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Research



# Plasticity in expression clarifies the ecological drivers and evolutionary history of cannibalism in invasive cane toads (*Rhinella marina*)

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When rapid evolutionary change occurs in a trait's expression, what role is played by proximate factors that induce variation in expression? We assessed drivers of variation in a recently evolved behaviour—targeted cannibalism by invasive cane toad tadpoles (*Rhinella marina*) in Australia—to better understand its evolution. Because targeted cannibalism probably evolved due to intense intraspecific competition, we first assessed effects of two proximate factors that influence resource availability—cannibal density and developmental stage—on rates of cannibalism. We then used published data to quantify evolutionary shifts in the impact of another potential driver of variation—tadpole mass. In laboratory trials, rates of cannibalism increased with cannibal development and rearing density, and cannibals reared at higher densities began consuming eggs earlier in embryonic development. Analyses of published data revealed that ancestral-range tadpoles were less cannibalistic than invasive conspecifics, and ancestral rates of cannibalism increased more slowly with larval mass. Thus, larval cannibalism is plastic in both ancestral and derived populations. Our work clarifies the ecological drivers and evolutionary history of targeted cannibalism and cannibal-induced defences in invasive Australian cane toad tadpoles and illustrates the

Supplementary material is available online at  
<https://doi.org/10.6084/m9.figshare.c.8160886>.

## 1. Introduction

The expression of behavioural traits can vary within individuals throughout development and among individuals within a population [1,2]. Often, trait expression is also affected by local environmental conditions [3,4]. In unpredictable environments, this behavioural flexibility may enhance fitness [5]. Consequently, behavioural phenotypes can shift rapidly in response to environmental change [6].

One such flexible and rapidly evolving behaviour is cannibalism, a geographically and taxonomically widespread trait exhibited by many vertebrates [7]. Expression of cannibalistic behaviour varies at both the individual and population level and is influenced by both intrinsic and extrinsic factors [7,8]. Cannibalistic behaviour probably intensifies when the fitness benefit it confers to an individual outweighs its cost to kin, and various factors modify this cost–benefit equation [7,8]. For example, cannibalism is often more intense in invasive populations than their native counterparts (e.g. [9,10]), potentially because release from natural enemies (e.g. parasites, predators and heterospecific competitors) renders intraspecific competition a more important determinant of individual viability in invasive populations [11].

Pond-breeding amphibian larvae are a model system for studies of the ecological drivers and evolution of cannibalism because cannibalistic behaviour varies among species on a gradient from non-expression to obligate expression [12,13], and shifts in expression can occur both within and among individuals in response to environmental conditions and larval body size (e.g. [14,15]). Propensity for cannibalism may also be influenced by genotype in some species [16]. In amphibian larvae, cannibalism appears to both reduce competition and provide nutrition to developing larvae [17,18]. Fixed, intense cannibalism is rare in amphibian larvae and largely limited to egg-eating dart frog tadpoles and the larvae of caudates and anurans that have evolved distinct cannibalistic phenotypes [12,13].

Because fixed, intense cannibalism is not known to occur in amphibian larvae without an associated cannibalistic phenotype aside from cases of obligate egg-eating, the rapid evolution of intense targeted cannibalism by the larvae of invasive cane toads (*Rhinella marina*) in Australia is surprising [19,20]. Cane toad tadpoles in Australia are attracted to bufadienolide toxins excreted by the eggs and early-developmental larvae of other cane toads and respond to the toxin with feeding behaviour, hence the term ‘targeted cannibalism’ [21,22]. Previous studies suggest that larval cannibalism is infrequent and opportunistic in native-range populations of cane toads but frequent and targeted in Australia [19,20]. Whether intensity of cannibalism is truly fixed—in both native and invasive cane toads—is unknown because DeVore *et al.* [19] did not assess whether rates of cannibalism varied with factors known to influence this behaviour in other amphibians. Thus, in the present study we explore the degree to which cannibalistic behaviour by the tadpoles of cane toads is fixed both in Australian invasive populations and in the species’ native range in South America. If the intensity of cannibalism is variable within or among populations, what can this tell us about the evolution of targeted cannibalism and cannibal-induced defences (eggs develop faster in Australia and some larvae accelerate development through vulnerable early larval stages when exposed to cannibals [19,20]) in invasive Australian cane toads?

