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Animal behaviour

Diet alters species recognition in juvenile toads

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Whether environmental effects during juvenile development can alter the ontogeny of adult mating behaviour remains largely unexplored. We evaluated the effect of diet on the early expression of conspecific recognition in spadefoot toads, *Spea bombifrons*. We found that juvenile toads display phonotaxis behaviour six weeks post-metamorphosis. However, preference for conspecifics versus heterospecifics emerged later and was diet dependent. Thus, the environment can affect the early development of species recognition in a way that might alter adult behaviour. Evaluating such effects is important for understanding variation in hybridization between species and the nature of species boundaries.

1. Introduction

Generally, during reproduction individuals should preferentially associate with conspecifics to minimize the risk of costly matings with heterospecifics [1]. Such conspecific preferences are often assumed to be invariant among individuals. Any preferences for heterospecifics are ascribed to mistakes during, or constraints on, species recognition [2]. Yet, if hybridization is sometimes beneficial, selection might favour individuals that facultatively express preferences for conspecifics versus heterospecifics depending on the prevailing fitness consequences of hybridization [3,4].

Whether discrimination of conspecifics from heterospecifics varies with either the environment or an individual's internal state remains largely unexplored [3–6], as are the effects of early development on such species recognition. Yet, evaluating whether early environmental effects alter species recognition is important, because this could promote context-dependent hybridization and gene flow between species [3,4,6,7].

To date, studies of early experience on species recognition have focused on learning effects [8,9]. Where species recognition is unlearned (as in our system described below), species recognition is thought to be insensitive to environmental effects. Yet, even in species where species recognition is unlearned, conspecific preferences could ultimately stem from environmental effects during early development [3,4,6].

We addressed these issues by evaluating how diet affects the development of conspecific preferences. Because diet affects an individual's internal state (e.g. by altering size, body condition, brain development and even sensitivity to other environmental cues), it can directly or indirectly affect the development and expression of preferences [10–14].

We used spadefoot toads, *Spea bombifrons*, as our study system. In part of its range, *S. bombifrons* co-occurs and hybridizes with a congener, *Spea multiplicata*. In sympatry, male and female *S. bombifrons* discriminate *S. bombifrons* calls from *S. multiplicata* calls. Male *S. bombifrons* preferentially associate with conspecifics

Table 1. Contrasts of size (SVL), condition and latency to choose for juveniles on a high versus low diet at six and 12 weeks.

age group	phenotypic measure	mean difference (\pm s.e.m.), high diet–low diet	t-value	d.f.	p-value
six weeks	SVL	2.37 (0.35) mm	6.75	118	<0.001
	condition	−0.01 (0.02) g mm ^{−1}	−0.39	118	0.70
	latency	−1.75 (1.46) min	−1.20	103	0.23
12 weeks	SVL	6.16 (0.56) mm	10.95	86	<0.001
	condition	0.11 (0.05) g mm ^{−1}	2.37	85	0.02
	latency	0.66 (1.61) min	0.41	76	0.69

during breeding [15]. Females, by contrast, facultatively alter their preferences for conspecifics depending on their body condition (which is potentially the product of early development [16] and foraging success in previous years), and pond depth (which varies with rainfall in a given year) [4]. Such flexible female choice appears to have evolved because hybridization with *S. multiplicata* (which is faster developing) is beneficial in shallow water: hybrid tadpoles develop rapidly and are therefore more likely to escape an ephemeral pond [4]. This is especially important for poor-condition females, which produce slower developing tadpoles [4]. Thus, whereas females prefer conspecific calls in deep water (where tadpoles have time to develop), in shallow water, females—particularly those in poor condition—are more likely to prefer *S. multiplicata* calls [4].

Because males and females preferentially associate with conspecifics, but female preferences are condition dependent, we sought to determine whether early diet affects species recognition in juvenile *S. bombifrons*. By determining whether juvenile diet affects conspecific preferences, we evaluated whether early diet can *potentially* alter adult preferences and the likelihood of hybridization.

