

The influence of visual and tactile stimulation on growth and metamorphosis in anuran larvae

I. ROT-NIKCEVIC,*† R. J. DENVER‡ and R. J. WASSERSUG*

*Department of Anatomy and Neurobiology, Dalhousie University, 5850 College St, Halifax, Nova Scotia, Canada B3H 1X5 and †Department of Molecular, Cellular and Developmental Biology, University of Michigan, 3077 Natural Science Bldg, Ann Arbor, Michigan, 48109–1048, USA

Summary

1. Sensory modalities that allow tadpoles to assess their environment, and subsequently mediate their development, are not well understood.
2. By putting clay model tadpoles into the tanks with live tadpoles we have enhanced tactile and visual stimuli for tadpoles of three species (*Rana sylvatica*, *Bufo americanus* and *Xenopus laevis*) in a controlled fashion. The goal was to determine whether visual and tactile cues in the absence of chemical signals influenced tadpole growth and development.
3. The response to enhanced visual and tactile stimuli was strong in *Rana*, intermediate in *Xenopus*, but absent in *Bufo* tadpoles. *Rana* tadpoles that experienced both stimuli enhanced developed the fastest and metamorphosed at the smallest body size. Development was slower in the treatments with only one stimulus enhanced, and slowest in the controls.
4. Our results suggest that tadpoles use both vision and mechanoreception for environment assessment, and that they are able to modify their growth and developmental rates in response to sensory enrichment.
5. Tadpoles exposed to the combination of visual and tactile stimulation showed the highest whole-body content of the stress hormone corticosterone, suggesting that the enhanced stimuli were experienced as stressful. Corticosterone is known to synergize with thyroid hormone to promote metamorphosis.

Key-words: Corticosterone, development, mechanoreception, tadpoles, vision

Functional Ecology (2005) **19**, 1008–1016
doi: 10.1111/j.1365-2435.2005.01051.x

Introduction

Amphibian larvae exhibit extreme plasticity in life-history traits in response to their environment. Two of the most plastic traits of amphibians, the timing and size at metamorphosis, are both influenced by various abiotic and biotic factors (Smith-Gill & Berven 1979; Berven & Chadra 1988; Newman 1989; Scott 1990; Hayes, Chan & Licht 1993; McCollum & Van Buskirk 1996). Unpredictable or deleterious (i.e. stressful) changes in habitat, such as pond desiccation, limited food resources, presence of predators or competition, can all affect the duration of the larval stage and metamorphosis (Newman 1994; Denver, Boorse & Glennemeier 2002). Plasticity in the timing of metamorphosis can be adaptive as it allows larval amphibians to respond to changes in the quality of their aquatic habitat (Stearns 1989; Newman 1992).

For anurans even tadpoles from the same clutch can show a wide range of body sizes and ages at transformation. The biological basis for this variation has been studied by molecular biologists (Shi 1994; Wang & Brown 1993; Denver, Pavgi & Shi 1997), who have identified many genes and genetic programmes activated at metamorphosis (Shi 2000). At the same time many ecologists have studied social and environmental factors that affect metamorphosis (Newman 1989; Hayes *et al.* 1993; Denver 1997, 1998). In amphibian species, for example, crowding of the larvae has been shown to slow growth and development (Wilbur & Collins 1973; Smith-Gill & Berven 1979; Berven & Chadra 1988; Scott 1990), and this effect is mediated by an increase in corticosterone (Glennemeier & Denver 2002a). Tadpoles of several anuran species accelerate metamorphosis in response to habitat desiccation (Newman 1989, 1992; Denver, Mirhadi & Phillips 1998). Denver (1998) showed that tadpoles of Western Spadefoot Toads (*Scaphiopus hammondi*) exposed to declining water levels accelerated metamorphosis compared with tadpoles raised in a constant high water

environment. What remains least investigated is the link between the two approaches, i.e. the mechanisms that allow tadpoles to sense ('read') their environment and subsequently mediate their developmental response to extrinsic factors, such as density or water depth.

Tadpoles are capable of using mechanoreception through the lateral line system, chemoreception (taste) and vision (see Lannoo 1999) to assess features of their habitat. Of these three sensory pathways (chemical, visual, mechanical) for environmental assessment, the reaction of tadpoles to chemical stimuli has been analysed the most. In response to chemical cues from predators tadpoles alter their phenotype; they become less active and develop a relatively small body and deep tail fin (Van Buskirk 2002; Van Buskirk, McCollum & Werner 1997; Relyea & Werner 1999, 2000; Relyea 2001). Isolated chemical cues from predators, e.g. fish, elicit a similar response in tadpoles as does the presence of the fish itself (Petranka, Kats & Sih 1987; Kats, Petranka & Sih 1988; Semlitsch & Reyer 1992; Kats & Dill 1998). Conversely in response to competitors, tadpoles become more active, and develop relatively smaller tails and larger bodies (Relyea 2002).

In contrast to chemical signalling, the relative importance of mechanoreception and vision in environment assessment has not been studied. Here we ask whether tadpoles can rely on visual cues to assess their habitat (such as the presence of tadpole-like objects) in a way that will alter their growth and development. Similarly, we asked whether tactile cues alone alter larval growth and development. We hypothesized that tactile cues may be of lesser importance than other cues in species that tend to school densely, such as *Bufo americanus*, since they are normally exposed to abundant tactile cues. Species that normally form schools with larger distances between neighbouring conspecifics, such as *Rana sylvatica* and *Xenopus laevis*, may be more responsive to both visual and tactile cues. In a similar way one can hypothesize that tadpoles living in small ponds with dense vegetation may rely less on visual cues.

