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„Oviposition and father presence reduce clutch cannibalism by female poison frogs“

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Sandra Spring, BSc

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Zusammenfassung

Einleitung: Das Fressen von Jungtieren durch adulte Artgenossen ist eine weit im Tierreich verbreitete Verhaltensweise. Mögliche Gründe für dieses kannibalistische Vorgehen sind zum Beispiel der Erhalt von Nährstoffen, eine Verringerung des Konkurrenzdrucks auf die eigenen Nachkommen sowie zusätzliche Paarungsmöglichkeiten. Bei einigen Arten neotropischer Pfeilgiftfrösche wurde Prädation arteigener Gelege bereits beobachtet, jedoch sind die zugrunde liegenden Motivationen und Mechanismen dieses Verhaltens noch kaum erforscht. Experimente bei der Art *Allobates femoralis* zeigten, dass männliche Frösche sich um terrestrische Gelege in ihrem eigenen Territorium kümmern, selbst wenn es nicht die eigenen sind, aber während einer Territorium-Übernahme Gelege im neuen Territorium fressen. Im Regelfall transportiert das Männchen die Kaulquappen nach dem Schlüpfen zu kleinen Wasserstellen. Fällt das Männchen jedoch aus, so wird der Kaulquappentransport von den Weibchen übernommen. Versuche im Labor haben gezeigt, dass Weibchen ihre eigenen Gelege an der exakten Position erkennen und in der Lage sind, sich diese über mehrere Wochen hinweg zu merken. Kannibalismus durch Weibchen wurde bisher noch nicht beobachtet. Ziel dieser Studie war zu untersuchen, ob und in welchen Situationen weibliche *A. femoralis* Frösche die Gelege anderer Weibchen fressen. Wir führten daher Experimente im Labor durch, in denen wir die Anwesenheit des Vaters beim fremden Gelege, den Reproduktionsstatus des Weibchens, sowie die Vertrautheit des Weibchens mit der Umgebung (bekanntes oder unbekanntes Terrarium) veränderten.

Ergebnisse: *Allobates femoralis* Weibchen fressen eindeutig Gelege anderer Weibchen. Dies geschah in unserer Studie häufiger, wenn das Weibchen kein eigenes Gelege produziert hatte und der Vater des fremden Geleges abwesend war. Ob sich die Weibchen in ihrem gewohnten oder einem neuen Terrarium befanden, hatte keinen Einfluss auf das Auftreten und die Häufigkeit von kannibalistischem Verhalten.

Conclusio: Unsere Ergebnisse zeigen, dass sowohl die Anwesenheit des Vaters als auch die zeitnahe Produktion eines eigenen Geleges das kannibalistische Verhalten der Weibchen reduzieren. Mögliche Gründe für kannibalistisches Verhalten können der Erhalt von Nährstoffen sowie die Steigerung der inklusiven Fitness im Vergleich zu anderen Weibchen umfassen. Die Tatsache, dass die Anwesenheit des Vaters im Terrarium/Territorium

ausreicht, um Kannibalismus durch Weibchen zu reduzieren, impliziert die wichtige Rolle von männlicher Territorialität für die Evolution von männlicher Brutpflege.

Schlüsselwörter: Kannibalismus, Oophagie, Heterokannibalismus, Brutpflege, Aggression, Dendrobatidae, *Allobates femoralis*.

Abstract

Background: The consumption of conspecific young by adult individuals is a common phenomenon across various animal taxa. Possible adaptive benefits of such behaviour include the acquisition of nutrients, decreased competition pressure on one's own offspring, and/or increased mating opportunities. Clutch cannibalism has previously been observed in several species of Neotropical poison frogs, but the underlying motivations and mechanisms were rarely investigated. Previous experiments in the poison frog *Allobates femoralis* have shown that males adopt and care for any clutch inside their own territory but cannibalize clutches when taking over a new territory. Paternal care includes the transport of hatched tadpoles to small bodies of water. Females are able to indirectly recognize their own clutch by location and transport their tadpoles in absence of the father. Cannibalism by *A. femoralis* females has previously not been observed. We experimentally investigated under what circumstances cannibalism of unrelated clutches by female *A. femoralis* would occur, by manipulating the presence of the clutch's father, the female's own reproductive state, and the female's familiarity with the environment.

Results: Females clearly cannibalize foreign clutches. This was most pronounced when the female had not produced an own clutch recently and the father of the foreign clutch was absent. The female's familiarity with the location had no significant influence on the likelihood of cannibalism to occur.

Conclusions: Our data indicate that both male presence and preceding oviposition reduce females' cannibalistic behaviour. The motivations for cannibalism might include a gain of nutritional benefits or enhancement of the female's inclusive fitness relative to others. As the father's presence at the clutch site/territory is sufficient to reduce cannibalism by females our findings further suggest a prominent role of male territoriality for the evolution of male parental care.

Keywords: Cannibalism, Oophagy, Heterocannibalism, Parental care, Aggression, Dendrobatidae, *Allobates femoralis*.

Introduction

Cannibalism, the act of consuming conspecific individuals (Allaby, 2010), is common across various animal taxa, including the consumption of male mating partners by females (sexual cannibalism; several arachnids and insects, e.g. praying mantis; Buskirk et al., 1984; Wise, 2006; Barry et al., 2008), the consumption of conspecific young by other young (e.g. salamander larvae; Pfennig et al., 1994; dendrobatid frog larvae; Brown et al., 2008), and the consumption of conspecific young by adult individuals (various taxa e.g. frogs, insects, fish and birds; Summers, 1989; Elgar and Crespi, 1992; Schausberger and Croft, 2001; Klug et al., 2006; Smith and Munro, 2008). Two types of cannibalism of juveniles by adult individuals can be distinguished: filial cannibalism (cannibalizing genetically related individuals) and heterocannibalism (cannibalizing genetically unrelated individuals; Rohwer, 1978). While such destructive behaviour, in particular filial cannibalism, may seem maladaptive from an evolutionary perspective, a couple of benefits have been demonstrated to be associated with cannibalism. Primarily, cannibalistic individuals are expected to benefit from a gain of nutrients (Hrdy, 1979; Ebensperger, 1998). For example, Argentine anchovies, *Engraulis anchoita*, cannibalize conspecific eggs and use them as additional energy source when their principal food is scarce (Pájaro et al., 2007). The Australian pelican, *Pelecanus conspicillatus*, feeds on various small animals including birds and conspecific nestlings without discrimination (Smith and Munro, 2008). However, killing other individuals' offspring does not necessarily involve consumption thereof. In several species it has been shown that killing of others' offspring serves to eliminate or reduce intraspecific competition of the perpetrator's own offspring (Hrdy, 1979; Polis, 1980; Crump, 1992). In meerkats, *Suricata suricatta*, a pregnant dominant female kills pups of subordinate females, in order to increase the helper-to-pup-ratio for her own offspring (Clutton-Brock et al., 2001). However, also pregnant subordinates kill pups from other subordinates and even from the dominant female for the same reason (Young and Clutton-Brock, 2006). Another benefit of killing conspecific offspring is to gain increased mating opportunities, as is the case in many mammals, for example lions, because females become receptive again sooner if their offspring is killed (Palombit, 2015). In wattled jacanas, *Jacana jacana*, both parents care for their offspring. When a breeding female has disappeared, another female kills the former female's brood and repeatedly mates with the father of the killed brood (Emlen et al., 1989).

