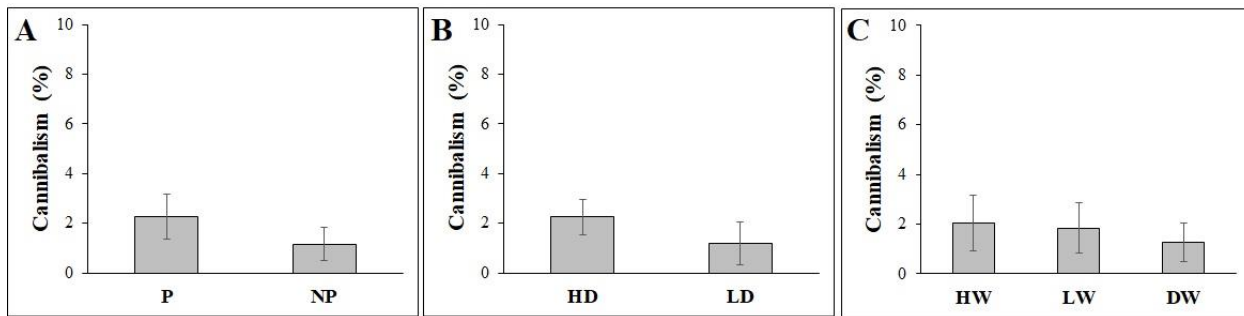


FIGURE 3. The percentage of cannibalism in A) the presence of predator cues (P), the absence of predator's cues (NP); B) the high density (HD), the low density (LD); C) the high-water level (HW), the low water level (LW), the decreasing water level (DW).

TABLE 1. Summary statistics of multivariate analysis of variance (MANOVA) for predator cues, water level and density effects on head cannibalism.

Head cannibalism	Wilks' lambda	df	F	p-value
Predator cues	0.99	1	0.35	0.55
Water level	0.99	2	0.77	0.46
Density	0.99	1	1.61	0.20
Predator cues × density	0.99	1	0.61	0.43
Predator cues × water level	0.99	1	0.44	0.63
Water level × density	0.99	2	0.65	0.52
Predator cues × water level × density	0.99	2	0.52	0.59
Predator cues × time	0.98	1	2.03	0.16
Water level × time	0.97	2	1.40	0.14
Density × time	0.99	1	0.11	0.63
Predator cues × density × time	0.98	1	2.03	0.15
Predator cues × water level × time	0.98	1	0.89	0.43
Water level × density × time	0.97	2	1.86	0.15
Predator cues × water level × density × time	0.98	2	0.89	0.41

The percentage of whole body cannibalism observed from high to low in HD.NP.LW (2.93 ± 0.54 %), HD.NP.DW (2.22 ± 0.38 %), LD.NP.DW (2.22 ± 0.65 %), HD.NP.HW (1.86 ± 0.30 %), HD.P.LW (1.59 ± 0.34 %), HD.P.DW (1.50 ± 0.51 %), HD.P.HW (1.15 ± 0.57 %), LD.NP.HW (0.88 ± 0.14 %), LD.P.LW (0.88 ± 0 %), LD.NP.LW (0.44 ± 0.29 %), LD.P.DW (0.44 ± 0 %) and LD.P.HW (0.44 ± 0 %) treatments, respectively (Fig. 4). The results of MANOVA indicated that water level factor independently had significant effect on total rate of whole-body cannibalism in *P. bedriagae* ($P \leq 0.04$) (Table 3). During the time, except significant effect of predator cue ($P \leq 0.05$), water level and density factors independently and interactively had not significant effect on rate of whole-body cannibalism (Table 3).

TABLE 2. Summary statistics of multivariate analysis of variance (MANOVA) for predator cues, water level and density effects on tail cannibalism.