2. Material and methods

We bred six pairs of males and females from sympatric populations near Portal, AZ, USA. Tadpoles were fed tadpole chow ad libitum. At metamorphosis, juvenile toads were randomly assigned to either a high food or low food diet. Sibships were evenly divided between diet treatments. Individuals were size matched so no initial differences between diet treatments existed.

From metamorphosis until six weeks old, juveniles on high diet were fed five 1/8" crickets daily; those on low diet were fed five 1/8" crickets three times per week. After six weeks, both high- and low-diet animals were fed crickets daily, but high-diet juveniles were also fed two waxworms three times per week. These treatments mimicked variation in the toads' natural diets [17].

At six and 12 weeks post-metamorphosis, each toad was tested for its responses to conspecific male calls versus *S. multiplicata* male calls using previous methods for testing adult behaviour [4,15]. Specifically, we placed each toad in the centre of a circular, water-filled wading pool (1.8 m diameter). Each toad was initially placed on a central platform (above water level) equidistant between two speakers broadcasting either conspecific or heterospecific calls. We tested juveniles at a water depth (30 cm) in which *adult* females prefer conspecifics [4].

We scored a toad as preferring a call stimulus if it approached and touched a speaker. The time to touch the speaker was

recorded as the latency to choose. The toad was scored as non-responsive if it did not choose a stimulus within 30 min.

We measured mass and snout–vent length (SVL) at six and 12 weeks. We regressed mass on SVL and used the resulting residuals as our measure of body condition [4]. Data are available in the electronic supplementary material.

3. Results

At six weeks, juveniles on the high diet were larger than those on a low diet, but of similar body condition (table 1). By 12 weeks post-metamorphosis, high-diet juveniles were larger and in better condition than low-diet juveniles (table 1).

Both six and 12 week juvenile toads engaged in phonotaxis. Indeed, 88% of six week juveniles and 90% of the 12 week juveniles responded to one of the stimuli, a pattern that was significant (six weeks: log-likelihood $\chi^2 = 75.93$, d.f. = 1, $n = 120$, $p < 0.001$; 12 weeks: log-likelihood $\chi^2 = 62.74$, d.f. = 1, $n = 87$, $p < 0.001$). Moreover, we found no clear dietary effects on the likelihood of expressing phonotaxis at six weeks (log-likelihood $\chi^2 = 2.78$, d.f. = 1, $n = 120$, $p = 0.10$) or at 12 weeks (log-likelihood $\chi^2 = 1.74$, d.f. = 1, $n = 87$, $p = 0.19$). We also found no diet effects on latency to choose a stimulus (table 1).

We examined whether juveniles preferred conspecific over heterospecific male calls. As a group, six-week-old toads showed no preference (binomial test exact $p = 0.28$, $n = 105$). We found no effect of diet, SVL or condition on preferences of six-week-old juveniles (diet: log-likelihood $\chi^2 = 1.94$, d.f. = 1, $n = 105$, $p = 0.16$; SVL: log-likelihood $\chi^2 = 0.33$, d.f. = 1, $n = 105$, $p = 0.57$; condition: log-likelihood $\chi^2 = 0.74$, d.f. = 1, $n = 105$, $p = 0.39$). Thus, although juveniles show phonotaxis as early as six weeks post metamorphosis, they were not yet discriminating between conspecific calls and heterospecific calls.

By contrast, 12-week-old toads as a group preferred conspecific calls to heterospecifics calls (binomial test exact $p = 0.04$; $n = 78$). However, we found a significant effect of diet (log-likelihood $\chi^2 = 6.60$, d.f. = 1, $n = 78$, $p = 0.01$; figure 1). Specifically, juvenile toads on a high diet preferred conspecific calls to heterospecific calls (binomial test exact $p = 0.002$, $n = 44$; figure 1), but those on a low diet did not (binomial test exact $p = 0.30$, $n = 34$; figure 1).