In summary, possible benefits of killing and/or consuming conspecific young include gaining nutrients, reduction of future competitors or gaining increased mating opportunities.

One potential risk of adults killing conspecific young is the accidental killing of related individuals (Elgar and Crespi, 1992) which may have considerable impact on one's own inclusive fitness. One possible mechanism to prevent consuming related offspring is kin recognition (Elwood, 1991). In two species of predaceous mites, *Phytoseiulus macropilis* and *P. persimilis*, females are able to directly recognize related larvae and cannibalize preferentially unrelated ones, but the exact mechanisms how degree of relatedness is perceived and assessed are not well investigated (Schausberger and Croft, 2001). Another way to avoid consuming related juvenile individuals is via indirect offspring discrimination, for example by location. Female red-spotted newts, *Notophtalmus viridescens*, remember the exact spatial location of their nest site, therefore being able to recognize their own eggs and preferentially cannibalize unrelated eggs (Gabor, 1996; Peterson, 2000). Although eating one's own offspring might seem maladaptive, it can be beneficial under certain circumstances. Males of the sand goby, *Pomatoschistus minutus*, care for their eggs and fan them to provide them with oxygen. If egg density is high, they cannibalize some of their eggs to enhance survival rate of the clutch (Klug et al. 2006). The consumption of one's own eggs in amphibians is mainly reported from plethodontid salamanders during parental care (e.g. *Desmognathus fuscus fuscus*; Noble and Evans, 1932; *Plethodon cinereus*; Peterson, 2000). For example, in *Desmognathus ochrophaeus* females eat their eggs if they are dead or infected with fungus to prevent infection of the remaining clutch (Tilley, 1972; Forester, 1979).

In amphibians, cannibalism across or within different life-cycle stages (egg-larva-metamorph-adult) is a common phenomenon, and hetero- and even filial cannibalism have been documented in various species. In some genera, for example *Xenopus* (McCoid and Fritts, 1980; Measey, 1998) and *Notophthalmus* (Burton, 1977; Morin, 1983; Gabor, 1996), adults and conspecific larvae inhabit the same habitat and adults feed on small prey including conspecific eggs and larvae. Particularly, the consumption of eggs is a valuable source of energy, since amphibian eggs are rich in nutrients (Crump, 1992). For example, adult eastern newts, *Notophtalmus viridescens*, are known to prey on eggs of other Amphibians like the tiger salamander, *Ambystoma tigrinum* (Morin, 1983). In other species,

individuals have been shown to mainly cannibalize eggs of conspecifics, like female California newts, *Taricha torosa* (Kaplan and Sherman, 1980; Marshall et al., 1990) or the coqui frog, *Eleutherodactylus coqui* (Townsend et al., 1984; Beard, 2007). The reason for cannibalism in those cases might again be a gain of nutrients. Egg predation in *E. coqui* is in great measure caused by conspecifics. Hence, males defend their nests, thereby increasing egg survival (Townsend et al., 1984). This suggests a strong link between predation, including cannibalism, and parental care, as for male *E. coqui* nest defence is necessary for high egg survival. Males of Fornasini's spiny reed frog, *Afrixalus fornasini,* construct folded-leaf nests, which can also be seen as a form of parental care to prevent clutch cannibalism by other adult frogs, as both males and females cannibalize eggs and developing larvae (Drewes and Altig, 1996).

In frog species that show parental care and reproduce primarily outside streams and ponds, like poison frogs, oophagy is very common (Kuzmin, 1991). Paternal care (male parental care) is widespread in dendrobatid frogs and is assumed to be the ancestral form of parental care in this group (Summers and McKeon, 2004). Many species of dendrobatid frogs have been observed to cannibalize conspecific eggs, but the underlying motivations and mechanisms were rarely studied. In some species, males have been shown to exhibit cannibalistic behaviour: e.g. male *Oophaga pumilio consume* unrelated eggs, presumably to make the female become receptive again sooner because parenting females are unlikely to mate (Weygoldt, 1980). However, clutch cannibalism has mainly been reported for females in several dendrobatid species both in captivity (Wells, 1978; Myers et al., 1984) and in the field (Summers, 1989). Females cannibalizing other females' clutches have been observed in *Dendrobates auratus*, where males provide their clutches with moisture and transport tadpoles individually. Males are highly polygynous; females compete for certain males and cannibalize eggs of other females to gain a monopoly on the male's paternal care (Wells, 1978; Summers, 1989; Summers, 1990). In captivity, adult *Oophaga arborea* females and possibly also males were found to cannibalize unrelated clutches, but the reasons for cannibalism in this species remain unclear (Myers et al., 1984). Even filial cannibalism occurs in some species. *Colostethus palmatus* males usually care for only one clutch at a time and attend it continuously. When the clutch gets manipulated experimentally, e.g. covered or removed to another site, or when the male was hindered to get to its clutch for two days, the male stops attendance and sometimes cannibalizes its own eggs (Lüddecke, 1999). Filial

cannibalism by females was observed in *Epipedobates tricolor*. Observations of one pair in captivity showed that the female didn't attend the clutch, unless the father was removed (Myers and Daly, 1983). When the father was removed directly after mating, the mother cared for her clutch, but when the father was removed some days after mating the female cannibalized its clutch. Egg cannibalism is described in many poison frog species, but it was rarely studied using manipulative experimental approaches, consequentially the underlying circumstances that either promote or inhibit clutch cannibalism by adult frogs still remain unclear (Kuzmin, 1991).

