

Underwater sound emission as part of an antipredator mechanism in *Ceratophrys cranwelli* tadpoles

Carolina Salgado Costa,¹ Mariana Chuliver Pereyra,¹ Leandro Alcalde,² Raúl Herrera,³ Vance L. Trudeau⁴ and Guillermo S. Natale¹

¹Departamento de Química, Facultad de Ciencias Exactas, CIMA, Universidad Nacional de La Plata, 47 y 115, La Plata, Buenos Aires, 1900, Argentina; ²ILPLA-CONICET, Sección Herpetología, 120 y 62, La Plata, Buenos Aires, 1900, Argentina; ³Fundación Óga, Guardias Nacionales N°19, San Nicolás, Buenos Aires, 2900, Argentina; ⁴Department of Biology, Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, ON, K1N 6N5, Canada

Keywords:

predator–prey interactions, cannibalism, antipredator mechanism, underwater sound emission, *Ceratophrys cranwelli* tadpoles

Accepted for publication:
16 April 2013

Abstract

Salgado Costa, C., Chuliver Pereyra, M., Alcalde, L., Herrera, R., Trudeau, V.L. and Natale, G.S. 2014. Underwater sound emission as part of an antipredator mechanism in *Ceratophrys cranwelli* tadpoles. — *Acta Zoologica* (Stockholm) 95: 367–374.

We report the emission of underwater sounds in the tadpoles of a second member of the family Ceratophryidae, *Ceratophrys cranwelli*. These tadpoles produce a short metallic-like sound, which consists of short trains of pulses at Gosner stages 25, 28, and 37. Experiment I was designed to record underwater sounds and their characteristics. Experiment II was designed to test: (i) if at higher densities (total number of tadpoles/L) but fixed predator–prey proportions *C. cranwelli* larvae are cannibalistic, (ii) if cannibalism increases at higher proportions of predators at a fixed density, and (iii) if tadpoles display a mechanism of intraspecific recognition that may diminish the frequency of cannibalism. Each treatment combines larvae of *C. cranwelli* (predator) with those of *Rhinella arenarum* (prey). The number of live and dead individuals was recorded during 72 h, and the following variables were calculated: time to eat the first and second prey, time without eating, time to eat a congener, and number of events of cannibalism. The results indicate that relative predator–prey availability affects the frequency of predation between conspecifics. We consider that an antipredator mechanism exists in *C. cranwelli* tadpoles and that the underwater sound is part of it.

Guillermo S. Natale, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CIMA, 47 y 115, La Plata, Buenos Aires 1900, Argentina-CONICET. E-mail: gnatale@quimica.unlp.edu.ar

Introduction

Intraspecific predation is a well-known process of both killing and eating an individual of the same species that may influence population structure, life history, behavior, and competition for mates and resources. In general, older animals are more voracious cannibals than younger ones (Polis 1981). This phenomenon, also defined as an intraspecific predation, can be found in a wide variety of animals and is frequently a response to different factors such as prey availability, stress, and high population density (Fox 1975).

Among amphibians, different types of signals may contribute to the detection of a predator. The roles of these signals (chemical, visual, acoustic, or hydraulic) in anuran larvae have been recently tested by Hettyey *et al.* (2012), who described a

decrease in the activity of tadpoles in the presence of predators. They concluded that chemical cues are the most important for the detection of a predator.

Animals have developed a wide variety of signals to communicate with conspecifics at different social levels (Searcy and Nowicki 2005). Considering recognition mechanisms, the three basic ones are the following: (i) kin recognition by familiarizing with conspecifics during early life stages; (ii) recognition by phenotype matching (Porter 1988), allowing an animal to recognize individuals not previously encountered; and (iii) the employment of recognition alleles without learning. Determining which mechanism is operating requires knowledge of the dispersal characteristics of tadpoles, type of egg deposition, and both larval population sizes and densities. For most species, the kin recognition system is consistent with

characteristics of larval ecology and behavior. Taking into account numerous experimental replicates in the laboratory and in the field, using thousands of individuals, anuran larvae of several species can discriminate between kin and nonkin. However, certain species have a fine-tuned kin recognition system while others cannot discriminate between kin and nonkin (Blaustein 1988).