After registering information from the habitat, no matter what sensory modalities used, anuran larvae need to transform the perceived sensory information into a developmental and/or behavioural response that increases their fitness in a given environment. Denver (1997) suggested that the tadpole's neuroendocrine system functions as a monitoring system, transducing environmental information into a physiological response, thus modifying the rate of development. The tadpoles of Western Spadefoot Toad (*Spea hammondi*) subjected to habitat desiccation exhibited elevated hypothalamic corticotropin-releasing hormone (CRH) content at the time when they responded developmentally to the declining water level (Denver 1996). Also, CRH injection elevated whole-body thyroxine, triiodothyronine and corticosterone content, the primary hormonal regulators of metamorphosis, while blockade of endogenous CRH action reduced hormone content and slowed pond drying-induced metamorphosis. These data sup-

port a central role for CRH as a neurohormonal transducer of environmental stimuli into the endocrine response, which modulates the rate of metamorphosis (Denver 1997; Boorse & Denver 2004).

The objectives of the current study were to elucidate sensory pathways that tadpoles can use in environment assessment and physiological response mechanisms regulating metamorphosis in anuran larvae. We ask here: How do tadpoles 'recognize' and sense environmental factors that may influence their rate of development? In a controlled laboratory setting we analysed the relative importance of tactile and visual stimuli, alone and in combination, on growth rates and time to metamorphosis in tadpoles of three species.

Materials and methods

ANIMALS

The experiment was performed with three species, Wood Frog (*Rana sylvatica*, LeConte), American Toad (*Bufo americanus*, Holbrook) and African Clawed Frog (*Xenopus laevis*, Daudin) to test whether potential differences in developmental responses are species-specific. These species show different schooling behaviours and differ in their population densities in the wild. American Toad tadpoles school very densely, compared with Wood Frog tadpoles, which school, but to a much lesser extent (Wassersug 1973; O'Hara & Blaustein 1982; Waldman 1984). *Bufo* schools are usually near or on the bottom and tadpoles are commonly in physical contact with each other. Schools formed by African Clawed Frog tadpoles are clusters of strongly polarized individuals suspended in midwater. *Xenopus* avoid contact with each other and maintain a spatial distance of at least a full body's width (Wassersug 1973). Their schools are often static. In contrast *Rana sylvatica* tadpoles primarily school when moving as an aggregate, but like *Xenopus* individuals *Rana* do not contact each other (Wassersug 1973).

In each experiment we raised 20 premetamorphic tadpoles per 10-l plastic tank (45 cm × 24 cm × 12 cm). We considered 20 tadpoles per 10 l as our low-density treatment; e.g. Glennemeier & Denver (2002a) used 8 tadpoles per 4 l as low density in *Rana pipiens*, and 40 tadpoles per 4 l as high density. Each treatment had three replicates (3 tanks = 60 tadpoles total). The experiment conducted in 2001 with *R. sylvatica* and *B. americanus* started when tadpoles reached Gosner developmental stage 29–31 (Gosner 1960), and with *X. laevis* at Nieuwkoop–Faber stage 50–52 (Nieuwkoop & Faber 1956), which corresponds to Gosner stage 28–30. Tadpoles were randomly assigned to experimental tanks. The experiment with *R. sylvatica* conducted in 2002 started when tadpoles reached Gosner developmental stage 27–28. Denver *et al.* (1998) showed that the minimum developmental stage to respond to habitat desiccation for *Scaphiopus hammondi* is Gosner stage 30–32.

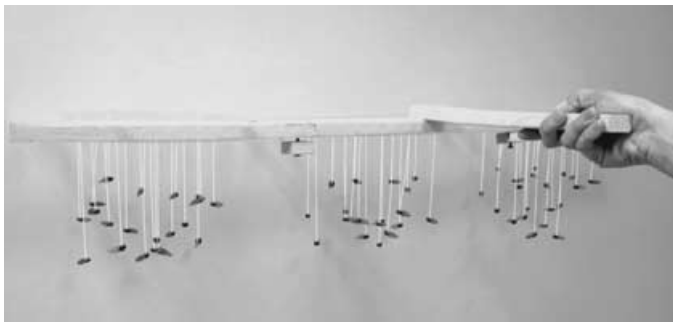


Fig. 1. A specially designed array of tadpole clay models on tips of the plastic rods. The artificial tadpoles were able to rotate on rods. By manually sliding the tool from one end of the tank to the other, the tadpole models approached and were free to contact live tadpoles. Each frame carried 20 artificial tadpoles, thus mimicking a doubling of the conspecific density in each tank. Rods were of different lengths so that the artificial tadpoles could be at different distances from the tank bottom.

Rana sylvatica eggs were collected on 26 April 2001, at Harrietsfield's Pond, Halifax County, Nova Scotia. Eggs from three medium size clutches hatched on 30 April in our laboratory. Four weeks later, when most of the larvae reached Gosner developmental stage 30, they were measured and randomly assigned to tanks for treatments. *Bufo americanus* tadpoles (approximately 1 week old) were collected at Crystal Crescent Beach, Halifax County, Nova Scotia, on 13 June 2001. The treatments started on 29 June 2001. *Xenopus laevis* eggs were obtained from our in-house breeding colony. Eggs from one clutch hatched on 19 August 2001 and the experiment started on 10 September 2001.

EXPERIMENTAL DESIGN

To visually and mechanically stimulate tadpoles in a controlled fashion, a specially designed array of clay models of tadpoles on the tips of the plastic rods was used (Fig. 1). The frame rested on top of the tank above water with the plastic rods immersed in the water. At the end of each rod was a clay tadpole. The clay models were made for each species individually to mimic in size, shape and colour the tadpoles of the species being tested (Fig. 2). The array of model tadpoles was drawn through the water to produce the enhanced tactile stimulus effect. The artificial tadpoles were able to rotate on the rods. By manually sliding the frame from one end of the tank to the other the tadpole models approached and were free to contact live tadpoles. Each tool carried 20 artificial tadpoles. Rods differed in length so that the artificial tadpoles could be at different distances from the tank bottom, reflecting where the tadpoles of the species being studied are found in the water column in nature. The models were mostly on the bottom for *B. americanus* and at various depths for *X. laevis* and *R. sylvatica* (see vS Hoff *et al.* 1999, and references therein).