Cannibalism has recently been demonstrated in male *Allobates femoralis* (Dendrobatidae), a non-toxic Neotropical frog that occurs in lowland forests of the Amazon basin and Guiana Shield (Amézquita et al., 2009). Individuals are diurnal and inhabit the forest floor. Males are highly territorial and actively defend their territories against calling intruders (Narins et al., 2003; Ringler et al., 2011). Females show site fidelity, but do not actively defend the area (Ringler et al., 2009; Ringler et al., 2011). The prolonged breeding period corresponds with the local rainy season (Gascon, 1991; Gottsberger and Gruber, 2004). *Allobates femoralis* has a polygamous mating system, where males and females have multiple mating partners (Ursprung et al., 2011). Mating occurs inside the male's territory after a long and complex courtship procedure (Montanarin et al., 2011), where eggs are finally deposited in the leaf litter. Females leave the oviposition site soon after mating, and the male remains with the clutch. About 15-20 days after oviposition the tadpoles hatch and are transported by the male to small pools of water, where larval development is completed (Ringler et al., 2013; Beck et al., 2017). Females only transport tadpoles if the male is absent at the time when tadpole transport is due (Ringler et al., 2015), in which case, they will only select their own clutch for transport based on its exact location (Ringler et al., 2016). A recent study has found that males become cannibalistic when taking over new territories. By systematically removing the former territory holder's offspring they thereby minimize risks and costs of misdirected parental care (Ringler et al., 2017). Although clutch cannibalism in poison frogs has mainly been reported for females, in *A. femoralis* cannibalism by females has previously never been observed – neither in the field nor in the lab. However, as females have been shown to be able to distinguish between their own and an unrelated clutch by exact location (Ringler et al., 2016), this would provide ideal prerequisites for selective cannibalism on non-related clutches to evolve.

We thus asked if *A. femoralis* females become cannibalistic when confronted with unrelated offspring. To answer this question, we designed an experiment, where we tested different scenarios that represent biologically relevant situations in which females might show cannibalistic behaviour. We hypothesized that females may become more cannibalistic when confronted with unrelated clutches (1) outside their home area (2) that are not guarded by the respective father and/or (3) in the absence of an own clutch.

Material and Methods

Setup/Housing

The experiments were conducted from February 10th, 2016 to December 21st, 2016 in the animal care facilities of the University of Vienna. Each experiment was performed in a standard glass terrarium with a floor area of 60 x 40 cm and 40 cm height. Each terrarium had the same furnishing and equipment, but arranged differently; the side walls and back wall were covered with xaxim and cork mats, the floor was covered with expanded clay pebbles. Dried oak leaves were provided as a substrate for egg clutches. Each terrarium contained a glass bowl of 12 cm diameter filled with approximately 350 ml of (reverse) osmosis water, a small plant, half a coconut shell as a hiding place, and a branch. The front of each terrarium was covered with fabric to prevent visual contact with other individuals and other disturbances. Climatic conditions were similar to natural conditions in French Guiana and standardized in all terraria through an automatic heating, lighting, and raining system. The temperature ranged from 19°C at night to 30°C during the day. Lights were on from 7 a.m. to 7 p.m. and humidity was 100% constantly. Feeding was conducted every second day for all frogs; feeding animals were wingless fruit flies.

Ethical note

The frogs used in this experiment were part of an ex situ laboratory population of the University of Vienna's animal care facilities. Original stock for this population was sampled and exported in compliance with all legal requirements from the responsible French authorities (DIREN: Arrete n°82 du 10.08.2012 and Arrete n°4 du 14.01.2013). The experimental procedures used for this study were approved by the ethics animal welfare committees of the University of Veterinary Medicine Vienna and the University of Vienna. They were strictly according to the current Austrian legislation and good scientific practice guidelines and followed the ASAB guidelines for treatment of animals in behavioural research and teaching.

Experimental Design

We randomly assigned females to one of the four test conditions, where we presented in all trials an unrelated clutch, but manipulated the presence of the father, the presence of an own clutch, and the familiarity of the female with the terrarium (figure 1). In each of the four conditions ten different females were tested. No individual participated twice in the whole experiment. In “home” we tested if females prey on unrelated clutches inside their home terrarium. To this end, females were temporarily (i.e. 5-10 min) removed from their terrarium and an unrelated clutch (i.e. a clutch from another breeding pair) placed inside before the female was returned. In “out” we tested if females cannibalize on unrelated clutches in an otherwise uninhabited foreign terrarium. Here, females were transferred into a new terrarium that contained an unrelated clutch but no father present. In “out M+” we tested if females cannibalize unrelated clutches in a new terrarium where the respective father was present. As after the first trials of “out M+”, we saw that females and males immediately started courting and produced a clutch, we added another condition to disentangle the effects of oviposition and male presence: In “out M-”, females were transferred to a new terrarium with an unrelated clutch and its father (like in “out M+”); however, the male was removed from the terrarium immediately after the two frogs had produced a clutch together.

