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Mother Chemical Stimulus and Aggregation Behaviour of Tadpoles in a Neotropical Foam-Nesting Frog

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Keywords: chemical communication | *Leptodactylus* | mother–offspring interactions | parental care | schooling behaviour

ABSTRACT

Aggregation behaviour is a common strategy among aquatic organisms to avoid predators and may result from social interactions mediated by signals and/or cues. Tadpoles of some species in the Neotropical genus *Leptodactylus* (Leptodactylidae) exhibit schooling behaviour and are escorted by an attending female. Here, we experimentally tested whether chemical skin secretion produced by attending females of *Leptodactylus macrosternum* triggers tadpole schooling behaviour. We assessed aggregation time of the tadpoles of *L. macrosternum* under three different chemical stimuli—male, non-attending female and attending female—and compared it to a control. We found that tadpoles only increased the time of aggregation under the presence of the chemical stimulus of the attending females. Tadpoles of other species from the *L. latrans* group, such as *L. insularum* and *L. luctator*, may present similar responses to the attending female chemical stimulus. The next steps would be to identify the chemical components used as signals or cues and to conduct additional behavioural experiments to better assess intra- and interspecific communication between tadpoles and attending females.

1 | Introduction

Aquatic organisms such as fish and tadpoles may show aggregation behaviour (Wells 2007; Ward et al. 2020). For anurans, social aggregation may result from conspecific attraction, and different classifications have been proposed (see Hoff et al. 1990). In general, aggregation of schools varies among species in terms of polarisation, synchrony of movements and distance between individuals (Hoff et al. 1990). Caldwell (1989) classified tadpole aggregation into three types: Type I behaviour, when tadpoles form loose aggregates in shallow water or on the bottom of ponds. Movements are slow and may or may not be polarised (e.g., some bufonids). Type II is characterised by polarised schools in midwater, slow movements and no contact among individuals (e.g., some phyllomedusines and microhylids). Type III behaviour includes polarised schools in the shape of a sphere and tadpoles in physical contact (e.g., *Boana geographica*, some ranids and rhacophorids). However, compared to fish aggregation behaviour, much less is known for anurans (Hoff et al. 1990). As for the frog genus *Leptodactylus*, recently, two types of aggregation behaviour have been described: a stationary aggregation and a constant movement aggregation (see Carrillo et al. 2024).

In aquatic habitats, schools that perform coordinated movements may have multiple benefits (Pavlov and Kasumyan 2000). The most conspicuous benefit of aggregation is the reduction of

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predation risk, by confusing the predators and/or creating a dilution effect over prey (Lehtonen and Jaatinen 2016). Aggregation behaviour may be the result of following favourable environmental conditions (temperature or light) or food, or it can be the result of a more complex social interaction mediated by signals and cues (Wells 2007; Mattey et al. 2018; Ward et al. 2020). These interactions may include conspecifics or individual identification depending on the context (Jacot et al. 2010; Coombes et al. 2018; Ward et al. 2020). Different types of signals are used for aggregation, such as visual or chemical (Ward et al. 2020), although chemical signals and cues are the most ancient and have particular advantages in aquatic environments (Bronmark and Hansson 2000).

Aggregation behaviour has been recorded for tadpoles from more than 12 families of anurans, including *Leptodactylus*, the type genus of Leptodactylidae (Wells 2007). In natural conditions, tadpoles of *Leptodactylus* show dense aggregation in schools with constant body contact and coordinated movements (Wells 2007). In laboratory conditions, they also present stationary aggregation (Carrillo et al. 2024). The aggregation behaviour of *Leptodactylus* tadpoles is usually accompanied by maternal care with coordinated movements between the attending female and its respective school (e.g., Wells and Bard 1988; Vaz-Ferreira and Gehrau 1975). For instance, during tadpole guidance, females perform "pumping behaviour" (sensu Wells and Bard 1988), hitting the water with their pelvis and producing waves. Such behaviour has been suggested as a mechanism of mechanical and/or chemical communication between mother and tadpoles (Wells and Bard 1988; Castro et al. 2013). Recent experiments with *L. podicipinus* showed the importance of chemical stimulus in mother–tadpole communication, which significantly favours the aggregation of tadpoles close to the attending females (Carrillo et al. 2024).