All tanks were kept in the same animal room, out of direct sunlight and under overhead lights. Photoperiod was kept constant 12:12 h light : dark for all tadpoles,



Fig. 2. Clay model of *Rana sylvatica* tadpole. The clay models were made for each species individually in an effort to mimic in size, shape and colour the tadpoles of the species being tested.

Table 1. Explanation of the experimental treatments

		During dark phase	During light phase
Model tadpoles	Moved	Treatment no. 1	Treatment no. 3
	Still	Tactile stimulus	Tactile + Visual Treatment no. 2 Visual stimulus

with lights switching on and off at 9.30 a.m. and 9.30 p.m., respectively. Water temperature ranged from 20 to 22 °C. *Rana sylvatica* and *B. americanus* tadpoles were fed *ad libitum* boiled lettuce and spinach leaves, and *X. laevis* tadpoles were fed alfalfa powder and yeast mixture. One-third of the water was gently syphoned and replaced with standing tap water every 3–4 days. Feeding and tank cleaning took the same amount of time for each tank, with as little disturbance as possible. Water in all tanks was aerated constantly.

By sliding the array of model tadpoles during the light and/or dark cycle, the live tadpoles were exposed to enhanced mechanical (i.e. tactile) stimulus alone (treatment 'T'), or enhanced visual (treatment 'V') stimulus alone, or a combination of both enhanced tactile and visual stimulation (treatment 'T + V') (see Table 1). Stimulation was repeated four times a day for each treatment, every day, until forelimbs had emerged (FLE) in 50% of all the tadpoles in one treatment, which corresponds to Gosner stage 42, and Nieuwkoop–Faber stage 58. Manipulations took place just before or after the lights were turned on or off (at 9.30 a.m./9.30 p.m.). For example, when the lights came on at 9.30 a.m., the treatment T + V was performed. It was repeated once, 20 min after the first stimulation. The array of model

tadpoles was left in the tanks (providing constant visual stimulus). At 9.00 p.m., before the lights went off, the treatment T + V was performed again, and then was repeated once 20 min after the first stimulation (9.20 p.m.).

For the treatment V the arrays of model tadpoles were constantly present in the tanks during the light phase without sliding movements, thus providing visual stimulus only. The focus was on creating a dynamic *vs* static environment in 'tactile only' *vs* 'visual only' treatments. Although model tadpoles were physically present in both the visual only and tactile only treatments, the mechanical stimulation from static objects clearly differed from the mechanical cues produced by moving tadpole models, thus distinguishing the tactile (i.e. the enhanced mechanical) stimulus in the 'tactile only' from the tactile stimulus in the 'visual only' treatment. When testing the effects of tactile cues we primarily studied the effects of enhanced water movements. However, the clay models resembled real tadpoles in their size and velocity and we expected that this produced tactile cues similar to those that would normally be elicited from real conspecifics.

At 9.30 p.m., when the lights went off, the treatment T was performed by moving the model tadpoles in the dark, i.e. providing tactile stimulus only. It was repeated once, 20 min after the first stimulation. The tool was left in the tank during the night, and at 9.00 a.m., before the lights went on, the treatment was performed again and then repeated 20 min after the first stimulation. The tool was removed from the tank before the lights went on.

The sliding movement was performed a fixed number of times to standardize treatments in each trial. The tool was moved the length of the tank 300 times during each stimulation, and each stimulation lasted 5 min (i.e. one sliding movement every second). To standardize the speed in all treatments every sliding movement was made to last 1 s. This corresponded to the speed of approximately 10 body lengths per second; i.e. fast swimming for tadpoles (see Wassersug & vS Hoff 1985; vS Hoff & Wassersug 1986). To avoid possible differences in the speed at which the tool is moved, the three replicates of each treatment were kept next to each other, and the frames of the tools connected. Thus they were being pulled together, at the same time and speed. Tanks were on a shelf 'hidden' behind a curtain, and it was possible to slide the tools while reaching inside the curtain with a single hand. This shielding was undertaken to minimize other visual stimuli, i.e. the experimenter's presence, that could be stressful to the tadpoles.

Two controls were used with each species. Control no. 1 (C1) consisted of three tanks without any model tadpoles or rods, in order to analyse the effects of the treatments. Control no. 2 (C2) was used to test for the possible effects of chemicals introduced into the water from the rods and model tadpoles. It consisted of three tanks with plastic rods left on the tank bottom, and clay tadpoles left in as a pile in the tank corner, covered (hidden) by cheesecloth. Cheesecloth was also placed in all the tanks with experimental treatments.

The treatments in the experiment with *R. sylvatica* lasted until 50% of tadpoles in all three experimental treatments reached FLE which occurred after 25 days. The *B. americanus* experiment was stopped when 50% of the tadpoles in at least one treatment reached FLE (28–32 days depending on the tank). The experiment with *X. laevis* tadpoles was terminated 50 days after it started, when only four tadpoles had reached FLE, because differences in developmental stages among tadpoles in the same tank were too large, and when the first larvae metamorphosed the other ones in the same treatment were still very far from FLE.

The experiment with *R. sylvatica* was repeated in the following year, 2002. In this year the experiment started with younger tadpoles, Gosner stage 27–28, and the treatments were run only until the first metamorphosed individual appeared in any of the treatments. In the previous year the individuals that metamorphosed first could not leave the tanks and drowned, thus they were lost for experimental purposes.

At the end of experiments all tadpoles were killed by adding MS 222 to the rearing water to a final concentration of 300 mg l⁻¹. They were staged, weighed and measured. In metamorphosed froglets the time to metamorphosis was determined as the number of days between the Day 1 (when the treatments started) and the day when the tadpole reached FLE.

Total body length, tail length, maximum tail fin height and tail muscle height were measured at the beginning of the experiment. The same set of body measurements, plus mass, were taken following the treatments. By comparing these data among tadpoles from different treatments we hoped to determine whether tadpoles respond to increased competition and crowding by altering their shape (as previously shown by McCollum & Van Buskirk 1996; Relyea 2000, 2001).

