



## Feeding regime and food availability determine behavioural decisions under predation risk in *Pleurodema thaul* (Anura: Leiuperidae) tadpoles

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Movement makes prey more vulnerable to predators. Antipredator responses usually have associated costs such as reduced feeding activity, and decisions made under predation risk may be affected by the feeding state of the prey. In this study, we evaluated whether food regime influences antipredator behavioural responses of *Pleurodema thaul* tadpoles (diet treatment) before adding food or water (incentive treatment) while the tadpoles were exposed to alarm cues. Under predation risk, normally fed tadpoles showed decreased overall activity, whereas poorly fed tadpoles spent more time feeding after food was provided. Tadpoles in all treatments decreased their swimming activity but not their feeding activity. Our study showed that swimming behaviour was more sensitive to predation risk than feeding behaviour. We suggest that the motivational state of tadpoles under different environmental conditions should be considered when behavioural patterns are analysed to understand short-term trade-offs between foraging requirements and predator avoidance.

*Key words:* behaviour, diet, food availability, foraging, predation risk, tadpoles

The degree of food deprivation determines the physiological and also the motivational state of animals (Kandel et al., 1997; Coleman et al., 2005; Leblond et al., 2011), the latter of which may be considered an internal source of activation of foraging behaviour (Kandel et al., 1997; Domjan, 2003; Coleman et al., 2005). Animals are often active to acquire resources, which as a consequence makes them vulnerable to predators (Gerritsen & Strickler, 1977; Gendron & Staddon, 1984). Foraging activity involves mainly two behaviours: the search for food, and feeding. Many studies have shown that the behavioural changes that mediate the trade-off between foraging and avoiding predators may vary as a function of the hunger state of prey, predation risk intensity, temperature and food availability (Lima & Dill,

1990; Horat & Semlitsch, 1994; Anholt & Werner, 1998; Fraker, 2008a, Lienart et al., 2014).

Once the prey recognises the risk, a short-term behavioural response is displayed to minimise the risk of predation. In this regard, anuran tadpoles show a range of behavioural responses, such as a decrease in activity and an increase in refuge use (Skelly, 1994; Mirza et al., 2006; Fraker, 2008b). *Pleurodema thaul* (Leiuperidae) is one of the most common anuran species in northwestern Patagonia, Argentina, breeding primarily in temporary ponds devoid of fishes and coexisting with predaceous insects that change in relative abundance along wetlands and years (Jara, 2010). These temporary ponds also present inter- and intra-annual variation in food resources for anuran tadpoles (Díaz Villanueva, 2006). Given that both internal (feeding state) and external (food availability) factors may interact to lead animals to adjust their behaviours in the presence of predation risk, the goal of this study was to assess whether feeding state (normal versus a quarter of normal food ration diet) and food availability (incentive cue: food or water) influences the behavioural responses to alarm cues (injured conspecifics). We analysed the feeding and swimming activity levels separately to examine how the energetic state of tadpoles can modulate the potential trade-off between active foraging and predator avoidance when the availability of food varies.

All animals were taken as eggs from Laguna Fantasma (41°05'33" S, 71°27'00" W; 794 m.a.s.l.), located 14 km SW from San Carlos de Bariloche downtown, Río Negro province, Argentina. Six clutches of *P. thaul* (Gosner stage 11–13; Gosner, 1960) were collected. Alarm cues were created by using additional tadpoles Gosner stage 32–35 after freezing (-8°C) and decapitation. Viscera were removed to avoid the scent from visceral contents. The tadpole tissue mass (1.06 g in total) was crushed using mortar and pestle and then suspended in 15 ml distilled water, filtered through filter floss (45 µm nitex), aliquoted into 1 ml doses and frozen at -20°C until use no more than two days later.

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**Table 1.** Diet treatment (normal vs quarter food ration) effects upon behavioural repertoire in *Pleurodema thaul* tadpoles during the pre-stimulus period.

