

Annotated Bibliography

My articles are organized based on their respective topics, starting with a broad overview of the behaviour (amplexus) and its subtypes, then going into changes in the prevalence of these subtypes through evolutionary time. Subsequent articles focus on the adaptations and strategies cane toads have acquired that function to maximize their reproductive fitness, concluding with a study on a particular case of amplexus which was not adaptive.

Overview of Amplexus

Duellman, W. E., & Trueb, L. (1994). Courtship and mating. *Biology of amphibians* (pp. 68-72). John Hopkins University Press.

Summary: Amplexus is the mating embrace performed in many Anurans, in which a male tightly grasps the female with his forelimbs, bringing their vents (the orifice which is the sole exit passage for the urinary, excretory, and reproductive systems of the organism) close together, and externally fertilizes her eggs. Duellman et al. (1994) describe six mainly researched amplexus positions in various Anuran species based on the positioning of the male and female's vents relative to each other (inguinal, axillary, cephalic, straddle, glued, and independent amplexus). Methods that place the anuran's vents closer together are more efficient in ensuring that fertilization occurs, as is true for the axillary position. However, due variation in body sizes and sexual dimorphism in these species, some organisms must adopt alternative methods if axillary amplexus cannot be achieved, such as the glued position practiced by the small Andean toads of the genus *Osomophryne*. The chapter describes amplexus as occurring near the oviposition site, or the location where a female will release her eggs into the external environment. Pairs can go from two hours to remaining in the amplexed position for several days. In species that do not aggressively defend territory, energy is allocated towards tactile courtship (typically by the male), and often a visual display such as in *Dendrobate auratus*, when a male prods and jumps on the back of the female. If a female is unreceptive when clasped by a male, she may twitch her arms, quiver her body, or display other agitated behaviours to attempt to dislodge the male. The authors note here that these courtship observations in frogs and toads do not provide insight into their significance in mating; however, they predict that these signals may induce ovulation in the female or facilitate communication between the pair in ways that are not fully understood.

Contribution: This chapter provides useful insight into the different positions used during amplexus and goes into depth describing the courtship behaviours employed by males (and the responses to these advanced by females) during this complex process. I included this text in my literature review because the illustrations of each amplexus type in conjunction with their descriptions allowed me to better visualize how the male is able to position himself behind the female according to their comparative body sizes to maximize reproductive success.

Evolution of Amplexus Subtypes

Carvajal-Castro, J. D., López-Aguirre, Y., Ospina-L, A. M., Santos, J. C., Rojas, B., & Vargas-Salinas, F. (2020). Much more than a clasp: Evolutionary patterns of amplexus

diversity in anurans. *Biological Journal of the Linnean Society*, 129(3), 652-663.
<https://doi.org/10.1093/biolinnean/blaa009>

Summary: Carvajal-Castro et al. (2020) aimed to understand the evolution of the diverse reproductive modes of anurans, specifically the mating position known as amplexus in which the male uses his forelimbs to tightly grasp the female, and to identify periods when transitions in this behavioural trait occurred. To explore these objectives, primary literature was compiled from Google Scholar and the Web of Science discussing amplexus types, with “amplexus”, “nuptial clasp”, “anura”, and “frogs” included as keywords. Selected publications were narrowed down further with preference for detailed accounts of amplexus behaviours. Evolutionary trends were characterized with a comparative phylogenetic framework using genetic data from Jetz & Pyron (2018), and the analysis was run 1000 times; the analyses included 685 species from 45 anuran families. The results supported previous literature in that inguinal amplexus, in which the male dorsally grasps the female from her waist, is basal for all Anurans; axillary amplexus, in which the male grasps the female from her armpits, was found to currently be the most frequent amongst species. 34 distinct evolutionary transitions between types of amplexus were found in the phylogenetic analysis. It was also determined that sexual dimorphism in these species was unrelated to amplexus type. This study was significant as it suggested that many more reproductive modes exist than previously thought, and it attempted to understand and explain the patterns in amplexus type emergence over anuran evolution. Additional research and field observations are required to further investigate these mating behaviours and the benefits they confer upon select species.

Contribution: Compared to previous phylogenetic studies on amplexus diversity in anurans, this study greatly expanded the dataset of species to 685 and explored the connection of amplexus evolution to sexual size dimorphism. It also provided context for how this behaviour is optimized in species that have many successful fertilizations per breeding season, such as cane toads, which are the focus of my literature review. For instance, the evolution of the male’s strengthened grip to the female in axillary amplexus is discussed as being a result of other uncoupled males trying to remove the amplexant male from female to take his spot.

Amplexus Adaptations and Reproductive Strategies

Clark, D. L., & Peters, S. E. (2006). Isometric contractile properties of sexually dimorphic forelimb muscles in the marine toad *Bufo marinus* Linnaeus 1758: Functional analysis and implications for amplexus. *Journal of Experimental Biology*, 209(17), 3448-3456. <https://doi-org.ezproxy.lib.ucalgary.ca/10.1242/jeb.02396>