To answer these questions, we first conducted experimental trials with invasive-range tadpoles to investigate effects of three factors known to influence cannibalistic behaviour in other amphibians—cannibal clutch of origin, developmental stage of cannibals and their victims, and rearing density of cannibals—on the expression of cannibalism in Australia. We then used published data to assess the ancestral state of responses to one potential driver (cannibal mass) and compare its effect between native and invasive populations. By integrating these two analyses, we hope to clarify the ecological drivers and evolutionary history of targeted cannibalism and cannibal-induced defences in Australian cane toads.

## 2. Methods

### 2.1. Study system

Cane toads are large bufonid anurans from northeastern South America that were translocated to Australia via the Caribbean and Hawai'i in 1935, and have since spread widely across tropical and subtropical parts of that continent [23,24]. Despite their primarily herbivorous diet, the tadpoles of Australian cane toads are voracious predators of conspecific eggs and pre-feeding larvae [19,20] (figure 1). Cannibalism is opportunistic and infrequent in native-range populations of the cane toad, but tadpoles from Australian populations engage in intense, targeted predation of conspecifics [19,20,22]. Targeted cannibalism occurs throughout the Australian distribution of cane toads and may have arisen in response to high larval densities and intense intraspecific competition in Australia [19]. Field experiments suggest that cannibalism by older cohorts can reduce clutch survival by over 99% in Australia, stimulating the rapid evolution of plastic, cannibal-induced developmental acceleration in pre-feeding larvae that are particularly vulnerable to cannibalism because they lack the protection of a jelly coat and are immobile [20,25].

### 2.2. Obtaining eggs

We hand-collected adult cane toads in 2023 from the Adelaide River floodplain (12°34'38" S, 131°18'55" E) in the Northern Territory of Australia, an area that was first colonized by invasive cane toads in 2005 [26]. After capture, we housed putative breeding pairs in 75 l tubs containing a shallow layer of bore water. We then administered a subcutaneous injection of leuprorelin acetate (Lucrin; Abbot Australasia, Kurnell, Australia) diluted in amphibian Ringer's solution (concentration = 0.25 mg ml<sup>-1</sup>) to stimulate amplexus and oviposition. We injected males with 0.25 ml of solution and females with 0.75 ml, and left pairs to spawn overnight. After approximately 12 h, we checked tubs for eggs.

### 2.3. Husbandry of eggs and pre-feeding larvae

We produced and reared three egg clutches (from three independent pairs of adult cane toads) synchronously for use as the cannibalistic cohort in trials. Upon discovery of eggs (i.e. <12 h post-deposition), we transferred them to clutch-specific 75 l tubs containing approximately 50 l of bore water. We fitted each tub with an aquarium bubbler for oxygenation. After 5 days, when immobile pre-feeding larvae had developed into free swimming tadpoles (i.e. at Gosner stage 25 [27]), we split each clutch into density treatments.

### 2.4. Density manipulation and tadpole husbandry

We haphazardly selected tadpoles from each of the three egg clutches and added them to one of two clutch-specific rearing tubs, each containing approximately 300 l of bore water. From each clutch, we allocated 300 tadpoles to a low-density tub (i.e. one tadpole per litre) and 1500 tadpoles to a high-density tub (i.e. five tadpoles per litre). Thus, we created six rearing tubs—one per density treatment per clutch. Our choice of treatment densities approximated *in situ* observations of densities of cane toad tadpoles in Northern Territory water bodies (A.T.F. 2023, personal observation). Throughout development, we added the same amount of food to all tubs to mimic low-density resource-rich ponds and high-density resource-poor ponds without inducing starvation. We fed tadpoles a 3:1 ratio of ground Vetafarm rabbit pellets (Vetafarm, Wagga Wagga, Australia) to Petbarn tropical fish flakes (Petbarn, Sydney, Australia) (see [28,29]), and provided each rearing tub with 450 mg of food per day. This resulted in *per capita* food availabilities of 1.5 mg per day and 0.3 mg per day in low-density and high-density treatments, respectively. We housed rearing tubs in a shaded outdoor location to allow exposure to fluctuations in ambient temperature and natural light–dark cycles and covered each tub with window-screen mesh to exclude insects. To maintain water quality, one-third of the volume of each tub was replaced with fresh bore water every fourth day.



**Figure 1.** In their invaded range in Australia, the tadpoles of cane toads (*Rhinella marina*) engage in intense cannibalism of conspecific eggs and pre-feeding larvae. Pictured is a late-developmental cane toad tadpole (Gosner stage 36) beginning to consume an egg laid by a conspecific. Photo by Etienne Littlefair (Wild Territory Images).