Tail cannibalism	Wilks' lambda	df	F	p-value
Predator cues	0.99	1	0.39	0.53
Water level	0.99	2	0.33	0.72
Density	0.98	1	2.05	0.15
Predator cues \times density	0.99	1	1.35	0.24
Predator cues \times water level	0.99	1	0.33	0.65
Water level \times density	0.99	2	0.42	0.65
Predator cues \times water level \times density	0.98	2	1.49	0.22
Predator cues \times time	0.99	1	0.17	0.68
Water level \times time	0.99	2	0.05	0.94
Density \times time	0.99	1	0.04	0.83
Predator cues \times density \times time	0.99	1	0.69	0.40
Predator cues \times water level \times time	0.99	1	0.02	0.97
Water level \times density \times time	0.99	2	0.05	0.94
Predator cues \times water level \times density \times time	0.99	2	0.13	0.88

TABLE 3. Summary statistics of multivariate analysis of variance (MANOVA) for predator cues, water level and density effects on whole body cannibalism.

Whole body cannibalism	Wilks' lambda	df	F	p-value
Predator cues	0.99	1	0.03	0.86
Water level	0.96	2	3.15	0.04
Density	0.99	1	0.63	0.43
Predator cues \times density	0.99	1	0.48	0.49
Predator cues \times water level	0.97	1	2.08	0.12
Water level \times density	0.99	2	0.16	0.85
Predator cues \times water level \times density	0.99	2	0.28	0.75
Predator cues \times time	0.96	1	5.55	0.05
Water level \times time	0.99	2	1.09	0.73
Density \times time	0.99	1	0.11	0.77
Predator cues \times density \times time	0.99	1	0.03	0.85
Predator cues \times water level \times time	0.98	1	0.80	0.44
Water level \times density \times time	0.99	2	0.46	0.63
Predator cues \times water level \times density \times time	0.99	2	0.72	0.48

The percentage of total cannibalism (including head, tail and whole body cannibalism) observed from high to low in HD.NP.LW (3.55 ± 5.99 %), LD.NP.DW (2.66 ± 3.37 %), HD.NP.DW (2.48 ± 3.79 %), HD.NP.HW (2.21 ± 2.69 %), HD.P.LW (1.95 ± 3.83 %), LD.NP.LW (1.77 ± 5.32 %), HD.P.DW (1.77 ± 2.17 %), HD.P.HW (1.50 ± 2.51 %), LD.NP.HW (0.88 ± 3.44 %), LD.P.LW (0.88 ± 2.34 %), LD.P.DW (0.44 ± 1.71 %) and LD.P.HW (0.44 ± 1.71 %) treatments, respectively (Fig. 4). The results of MANOVA indicated that predator cues, water level and density independently and interactively had not significant effect on rate of total cannibalism over time (Table 4).

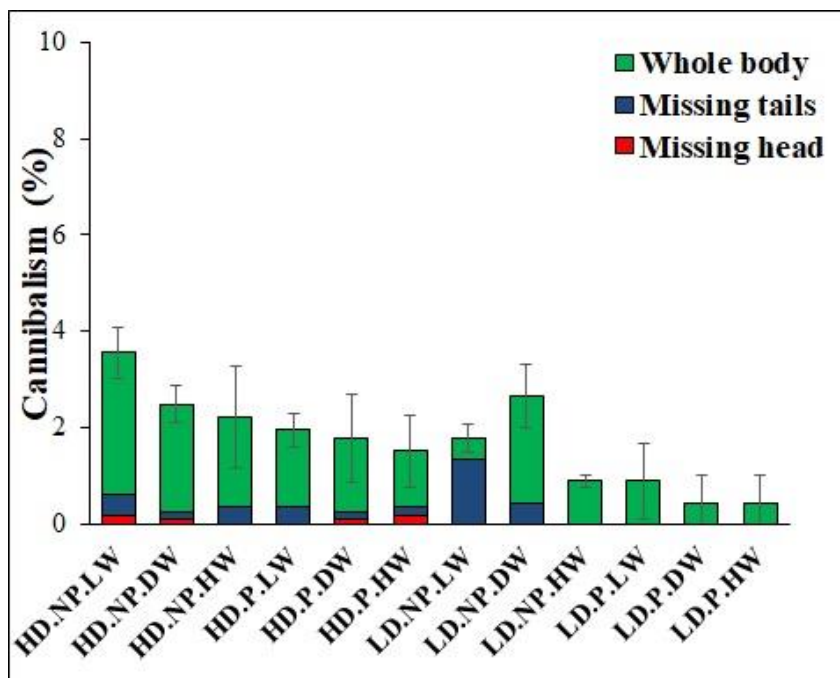


FIGURE 4. The percentage of head, tail and whole-body cannibalism in the different treatments. Abbreviation: presence of predator cues (P), absence of predator's cues (NP), high density (HD), low density (LD), high water level (HW), low water level (LW), decreasing water level (DW).