HORMONE EXTRACTIONS

Twenty *R. sylvatica* tadpoles per treatment that had reached FLE were kept on dry ice, staged, measured, individually wrapped in aluminium foil and stored at -80 °C for later extraction and analysis of whole-body corticosterone (CORT) content by radioimmunoassay (RIA). The extraction procedure is described by Hayes & Wu (1995) with modifications by Denver (1998), and the RIA for CORT was performed as described by Licht *et al.* (1983). Data on a possible increase in whole-body stress hormone content should clarify whether tadpoles assessed their environment as a stressful one, and whether a stressor was the initiator of the changes in time and size at metamorphosis, as has been shown in other species (see Denver *et al.* 2002 and references therein).

STATISTICAL ANALYSES

All analyses were run in STATISTICA (Statistica for Windows 1997) and SAS/Stat (2001). Body measurements were log-transformed to improve the linearity of

Table 2. Morphological traits in *Rana sylvatica* tadpoles following the treatments. Values are means \pm SE (range) (N = sample size)

	Stage	Body length (mm)	Tail length (mm)	Tail fin height (mm)	Tail muscle height (mm)	Body mass (g)
T1 (Tactile) $N = 57$	42.4 \pm 0.3 (39–46)	40.1 \pm 1.4 (13.8–51.9)	26.0 \pm 1.2 (3.7–35.8)	6.4 \pm 0.2 (2.5–8.5)	3.6 \pm 0.1 (2.2–4.7)	0.66 \pm 0.03 (0.23–1.05)
T2 (Visual) $N = 59$	41.5 \pm 0.3 (36–45)	41.1 \pm 1.0 (14.6–51.2)	26.3 \pm 0.9 (4.6–35.3)	5.4 \pm 0.2 (2.0–7.9)	3.4 \pm 0.1 (2.1–4.4)	0.57 \pm 0.02 (0.26–0.87)
T3 (Tactile + Visual) $N = 55$	42.6 \pm 0.2 (38–45)	39.4 \pm 1.0 (17.8–51.2)	25.9 \pm 0.9 (6.4–34.2)	5.1 \pm 0.2 (2.7–6.7)	3.4 \pm 0.1 (2.52–4.3)	0.54 \pm 0.01 (0.39–0.86)
C1 (Treatments) $N = 58$	41.3 \pm 0.2 (37–45)	46.8 \pm 1.0 (18.4–55.7)	30.3 \pm 0.9 (5.0–37.2)	6.8 \pm 0.2 (2.2–8.8)	4.0 \pm 0.1 (2.6–4.9)	0.78 \pm 0.02 (0.55–1.19)
C2 (Material) $N = 55$	41.5 \pm 0.3 (36–46)	43.3 \pm 1.2 (15.8–55.5)	29.5 \pm 1.0 (4.1–36.5)	6.7 \pm 0.3 (2.72–8.5)	4.0 \pm 0.1 (1.6–5.4)	0.71 \pm 0.02 (0.30–1.11)

pairwise relationships between measured traits. After log-transformations the distribution was checked visually by assessing quantile–quantile plots, and the assumptions of normality were met. To analyse the differences between treatments the linear mixed effects model was used. The model has two components – fixed effects (treatment and stage) and random effects (tank). The treatment effect was tested over the effect of tank nested within the treatment, and tank was used as a random factor. The random effects in the mixed model allow for the correlation between tadpoles within a tank. Differences between treatments in morphological traits were tested using analyses of covariance (ANCOVA) with stage as covariate. To control for the curved relationship between stage and total body length we used the quadratic effect of stage in addition to the linear effect. Where significant differences were found with the ANCOVA a Tukey–Kramer test was used to compare treatments' means. Differences between treatments in developmental stages were tested using analyses of variance (ANOVA). All statistical results were considered significant as an alpha of 0.05.

Results

RANA SYLVATICA (2001)

The first metamorphosed individual appeared in the T + V treatment and the largest number of tadpoles (44) with FLE at the termination of the experiment was found in this treatment. In the T and the V treatments the number of metamorphosed animals was 35 and 34, respectively, but the differences in stage (see Table 2) between the three treatments were not significant (ANOVA, df (1, 10), T vs V: $F = 1.66$, $P = 0.23$; T vs T + V: $F = 0.05$, $P = 0.83$; V vs T + V: $F = 2.25$, $P = 0.16$). The control treatments had the smallest number of metamorphosed individuals, 26 in C1 and 28 in C2. The average developmental stage was significantly greater in the three experimental treatments compared to the controls (e.g. ANOVA, df (1, 10), T + V vs C1: $F = 5.76$, $P = 0.04$; T + V vs C2: $F = 5.62$, $P = 0.04$).

In contrast, body size was the smallest in tadpoles in the T + V treatment. They had the smallest total body length, tail length and body mass compared with all the other experimental groups (see Table 2); ANCOVA: **Total body length**, T + V vs T and T + V vs V: df (1, 251), $F > 2.89$, $P < 0.04$; T + V vs controls: $F > 11.56$, $P < 0.001$. **Tail length**: T + V vs T, and T + V vs V: df (1, 242), $F > 5.47$, $P < 0.03$; T + V vs controls: $F > 9.99$, $P < 0.002$. **Body mass**: df (1, 251), T + V vs T: $F = 3.06$, $P = 0.04$; T + V vs V: $F = 11.49$, $P < 0.001$; T + V vs C1: $F = 5.90$, $P = 0.02$; T + V vs C2, $F = 18.15$, $P < 0.001$.

Tadpoles in the T treatment had shorter total body length than tadpoles in the V treatment (ANCOVA, df (1, 251), $F = 15.37$, $P < 0.001$), shorter tail length (df (1, 242), $F = 17.39$, $P < 0.001$), but similar body mass (df (1, 251), $F = 2.99$, $P = 0.08$).

Across all five experimental groups tadpoles did not show significant differences in either tail fin height (ANCOVA, df (1, 228), $F = 0.44$, $P = 0.78$) nor tail muscle height (ANCOVA, df (1, 215), $F = 1.14$, $P = 0.26$).