## 2.5. Cannibalism trials

We conducted six cannibalism trials to assess effects of clutch, rearing density and developmental stage on intensity of cannibalism. We conducted one trial early in tadpole development (i.e. Gosner stages 26–29 [27]), one midway through development (i.e. stages 32–35), and one late in development (i.e. stages 36–39) for both high- and low-density tadpoles. Because food availability affects larval developmental rates [29], we could not test high-density and low-density tadpoles at each range of Gosner stages in synchrony. Thus, we conducted one trial at each developmental stage and density (i.e. six rather than three trials).

Twenty-four hours prior to each trial, we haphazardly selected 10 tadpoles from each clutch-specific rearing tub at the desired density, anaesthetized them in a 1 : 1 solution of 1% MS 222 and bore water, and staged them under a dissecting microscope to estimate Gosner stages [27] of the non-anaesthetized experimental tadpoles. We then haphazardly selected another 10 tadpoles from each clutch-specific rearing tub at one of the specified densities ( $n = 10$  tadpoles per clutch,  $n = 30$  tadpoles per trial). We housed the latter tadpoles individually indoors (room ambient temperature approx. 30°C) in 1 l plastic tubs containing 1 l of bore water and covered with mesh (hereafter called ‘experimental tubs’). We allowed tadpoles to acclimate to experimental conditions during this period and fasted them to standardize appetites among individuals and between density treatments.

We produced one clutch of cane toad eggs for use as prey in each trial. Thus, throughout the experiment, we produced six clutches of eggs to serve as the cannibalized cohort (one for each of the six cannibalism trials) from six independent pairs of adult cane toads. To do so, we collected adult toads, injected them with leuprorelin acetate solution, and allowed pairs to spawn through the night. The following morning (i.e. < 12 h post-deposition), we added 10 eggs to each experimental tub and constructed 10 control tubs containing only bore water and 10 eggs ( $n = 400$  eggs per trial). To assess the impact of development of the cannibalized cohort on rates of cannibalism, we recorded the number of eggs and pre-feeding larvae in each tub at 12 h intervals during trials. We considered eggs and pre-feeding larvae to have been cannibalized if they were either fully or partially consumed. We concluded trials when pre-feeding larvae developed into free-swimming tadpoles (at which time they cease to be vulnerable to cannibalism [20,22,25]) or when all eggs and pre-feeding larvae had been cannibalized. Maximum duration of trials was 96 h. Following completion of each trial, we returned tadpoles from the older cohort to source tubs and counted any remaining unviable eggs and surviving tadpoles from the younger cohort. We then euthanized all remaining tadpoles from the younger cohort via immersion in 1% MS 222.

## 2.6. Effects of tadpole origin and size on rates of cannibalism

We used publicly available data from DeVore *et al.* [19] to assess effects of tadpole mass and geographic origin (i.e. invasion history) on intensity of cannibalism. DeVore *et al.* [19] reared multiple clutches of cane toad tadpoles from each of multiple native (French Guianese) and invasive (Australian) populations in captivity under standardized conditions. They then conducted cannibalism trials in which they exposed individual toad tadpoles to 10 pre-feeding toad larvae for 24 h to compare rates of cannibalism between native and invasive Australian cane toad populations [19]. However, those authors did not investigate geographic differences in intensity of cannibalism within Australia or potential differences in mass-specific rates of cannibalism between native and invasive populations (see [19]). For statistical analyses, we used data on the intensity of cannibalism (measured as the number of conspecifics consumed by a tadpole in 24 h), body mass and clutch of origin of tadpoles derived from toad populations in French Guiana (FG) and three Australian states—Queensland (QLD), the Northern Territory (NT) and Western Australia (WA). State of origin can be used as a proxy of ‘years since invasion’ in this system (e.g. [30,31]).

We recognize that our laboratory trials used a different measure of tadpole development than those of DeVore *et al.* [19]. There is debate as to whether our measure (Gosner stage) and theirs (mass) are sufficiently correlated to function as the same predictor or if the two constitute independent potential drivers of variation. Because neither our experiment nor that of DeVore *et al.* [19] recorded both the Gosner stage and mass of putative cannibals, we could not resolve this debate. Thus, we opted to treat Gosner stage and mass as separate predictors and interpret them as such.