Behaviour (s)	Mean±SE		Test of Between-Subject Effects	
	NR diet	QR diet	Statistic	p-value
Swimming	80.23±14.54	60.61±11.51	$F_{1,30}=1.08$	0.307
Feeding	2.50±0.82	4.74±1.94	$U=119.5$	0.761
Overall Activity	82.73±14.6	65.35±11.68	$F_{1,30}=0.83$	0.369

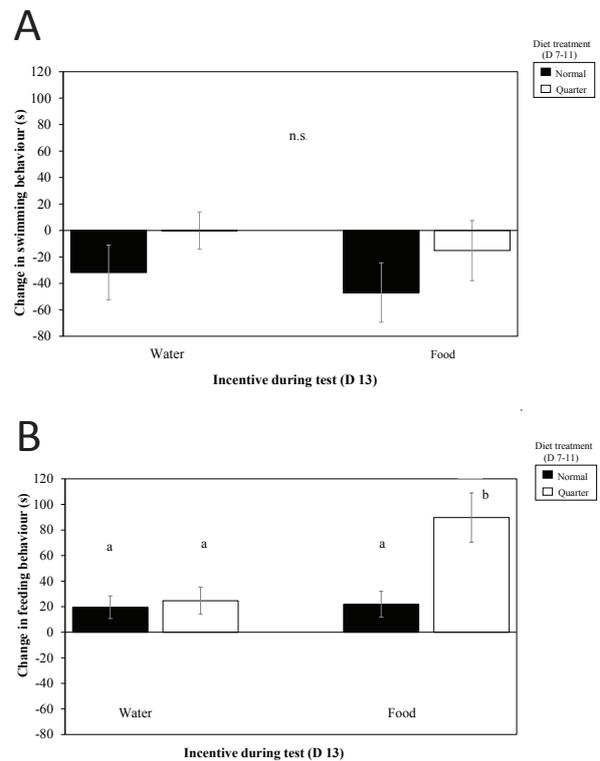
After collection, the six *P. thaul* clutches were placed in plastic bowls (15 L) filled with well water indoors at controlled photoperiod (12:12 L:D) provided by two fluorescent lamps (Philips daylight, TLT 40W/54RS) at 17±0.5°C. After hatching, 40 tadpoles were individually assigned to plastic cups (10 cm in diameter) with 250 ml of well water. From the first post-hatching day (day 1) until day 5, tadpoles were fed every other day with 1 ml of food which consisted of a freshly prepared mix of 0.05 g of fish food (VitaFish®) with 25 ml of algal culture (*Scenedesmus* sp. and *Chlamydomonas* sp.). On days 2, 4 and 6, plastic cups were cleaned and well water was added to complete 250 ml.

To examine the effect of feeding regime and food availability on the behavioural response of *P. thaul* tadpoles, the experiment followed a 2×2 factorial design: diet treatment (normal food ration and quarter food ration) and incentive cue treatment during evaluation (food or water). The diet treatments started on post-hatching day 7 when tadpoles ( $n=40$ ) were assigned to one of the two diet treatments. On days 7, 9 and 11, tadpoles were fed with 1 ml of the corresponding food solutions: "normal food ration (from now on NR)", which was prepared as that used for the maintenance of tadpoles, or "a quarter of normal food ration (from now on QR)", which consisted of a solution of normal food diluted with distilled water to 25%. On days 8 and 10, cups were gently cleaned with a bottom cleaner and well water was added to achieve 250 ml. On day 12, tadpoles from both diet treatments were maintained without food. The results were derived from a total of 32 tadpoles (eight tadpoles were inactive during the pre-stimulus period). The final number of tadpoles per group defined by the factorial design (diet treatment x incentive cue) was as follows: normal food-food ( $n=8$ ); normal food-water ( $n=9$ ); low food-food ( $n=7$ ); low food-water ( $n=8$ ).

On day 13, individual tadpoles corresponding to NR and QR diet treatments were randomly assigned to food or water incentive cue treatments. Food incentive was the same as "normal food ration". For the water incentive treatment, we added well water at room temperature to mimic the action of adding food. Containers were 10-cm diameter plastic cups filled with 250 ml of well water. Experimental trials took place from 1200 to 1700 hours. Tadpoles were allowed to acclimatise for 5 minutes. We first recorded tadpoles for 10 min (pre-stimulus period), then added the incentive treatment (1 ml of water or food) and allowed tadpoles to remain undisturbed for 2 minutes. Tadpoles in all treatments were then exposed to alarm cues (1 ml), and their activities were recorded

for 10 more minutes (post-stimulus period) using a digital video camera (Sony HDR-XR200V; 30 frames s<sup>-1</sup>) placed 0.70 m above the plastic cups. At the end of the experiment, tadpoles were weighed and their Gosner stages determined.