Acoustic signals play a fundamental role in anurans for species recognition, male spacing, and mating (Wells 1977). Diverse types of sounds are produced by adult frogs, the best known being advertisement calls which are produced by sexually active males (Littlejohn 1977; Wells 1977; Duellman and Trueb 1994) and distress calls which are produced by both sexes at any postmetamorphic stage in occasion of any disturbance (Duellman and Trueb 1994; Toledo *et al.* 2005; Toledo and Haddad 2009). Sound emission is rare in females but when it occurs, the calls differ from that of males (Dixon 1957; Heinzmann 1970; De Kokubum *et al.* 2009).

Among anuran larvae, sound production remained unknown until the description of sounds for two unrelated species with macrophagic predator larvae: the Ornate horned frog *Ceratophrys ornata* (Anura: Ceratophryidae) (Natale *et al.* 2011) and Azzurra's canyon frog *Gephyromantis azzurrae* (Anura: Mantellidae) (Reeve *et al.* 2011). The main findings of Natale *et al.* (2011) are the following: (i) larval sound was considered a distress call (Barrio 1963; Duellman and Trueb 1994), (ii) such calls are produced when a larvae of *C. ornata* is touched or bitten by a conspecific or when touched experimentally by an object and is generally accompanied by a contraction at the body–tail junction, and (iii) larval sound parameters are very similar to those produced by froglets. The main findings of the work of Reeve *et al.* (2011) are: (i) sound emission in *Gephyromantis azzurrae* larvae could be an aggressive signal toward conspecifics during competitive feeding and (ii) the sound is produced during a rapid closure of the previously wide-open jaw sheaths, often during an upward movement of the body.

The function of the interactions between conspecific larvae has been hypothesized for both these species, but neither appropriately tested nor demonstrated (Natale *et al.* 2011; Reeve *et al.* 2011). This raises three major questions: (i) Do tadpoles of related species also produce sounds? (ii) Do tadpoles in the predator role distinguish between conspecifics and other species? (iii) What is the influence of density and relative prey availability on the frequency of cannibalism?

The aims of the present work are therefore twofold: to determine if tadpoles of *Ceratophrys cranwelli* (Anura: Ceratophryidae) also produce underwater sounds, and to determine if a mechanism of intraspecific recognition that diminishes the frequency of predation between conspecifics exists. In this species, we therefore tested if (i) at higher densities but fixed predator–prey proportions, *C. cranwelli* larvae exhibit more events of cannibalism; (ii) if cannibalism increases at higher proportions of predators at a fixed den-

sity; and (iii) if a mechanism of intraspecific recognition capable to diminish the frequency of predation between conspecifics does exist.

Materials and Methods

Scientific names and taxonomic classifications were updated according to Frost (2013).

Study species

Ceratophrys cranwelli and other species of the genus are explosive breeders. Their macrophagic carnivorous tadpoles develop in highly ephemeral ponds experimenting extremely rapid morphological development, and possess several rows of keratinized ‘teeth’ and heavily keratinized jaw sheaths as an adaptation to tear and kill prey (Wild 1997). They feed on microalgae, crustaceans, insects, and rotifers. Tadpoles in this genus will pursue and engulf entire small preys. Larger preys are grabbed at any part of the body and swallowed piecemeal (Vera Candioti 2005). Furthermore, they have simplified internal oral structures (Wassersug and Heyer 1988) and a short alimentary tract with an adult-like stomach (Ulloa Kriesler 2000).

Breeding and maintenance

On December 6th, 2006, 20 *C. cranwelli* tadpoles were collected in Formosa Province (24°20'44.5"S, 61°06'54.5"W) and maintained in the laboratory till metamorphosis (which occur between December 12th, 2006 and January 23rd, 2007). Organisms were housed individually in a 1-m² terrarium containing soil and water simulating their natural habitat, at 25 °C ± 1 with a photoperiod of 16L : 8D (Salgado Costa *et al.* 2011). Two adult females (mean body weight 202 g) and two males (mean body weight 117 g) were injected with AMPHIPLEX (0.4 µg/g GnRH-A + 10 µg/g MET; Trudeau *et al.* 2010) on June 9th, 2010, and 25 h later, both pairs laid 1200 fertile eggs. Eggs and tadpoles were thereafter reared under the same controlled conditions as adults but using dechlorinated tap water (pH 7.6–8.3; hardness, 180–250 mg CaCO₃/L) with continuous aeration.