Head width size at 13th week of experiment measured from high to low in LD.P.LW (8.43 ± 0.6 mm), LD.P.HW (8.38 ± 0.45 mm), LD.P.DW (7.83 ± 1.01 mm), HD.P.LW (6.98 ± 0.21 mm), LD.NP.HW (6.97 ± 0.37 mm), LD.NP.DW (6.85 ± 0.68 mm), HD.P.HW (6.71 ± 0.77 mm), HD.P.DW (6.63 ± 0.06 mm), HD.NP.DW (6.51 ± 0.09 mm), LD.NP.LW (6.41 ± 0.53 mm), HD.NP.HW (6.23 ± 0.33 mm), HD.NP.LW (6.20 ± 0.20 mm) treatments, respectively. Also, the size of head width measured in 29th week of experiment and observed from high to low in LD.NP.HW (9.60 ± 0.39 mm), LD.NP.DW (9.30 ± 0.6 mm), HD.P.LW (9.30 ± 0.36 mm), HD.P.DW (9.27 ± 0.03 mm), LD.NP.LW (9.07 ± 0.41 mm), HD.P.HW (9.01 ± 0.08 mm), HD.NP.DW (8.84 ± 0.08 mm), HD.NP.LW (8.80 ± 0.44 mm) and HD.NP.HW (8.42 ± 0.33 mm) treatments, respectively. LD.P. LW, LD.P. DW and LD.P. HW treatments

TABLE 4. Summary statistics of multivariate analysis of variance (MANOVA) for predator cues, water level and density effects on total cannibalism.

Total cannibalism	Wilks' lambda	df	F	p-value
Predator cues	0.99	1	0.01	0.92
Water level	0.97	2	1.76	0.17
Density	0.99	1	0.01	0.91
Predator cues × density	0.99	1	1.33	0.25
Predator cues × water level	0.99	1	0.82	0.44
Water level × density	0.99	2	0.45	0.63
Predator cues × water level × density	0.99	2	0.78	0.45
Predator cues × time	0.96	1	15.01	0.02
Water level × time	0.99	2	0.23	0.79
Density × time	0.99	1	0.05	0.82
Predator cues × density × time	0.99	1	0.42	0.51
Predator cues × water level × time	0.99	1	0.43	0.64
Water level × density × time	0.99	2	0.53	0.58
Predator cues × water level × density × time	0.99	2	0.75	0.47

TABLE 5. Summary statistics of multivariate analysis of variance (MANOVA) for effects of predator cues, water level and density on the size of head width.

Head width	Wilks' lambda	df	F	p-value
Predator cues	0.90	1	49.91	0.0001
Water level	0.78	2	62.74	0.0001
Density	0.88	1	56.22	0.0001
Predator cues × density	0.93	1	31.43	0.0001
Predator cues × water level	0.96	1	8.06	0.0004
Water level × density	0.98	2	4.38	0.01
Predator cues × water level × density	0.92	2	17.65	0.0001
Predator cues × time	0.86	1	64.24	0.0001
Water level × time	0.95	2	63.71	0.0001
Density × time	0.89	1	53.79	0.0001
Predator cues × density × time	0.82	1	95.88	0.0001
Predator cues × water level × time	0.99	1	1.09	0.33
Water level × density × time	0.98	2	3.03	0.04
Predator cues × water level × density × time	0.96	2	7.61	0.0006

performed metamorphosis on 29th week. The results of MANOVA indicated that predator cues, water level and density independently and interactively had significant effect on head width (Table 5).