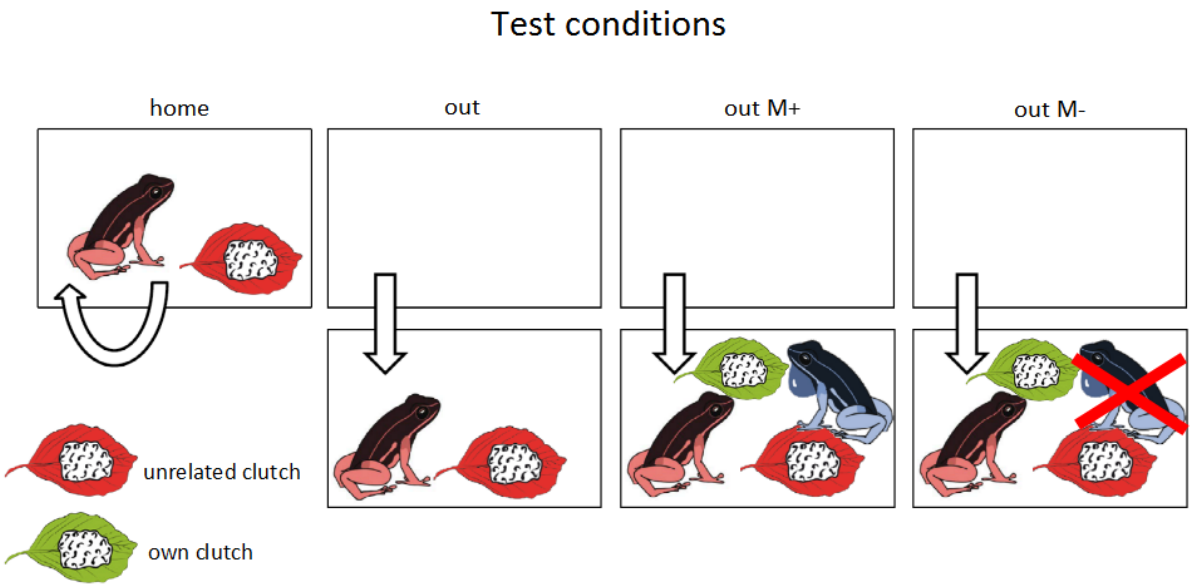

Figure 1. Experimental design (figure modified after Ringler et al., 2017). “Home”: an unrelated clutch (red) was placed inside a female’s (red) home terrarium while the female was temporarily removed. “Out”: females were transferred into a new terrarium with an unrelated clutch inside, but no guarding male. “Out M+”: females were transferred into a new terrarium with an unrelated clutch and a male (blue) inside, they produced an own clutch (green) together. “Out M-”: like “Out M+”, but the male was removed after they had produced an own clutch together.

Females were caught from their terraria with transparent plastic bags and then returned or transferred to the same or a new terrarium, depending on the condition, to assure equal handling effects across all trials. There was no significant difference in neither the developmental stage, measured in days after oviposition (Kruskal-Wallis test; $\chi^2 = 0.296$, df = 3, $p = 0.961$), nor in the number of tadpoles per clutch (Kruskal-Wallis test; $\chi^2 = 0.496$, df = 3, $p = 0.920$) in the clutches used for experimentation across all the conditions. All experiments were filmed by using digital full-HD video surveillance cameras (IndigoVision, BX400 HD Minidome) that were installed on top of the terraria. Cameras filmed from 7 am to 7 pm, corresponding to the duration of lighting in the terraria. The zoom and focus were adjusted so that the whole clutch and surrounding area were fully visible. Every other day the clutches were additionally inspected directly in the terraria and tadpoles were counted to verify the count from camera recordings. The videos were visually inspected via the

computer program IndigoVision Control Center and the following behaviours and parameters were transcribed: the occurrence of cannibalism per female (yes/no; in summary referred to as the number of cannibalistic females), the number of cannibalistic events, the sum of consumed tadpoles per female, the degree of cannibalism in categories (none/partial/complete clutch cannibalism), the percentage of the clutch that was cannibalized per female, and eventual tadpole transport.

Analysis

Significant differences across conditions regarding the parameters occurrence of cannibalism (yes/no) and degree of cannibalism were tested with Chi-square tests. We used Kruskal-Wallis tests to test for significant differences across conditions regarding the following parameters: the number of cannibalistic events per female, sum of tadpoles consumed by a female, and the percentage of the foreign clutch that was consumed by females. If omnibus tests showed statistically significant differences across groups, post-hoc analyses were performed for which the p-values were adjusted using false-discovery-rate (fdr) corrections to reduce the Type 1 error rate. To test for correlations between the sum of consumed tadpoles per female and the percentage of the clutch that was cannibalized we used Spearman's rank correlation test.

Results

The overall level of clutch cannibalism was high (N = 25 out of 40 trials, 62,5%). It was strongly reduced by the presence of the father and by oviposition (N = 2 out of 10 females, condition “out M+”). Females never transported any of the unrelated tadpoles (N = 0 out of 40 trials).

We found significant differences across conditions for all tested parameters. These include the number of cannibalistic females (Chi-square test; $\chi^2 = 15.68$, df = 3, $p = 0.001$), the number of cannibalistic events (Kruskal-Wallis test; $\chi^2 = 13.60$, df = 3, $p = 0.003$), sum of tadpoles consumed by a female (Kruskal-Wallis test; $\chi^2 = 14.47$, df = 3, $p = 0.002$), the percentage of the foreign clutch that was consumed (Kruskal-Wallis test; $\chi^2 = 14.50$, df = 3, $p = 0.002$), and the degree of cannibalism (Chi-square test; $\chi^2 = 17.29$, df = 6, $p = 0.008$). Since there was a high correlation between the sum of consumed tadpoles and the percentage of the clutch that was consumed per female (Spearman’s rank correlation test; N = 40, $\rho = 0.98$, $p < 0.001$), we only considered the variable “sum of consumed tadpoles” in all following analyses.

Cannibalism was more often observed in the two conditions completely without a male (8 of 10 females in “home” and 10 of 10 females in “out”, figure 2). The number of cannibalistic females was highest in a new terrarium without a male (“out”), and lowest when a male was present throughout the experiment (2 of 10 females in “out M+”, figure 2). The two cannibalistic individuals in “out M+” were the only two in this condition that did not produce an own clutch during the course of the experiment. Half of the females were cannibalistic when they were in a new terrarium with a male that was removed after they produced an own clutch together (5 of 10 females in “out M-”, figure 2).