Eggs from each spawning were maintained under two different experimental conditions. For Experiment I, eggs from one clutch were placed in a common 120-L aquarium (10 eggs/L) under controlled conditions until they reached stages 25, 28, and 37 (Gosner 1960). For Experiment II, tadpoles from the other clutch were raised in the conditions previously mentioned until stage 37. Thereafter, they were housed individually in 0.5-L polypropylene containers (*n* = 210). Individuals of both experiments were fed *ad libitum* with several mixed food items: *Tubifex* sp., *Daphnia* sp., liquefied lettuce, pieces of fish, heart and liver of cow, and dog food (Purina dog chow®, Société des Produits Nestlé S.A., Vevey, Switzerland).

Experimental design

Experiment I was designed to record underwater sounds, and their characteristics and variations during *C. cranwelli* larval development, taking into account three representative stages. Experiment II was designed to test three hypotheses: (i) The density effect hypothesis (DEH) predicts that at higher densities (total number of tadpoles/L) but fixed predator–prey proportions, *C. cranwelli* larvae will exhibit more events of cannibalism; (ii) The relative prey availability hypothesis (RPAH) predicts that cannibalism will be higher at a higher proportion of predators (=lower proportion of preys) but at a fixed density; and (iii) if *C. cranwelli* tadpoles display a mechanism of intraspecific recognition capable to diminish the frequency of predation between conspecifics at the presence of prey items.

Experiment I: description of sounds produced by *C. cranwelli* larvae

Tadpoles reared in the common aquarium and at stages 25, 28, and 37 were used for sound-recording experiments. At the end of the experiment, larvae were all anesthetized (250 mg/L benzocaine) and fixed in 10% v/v formalin. Each individual was measured using a digital caliper to the nearest 0.01 mm (body length and total length, both in mm) and weighed (g) using a digital balance to the nearest 0.001 g. All morphometric measurements (=variables) were employed to evaluate the relationship to acoustic variables.

Both audio and video recordings of the tadpoles were made simultaneously in plastic containers (33 × 23 × 7.5 cm). Intraspecific interactions were recorded three times at each of the larval stage (25, 28, and 37) for one hour per session. Interspecific (=predator–prey) interactions were recorded once per larval stage for one hour per session. Each session consisted of recording intraspecific interactions (ten *C. cranwelli* tadpoles per liter) or interspecific interactions (five *C. cranwelli* tadpoles with five *Rhinella arenarum* tadpoles per liter) of individuals placed in 1-L polypropylene trays (21.5 × 15.5 × 3.5 cm). Video recordings were made using a Handycam Sony DCR-HC28. Underwater sounds were recorded using the same methodology as in Natale *et al.* (2011). An Audio-Technica ATR55 directional microphone was covered with a latex sheet to protect it from water (preliminary studies determined that the latex covering did not affect sound recordings). The microphone was then connected to an M-Audio Fast Track interface coupled with a notebook (HP 540). Audio recordings were digitalized and analyzed using Adobe Audition 1.5 (Adobe Systems Inc.) software at a sampling rate of 44.1 kHz and 16-bit resolution. Frequency information was obtained through fast Fourier transformation (FFT size 2048 algorithms) at Blackman–Harris window function. Figures of oscillograms were obtained with SoundRuler software (Gridi-Papp 2003–2007).

We analyzed 11 underwater sounds (each belonging to a different individual) per tadpole stage, and the arithmetic mean of the following sound variables was calculated: call duration (Cd) expressed in seconds (s), number of pulses (Np), pulse duration (Pd) (s), number of interpulses (Nip), interpulse duration (=interpulse interval) (Ipd) (s), and dominant frequency (Df) expressed in Hertz (Hz). The sound terminology follows Schneider and Sinsch (2006).