## 2.7. Statistical analyses

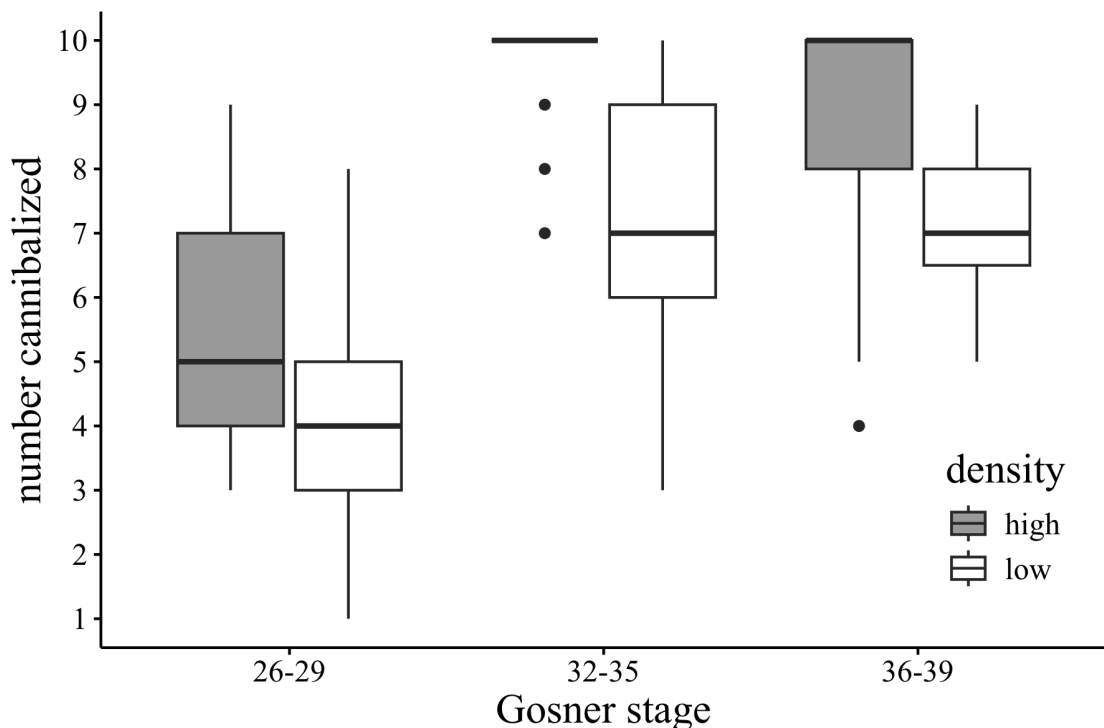
We conducted all analyses in R version 4.4.3 [32]. Data and R scripts—including all relevant packages and functions—are available in the electronic supplementary material.

### 2.7.1. Analyses of novel experimental data

To investigate the interactive effects of cannibal tadpole clutch, cannibal rearing density, and developmental stages of the cannibal and cannibalized cohorts on intensity of cannibalism (defined here as the number of conspecifics cannibalized before reaching the free-swimming tadpole stage), we used a Poisson-distributed generalized linear model. We excluded late-stage cannibals in which at least one forelimb emerged during trials (i.e. those that reached Gosner stage 42) from this analysis because larval mouthparts atrophy and feeding ceases at this stage [27]. We then conducted a time-series analysis of the interactive effects of cannibal tadpole clutch, cannibal rearing density, cannibal developmental stage and egg development on rates of cannibalism throughout the trial period using a repeated measures negative binomial generalized linear mixed model. Replicate ID was included as a random effect in the model to account for repeated measures. We excluded late-stage tadpoles that developed at least one forelimb during trials from this analysis after the timepoint of first limb emergence.

### 2.7.2. Analyses of published experimental data

Using data from DeVore *et al.* [19], we assessed the interactive effects of invasion history and tadpole mass on intensity of cannibalism (defined here as the number of pre-feeding larvae consumed by a cannibal in 24 h) with a negative binomial generalized linear mixed model (GLMM) and a zero-inflated Poisson (ZIP) mixed model. We used the negative binomial GLMM to compare intensity of cannibalism among tadpoles from different invasive populations (QLD, NT and WA) and the ZIP mixed model to compare native French Guianese (FG) tadpoles with invasive Australian tadpoles because cannibalism intensity often equalled 0 in experimental replicates from FG. We included tadpole clutch of origin as a random effect in both models because the developmental stage at which each potential cannibal was exposed to pre-feeding larvae was not recorded nor standardized among trials. Thus, parentage effects and their interpretation were confounded with developmental effects. We included only tadpoles with masses  $\leq 150$  mg in both models to control for differences in the number of large tadpoles tested among populations.