Leptodactylus macrosternum is a species from the *L. latrans* group, which has a wide distribution across the open diagonal of South America, from the Llanos of Venezuela to northern Argentina, including Trinidad and Tobago (Magalhães et al. 2020; Frost 2024). The species has an explosive breeding pattern, with reproduction always restricted to the onset of the rainy season (Prado et al. 2005; Dixon and Staton 1976). Females guard the foam nests containing the eggs and subsequently attend large schools of tadpoles that follow the attending females without visual signalling (Figure 1; Prado et al. 2000). The complex maternal behaviour in *Leptodactylus* species of the *L. latrans* and *L. melanonotus* groups includes protection of offspring against predators, channel digging and tadpole guidance (see Carrillo et al. 2023). However, signals/cues involved in mother–offspring communication are still poorly understood (Carrillo et al. 2024).

Here, we experimentally tested whether attending females produce a chemical stimulus that encourages tadpole schooling behaviour. Thus, we designed an experiment comparing chemical stimuli of the attending females of *L. macrosternum* with those emitted by conspecifics (males and non-attending females) that may elicit tadpoles' aggregation. We expected that tadpoles of *L. macrosternum* would increase the time of aggregation exclusively with the presence of the chemical stimulus of the attending female, while showing similar aggregation times between control, male and non-attending female stimuli.

FIGURE 1 | Female of *Leptodactylus macrosternum* attending a school of tadpoles in Rio Grande do Norte state, northeastern Brazil. Photo by Diego Santana.

2 | Methods

Field work took place in a Cerrado area during the rainy seasons of 2020 and 2021 (November to March), in the municipality of Alcinópolis (18°19′27.1″ S 53°42′22.0″ W), state of Mato Grosso do Sul, central Brazil. We captured two attending females of *Leptodactylus macrosternum* with their tadpole schools. One school had 692 tadpoles at developmental stage 25 (sensu Gosner 1960). The second school had 395 tadpoles, of which 378 were at developmental stage 28 and 17 at developmental stage 26. For the experiments, we collected two additional adults, one male and one female. After all the experiments, individuals were anaesthetised, euthanised and housed at the Zoological Collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP—14709, 14660, 16238, 16239, 16242, 19654). Field sampling and experiments were approved by the Brazilian environmental agencies (licences IMASUL 71/400151/2018; SISBIO 49080-5) and by the Ethics Committee on Animal Use (CEUA/UFMS 1.085/2019), respectively.

In the laboratory, we extracted the adults' skin secretion with a cotton ball using a mild electric stimulation (see Tyler et al. 1992) and used a fresh sterile cotton ball as a control. Skin secretions were used in the experiment because of the observations of *Leptodactylus* tadpoles scraping attending females back and legs (Vaz-Ferreira and Gehrau 1975; Hoffmann 2006; Castro et al. 2013; Carrillo et al. 2022). Additionally, we have histological and proteomic data suggesting skin adaptations in attending female's skin during parental care (Juan F. C. Carrillo, unpublished data). For each school ($N = 2$), we conducted six trials of each treatment, using one male, one non-attending female and the school mother, with a total of 12 replicates per treatment and 48 in total, including the control.

For each experiment, we placed five *L. macrosternum* tadpoles from the same school in Petri dishes (150 mm of diameter) filled with fresh rainwater (changing it for each trail), guaranteeing

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enough vertical water space for swimming (15 mm). After 3 min of habituation in the Petri dish, we placed the corresponding stimulus (cotton ball) fixed to the border. For each replicate, we used different tadpoles.

For the purpose of this experiment, we used two tadpoles' interactions as indicators of aggregation behaviour (Hoff et al. 1990; Pavlov and Kasumyan 2000): tadpoles moving together (coordinated swimming) and tadpoles in contact (individuals physically touching). Therefore, we defined aggregation time as the time that three or more tadpoles remained in contact or moved together in the same direction with minimal distance among them.