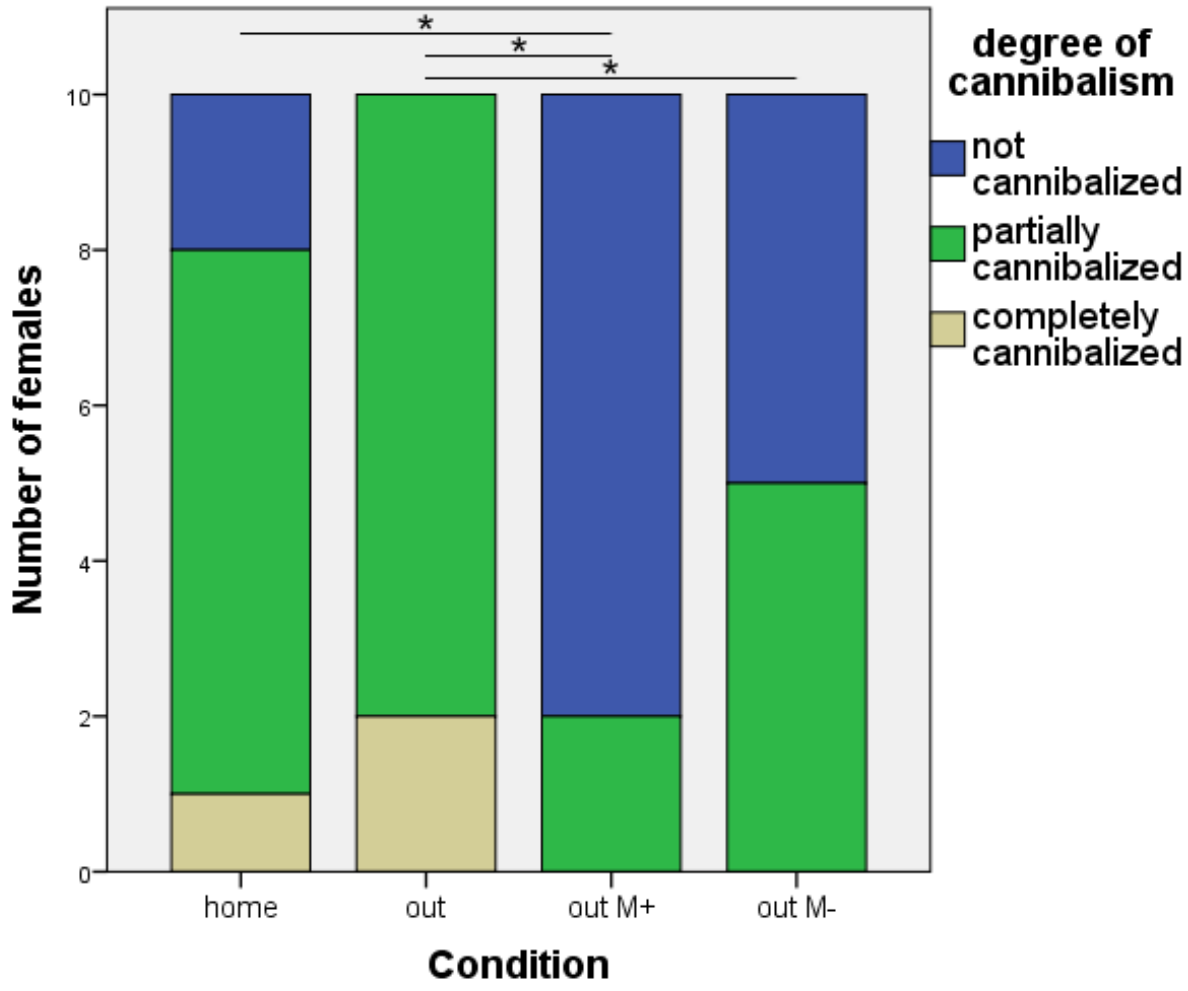

Figure 2. Degree of cannibalism (categories: clutch not / partially / completely cannibalized) across all four test conditions.

Pairwise comparisons revealed that the biggest differences in all parameters tested were found between the condition where females were outside their home tank and without any male ("out") and the condition where they were moved to a foreign tank with a male present throughout ("out M+"): In the absence of a father, there were significantly more females cannibalizing clutches (Chi-square-Post hoc test, $p = 0.004$; figure 2), more cannibalistic events (Wilcoxon Rank Sum test, $p = 0.011$; figure 3), higher numbers of consumed tadpoles (Wilcoxon Rank Sum test, $p = 0.007$; figure 4), and the degree of cannibalism was significantly higher (Chi-square-Post hoc test, $p = 0.004$; figure 2) than when the father was present.

Further, there were significant differences between conditions “out” and “out M-”: When females were in a new terrarium without a male (“out”) the number of cannibalistic events (Wilcoxon Rank Sum test, $p = 0.035$), the sum of tadpoles consumed by females (Wilcoxon Rank Sum test, $p = 0.031$), and the degree of cannibalism (Chi-square-Post hoc test, $p = 0.046$) were significantly higher than when in a new terrarium with an own clutch but where the father had been removed (“out M-”). We further found a trend for the number of cannibalistic females being higher in “out” than in “out M-” (Chi-square-Post hoc test, $p = 0.065$).