**Figure 2.** Total number of cane toad eggs and pre-feeding larvae cannibalized by conspecific tadpoles at early, intermediate, and late developmental stages (i.e. Gosner stages 26—29, 32—35 and 36—39, respectively) raised at high versus low densities (i.e. 5 tadpoles per litre and 1 tadpole per litre, respectively). We exposed individual tadpoles to 10 conspecific eggs and concluded trials when the younger cohort reached the free-swimming tadpole stage.

### 2.7.3. Assessment of predictor significance

For all models, we assessed predictor significance with Wald chi-squared tests. Where applicable, we followed these tests with post-hoc Tukey tests to assess the locations of significant differences within categorical predictors.

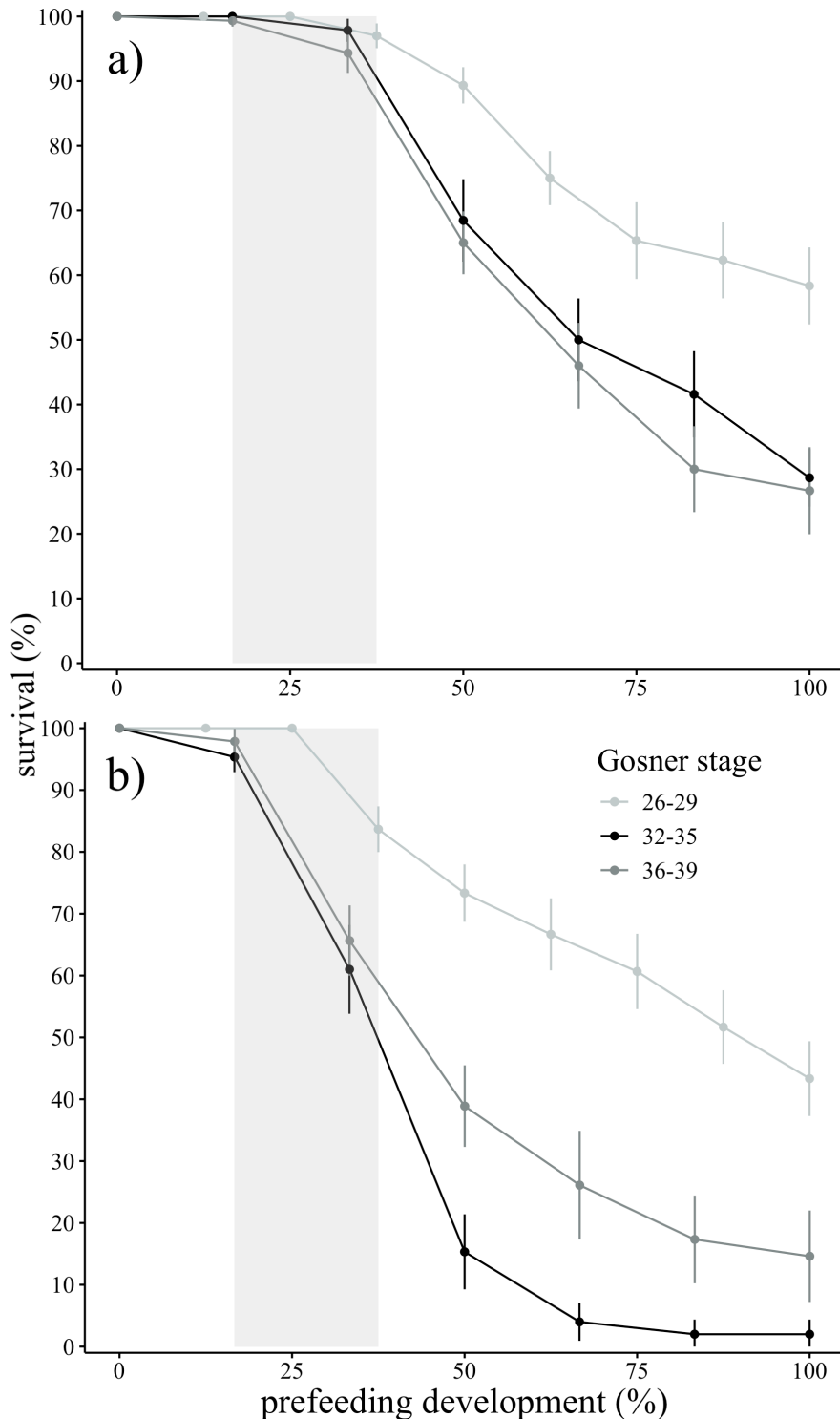
## 3. Results

### 3.1. Tadpole clutch of origin, rearing density and ontogeny

Tadpoles reared at higher densities cannibalized more eggs and pre-feeding larvae ( $\mu_{\text{high}} = 7.88 \pm 0.55$ ,  $\mu_{\text{low}} = 