Experiment II: Intraspecific recognition by acoustic signals

Larvae of *C. cranwelli* at stage 37 (mean body length = 17.21 mm, mean total length = 37.16 mm, mean body weight = 1.032 g, $n = 10$) reared in the individual containers were placed in 1-L polypropylene trays (21.5 × 15.5 × 3.5 cm) with stage 37 *R. arenarum* tadpoles (mean body length = 11.87 mm, mean total length = 28.43 mm, mean body weight = 0.715 g, $n = 10$).

The experimental design consisted of three treatments to test DEH, five treatments to test the RPAH hypothesis, and five replicates per treatment. Each treatment combines larvae of *C. cranwelli* (*Cc*, predator) with those of *R. arenarum* (*Ra*, prey) according to the following densities at the same predator–prey proportion: 2*Cc*-2*Ra*; 5*Cc*-5*Ra*; 10*Cc*-10*Ra*, and the following predator–prey proportions at the same density: 2*Cc*-8*Ra*; 4*Cc*-6*Ra*; 5*Cc*-5*Ra*; 6*Cc*-4*Ra*; 8*Cc*-2*Ra*. All the experiments lasted 72 h. The number of live and dead individuals was recorded during this period. In total, 28 measurements were made: the first six measurements every hour and the remaining ones every three hours till the end of the experiment. The obtained data allowed us to calculate the following variables: (i) time to eat the first prey, (ii) time to eat the second prey, (iii) time without eating (after depletion of all preys), (iv) time to eat a congener, and (v) number of events of cannibalism.

Based on preliminary laboratory observations, we determined that there are ‘normal’ and ‘high’ appetite periods for *C. cranwelli* larvae. We define the ‘normal’ appetite period from 0 to 12 h as being the time they spent to eat when they were not starving. Although at the end of this time, there were no more prey items available, predation between conspecifics did not occur. The ‘high’ appetite period began from the end of the normal period and was characterized by the occurrence of events of predation over conspecifics.

Statistical analysis

For experiment I, analysis of variance (ANOVA) was performed in order to compare underwater sound variables between the selected tadpole stages (25, 28, and 37). Regression and correlation analyses were performed between stages and bioacoustic variables (Cd, Np, Pd, Nip, Ipd, and Df), and between morphometric (body length, total length, and body weight) and bioacoustic variables (Cd, Np, Pd, Nip, Ipd, and Df). Tests of significance for regression and correlation were

performed following Zar (2010). Finally, a two-tailed paired-sample *t*-test was performed in order to compare the underwater sound variables Cd, Np, Ipd, and Df of *C. cranwelli* tadpoles at stages 28 and 37 with previously published data from *C. ornata* (Natale *et al.* 2011) at the same stages. The level of significance chosen was 0.05 for all tests.

For experiment II, ANOVA was performed in order to compare the evaluated variables (time to eat the first prey, time without eating, and time to eat a congener) between the different densities of each treatment. Moreover, in order to corroborate the DEH and the RPAH, both regression and correlation analyses were performed between densities (2Cc-2Ra; 5Cc-5Ra; 10Cc-10Ra) and the evaluated variables (time to eat the first prey, time without eating, time to eat a congener, and number of events of cannibalism), and between predator–prey proportions (2Cc-8Ra; 4Cc-6Ra; 5Cc-5Ra; 6Cc-4Ra; 8Cc-2Ra) and the evaluated variables (time to eat the first prey, time to eat the second prey, time without eating, time to eat a congener, and number of events of cannibalism). Tests of significance for regression and correlation were performed following Zar (2010). The level of significance chosen was 0.05 for all tests.

Finally, in order to determine the existence of a mechanism of recognition that diminishes the frequency of predation between conspecifics, the number of events of cannibalism was compared among the different densities and predator–prey proportions considering the normal and high appetite period. Similarly, a two-tailed *t*-test for difference between means was performed between the mean of the variable time to eat the first prey and the mean of the variable time to eat a conspecific.