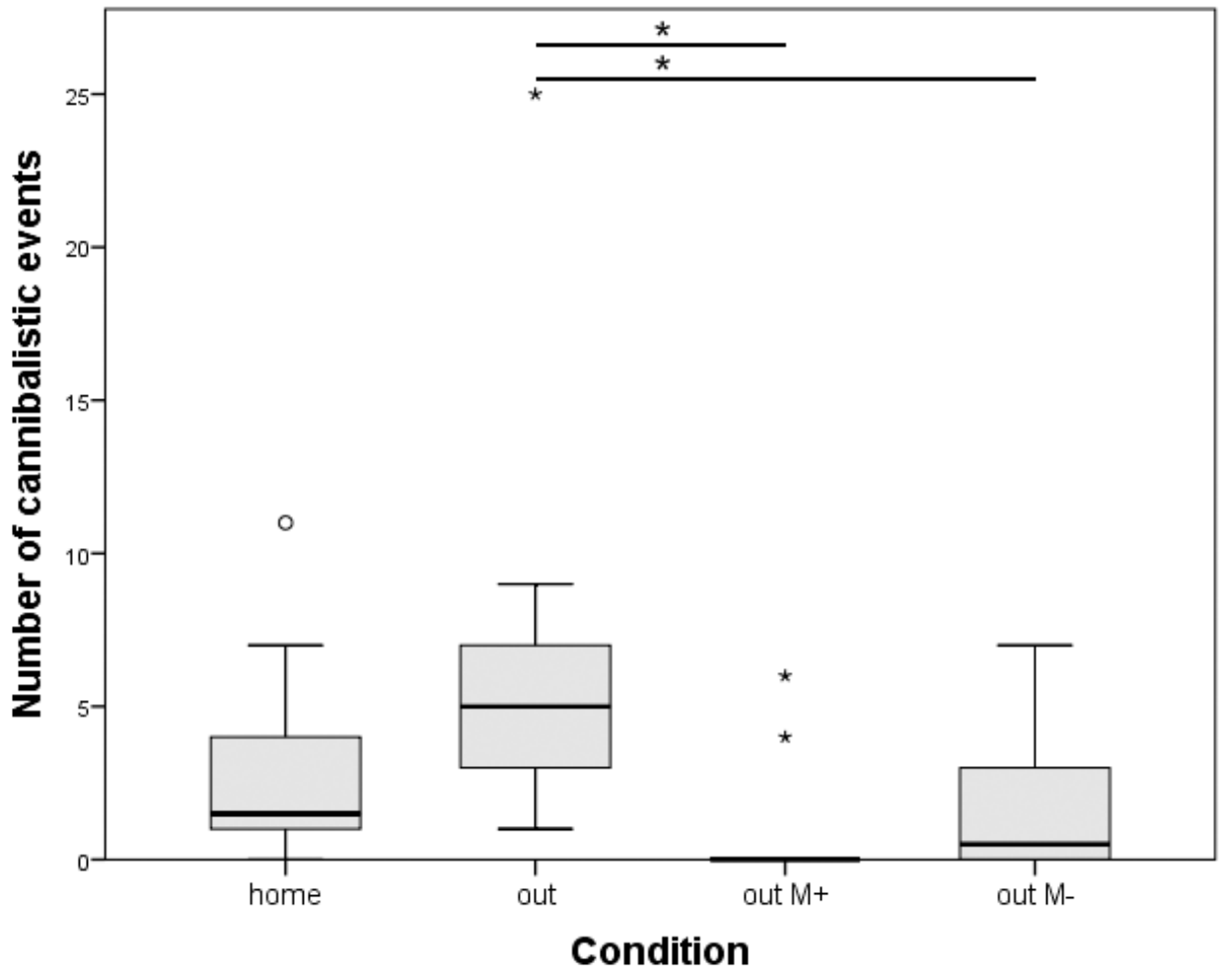

Figure 3. Number of cannibalistic events across all four test conditions.

When females were in their home terrarium (“home”) the sum of cannibalized tadpoles was significantly lower (Wilcoxon Rank Sum test, $p = 0.050$) than in a new terrarium without a

male (“out”). Between those conditions no significant differences were found for the number of cannibalistic females (Chi-square-Post hoc test, $p = 0.474$), cannibalistic events (Wilcoxon Rank Sum test, $p = 0.093$), and the degree of cannibalism (Chi-square-Post hoc test, $p = 0.582$).

No significant differences were found between the conditions “home” and “out M-” (cannibalistic females, Chi-square-Post hoc test, $p = 0.420$; cannibalistic events, Wilcoxon Rank Sum test, $p = 0.332$; sum of consumed tadpoles per female, Wilcoxon Rank Sum test, $p = 0.509$; degree of cannibalism, Chi-square-Post hoc test, $p = 0.420$).

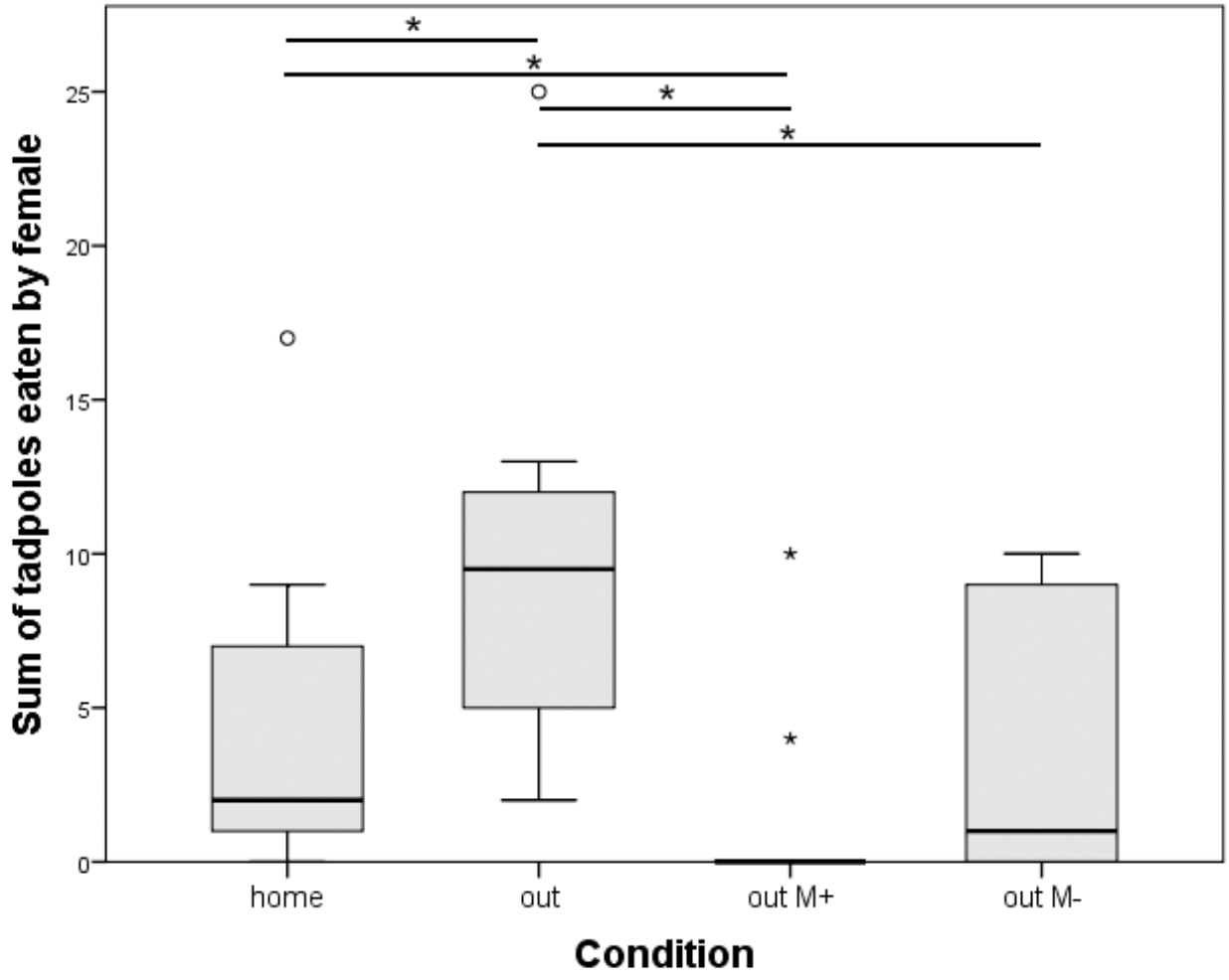

Figure 4. Sum of tadpoles eaten by female across all four test conditions.