6.03 \pm 0.51$ ;  $\chi^2 = 18.69$ ,  $p < 0.001$ ; **figure 2**) and cannibalism intensity was higher in tadpoles at later developmental stages ( $\Delta\mu_{\text{mid-early}} = 3.65$ ,  $z = 7.60$ ,  $p < 0.001$ ;  $\Delta\mu_{\text{late-early}} = 2.87$ ,  $z = 5.15$ ,  $p < 0.001$ ; **figure 2**). Mean intensity of cannibalism did not differ between late and mid-stage tadpoles ( $\Delta\mu = -0.78$ ,  $z = -1.183$ ,  $p = 0.461$ ; **figure 2**) and was not significantly associated with tadpole clutch ( $\chi^2 = 1.333$ ,  $p = 0.514$ ). The average number of eggs and pre-feeding larvae consumed ranged from 4.2 (42% of those available) in early-stage tadpoles reared at low densities to 9.8 (98%) in mid-stage tadpoles reared at high densities. Among control treatments, the mean number of eggs that survived to the free-swimming tadpole stage ranged from 6.1 (61%) to 9.9 (99%).

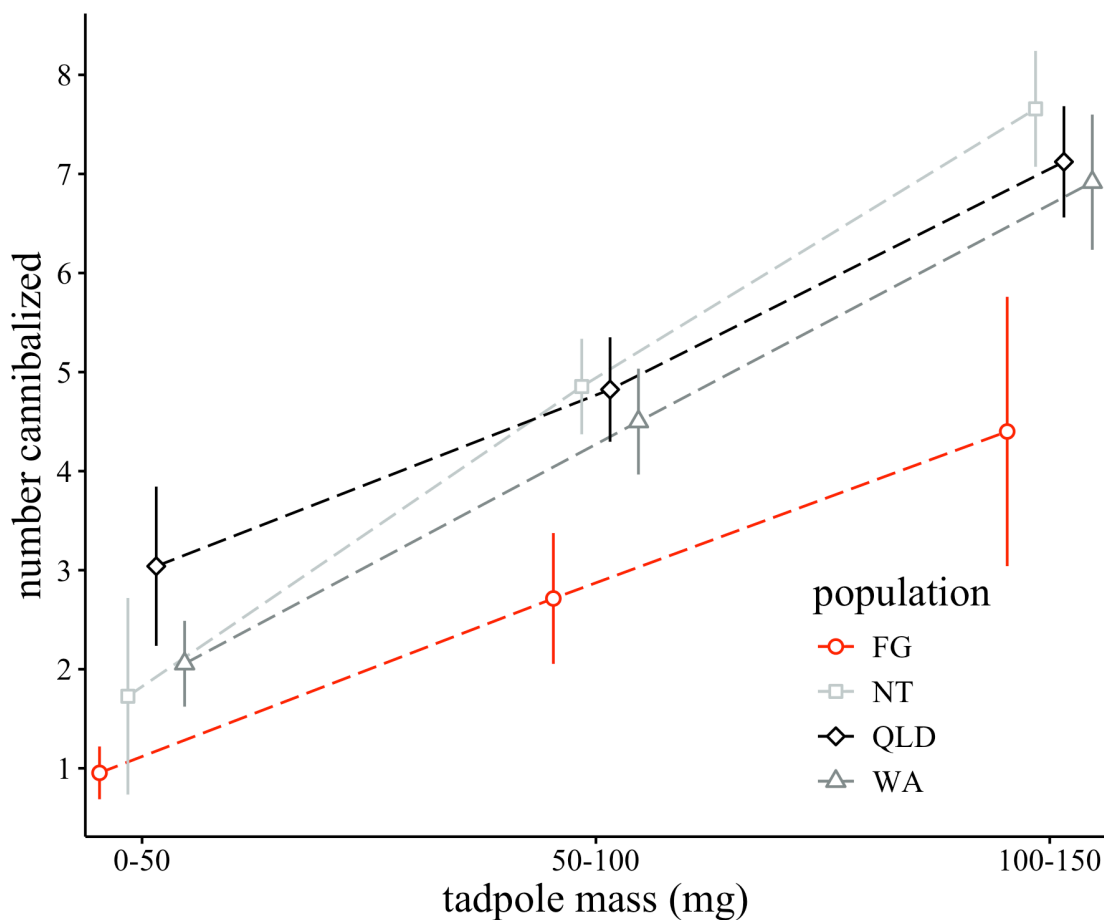
### 3.2. Developmental stages of eggs and pre-feeding larvae