Results

Experiment I

Although underwater sounds were only recorded at three stages of development, sound production was verified for all the larval periods of *C. cranwelli* tadpoles until stage 42. This developmental stage is the beginning of the metamorphic climax and coincides with the loss of keratinized teeth. The earliest age that sounds were produced was 72-h posthatch (stage 25), which is the same stage at which the

formation of anuran mouthparts is essentially complete (Duellman and Trueb 1994).

The bioacoustic parameters of the spectral analyses and oscillograms of the sounds produced by larvae at stages 25, 28, and 37 are shown in Table 1 and Fig. 1, respectively. At these stages, the underwater sounds of *C. cranwelli* larvae consist of short trains of pulses. Sound emission occurs when a *C. cranwelli* tadpole is touched or bitten by a conspecific larva or when touched experimentally by an object to simulate an attack. The sound is accompanied by a simultaneous (0.04 s) fast tail movement toward the body, followed by a rapid escape of the attacked larva in the opposite direction from the predator (=stressor) (see Movie S1).

ANOVA results considering call duration and pulse duration showed significant differences ($P < 0.05$) among stages 25 and 37. Considering dominant frequency, ANOVA results indicate highly significant differences ($P < 0.01$) among stages 25–37 and stages 28–37. There is a positive correlation between call duration ($P < 0.05$, $r = 0.44$) and pulse duration ($P < 0.01$, $r = 0.46$) with stages, body length, total length and body weight, and a negative correlation ($P < 0.01$, $r = -0.62$) between dominant frequency with stages, body length, total length, and body weight.

The two-tailed paired-sample *t*-test showed no significant differences between call duration ($P = 0.2506$), number of pulses ($P = 0.2451$), interpulse duration ($P = 0.1386$), and dominant frequency ($P = 0.1346$) of *C. ornata* (data from Natale *et al.* 2011) and *C. cranwelli* stage-specific underwater sounds variables.

Experiment II

Testing the density effect hypothesis. During the normal appetite period, no events of cannibalism were observed. The only two cases of cannibalism occurred during the high appetite period in treatments 2Cc-2Ra and 5Cc-5Ra (Table 2). The ANOVA showed significant differences ($P < 0.05$) between densities 2Cc-2Ra (=4 tadpoles/L) and 10Cc-10Ra (=20 tadpoles/L) considering the variable time to eat the first prey. Regression and correlation analyses showed a negative correlation between the time to eat the first prey ($P < 0.05$, $r = -0.2624$) with all the densities used to test the DEH.

Table 1 Arithmetic mean \pm standard deviation (SD) for underwater sound variables at each selected Gosner stage (Gs)

Gs	<i>n</i>	Cd (s)	Np	Pd (s)	Nip	Ipd	Df (Hz)
25	11	0.029 \pm 0.022 ^a	8 \pm 5	0.001 \pm 0 ^a	7 \pm 5	0.002 \pm 0.001	3871 \pm 1369 ^b
28	11	0.045 \pm 0.011	12 \pm 3	0.002 \pm 0.001	11 \pm 3	0.002 \pm 0.001	3204 \pm 537 ^b
37	11	0.051 \pm 0.018 ^a	9 \pm 3	0.004 \pm 0.005 ^a	8 \pm 3	0.002 \pm 0.001	2258 \pm 248 ^b

n, number of calls analyzed; Cd, call duration in seconds (s); Np, number of pulses; Pd, pulse duration; Nip, number of inter-pulses; Ipd, interpulse duration; Df, dominant frequency in Hertz (Hz).

^aSignificant differences ($P < 0.05$).

^bHighly significant differences ($P < 0.01$).