WHOLE-BODY CORTICOSTERONE CONTENT

The whole-body CORT content in *R. sylvatica* tadpoles was significantly greater in tadpoles exposed to a combination of enhanced tactile and visual stimuli than in tadpoles in any other experimental group (Table 3)

Table 3. Whole-body content of stress hormone corticosterone (CORT) in *Rana sylvatica* tadpoles, following the treatments. Values are means \pm SE (range) (N = sample size). Corticosterone content is given in ng/g body mass

Treatment	N	CORT
T1 (Tactile)	7	5.42 \pm 1.12 (1.52–9.91)
T2 (Visual)	5	3.72 \pm 1.58 (1.39–9.95)
T3 (Tactile + Visual)	6	10.82 \pm 1.07 (7.53–13.92)
C1 (Treatments)	6	1.96 \pm 0.40 (0.95–3.80)
C2 (Material)	7	5.11 \pm 1.25 (0.64–9.09)

Table 4. Morphological traits in *Bufo americanus* tadpoles following the treatments. Values are means \pm SE (range) (N = sample size)

	Stage	Body length (mm)	Tail length (mm)	Tail fin height (mm)	Tail muscle height (mm)	Body mass (g)
T1 (Tactile) $N = 51$	40.6 \pm 0.6 (33–46)	23.4 \pm 0.4 (16.6–27.2)	12.9 \pm 0.3 (6.0–15.6)	3.5 \pm 0.1 (1.9–5.0)	1.8 \pm 0.1 (1.1–2.3)	0.16 \pm 0.01 (0.06–0.25)
T2 (Visual) $N = 48$	39.8 \pm 0.6 (32–46)	22.6 \pm 0.4 (16.8–26.0)	12.6 \pm 0.4 (6.6–15.6)	3.5 \pm 0.1 (2.1–4.7)	1.5 \pm 0.1 (1.0–2.4)	0.15 \pm 0.01 (0.09–0.21)
T3 (Tactile + Visual) $N = 58$	41.5 \pm 0.5 (32–46)	22.9 \pm 0.4 (17.3–26.5)	12.7 \pm 0.3 (9.0–15.6)	3.6 \pm 0.2 (2.2–4.9)	1.6 \pm 0.1 (1.1–2.0)	0.15 \pm 0.01 (0.07–0.20)
C1 (Treatments) $N = 44$	38.8 \pm 0.6 (32–46)	23.1 \pm 0.4 (16.3–26.4)	12.8 \pm 0.2 (9.0–14.3)	3.8 \pm 0.1 (2.9–4.7)	1.6 \pm 0.1 (1.1–2.3)	0.16 \pm 0.01 (0.07–0.21)
C2 (Material) $N = 43$	38.9 \pm 0.5 (35–45)	22.5 \pm 0.2 (19.9–26.2)	12.2 \pm 0.2 (9.2–15.7)	3.7 \pm 0.1 (2.5–4.9)	1.7 \pm 0.0 (1.2–2.2)	0.15 \pm 0.01 (0.09–0.26)

(ANOVA, df (1, 26), T + V *vs* T: $P = 0.02$; T + V *vs* V: $P = 0.004$; T + V *vs* C1: $P = 0.01$; T + V *vs* C2: $P < 0.001$). Tadpoles in the T + V treatment were the ones that had the fastest developmental rate. The lowest whole-body CORT was found in tadpoles in the control group without any cues (C1), i.e. the group that showed the slowest development. The remaining groups had intermediate levels of whole-body CORT, and the between group differences were not statistically significant ($P = 0.20$).

RANA SYLVATICA (2002)

To avoid losing experimental animals because of drowning of metamorphosing froglets, the experiment with *R. sylvatica* in the following year (2002) was stopped as soon as the first metamorphosed froglet appeared. The majority of tadpoles were still at early developmental stages. The mean developmental stage ranged from 34.16 ± 0.37 in C1, to 35.22 ± 0.26 in the T treatment and the differences between treatments were not statistically significant (ANOVA, df (4, 10), $F = 1.87$, $P = 0.21$).

In this year the only significant difference found was in body mass. Similar to the previous year, tadpoles in the control group C2 were the heaviest (mean: 0.51 ± 0.02 g) followed by the control group C1 (mean: 0.48 ± 0.02 g). Tadpoles in the control groups were significantly heavier than animals in the treatments with enhanced stimuli (ANCOVA, df (1, 245), $P < 0.05$ in all pairwise comparisons). Among the experimental treatments, body mass ranged from 0.42 ± 0.02 g in the T treatment, to 0.44 ± 0.02 g in the T + V treatment. The differences were not statistically significant (ANCOVA, df (1, 245), $P > 0.05$ in all pairwise comparisons).

BUFO AMERICANUS

There were no statistically significant differences between treatments for mean developmental stage (ANCOVA, df (4, 10), $F = 1.94$, $P = 0.18$). However, a similar pattern to the one in *Rana* tadpoles was observed, as the first metamorphosed individual, and the largest number of tadpoles (32) that reached FLE stage, were found in the T + V treatment. In the V and the T treatments the

number of metamorphosed animals was 29 and 27, respectively. The controls had the slowest developmental rate and the smallest number of metamorphosed animals; i.e. 25 in C1 and 26 in the C2. Differences in morphological traits (Table 4) were not statistically significant either (ANCOVA, $F < 1.23$, $P > 0.3$ for all traits).

XENOPUS LAEVIS

For *X. laevis* tadpoles treatments were stopped after 50 days. At that point only four tadpoles had reached the FLE stage, two of which were in the T + V, and one in each V and C1 treatments. Differences between groups were not statistically significant (ANOVA, df (4, 10), $F = 3.32$, $P = 0.06$). **Body mass** was significantly greater in control groups than in all three experimental treatments with enhanced stimuli (Table 5). For example, ANCOVA, df (1, 220), T *vs* C1: $F = 6.10$, $P = 0.01$; T + V *vs* C1: $F = 4.94$, $P = 0.02$. Similarly, the **total body length** was greater in tadpoles from the three treatments with enhanced cues than in the control tadpoles (i.e. ANCOVA, df (1, 220), T + V *vs* C1: $F = 4.16$, $P = 0.04$; T + V *vs* C2: $F = 8.76$, $P = 0.004$; V *vs* C2, $F = 10.82$, $P = 0.001$). Tail length, tail fin height and tail muscle height were greater in control tadpoles than in tadpoles from tactile stimuli ($P < 0.01$ for all pairwise comparisons).