Patterns of cannibalism over time were affected by interactions between egg developmental stage and tadpole-rearing density ( $\chi^2 = 49.15$ ,  $p < 0.001$ ), egg developmental stage and tadpole developmental stage ( $\chi^2 = 37.29$ ,  $p < 0.001$ ), and tadpole-rearing density and clutch ( $\chi^2 = 6.12$ ,  $p = 0.047$ ). Nonetheless, some general patterns in rates of cannibalism were similar among all trials. Few tadpoles (9.4%) broke the jelly coating to consume early developmental-stage eggs, and cannibalism intensified as the jelly coat around late-stage eggs dissolved and those eggs developed into pre-feeding larvae (**figure 3**).



**Figure 3.** Average percentage survival of eggs and pre-feeding larvae of cane toads exposed to early, intermediate and late developmental-stage cannibalistic tadpoles (i.e. Gosner stages 26–29, 32–35 and 36–39, respectively) raised at (a) low *versus* (b) high densities (i.e. 1 tadpole per litre and 5 tadpoles per litre, respectively). Points are derived from observations at 12 h intervals throughout egg and pre-feeding larval development and represent the average percentage of eggs and larvae not consumed by cannibalistic tadpoles. Pre-feeding development (%) refers to the development of the cannibalized cohort from early egg (0%) to free-swimming tadpole (100%). The shaded portion of the graph encompasses the transition period during which eggs (Gosner stages 0–16) developed into pre-feeding larvae (Gosner stages 17–25). We exposed individual tadpoles to 10 conspecific eggs and concluded trials when the younger cohort reached the free-swimming tadpole stage.

Rates of consumption then remained high until either most eggs and pre-feeding larvae had been consumed, or larvae reached the free-swimming tadpole stage at which they were no longer vulnerable



**Figure 4.** Interactive effects of the mass and population of origin of cane toad tadpoles on the number of pre-feeding cane toad larvae cannibalized. Points represent the average number of pre-feeding larvae consumed by tadpoles from different populations. Point shape and line colour denote population of origin. Tadpoles from both native (FG = French Guiana) and invasive populations (NT = Northern Territory, QLD = Queensland and WA = Western Australia) were tested. Data are from DeVore *et al.* [19], who exposed individual tadpoles to 10 pre-feeding larvae for 24 h.

to cannibalism (figure 3). Tadpoles reared at high densities began cannibalizing conspecifics earlier in egg development and did so more intensely than did those raised at low densities, and mid- to late-stage tadpoles began cannibalizing conspecifics earlier in egg development and did so more intensely than did early-stage tadpoles (figure 3). The impact of rearing density on rates of cannibalism through time differed among clutches, as evidenced by a significant interaction between tadpole rearing density and clutch.

### 3.3. Tadpole mass and invasion history

In invasive populations of cane toads, the number of pre-feeding larvae consumed by a tadpole increased with increasing tadpole mass ( $\chi^2 = 198.51$ ,  $p < 0.001$ ) and was not significantly affected by invasion history ( $\chi^2 = 0.53$ ,  $p = 0.766$ ) or the interaction between tadpole mass and invasion history ( $\chi^2 = 3.36$ ,  $p = 0.186$ ). When comparing native and invasive populations, intensity of cannibalism was affected by the interaction of status (i.e. native *versus* invasive) and mass ( $\chi^2 = 11.14$ ,  $p < 0.001$ ; figure 4). The number of pre-feeding larvae consumed increased with tadpole mass in both native and invasive populations ( $\chi^2 = 287.37$ ,  $p < 0.001$ ; figure 4), and mean intensity of cannibalism was lower in native tadpoles ( $\Delta\mu = 3.08$ ,  $\chi^2 = 20.33$ ,  $p < 0.001$ ). However, the number of pre-feeding larvae consumed increased more rapidly with increasing tadpole mass in invasive than in native populations (figure 4).

## 4. Discussion

Our experimental trials and analyses of published data demonstrated that the intensity and developmental timing of cannibalism by Australian toad larvae are influenced by multiple factors. In our experimental trials, tadpoles raised at high densities were more cannibalistic, and older, more developed tadpoles consumed more conspecifics than did young tadpoles. Additionally, tadpoles raised at high densities cannibalized recently laid as well as older eggs whereas their low-density counterparts did not begin consuming eggs until those eggs were close to hatching. Analyses of data from experimental trials conducted by DeVore *et al.* [19] revealed that the intensity of cannibalism by a tadpole is also influenced by its mass. These findings are consistent with known effects of density and resource conditions and size- and age-class distributions on intensity of cannibalism in larval amphibians [33,34]. Thus, cannibalism in invasive Australian cane toads, while more intense than in native-range toads, is a plastic behaviour. Having established this plasticity, we then used published data to assess whether one driver of variation in intensity of cannibalism—mass—is ancestral in larval cane toads.