When females were in a new terrarium with an own clutch and the father being present ("out M+") the sum of consumed tadpoles was significantly lower (Wilcoxon Rank Sum test, $p = 0.050$) than when females were in their home terrarium without a male or an own clutch ("home"). There was also a significant difference in degree of cannibalism (Chi-square-Post hoc test, $p = 0.046$; figure 2) between those conditions, being higher in "home" than in "out M+". Results showed a trend that in "home" more females were cannibalistic (Chi-square-Post hoc test, $p = 0.065$) than in "out M+" and did this at higher rates (number of cannibalistic events, Wilcoxon Rank Sum test, $p = 0.071$).

Between conditions "out M+" and "out M-" there were no significant differences for the number of cannibalistic females (Chi-square-Post hoc test, $p = 0.420$), number of cannibalistic events (Wilcoxon Rank Sum test, $p = 0.321$), sum of tadpoles (Wilcoxon Rank Sum test, $p = 0.298$), and the degree of cannibalism (Chi-square-Post hoc test, $p = 0.420$). Complete clutch cannibalism was only present in the two conditions where no male was present at any time during the experiment (conditions "home" and "out"; figure 2).

Discussion

In this study we clearly demonstrate selective clutch cannibalism in female *Allobates femoralis*. Cannibalism was generally reduced by both, the presence of the father and preceding oviposition by the tested female. Unrelated clutches were never transported by females.

Nutritional benefits

We suggest that one of the reasons for a female to cannibalize foreign clutches is to obtain valuable nutrients for producing own clutches, as amphibian eggs are rich in phosphorus and calcium (Crump, 1992), and egg production is likely to be energetically costly (Trivers, 1972). In many different frog taxa, it has been shown that conspecific eggs are part of adults' diet. For example, adult female African clawed frogs, *Xenopus laevis*, feed on small prey including conspecific eggs when they are highly abundant in the pond (Measey, 1998). *Eleutherodactylus coqui* has been documented to cannibalize conspecific eggs (Townsend et al., 1984; Beard, 2007) in order to gain nutrients. Also, in adult *Rana ridibunda* prey items include conspecific eggs and other life history stages (Ruchin and Ryzhov, 2002). However, we can exclude that nutritional aspects alone were responsible for the observed results in our study. All females in our study received equal and sufficient amounts of food over the course of the experiment, and cannibalism was considerably reduced by male presence and preceding oviposition. *Allobates femoralis* females also never cannibalized their own clutches in our study.

Reproductive benefits

Beside nutritional benefits, also other benefits of cannibalism, such as simultaneously increasing the own reproductive success relative to others (Crump, 1992), could have promoted clutch cannibalism in female poison frogs. In species where adults are able to recognize their own offspring, selective cannibalism of unrelated clutches can be a powerful mechanism to prevent other individuals from successful reproduction as well as to monopolize mating events within a population (e.g. *D. auratus*, Summers, 1990). For

example, females of some mite species are able to directly recognize their own offspring and selectively cannibalize unrelated larvae (Schausberger and Croft, 2001). A previous study has shown that female *A. femoralis* indirectly recognize own clutches by remembering the exact oviposition site (Ringler et al., 2016). Clutches in other locations are either ignored or cannibalized (Ringler et al., 2016; this study). A similar pattern is also found in other animal taxa. For example, in red-spotted newts, *Notophtalmus viridescens,* females indirectly recognize their own clutch by remembering the exact spatial location of their nest site (Peterson, 2000). They cannibalize unrelated clutches rather than their own clutch. Aside from providing the female with valuable nutrients and energy, this behaviour is assumed to also increase their own reproductive success relative to others (Gabor, 1996). As females in our study selectively cannibalized only unrelated clutches, we suggest that cannibalistic *A. femoralis* females likewise gain nutritive as well as indirect fitness benefits.

Cannibalizing foreign clutches may enhance the survival of one's own offspring, in situations where cannibalism reduces the future tadpole population size, and therefore the number of intraspecific competitors. Thus, the amount of food per individual tadpole is increased (Polis, 1980). Further, in some frog species the free-living aquatic tadpoles can be cannibalistic and feed on conspecific tadpoles that live in the same pool of water. Thus, there is a risk of reduced offspring survival for females if their mate deposits tadpoles from multiple females in the same pool (Summers and McKeon, 2004). In species where tadpoles feed on other tadpoles, as it is the case for example in *D. auratus*, cannibalizing other females' clutches could reduce future cannibalism in own offspring by other tadpoles (Summers and McKeon, 2004). However, tadpoles of *A. femoralis* are not cannibalistic (pers. obs. E. Ringler) and are usually distributed across different pools of water by males (Ringler et al., 2013). We consequentially doubt that the motivation of female *A. femoralis* to destroy foreign clutches is to reduce the risk of cannibalism among tadpoles. Further, the extent of cannibalism by females is rarely sufficient to considerably reduce future tadpole competition, since tadpoles are distributed across different pools of water (Ringler et al., 2013) and also frequency of clutch cannibalism by females in the field presumably is rather low.