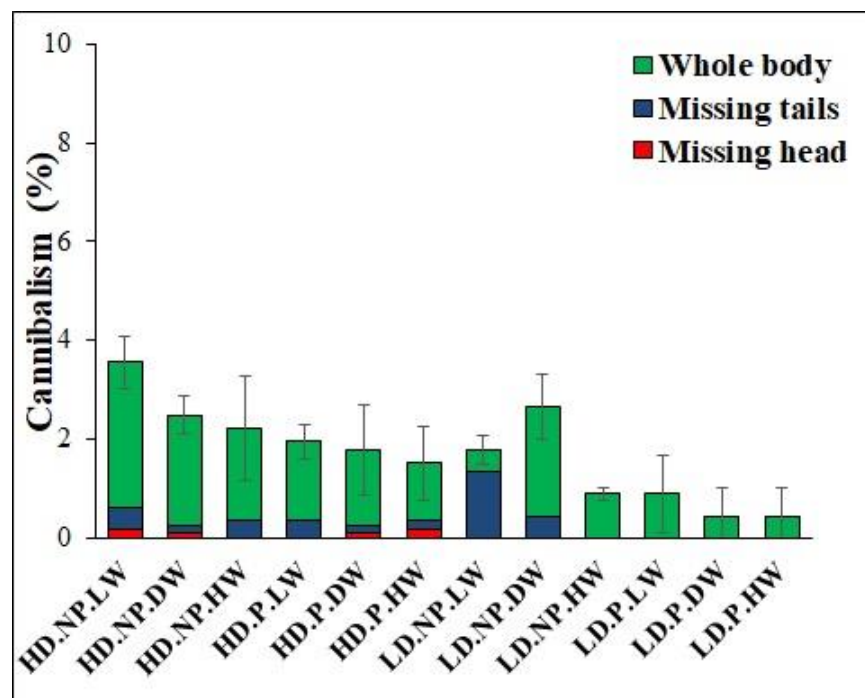


FIGURE 4. The percentage of head, tail and whole-body cannibalism in the different treatments. Abbreviation: presence of predator cues (P), absence of predator's cues (NP), high density (HD), low density (LD), high water level (HW), low water level (LW), decreasing water level (DW).

DISCUSSION

Predator cues play a crucial role in the antipredator behavior of amphibians, particularly in the presence of non-contact predators (Rajchard, 2006). Response to chemical alarm signals has been documented for frog tadpoles and several species of salamander (Marquis et al. 2004). Tadpole activity can be affected by predators, leading to reduce the rate of larval growth (Mogali et al. 2011, 2016). Similarly, to interspecific

interactions, antipredator responses can be exhibited in the presence of cannibalistic conspecifics (Kats et al. 1994). Chivers et al. (1997) showed that older larvae of long-toed salamanders exhibited antipredator behavior towards conspecifics possessing the cannibal morphology but not toward conspecifics possessing the typical morphology and observed reduced activity in their presence. In our experiment, significantly the highest rate of total cannibalism documented in presence of predator cues in *P. bedriagae* larvae. The results confirmed a significant increase in head width observed in all treatments where predator cues were present over time.

Previous studies have indicated that cannibalism is often observed among breeding amphibians in temporary water bodies (Wissinger et al. 2010). Such habitats, can dramatically fluctuate in response to changes in environmental conditions (Brooks, 2004). Low precipitation combined with high temperatures can lead to the decreased pond size and rapid pond drying (Brooks, 2004; Jefferson et al. 2014). These restrictions can force larvae into feeding aggregations, increase the degree of intraspecific competition, and lead to aggression and intraspecific predation (Jefferson et al. 2014). Therefore, Cannibalistic behavior of populations is increased to reduce the volume of water and accelerate the growth period because of the decrease in water soluble oxygen and the intraspecific competitions (Mori & Natuhara, 2004). Anderson et al (2013) suggested that predation risk and cannibalism increased in temporary ponds compared to permanent ponds in *Ambystoma talpoideum* larvae. Wildy et al. (1999) indicated cannibalism was not observed in either fast-drying or slow-drying conditions in *Ambystoma macrodactylum*.

Studies suggest that cannibalism is not simply a response to pond drying itself, but is a response to the limited food levels and the increased size variation that can occur when ponds dry up. However, research on the effects of water-level fluctuations on cannibalism rates is scarce. Moradi et al. (2019) reported the highest total cannibalism rate in low water levels in *Bufo variabilis*, but found that water level independently had no significant effect on the total cannibalism rate in *B. variabilis* larvae over time. In our study, we documented the highest total cannibalism rate in *P. bedriagae* observed in the high-water level. The results of MANOVA showed that, except for the significant effect of water level on whole body cannibalism, this factor independently had no significant effect on head, tail and total cannibalism rate over time (Tables 1-4). However, water level independently had significant effect on increase of head width over time (Table 5; Fig. 5).