Using data from DeVore *et al.* [19], we compared the effect of mass on cannibalism rate between cane toad tadpoles from native-range and Australian populations. Although Australian tadpoles were more cannibalistic than their native counterparts, rates of cannibalism increased with tadpole mass in both populations. Thus, an increase in rate of cannibalism with increasing tadpole mass is the ancestral condition in cane toads, but the slope of this mass–cannibalism relationship has shifted. At the same body mass, Australian toad larvae consumed more eggs and hatchlings than did the larvae of native-range cane toads, and this geographic disparity increased with tadpole mass. The mechanism underlying this shift may be the evolution of a behavioural response to bufadienolide toxins. Australian toad tadpoles are strongly attracted to the bufadienolide toxins excreted by conspecific eggs and pre-feeding larvae, and the presence of bufadienolides triggers intense feeding behaviour [21,22]. Thus, mass-specific rates of cannibalism in Australian toad tadpoles may have increased relative to their native-range counterparts because an Australian toad tadpole of the same mass as a tadpole from the species' native range can more easily locate eggs and pre-feeding larvae and recognize them as food and therefore may consume more vulnerable conspecifics.

Understanding the effects of these drivers of variation—and the ancestral state of responses to one such driver—modifies our understanding of the ecological drivers and evolution of targeted cannibalism and cannibal-induced defences in the larvae of Australian cane toads. Our work demonstrates that cannibalism by Australian toad larvae is not a highly canalized behaviour and—like cannibalism by larvae of other pond-breeding amphibians—instead reflects effects of both intrinsic (i.e. body size, developmental stage) and extrinsic (i.e. density, resource conditions) factors. The presence of an effect of body size in both native (ancestral) and Australian (derived) populations suggests that at least some of this variation is ancestral and that further studies comparing the other identified drivers of variation between native and invasive populations are needed to fully elucidate the evolutionary history of targeted cannibalism. These proximate drivers of variation also clarify the evolution of cannibal-induced defences in the eggs and larvae of Australian cane toads. The duration of the egg stage is reduced in Australian cane toad populations, and pre-feeding larvae accelerate development (at the cost of reduced subsequent growth rate) if they detect older conspecifics [19,20,35]. Both recent adaptations accelerate development, putatively reducing the duration of a developing egg and larva's vulnerability to predation. Our experimental data provide evidence for one critical assumption of that adaptationist hypothesis: high larval densities in Australia regularly elicit cannibalism of eggs that are early in embryonic development (a phenomenon reported as a rarity in DeVore *et al.* [19]), not simply eggs that have reached the pre-feeding stages. The consequent increase in vulnerability of early-stage eggs would thus be expected to also impose selection for more rapid development during the egg stage and not solely the especially vulnerable hatchling stage, as suggested by DeVore *et al.* [19,20].

Investigating drivers of variation in recently evolved traits—as opposed to identifying simple shifts in average phenotypes—allows for more nuanced interpretations of their ecological dynamics and evolutionary histories. This is especially true for behavioural traits, in which expression is typically highly plastic in response to the local environment and variable with respect to ontogeny [1,4]. After drivers of variation in a trait have been identified, their effects can be compared among ancestral and derived populations to aid in unravelling its evolutionary history. Invasive species are excellent models for these investigations, especially if we can unambiguously identify native-range source populations. Our investigation of variation in targeted cannibalism by invasive cane toad larvae and subsequent

interrogation of the ancestral state of one of these sources of variation illustrate the importance of considering proximate drivers of variation when studying rapidly evolved traits.

**Ethics.** All procedures were approved by the Macquarie University Animal Ethics Committee (AEC Project ID 13169).

**Data accessibility.** Data and all code for analyses are available in associated supplementary information files [36].

**Declaration of AI use.** We have not used AI-assisted technologies in creating this article.

**Authors' contributions.** A.T.F.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, writing—original draft, writing—review and editing; J.L.D.: investigation, methodology, resources, writing—review and editing; M.C.: investigation, methodology, resources, writing—review and editing; S.D.: investigation, methodology, resources, writing—review and editing; R.S.: conceptualization, funding acquisition, project administration, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

**Conflict of interest declaration.** We declare we have no competing interests.

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