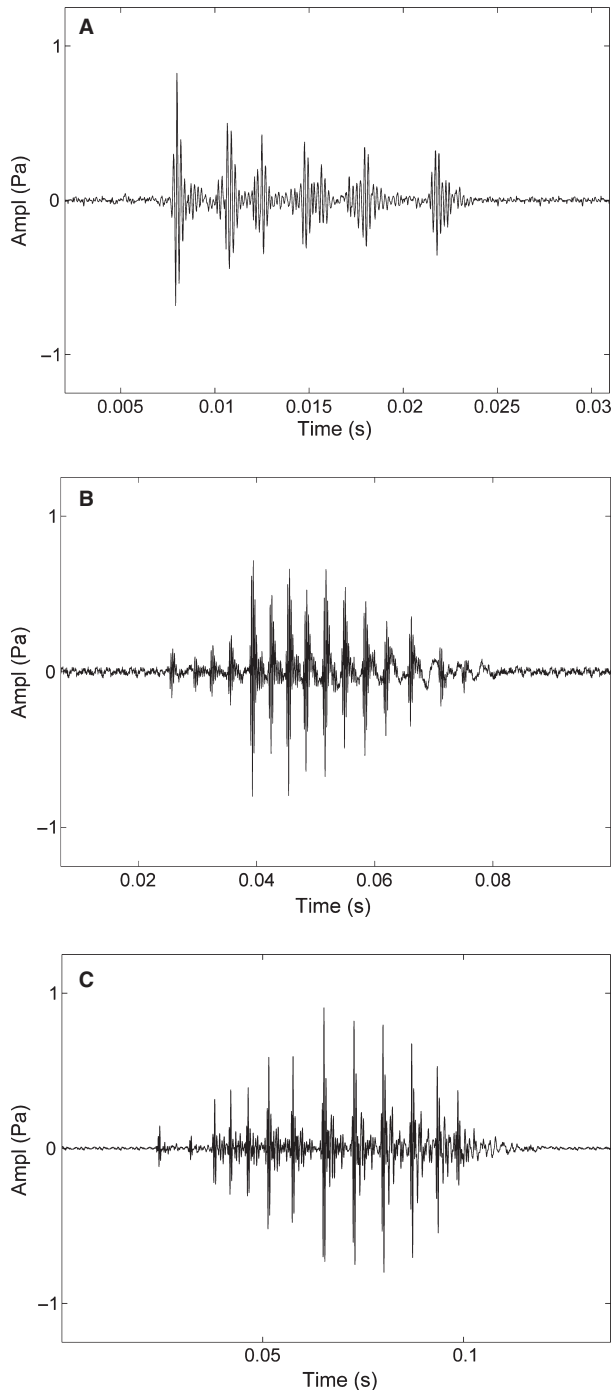


Fig. 1—Oscillograms of underwater sounds produced by *Ceratophrys cranwelli* larvae at Gosner stages 25 (A), 28 (B), and 37 (C); Ampl: amplitude in Pascal (Pa), time in seconds (s), frequency in kilohertz (kHz = 10^3 Hz).

Testing the relative prey availability hypothesis. Regression and correlation analyses considering predator–prey proportions showed there is a positive correlation between the time with-

out eating ($P < 0.01$, $r = 0.5839$) and the number of cases of cannibalism ($P < 0.01$, $r = 0.9837$) with all the proportions used to test the RPAH.

Testing the hypothesis that a mechanism of intraspecific recognition in C. cranwelli tadpoles exists. Considering all treatments and the obtained data (Tables 2 and 3), we registered six cases of cannibalism that occurred only during the high appetite period. These cannibalistic acts occur in 100% of the cases after depletion of *R. arenarum* larvae. Cannibalism is absent in the normal appetite period. The two-tailed *t*-test showed highly significant differences ($P < 0.01$) between the time to eat the first prey (time average = 160 min, normal appetite period) and the time to eat a congener (time average = 900 min, high appetite period). All the prey items were consumed at an average of 360 min in 74% of the cases during the normal appetite period, while the remaining 26% finished their prey items during the high appetite period.

Discussion

We report the existence of an underwater larval sound emission system in a second member of the family Ceratophryidae. As described for *Ceratophrys ornata* tadpoles, *C. cranwelli* larvae also produce a short metallic-like underwater sound, which consist of short trains of pulses during all the studied developmental stages. In both species, sound emissions are produced always by the attacked larva (prey) in the context of intraspecific interactions. These sounds are accompanied by a fast tail movement toward the body, followed by a rapid escape from the predator (attacker). The positive correlation between call duration and pulse duration with body weight in *C. cranwelli* is in agreement with the observations of Natale *et al.* (2011) for *C. ornata* tadpoles. In addition, the negative correlation between dominant frequency with body weight concurs with Natale *et al.* (2011), and has also been reported in most adult anurans (Ryan 1988; Gerhardt 1994). We also