We recorded swimming and feeding activity levels for each tadpole. Swimming was defined as a wavy tail movement that involved displacement of the tadpole from one place to another, whereas feeding was defined as the action of repeatedly touching either the bottom or the walls of the cup with the mouth, combined with tail waving, as described by Horat & Semlitsch (1994) and Steiner (2007). To obtain behavioural variables from the videos recorded, an observer blinded to the treatments measured total duration (in seconds) of each behaviour using the software Fox-Pro v.2.0 (Fox Holdings), generating the behavioural variable "overall



**Fig. 1.** Mean change (±SE) in (A), swimming behaviour and (B), feeding behaviour (in seconds) from the pre-stimulus baseline for tadpoles exposed to water and food incentive treatments during the test (day 13). During the post-stimulus period, tadpoles in all treatments were exposed to alarm cues. n.s.: not significant. Different letters denote significant differences.

activity” (swimming + feeding). To study the effect of diet treatment, we used the total duration as dependent variable. To evaluate each type of behaviour under predation risk, we calculated the change in behaviour from the pre-stimulus baseline (post-pre) and used these scores as our response variables. Additionally, we performed correlations between total duration of each type of behaviour and mass (g) of tested tadpoles to test for size effects on the behavioural responses in both the pre- and post-stimulus periods. Data on the swimming and overall activity met assumptions of normality and homoscedasticity. Data on the feeding activity, tadpole mass and Gosner stage did not meet those assumptions. Hence, we analysed these data by non-parametric tests. A rejection criterion of  $p < 0.05$  was adopted for all analyses.

At the end of the experiment, body mass and Gosner stage of tadpoles were significantly affected by diet treatment [Mann-Whitney U tests,  $U=48$ ,  $p < 0.003$  and  $U=64.5$ ,  $p < 0.018$ , respectively]. Normally fed tadpoles weighed more [ $0.053 \pm 0.006$  g, Gosner stage  $28.41 \pm 0.19$ ] than tadpoles at QR food treatment [ $0.028 \pm 0.004$  g, Gosner stage  $27.40 \pm 0.32$ ]. The diet treatment exerted no significant effect upon behavioural repertoire during the pre-stimulus period (Table 1). We found a significant correlation between each behaviour activity level and tadpole mass during the pre-stimulus period ( $n=32$ ; swimming:  $r_s=0.52$ ,  $p < 0.002$ ; feeding:  $r_s=0.38$ ,  $p < 0.03$ ; overall activity levels:  $r_s=0.52$ ,  $p < 0.002$ ). Correlations between behaviour activity level and mass during the post-stimulus period were not significant [Spearman correlations, swimming:  $r_s=0.31$ ,  $p=0.083$ ; feeding:  $r_s=0.27$ ,  $p=0.135$ ; overall activity levels:  $r_s=0.25$ ,  $p=0.160$ ].

When experiencing alarm cues, tadpoles which previously experienced QR food treatments showed higher overall activity levels (two-way ANOVA,  $F_{1,28}=9.85$ ,  $p < 0.004$ ; NR,  $-18.29 \pm 17.25$  s; QR,  $47.78 \pm 12.68$  s), without significant effects of incentive stimulus ( $F_{1,28}=0.74$ ,  $p=0.398$ ) or the interaction between both factors ( $F_{1,28}=2.09$ ,  $p=0.159$ ). Diet ( $F_{1,28}=2.43$ ,  $p=0.131$ ) and incentive treatments ( $F_{1,28}=0.55$ ,  $p=0.464$ ) showed no significant effects on swimming activity (Fig. 1A). The interaction between both factors was not significant ( $F_{1,28}=0.00$ ,  $p=0.998$ ). The diet and incentive treatments showed significant differences with respect to feeding activity (Kruskal-Wallis,  $H_{3, n=32}=10.11$ ,  $p < 0.017$ , Fig. 1B). Pairwise comparisons revealed that tadpoles from QR diet treatment which received food as incentive exhibited a higher feeding activity than other groups (Fig. 1B), with a 51% rise in overall activity for the QR group (Fig. 1A, B).