In some animal species, the killing of conspecific young mainly serves to make a prospective mating partner that is currently parenting, become receptive sooner. This mechanism has been documented in various taxa including mammals (Palombit, 2015), birds (Emlen et al., 1989), and also frogs (Weygoldt, 1980). Clutch cannibalism by *Oophaga pumilio* males has been hypothesized to serve to make the female become receptive again sooner, as parenting females are unlikely to mate (Weygoldt, 1980). In *D. auratus*, egg cannibalism by females may serve to increase the amount of paternal care of the female's own clutch (Summers, 1990). If parental investment in single clutches is reduced with increasing number of simultaneously present clutches ("dilutable benefits", Tazzyman et al., 2012), cannibalism on unrelated clutches could function to secure the male's paternal effort for the females' own clutches (Wells, 1978; Parental quality hypothesis, Summers, 1989). However, *A. femoralis* females have never been observed to fight for males or mating opportunities. Further, males were observed to care for up to five clutches simultaneously (Ursprung et al., 2011) and they do not specifically manipulate or actively defend their clutches (pers. obs. E. Ringler, this study). In our study, we only included males that had only one single clutch sired previously, in order to provide standardized conditions across trials. Thus, we cannot make inferences whether paternity quality is reduced with increasing number of clutches, and if clutch cannibalism by females is in turn a meaningful measure to ensure minimum levels of paternal care. However, we speculate that increasing number of clutches (at least in the range of naturally observed maximum numbers) does not reduce the respective parental care each single clutch receives to a relevant degree, and also does not limit the number of mating opportunities, as males are able to attend several clutches at a time. Therefore, we think that ensuring a certain amount of parental care by the father might not be a primary motivation for cannibalizing foreign clutches by *A. femoralis* females.

Factors reducing clutch cannibalism

Allobates femoralis, clutches are laid inside male territories (Ringler et al., 2017), which are heavily defended against conspecific calling intruders (Narins et al., 2003; Ringler et al., 2011). In our study, cannibalism was generally higher in conditions where no male was present at any time during the experiment, showing the impact of male presence on clutch cannibalism by females in our study. In general, egg-guarding by parent individuals can be a

powerful strategy to reduce clutch cannibalism and/or predation (Tilley, 1972). In many frog species, males defend their territories and/or their clutches from approaching predators. Male *Eleutherodactylus coqui* actively defend their nests to reduce heterocannibalism, which constitutes a major threat for offspring survival (Townsend et al., 1984; Beard, 2007). In *Allobates femoralis*, male presence alone was enough to prevent females from preying upon unrelated clutches, as active protection of clutches by fathers was never observed, neither in any previous nor in this study. Consequentially, territoriality in *A. femoralis* males might simultaneously serve to protect clutches against cannibalistic conspecifics. A similar mechanism was proposed for *Cophixalus parkeri*, where males defend their nest sites and thereby possibly reduce clutch cannibalism by other individuals (Simon, 1983). These findings propose that male territoriality might be an important prerequisite for the evolution of male parental care, and that active defence of clutches may be interpreted as a more derived and complex form of territorial defence behaviour.

In *A. femoralis*, clutch cannibalism by females was generally reduced by both, the presence of a male and also preceding oviposition. We were not able to test for the effects of male presence alone, as when a female was in a terrarium with a male they immediately produced a clutch together. When males were completely absent, and females had no own clutch, levels of cannibalism were higher than in a terrarium with a male and an own clutch. When females had produced an own clutch and the male was removed afterwards, cannibalism rates were intermediate to those of the conditions with males either entirely present or absent. Interestingly, the two females that cannibalized on the foreign clutch when a male was present were also the only two out of 10 from this trial that had not produced an own clutch previously, highlighting the prominent role of oviposition in mediating aggressive and affiliative behaviours in *A. femoralis* females. Similar observations typically are known from studies in mammals, where females generally show aggressive behaviour towards unrelated pups, but switch into affiliative behaviour soon after mating (Elwood, 1977; Parmigiani et al., 1994; Keverne and Curley, 2004). For example, female house mice, *Mus domesticus*, generally kill unrelated pups, but when they are lactating, they do not attack unrelated pups that are of similar age as their own offspring, and even adopt new-born pups (Parmigiani et al., 1994). Also, in Mongolian gerbils, *Meriones unguiculatus*,

unmated females cannibalize foreign pups, but in turn provide maternal behaviour towards foreign offspring during late pregnancy (Elwood, 1977). A similar relation between cannibalism and parenting was also previously found in frogs. Adult *Cophixalus parkeri* cannibalize clutches only when they are not breeding; thereby reducing the risk of accidental filial cannibalism (Simon, 1983). Considering the distant relationship of mammals and frogs and their different forms of parental care and mating systems (Prates and Guerra, 2005), it is interesting that similar behavioural responses are observed in both groups during pregnancy, oviposition, or raising of own young. Possibly similar hormonal and/or neuronal networks might be responsible for regulating parental and cannibalistic behaviour across vertebrates (O'Connell and Hofmann, 2011). In mammals, the hormonal pathways underlying parental behaviour are relatively well investigated. Oxytocin and prolactin have been shown to be important regulators of affiliative behaviours, including parental care. Oxytocin secretion induces maternal care and reduces aggression (Wise and Pryor, 1977; Keverne and Curley, 2004; Ferris, 2005). Prolactin levels increase after an infant is born (Ziegler, 2000) and reduce aggression in the parent; in turn suppression of prolactin leads to increased aggressive behaviour towards offspring (Wise and Pryor, 1977). In contrast, the neuroendocrinal regulation of parental behaviour in amphibians is still poorly understood (O'Connel and Hofmann, 2010). The few studies on hormonal regulation of aggressive and parental behaviour in anurans provide rather inconclusive results (Schulte and Summers, 2017). In our study, the finding that cannibalism was reduced after oviposition in *A. femoralis* females suggests hormonal changes in females after mating, which might prevent females from being cannibalistic in the presence of their own clutches. The observed reduction in cannibalistic behaviour in the presence of a male, as discussed above, might likewise be linked to hormonal changes in females. When confronted with a courting male, females might immediately turn into a reproductive mode, where affiliative behaviours are promoted and any aggressive behaviour gets suppressed. Future studies should identify the distinct hormonal and neuronal mechanisms underlying parental decision-making in *A. femoralis*, which will also provide valuable insight into the evolution of parental behaviours across vertebrates.

Conclusion

In summary, we found selective clutch cannibalism by *A. femoralis* females. Both, male presence and oviposition reduced the extent and frequency of clutch cannibalism, possibly mediated by hormonal and/or neuronal responses. Cannibalistic behaviour could be triggered by nutritional motivations and/or serve to ensure a certain amount of parental care by the father and/or to enhance the female's inclusive fitness. The father's simple physical presence reducing clutch cannibalism by females highlights the important role of female aggression and male territoriality for the evolution of male parental care.

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