Discussion

Tadpoles of all three species that had experienced increased tactile plus visual stimuli progressed through developmental stages the fastest. However, these differences were statistically significant only in *R. sylvatica* tadpoles. The probable explanation is that in species that form dense schools, such as *B. americanus*, increased tactile and/or visual stimulation does not have the same effect as in the species that normally occur at lower density and do not exhibit strong schooling behaviour, such as *R. sylvatica*. The differences in timing of metamorphosis were not significant among the treatments in which tadpoles were exposed to only one type of stimulus, either visual or tactile. This suggests that *Rana* tadpoles use *both* vision and mechanoreception to assess features of their habitat, and they are able to accelerate

Table 5. Morphological traits in *Xenopus laevis* tadpoles following the treatments. Values are means \pm SE (range) (N = sample size)

	Stage	Body length (mm)	Tail length (mm)	Tail fin height (mm)	Tail muscle height (mm)	Body mass (g)
T1 (Tactile) $N = 52$	52.2 \pm 0.3 (48–55)	30.0 \pm 0.6 (18.3–37.8)	19.5 \pm 0.4 (11.7–25.1)	3.1 \pm 0.1 (2.0–4.3)	2.1 \pm 0.1 (1.25–2.88)	0.17 \pm 0.01 (0.05–0.28)
T2 (Visual) $N = 53$	52.9 \pm 0.4 (47–65)	30.0 \pm 0.8 (10.9–41.3)	19.8 \pm 0.5 (11.9–29.4)	3.0 \pm 0.1 (1.7–4.2)	2.2 \pm 0.1 (1.6–2.9)	0.16 \pm 0.01 (0.03–0.29)
T3 (Tactile + Visual) $N = 53$	53.7 \pm 0.3 (50–64)	32.1 \pm 0.6 (17.1–41.9)	20.7 \pm 0.4 (6.8–26.7)	3.3 \pm 0.1 (1.8–4.5)	2.4 \pm 0.1 (1.5–3.6)	0.18 \pm 0.01 (0.05–0.34)
C1 (Treatments) $N = 31$	54.0 \pm 0.4 (50–61)	33.7 \pm 1.0 (22.4–44.9)	21.9 \pm 0.7 (14.0–28.5)	3.4 \pm 0.1 (2.3–5.1)	2.5 \pm 0.1 (1.7–3.9)	0.22 \pm 0.02 (0.07–0.50)
C2 (Material) $N = 57$	53.2 \pm 0.3 (48–58)	33.3 \pm 0.6 (22.3–43.0)	21.3 \pm 0.4 (13.9–27.7)	3.5 \pm 0.1 (2.4–5.5)	2.5 \pm 0.1 (1.5–3.3)	0.21 \pm 0.01 (0.08–0.38)

development in response to a sensory-enriched environment (increased density, competition).

Growth rates were also affected by the experimental sensory enhancements in *R. sylvatica* and *X. laevis* tadpoles. *Rana* tadpoles exposed to the strongest stimulus, i.e. the combination of visual plus tactile, showed the slowest growth rate. These results are consistent with previous studies that showed that increased competition decreases growth rate in anuran larvae (e.g. Wilbur & Collins 1973; Smith-Gill & Berven 1979; Berven & Chadra 1988; Scott 1990). *Rana* tadpoles exposed to the visual stimulus alone were significantly smaller than those exposed to the tactile stimulus alone. This may suggest that Wood Frog tadpoles rely more on their vision than mechanoreception when assessing features of their habitat. *Xenopus* tadpoles probably use both sensory systems to a similar extent, since tadpoles in the three experimental treatments only differed in their body measurements from the control tadpoles, but not among themselves.

Our results reveal strong differences between species in their susceptibility to environmental changes, and in the way they respond to these changes. The species-specific differences may result from differences in schooling behaviour and densities at which species normally occur in natural populations. It is not surprising that *Bufo* tadpoles are not affected by the increase in the visual and tactile cues since these cues are redundant in their natural habitats. *Bufo* probably primarily relies on olfactory cues when assessing its environment. On the other hand, *Rana* tadpoles that do not form tight, high-density schools are more susceptible to tactile cues coming from moving objects, as well as to visual cues. It is interesting that *Rana* tadpoles responded to enhanced environment by changing both their development and growth rates. However, *Xenopus* tadpoles, which also exhibited significant response to the experimental treatments as well, only adjusted their growth but not their developmental rate to changes in sensory input. These differences imply species-specific trade-offs between growth and developmental rates, as well as species-specific thresholds for visual and tactile cues.

An advantage to amphibians in having a larval stage is that it offers an opportunity to exploit temporary, productive habitats suitable for fast growth (Wassersug 1975; Wilbur 1980; Newman 1994). When environmental conditions deteriorate (e.g. pond desiccation, increased competition, decreased food availability) tadpoles escape by metamorphosing, as predicted by different ecological models (e.g. Werner 1986; Alford & Harris 1988). The tadpoles in our treatments with enhanced tactile plus visual stimuli which were designed to mimic stimulatory cues characteristic of increased competition should escape the sensory 'overload' by increasing their developmental rate and metamorphosing earlier and at smaller body sizes than tadpoles in other treatments. Tadpoles exposed to an enhancement of one stimulus alone, visual or tactile, reacted less intensely to a perceived change in their habitat. Tadpoles in the control treatments without any enhanced stimulation had longer larval periods, and therefore more opportunity for growth.