This diet effect on conspecific preferences was mediated in part by body size. Across treatments, SVL, but not condition, significantly predicted preference for conspecific calls (SVL: log-likelihood $\chi^2 = 4.05$, d.f. = 1, $n = 78$, $p = 0.04$; condition:

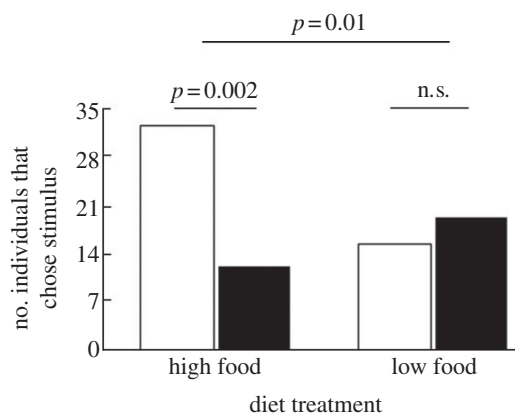


Figure 1. Diet affects conspecific preferences in 12 week juvenile toads. Juveniles on a high food diet preferred conspecific calls, whereas juveniles on a low food diet did not. White bars denote responses to conspecific calls and black bars denote responses to heterospecific calls.

log-likelihood $\chi^2 = 0.05$, d.f. = 1, $p = 0.82$; $n = 77$), which suggests a size threshold for conspecific preference.

However, within diet treatment, SVL did not predict preference (high-diet SVL: log-likelihood $\chi^2 = 0.01$, d.f. = 1, $n = 44$, $p = 0.91$; low-diet SVL: log-likelihood $\chi^2 = 0.01$, d.f. = 1, $n = 34$, $p = 0.90$). Moreover, when we restricted our analysis to toads that were between the 25th and 75th quartiles for SVL (i.e. where size between the diet treatments was more overlapping), we found a strong effect of diet, but not SVL, on conspecific preference (diet: likelihood $\chi^2 = 4.14$, d.f. = 1, $n = 30$, $p = 0.04$; SVL: log-likelihood $\chi^2 = 1.72$, d.f. = 1, $n = 30$, $p = 0.19$). These results suggest that the development of conspecific preference is also altered directly by diet.

4. Discussion

Juvenile toads expressed phonotaxis as early as six weeks (as found in another frog species [18]), but the expression of phonotaxis was independent of diet.

Preferences for conspecifics emerged at 12 weeks, but only among high-diet toads. Because the auditory system of rapidly developing frogs (such as spadefoots) may not be fully

developed at metamorphosis [19], diet could alter the development of species recognition through effects on the development of the auditory system. However, this possibility is unlikely to fully explain our findings. Juveniles displayed phonotaxis regardless of diet, indicating that their auditory system is sufficiently developed for phonotaxis. Moreover, although size predicted preference, when size was controlled, diet still affected conspecific preferences. Additionally, noradrenaline levels in the auditory system of six-week-old *S. bombifrons* differentiate conspecific and heterospecific calls independently of diet [20], suggesting that diet does not affect call perception. Finally, in response to conspecific calls, one-week-old *S. multiplicata* express enhanced monoamine activity in the tegmentum, a mid-brain region involved in phonotaxis [21]. Thus, diet may not simply delay auditory system development.

Regardless of mechanism, our results suggest that diet could affect the ontogeny of species recognition. Because we did not evaluate the effects of juvenile diet on adult species recognition, we cannot establish whether different preferences in our treatment groups reflect an individual's immediate state or whether they reflect shifts in ontogeny that might have long-term consequences for adult behaviour. Nevertheless, our results highlight the *potential* for such effects to arise.

Evaluating the life stage at which environmental effects modify species recognition has important ramifications. Early experience during critical developmental periods could generate long-lasting effects on species recognition, resulting in stable lifetime preferences within individuals but striking variation among individuals (mimicking a genetic polymorphism). Alternatively, an individual's contemporary environment could affect its immediate expression of species recognition, such that the degree to which an individual preferentially associates with conspecifics (and, therefore, hybridization) becomes closely tied to environmental stochasticity. Future work is needed to determine how—and at what point in ontogeny—environmental influences affect species recognition and, consequently, hybridization.

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