We then recorded the tadpoles' behaviour on video for 7 min. From the videos, we obtained the aggregation time (cumulative time of tadpoles' aggregation) using the Behavioural Observation Research Interactive Software BORIS (Friard and Gamba 2016). For each trial (males, non-attending females and attending females), we fitted a linear mixed effect model in R 4.0.4 (R Core Team 2023), with aggregation time as the response variable, treatment (males, non-attending females, attending females and control; where the control was the reference level) as a fixed factor and school ID as a random intercept, using the *nlme* package (Pinheiro et al. 2021).

3 | Results

Under unconditioned water (control), median (±SD) aggregation time of tadpoles of *L. macrosternum* was 6.48 ± 4.52 s (range = 2.02–18.52). Under conditioned water with attending female secretion, median aggregation time of tadpoles was 25.85 ± 29.82 s (range = 0.18–89.61); under conditioned water with male secretion, median aggregation time was 16.19 ± 14.67 s (range = 2.40–51.78); and under conditioned water with non-attending female secretion, median aggregation time of tadpoles was 10.50 ± 11.89 s (range = 0.00–33.89) (Figure 2). Despite the observed differences among treatments, aggregation time of *L. macrosternum* tadpoles increased significantly only in response to chemical stimulus from the attending female (compared to the control: $t = 3.52$, $p < 0.001$). By contrast, aggregation time did not change significantly under chemical stimuli from males ($t = 1.69$, $p = 0.09$) or non-attending females ($t = 0.51$, $p = 0.60$) when each was compared to the control. Full model details are present in Supporting Information.

4 | Discussion

We found that the aggregation time of tadpoles of *L. macrosternum* increased exclusively in water containing skin secretions of the attending female. Despite our limited sample size, our results confirm the importance of chemical communication between mother and tadpoles, as previously suggested for *Leptodactylus insularum*, another species in the *L. latrans* species group (Wells and Bard 1988). Experiments with *L. podicipinus* (*L. melanonotus* species group) have shown the importance of chemical stimulus from the attending females compared with other types of stimuli, such as visual and tactile, including chemical stimuli from conspecifics (males and non-attending females; Carrillo et al. 2024). Besides aggregation behaviour, chemical stimuli from attending females of *L. podicipinus* have also been proven to be important for tadpole attraction (Carrillo et al. 2024).

Social aggregation has multiple features based on tadpoles' interactions, including coordinated movements, polarisation and physical contact (Hoff et al. 1999). However, the role of the attendant parent on aggregation behaviour has only been described in *L. podicipinus*, a species from the *L. melanonotus* group