Our findings of increased whole-body corticosterone content in tadpoles exposed to enhanced tactile plus visual stimuli suggest that these animals experienced their environment as stressful, and responded by elevating stress hormone production. Hayes (1997) reported an increase in corticosterone content in *Bufo boreas* tadpoles held at high densities, and Glennemeier & Denver (2002a) showed that increased density, separated from food limitation, was associated with elevated corticosterone content. Exogenous corticosterone has been shown to slow tadpole growth (Wright *et al.* 1994; Hayes *et al.* 1993; Glennemeier & Denver 2002b) and to potentiate the metamorphic actions of thyroid hormone (Kikuyama *et al.* 1993; Hayes 1995). Our results concur with the findings of Glennemeier & Denver (2002b) that corticosterone is a proximate mediator of the growth response to competition in tadpoles.

The lesser extent of significant differences found in *R. sylvatica* tadpoles in 2002 experiment is probably due to an early developmental stages of tadpoles at the end of experiment. We suspect that the amount of time was insufficient for the morphological traits to develop to a

statistically significant level. However, the developmental trend was similar to the one observed in the previous year.

Concluding points:

1. Tadpoles use both visual and mechanical stimuli to assess features of their environment.
2. The three species studied differ in how much they use different sensory modalities for habitat assessment.
3. Enhanced visual and mechanical cues are interpreted by tadpoles as stressful stimuli, and they modify the production of stress hormones such that the timing of metamorphosis is altered.

Our results show that habitat assessment in tadpoles relies on both visual and tactile cues, and that tadpoles are able to modify their growth and developmental rates in response to the types and intensity of sensory input. However, the extent to which tadpoles respond to environmental cues is species-specific, and may correlate with a species' propensity to occur at high density; i.e. to aggregate or school. In a separate experiment we showed that tadpoles changed their growth patterns, developmental rate and behaviour when exposed to mirror images of themselves (Rot-Nikcevic, Taylor & Wassersug, in press). Although our mirror study eliminates tactile cues, we cannot say with certainty how well tadpoles see the mirror images. In the study presented here we are sure that tadpoles see clay models, and that the visual and tactile cues are strongly present. Whether tadpoles see the models as realistic conspecifics, or whether they can distinguish between touch from another tadpole and a model remains uninvestigated. More work using models, mirrors or both is necessary to understand what the tadpole's eye tells the brain and how that information is transduced into a developmental response.

Acknowledgements

We thank Chris Taylor and Kerri Oseen for their help with experimental treatments, and Stephen Whitefield for assisting us with Adobe PhotoShop. We are very grateful to Erica Crespi for providing technical assistance and for helpful comments on the manuscript. Wade Blanchard assisted with statistical analysis. This work was supported by the Natural Sciences and Engineering Research Council of Canada (awards to I.R.N. and R.J.W.), and by the I. W. Killam Memorial Scholarship (to I.R.N.). Partial support for this project was provided by National Science Foundation of the USA grants IBN9974672 and IBN0235401 (to R.J.D.).

References

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

Berven, K.A. & Chandra, B.G. (1988) The relationship among egg size, density and food level on larval development in the wood frog (*Rana sylvatica*). *Oecologia* **75**, 67–72.

Boorse, G.C. & Denver, R.J. (2004) Expression and hypophysiotropic actions of corticotropin-releasing factor in *Xenopus laevis*. *General and Comparative Endocrinology* **137**, 272–282.

Denver, R.J. (1996) Neuroendocrine control of amphibian metamorphosis. *Metamorphosis: Postembryonic Reprogramming of Gene Expression in Amphibian and Insect Cells* (eds L.I. Gilbert, J.R. Tata & B.G. Atkinson), pp. 434–464. Academic Press, San Diego, CA.

Denver, R.J. (1997) Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. *Hormones and Behaviour* **31**, 169–179.

Denver, R.J. (1998) Hormonal correlates of environmentally induced metamorphosis in the western spadefoot toad *Scaphiopus hamondii*. *General and Comparative Endocrinology* **110**, 326–336.

Denver, R.J., Pavgi, S. & Shi, Y.-B. (1997) Thyroid hormone-dependent gene expression program for *Xenopus* neural development. *Journal of Biological Chemistry* **272**, 8179–8188.

Denver, R.J., Mirhadi, N. & Phillips, M. (1998) Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology* **79**, 1859–1872.

Denver, R.J., Boorse, G.C. & Glennemeier, K.A. (2002) Endocrinology of complex life cycle: amphibians. *Hormones, Brain and Behaviour* (eds D. Pfaff, A. Arnold, A. Etgen, S. Fahrbach, R. Moss & R. Rubin), pp. 469–513. Academic Press, San Diego, CA.

Glennemeier, K.A. & Denver, R.J. (2002a) Role for corticoids in mediating the response of *Rana pipiens* tadpoles to intraspecific competition. *Journal of Experimental Zoology* **292**, 32–40.

Glennemeier, K.A. & Denver, R.J. (2002b) Small changes in whole-body corticosterone content affect larval *Rana pipiens* fitness components. *General and Comparative Endocrinology* **127**, 16–25.

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

Hayes, T.B. (1995) Interdependence of corticosterone and thyroid hormones in larval toads (*Bufo boreas*). I. Thyroid hormone-dependent and independent effects of corticosterone on growth and development. *Journal of Experimental Zoology* **271**, 95–102.

Hayes, T.B. (1997) Steroids as potential modulators of thyroid hormone activity in anuran metamorphosis. *American Zoologist* **37**, 185–194.

Hayes, T.B. & Wu, T.H. (1995) Interdependence of corticosterone and thyroid hormones in larval toads (*Bufo boreas*). I. Thyroid hormone-dependent and independent effects of corticosterone on growth and development. *Journal of Experimental Zoology* **271**, 95–102.