Habitat desiccation can change a series of environmental factors, including increasing the density of tadpoles (Brady & Griffiths, 2000; Enriquez-Urzelai et al. 2013). Many studies suggest that density is the principal factor affecting aggression (Collins & Cheek, 1983; Nishihara, 1996). Higher densities provide more opportunities for encounters between conspecifics, which can lead to increased aggression. For example, Thibault (1974) observed that increasing density of *Poeciliopsis monacha* negatively affected individuals within the population and stimulated cannibalistic behavior. Collins and Cheek (1983) indicated that high density influenced the production of cannibal morphs in larval Arizona tiger salamanders (*A. t. nebulosum*). In addition, there is a strong interaction between density and food level. Food availability and conspecific density can influence the expression of intraspecific aggression and cannibalistic behavior (Collins & Cheek, 1983; Wildy et al. 2001; Vaissi & Sharifi, 2016). Wildy et al. (2001), showed that the highest incidence of cannibalism was observed in the low food/high density treatment, while the lowest incidence was observed in high food/low density treatments. Maneti et al. (2015) found that aggression among larval European fire salamanders (*Salamandra salamandra*) increased with density and dietary stress, and this increased aggression enhanced the probability of intraspecific attacks and cannibalism. In cane toads, food quality can also conduct populations towards cannibalism and play a major role (Babbitt & Meshaka, 2000). According to Moradi et al. (2019), the highest total cannibalism rate in *Bufo variabilis* was observed at low density. This factor independently and interactively (temperature, water level and density) had a significant effect on cannibalism rate. In our study, the highest total cannibalism rate was observed at high density in *P. bedriagae*, but this effect was not significant (Table 5; Fig. 5).

With growing awareness of the complexity of natural systems, it has become generally accepted that controlled multi-factorial experimental studies are necessary as the combined effect of multiple

environmental factors cannot always be predicted from single-factor studies (Alton & Franklin, 2017). Vaissi and Sharifi (2016) examined the effects of food availability and density on agonistic interactions of larval *N. microspilotus* and found that conspecific density and food limitation were associated with increased biting. Pizzatto and Shine (2008) demonstrated that juvenile *Bufo marinus* consumed their smaller conspecifics with the switch from insectivory to cannibalism exhibited during dry seasons and at high densities of small conspecifics. Moradi et al. (2019) indicated that the interactive effects of temperature, water level and density factors had significant effect on cannibalism rate in *B. variabilis* larvae over time. In our study, we found that predator cues, water level and density interactively had no significant effect on head, tail, whole body and total cannibalism rates. However, the interaction of these three factors had a significant effect on the increase of head width over time.

Cannibalistic amphibian larval morphs, which have greater head widths than typical morphs and therefore have larger mouths, provide the basis for the construction of a simple model of cannibalism that describes the growth dynamics of body shape (Wakano et al. 2002). As the relative head sizes of interacting individuals determine the occurrence of cannibalism, the situation is frequency-dependent (Wakano et al. 2002). For example, Pierce et al. (1983) shown that head size does not increase at a faster rate relative to body size in cannibals, but cannibals begin with a larger head early in ontogeny. Nyman et al. (1993) examined body size and head dimensions in relation to cannibalism in autumn-breeding salamander larvae, *Ambystoma annulatum*. A comparison of allometric relationships in cannibals and non-cannibals indicated that cannibals had proportionally broader heads, although the differences were less than those observed between cannibal and non-cannibal morphs of the tiger salamander larvae, *A. tigrinum*. Nishihara-Takahashi (1999) supported the hypothesis that a wide head is an adaptation against frequent cannibalism in larval *Hynobius retardatus*. McLean et al. (2016) suggested environmental factors, such as density inducing the cannibalistic morphism may be more complex than previously thought. They identified a scenario where the presence of an invasive competitor/prey species might have induced the cannibal morph phenotype. In our study, the rate of cannibalism was low in all treatments; as a result, there was no significant relationship between larger head size and higher cannibalism rates.

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