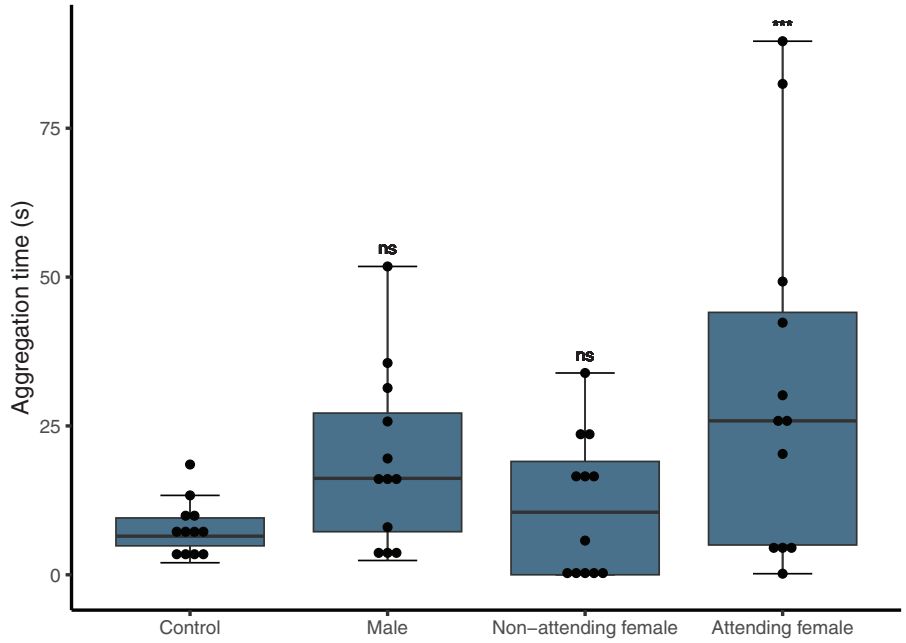

FIGURE 2 | Aggregation time of *Leptodactylus macrosternum* tadpoles under the control (sterile cotton ball) and chemical stimuli of different conspecifics: Males, non-attending female and attending females. Boxes indicate the interquartile range (IQR), with the central line depicting the median and the whiskers extending to 1.5 × IQR. Points denote the individual data. ***$p < 0.001$; ns, non-significant.

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(Carrillo et al. 2024). We now extend the effect of attending female skin secretion on tadpoles' aggregation of *L. macrosternum*, a species from the *L. latrans* group. In general, social aggregation is considered a defensive behaviour for tadpoles (Hoff et al. 1990; Wells 2007), but the specific functions in a parental care context remain unexplored. Behaviours such as physical contact and skin scraping of tadpoles on attending females, observed in several *Leptodactylus* species with parental care and aggregation (Vaz-Ferreira and Gehrau 1975; Hoffmann 2006; Castro et al. 2013; Carrillo et al. 2024), require further experimental studies to fully understand the context and function of each behaviour.

The waves produced by the pumping behaviour performed during maternal care in *Leptodactylus* species (Carrillo et al. 2023) have been suggested to be important to spread the chemical secretion produced by the attending females (Carrillo et al. 2024). Within the *L. latrans* group, besides *L. macrosternum*, pumping behaviour has been described for *L. insularum* and *L. luctator* (Castro et al. 2013; Rodrigues et al. 2011; Wells and Bard 1988), so it is expected that their tadpoles will present similar behaviour to chemical stimuli of attending females. Additionally, other species of the group also present maternal care of eggs and tadpoles—including *L. latrans* and *L. paranaru*—and the absence of records of pumping behaviour for these species may be due to the lack of behavioural observations (see Carrillo et al. 2023).

Tadpoles seem to equally follow familiar and unfamiliar attending females, at least in *L. podicipinus* (Carrillo et al. 2024); therefore, there is no reason to believe that skin secretions are different among attending females. Additionally, the recognition of heterospecific attending females by tadpoles remains underexplored, although observations on mixed schools of *L. luctator* and *L. podicipinus* indicate that tadpoles and females are unable to recognise heterospecific individuals (Rodrigues et al. 2011). Alternatively, females and tadpoles might benefit from larger schools, whether conspecific or heterospecific ones, due to the dilution effect and decrease in predation risk, which deserve further investigation (Carrillo et al. 2024). Thus, the next steps include assessing the composition of the chemical compounds produced by attending females, as well as how specific they are. Elucidating the specificity of the chemical stimulus will help to better understand the communication between tadpoles and attending females in *Leptodactylus* species. Moreover, other possible signals/cues that tadpoles might use during schooling behaviour (e.g., visual, tactile or acoustic) need to be examined to advance our understanding of the mechanisms underlying collective behaviour and movement patterns in tadpole schools (see Wells and Bard 1988; Vaz-Ferreira and Gehrau 1975).

Author Contributions

Juan F. C. Carrillo: conceptualization, data curation, formal analysis, methodology, writing – original draft. **Zaida Ortega:** formal analysis, methodology, writing – review and editing. **Diego J. Santana:** conceptualization, investigation, methodology, writing – review and editing. **Cynthia P. A. Prado:** conceptualization, investigation, methodology, writing – review and editing.

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Ethics Statement

All the animals were captured under SISBIO Brazilian Licence (68071-4) and IMASUL Licence (71/400115/20218). The experiments were conducted under bioethical approval of CEUA/UFMS from Universidade Federal de Mato Grosso do Sul, Brazil (ref. 1.085/2019).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analysed during this study are included in this published article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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