Table 2 Arithmetic mean \pm standard deviation (in min) of each variable under each treatment. The number of cases is in parenthesis

Variables/ Treatments	2Cc-2Ra	5Cc-5Ra	10Cc-10Ra
1° p	395 \pm 244 (5) ^a	197 \pm 72 (5) ^a	149 \pm 33 (5) ^a
2° p	–	–	–
We	3220 \pm 489 (5)	3368 \pm 321 (5) ^b	3530 \pm 181 (5)
Cong	2600 \pm 0 (1)	2150 \pm 0 (1)	0 \pm 0 (0)
C	1	1 ^b	0

Cc, *Ceratophrys cranwelli*; Ra, *Rhinella arenarum*; 1° p, time to eat the first prey; 2° p, time to eat the second prey; We, time without eating; Cong, time to eat a congener; C, number of cases of cannibalism.

^aSignificant differences ($P < 0.05$).

^bHighly significant differences ($P < 0.01$) considering the three treatments (2Cc-2Ra; 5Cc-5Ra; 10Cc-10Ra) to test the density effect hypothesis.

Table 3 Arithmetic mean \pm standard deviation (in min) of each variable under each treatment. The number of cases is in parenthesis

Variables/ Treatments	2Cc-8Ra	4Cc-6Ra	5Cc-5Ra	6Cc-4Ra	8Cc-2Ra
1° p	109 \pm 57 (4)	134 \pm 71 (5)	197 \pm 72 (5) ^a	77 \pm 16 (5)	60 \pm 44 (5)
2° p	461 \pm 206 (8)	402 \pm 139 (10)	–	–	–
We	943 \pm 691 (4) ^b	3172 \pm 293 (5) ^b	3368 \pm 321 (5) ^b	3836 \pm 68 (5) ^b	3896 \pm 88 (5) ^b
Cong	0 \pm 0 (0)	0 \pm 0 (0)	2150 \pm 0 (1)	0 \pm 0 (0)	1553 \pm 768 (4)
C	0 ^b	0 ^b	1 ^b	0 ^b	4 ^b

Cc, *Ceratophrys cranwelli*; Ra, *Rhinella arenarum*; 1° p, time to eat the first prey, 2° p, time to eat the second prey; We, time without eating; Cong, time to eat a congener; C, number of cases of cannibalism.

^aSignificant differences ($P < 0.05$).

^bHighly significant differences ($P < 0.01$) considering the five treatments (2Cc-8Ra; 4Cc-6Ra; 5Cc-5Ra; 6Cc-4Ra; 8Cc-2Ra) to test the relative prey availability hypothesis.

compared the stage-specific underwater sound variables of call duration, number of pulses, interpulse duration, and dominant frequency of *C. cranwelli* tadpoles with those of *C. ornata*. We conclude that both species of Ceratophryidae have very similar bioacoustic parameters.

An important report on underwater sound production was published by Reeve *et al.* (2011), who provided data for *Gephyromantis azzurrae* tadpoles. This is a species from Madagascar that belongs to the family Mantellidae and is unrelated to the genus *Ceratophrys*. These authors hypothesized that the sounds are an aggressive signal toward conspecifics during prey capture. They reported that sound emission frequency is related to long periods of time without food ingestion, being the sound produced by a rapid closure of the jaw sheaths, often during an upward movement of the body. The context of sound production in *G. azzurrae* is clearly different from the described for *C. cranwelli* and *C. ornata* tadpoles. We demonstrate that *C. cranwelli* tadpoles search for their prey items as adults do, but larvae never emit sounds during a predatory act toward a conspecific. They only produce sounds in the case of physical interactions with conspecifics or when being touched experimentally by an external object to simulate an attack.

Regarding the density effect hypothesis (DEH), the absence of correlation between the number of cases of cannibalism with densities (total number of tadpoles/L at fixed predator–prey proportions) allows us to reject this hypothesis. However, the obtained data allow us to affirm that at higher densities, the time *C. cranwelli* tadpoles spend to eat the first prey diminishes, being related to a high number of predator–prey interactions.