Our main findings are that swimming behaviour was influenced by predation risk regardless of the feeding regime or food availability, whereas feeding behaviour drove tadpoles to take risks when food was available only under the QR food regime. As expected, we found that diet affected the mass of tadpoles. However, the lack of differences in behaviour activities between tadpoles with different diets during the pre-stimulus period revealed that poorly fed *P. thaul* tadpoles did not reach a motivational or physiological state to change their baseline behaviour (see also Fraker, 2008a). Moreover,

our results revealed a positive correlation between activity and size for all tadpoles during the pre-stimulus period similar to that found by Eklöv & Werner (2000) in the absence of predators. As the feeding regime did not affect baseline behaviours, the positive correlation between size and behaviour in the absence of predation risk cannot be explained by the feeding state of tadpoles.

Feeding regime had notable effects on the behavioural repertoire when tadpoles were exposed to alarm cues. During the post-stimulus period, only tadpoles under NR diet showed antipredator response by decreasing their overall activity. This result is in agreement with the general prediction that the strength of a prey's antipredator response should decrease with declining energetic reserves (Houston et al., 1993; Coleman et al., 2005). Our results suggest that tadpoles corresponding to the QR treatment suffered from hunger, and that they were exposed to the risks imposed by both predation as well as starvation (McNamara & Houston, 1994; Lima & Bednekoff, 1999; Coleman et al., 2005). Similarly, activity levels of unfed *Rana esculenta* and *R. lessonae* tadpoles were higher than those of fed tadpoles under predation risk generated by fish aroma (Horat & Semlitsch, 1994), and in *R. clamitans* tadpoles the level of hunger influenced the duration of the antipredator response (Fraker, 2008a).

Our results provide more coherent information when both behaviours (swimming and feeding) are considered separately. The increase in overall activity shown by tadpoles from QR food treatments during exposure to alarm cues appears as an effect of increased efforts for feeding. This suggests that feeding state alone cannot explain the increased activity in QR tadpoles under risk of predation. For QR tadpoles, the trade-off between avoiding predation and feeding appears to lean toward taking the risk of predation when motivation of food is present; tadpoles can expose themselves to predation at even low levels of starvation (McNamara & Houston, 1987). Furthermore, we expected tadpoles under the QR food regime to swim more in search for food although this was not supported by the data.

Our results indicate that swimming behaviour is apparently more sensitive to predation risk than feeding. First, starvation risk may always be present, as high food availability does not validate the assumption that tadpoles are not in danger of starving (McNamara & Houston, 1987). The differential sensitivity to predation risk of each type of behaviour may also be explained by the adaptive behaviour of predators and prey (Abrams, 1991; McNamara & Houston, 1994). Activity and space use in tadpoles is related to the degree of risk imposed by predators (Eklöv & Werner, 2000). The main predators of *P. thaul* tadpoles are ambushing insects (Jara & Perotti, 2010), and we suggest that tadpoles may primarily modify their swimming behaviour because it makes them more conspicuous or detectable (see also Steinberg et al. 2014 for a study on anole lizards). Taken together, our study adds evidence to the significance of motivational state of animals to understand how behavioural patterns reflect the balance of trade-offs between foraging requirements and predator avoidance.

**Acknowledgements:** We are grateful to FG Jara for his help in the field. We acknowledge to anonymous reviewers that helped to considerably improve the early version of the manuscript. This work was supported by Universidad Nacional del Comahue (UNCo) B 166, Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT) PICT 13-2384-Prestamo BID- to MP and Consejo Nacional de Investigaciones Científicas y Técnicas PIP-11220110100782 to MGP. All treatments followed the ethical norms imposed by the Administración de Parques Nacionales-Argentina, N° 1231, and samplings in Laguna Fantasma were authorised by Subsecretaría de Medio Ambiente of San Carlos de Bariloche (PM N° 2012).

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Accepted: 1 June 2015