Hayes, T., Chan, R. & Licht, P. (1993) Interactions of temperature and steroids on larval growth, development, and metamorphosis in a toad (*Bufo boreas*). *Journal of Experimental Zoology* **266**, 206–215.

vS Hoff, K. & Wassersug, R.J. (1986) The kinematics of swimming in larvae of the clawed frog, *Xenopus laevis*. *Journal of Experimental Biology* **122**, 1–12.

vS Hoff, K., Blaustein, A.R., McDiarmid, R.W. & Altig, R. (1999) Behaviour: interactions and their consequences. *Tadpoles: the Biology of Anuran Larvae* (eds R.W. McDiarmid & R. Altig), pp. 149–169. University of Chicago Press, Chicago, IL.

Kats, L.B. & Dill, L.M. (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**, 361–394.

Kats, L.B., Petranka, J.W. & Sih, A. (1988) Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* **69**, 1865–1870.

- Kikuyama, S., Kawamura, K., Tanaka, S. & Yamamoto, K. (1993) Aspects of amphibian metamorphosis: hormonal control. *International Review of Cytology* **145**, 105–148.
- Lannoo, M.J. (1999) Integration: nervous and sensory systems. *Tadpoles: the Biology of Anuran Larvae* (eds R.W. McDiarmid & R. Altig), pp. 149–169. University of Chicago Press, Chicago, IL.
- Licht, P., McCreery, B.R., Barnes, R. & Pang, R. (1983) Seasonal and stress related changes in plasma gonadotropins, sex steroids and corticosterone in the bullfrog, *Rana catesbeiana*. *General and Comparative and Endocrinology* **50**, 124–145.
- McCollum, S.A. & Van Buskirk, J. (1996) Costs and benefits of a predator induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* **50**, 583–593.
- 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. *Biosciences* **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.
- Nieuwkoop, P.D. & Faber, J. (1956) *Normal Table of Xenopus Laevis*. North Holland Publishing, Amsterdam.
- O'Hara, R.K. & Blaustein, A.R. (1982) Kin preference behaviour in *Bufo boreas* tadpoles. *Behavioural and Ecological Sociobiology* **11**, 43–49.
- Petranka, J.W., Kats, L.B. & Sih, A. (1987) Predator–prey interaction among fish and larval amphibians: use of chemical cues to detect predatory fish. *Animal Behaviour* **35**, 420–425.
- Relyea, R.A. (2000) Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. *Ecology* **81**, 2278–2289.
- Relyea, R.A. (2001) Morphological and behavioural plasticity of larval anurans in response to different predators. *Ecology* **82**, 541–554.
- Relyea, R.A. (2002) Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs* **72**, 523–540.
- Relyea, R.A. & Werner, E.E. (1999) Quantifying the relation between predator-induced behaviour and growth performance in larval anurans. *Ecology* **80**, 2117–2124.
- Relyea, R.A. & Werner, E.E. (2000) Morphological plasticity of four larval anurans distributed along an environmental gradient. *Copeia* **2000**, 178–190.
- Rot-Nikcevic, I., Taylor, C. & Wassersug, R. (2005) The role of images of conspecifics as visual cues in the development and behavior of larval anurans. *Behavioral Ecology and Sociobiology* (in press).
- Scott, D.E. (1990) Effects of larval density in *Ambystoma opacum*: an experiment in large-scale field enclosures. *Ecology* **71**, 296–306.
- Semlitsch, R.D. & Reyer, H.U. (1992) Modification of anti-predator behaviour in tadpoles by environmental conditioning. *Journal of Animal Ecology* **61**, 353–360.
- Shi, Y.-B. (1994) Molecular biology of amphibian metamorphosis: a new approach to an old problem. *Trends in Endocrinology and Metabolism* **5**, 14–20.
- Shi, Y.-B. (2000) *Amphibian Metamorphosis: from Morphology to Molecular Biology*. John Wiley & Sons, Inc., Toronto.
- Smith-Gill, S.J. & Berven, K.A. (1979) Predicting amphibian metamorphosis. *American Naturalist* **113**, 563–585.
- Stearns, S.C. (1989) The evolutionary significance of phenotypic plasticity. *Bioscience* **3**, 436–445.
- Van Buskirk, J. (2002) A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *American Naturalist* **160**, 87–102.
- Van Buskirk, J., McCollum, S.A. & Werner, E.E. (1997) Natural selection for environmentally-induced phenotypes in tadpoles. *Evolution* **52**, 1983–1992.
- Waldman, B. (1984) Kin recognition and sibling association among wood frog (*Rana sylvatica*) tadpoles. *Behavioural and Ecological Sociobiology* **14**, 171–180.
- Wang, Z. & Brown, D.D. (1993) Thyroid hormone-induced gene expression program for amphibian tail resorption. *Journal of Biological Chemistry* **268**, 1270–1278.
- Wassersug, R. (1973) Aspects of social behaviour in anuran larvae. *Evolutionary Biology of the Anurans. Contemporary Research on Major Problems* (ed. J.L. Vial), pp. 273–297. University of Missouri Press, Columbia.
- Wassersug, R.J. (1975) The adaptive significance of the tadpole stage with comments on the maintenance of complex life cycles in anurans. *American Zoologist* **15**, 405–417.
- Wassersug, R.J., & vS Hoff, K. (1985) The kinematics of swimming in anuran larvae. *Journal of Experimental Biology* **119**, 1–30.
- Werner, E.E. (1986) Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *American Naturalist* **128**, 319–341.
- Wilbur, H.M. (1980) Complex life cycles. *Annual Review of Ecology and Systematics* **11**, 67–93.
- Wilbur, H.M. & Collins, J.P. (1973) Ecological aspects of amphibian metamorphosis. *Science* **182**, 1305–1314.
- Wright, M.L., Cykowski, L.J., Lundrigan, L., Hemond, K.L., Kochan, D.M., Faszewski, E.E. & Anuszewski, C.M. (1994) Anterior pituitary and adrenal cortical hormones accelerate or inhibit tadpole hindlimb growth and development depending on stage of spontaneous development of thyroxine concentration in induced metamorphosis. *Journal of Experimental Zoology* **270**, 175–188.

Received 30 March 2005; revised 28 June 2005; accepted 9 July 2005