Considering the relative prey availability hypothesis (RPAH), we are able to confirm that the number of cannibalistic acts increases at higher predator–prey proportions. Moreover, relative predator–prey availability affects the frequency of predation between conspecifics (=number of cases of cannibalism). We therefore do not reject the RPAH. The frequency of predation between conspecifics significantly diminishes when prey items of another species are available. We conclude that predator–prey proportions have an effect on the incidence

of cannibalism. Moreover, cannibalistic acts always occurred during the high appetite period.

Together our results suggest that *C. cranwelli* tadpoles possess a mechanism which influences predation between conspecifics (=cannibalism) under normal appetite periods. The mechanism is probably complex and could involve other cues (e.g., visual) like those described for some anuran larvae (Hettzey *et al.* 2012). Taking into account the present research, we could only detect two components which would probably be part of the mechanism and which occurred simultaneously when a *C. cranwelli* larva is attacked. These are (i) the emission of underwater sounds, and (ii) a fast tail movement toward the body. We can affirm that the described mechanism is highly effective because (i) cannibalistic acts do not occur under normal appetite periods in all the evaluated circumstances (different densities and predator–prey proportions), and (ii) the displayed mechanism has been observed thousands of times (~1500) between pairs of *C. cranwelli* larvae and cannibalism was absent in all cases. We also demonstrate that an attacked tadpole of *C. cranwelli* displays the mechanism, and the tadpole in the predator role is able to react accordingly. In order to elucidate if other cues are involved in the described mechanism, some independent experiments should be performed in future studies.

When *C. cranwelli* tadpoles are in the normal appetite period, no events of cannibalism were registered but intraspecific interactions do occur. After finishing all prey items and under long periods of hunger (=high appetite period), cannibalistic acts appear, although tadpoles activate the described mechanism. We therefore are able to speculate the following: (i) cannibalistic acts occur on an individual stressed prey that is not able to properly activate the mechanism; (ii) the attacker is so hungry that the described mechanism is not perceived by the predator.

Considering the different anuran tadpole signals that may contribute to predator detection (Hettzey *et al.* 2012), acoustic cues may have an important role in the described mechanism. But if such sound has a significant role, other tadpoles in the prey role should sense the presence of the predator, and tadpoles in the predator role should have a recognition sys-

tem. We cannot yet discount the existence of an intraspecific mechanism of recognition in *C. cranwelli* tadpoles like those described by Blaustein (1988). If such recognition does not really happen, any non-conspecific predator should activate the mechanism. The latter possibility is consistent with our experiments indicating that *C. cranwelli* larvae also make sounds when touched by an external object to simulate an attack. Such an observation opens the possibility to determine if *C. cranwelli* would respond similarly to other predators such as fish or dragonfly larvae. We propose that the described mechanism is an antipredator response that involves underwater sounds. This fits the general characteristics of a distress call described for adult anurans. Such a defensive vocalization is characterized by a high-pitched whistle, generally emitted with the mouth open and serves to repel predators when being subdued (Toledo *et al.* 2005; Toledo and Haddad 2009).

Only three anuran species are known to call underwater during larval stages. Considering the phylogenetic kinship, we can affirm that underwater emission of sound is a character that occurs in distant groups, but we are not able to reveal if it is an ancestral or a derived character. Therefore, it will be important to determine which other anuran species produce underwater sounds in tadpole stages. Such analyses will elucidate whether sound production is an evolutionary acquisition in response to food habits, or if it may have other functions in anuran tadpoles.

Acknowledgements

Special thanks to Dr. Alicia E. Ronco, director of Centro de Investigaciones del Medio Ambiente (CIMA), for supporting this research. We are also grateful to Miguel Tejedo for collaborating in the design of the experiments, the statement of the hypothesis, and the purchase of containers. CSC acknowledges CONICET for a doctoral scholarship (2011). Funding from the University of Ottawa Research Chair in Neuroendocrinology is acknowledged by VLT with appreciation.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Movie S1. Intraspecific interaction between two Gs 37 *Ceratophrys cranwelli* tadpoles. Note that the attacked larva displayed the described antipredator mechanism and cannibalism (=intraspecific predation) does not occur.