Summary: Male anurans use their forelimbs to tightly grasp a female and externally fertilize her eggs in the position known as amplexus; this has likely provided selection for larger forelimb muscles in males. Certain forelimb muscles in male bullfrogs (*Rana catesbeiana*) were shown to have different contractile properties (force, speed, fatigability) than females, which may have evolved to facilitate amplexus. Clark et al. (2006) extended this study to the species *Bufo marinus* (a distant relative of the bullfrog) to determine if these results are shared by other male anurans, or if different species achieve amplexus through varied muscle modifications. The isometric contractile properties of three muscles were studied: the abductor indicus longus (AIL)

and flexor carpi radialis (FCR) (sexually dimorphic muscles), and the extensor carpi ulnaris (ECU) (control muscle). The wrist and elbow mean joint angle changes for male and female toads were used to estimate their ranges of physiological muscle lengths. Experimental muscles were then excised from anesthetized toads and attached to an isometric strain gauge; electrodes delivered suprathreshold stimuli to the fibers, and the twitch and tetanic forces were recorded along with their half-relaxation times. In males, the AIL and FCR muscles had significantly greater masses and cross-sectional areas than in females. Though twitch force means did not differ between the sexes, male dimorphic muscles did produce significantly larger tetanic forces (sustained contractions) which was a contrary result to the bullfrog study. Male muscles were also less fatigable, correlated with longer relaxation times that maintained high levels of force between stimulus trains, which may allow males to maintain amplexus for prolonged periods. The similarity in muscular properties between bullfrogs and cane toads suggests their inheritance from a common ancestor or convergent evolution. Future research can investigate the mechanisms by which these dimorphic muscles become structurally differentiated in both sexes.

Contribution: This article highlights a particular adaptive trait in male cane toads that enhances his ability to maintain amplexus that may be shared by other Anurans. Like the previous work that demonstrated differences in forelimb muscle contractile properties in male and female bullfrogs, this aspect of size dimorphism was also shown to exist within cane toads. However, there were several features that were not observed in cane toads, such as the male bullfrog AIL producing significantly more force at lower frequencies of electrical stimulation. Clark et al. (2006) thus did not predict a physiological difference between male and female muscle tissue.

Bowcock, H., Brown, G. P., & Shine, R. (2013). Sexual selection in cane toads *Rhinella marina*: A male's body size affects his success and his tactics. *Current Zoology*, 59(6), 747-753. <https://doi.org/10.1093/czoolo/59.6.747>

Summary: A male anuran's body size can influence his sexual tactics and play a key role in the mating systems within its species. Bowcock et al. (2013) investigated the effects of a male's size on his reproductive behaviour and success (in particular, his mate choice) in an invasive population of cane toads (*Rhinella marina*) from the wet-dry tropics of northern Australia. Males belonging to three different size classes (with a 30g difference between each class) were injected with leuprorelin acetate (a synthetic hormone) three hours prior to the experiment to induce reproductive readiness, then housed communally with excess females in large plastic tubs. The results revealed that larger body size enhanced a male toad's ability to displace a smaller rival from amplexus (winning 10 of 12 encounters), likely owed to greater physical strength. Larger males were also more tenacious, as more force was required to dislodge them from females as compared to smaller males. Researchers also found that a male's size affected his mate-choice criteria; larger males tended to amplex larger females, though this size-selectivity would disappear when the solitary male encountered a target female that was already engaged in amplexus, as he was perhaps unable to evaluate her size in that situation. Though males were seen trying to amplex with pairs more often than with solitary toads, this bias was not proven statistically significant. Thus, a male cane toad's body size affects both his ability to defeat rivals in physical struggles over females, and the criteria he uses when selecting female targets to mate with, depending on the mating circumstance. As noted by the authors, sexual selection and

behaviour in cane toads remains poorly studied; future research can extend these laboratory studies into field observations and look for size-associative amplexus patterns in natural toad populations.

Contribution: The findings in this study supported previous literature in that there is a large-male advantage apparent in amplexus takeovers, and that males bufonids are unable to visually discriminate conspecific sex. Though the correlations between a male toad's body size and his ability to dislodge smaller males are straightforward, this paper was worth including in my literature review because the selection for size-associative mating is well explored; all males benefit from mating with larger females since they produce more eggs, however larger males will be ultimately successful in amplexing females and withstanding attacks from other males in this position until fertilization.

Clarke, G. S., Shine, R., & Phillips, B. L. (2019). Whispers on the wind: Male cane toads modify mate searching and amplexus tactics based on calls from other males. *Animal Behaviour*, 153, 131-136. <http://doi.org/10.1016/j.anbehav.2019.05.008>

Summary: Male anurans can produce advertisement calls that function to attract reproductively capable females from their species. As an outcome of intense intersexual selection, rival males can eavesdrop on these calls and change their own tactics to better compete for female mates. Clarke et al. (2019) investigated whether male cane toads (*Rhinella marina*) modify their mate-searching and amplexus behaviours in response to conspecific calls and movements. Sexually active toads were monitored in outdoor arenas while researchers played recordings of either male advertisement calls, the sound of a lawnmower (with similar acoustic structure to a toad chorus, though biologically irrelevant), or silence in three randomized sequences. The resulting movements and inclination of males to initiate amplexus were quantified. The results agreed with the author's predictions in that male toads were seven times more likely to engage in amplexus during a toad chorus than during the control sound or silence, which the authors hypothesize was due to males changing their focus from foraging and avoiding predation to mate searching after hearing calls from conspecifics. On average, males also moved twice as much during a toad chorus as compared to silence or the control. Visual cues were also shown to influence propensity to initiate amplexus, as males were 4.6 to 16 times more likely to amplex a moving target animal (whether male, female, or an amplexant pair) than a sedentary one. This study demonstrated that male toads dramatically alter their behaviour in response to acoustic and visual information from their conspecific rivals and suggests that they pay substantial attention to these elicited signals, while using them to optimize their own reproductive success. Future research on the responses of male cane toads to different environmental stimuli can be used to manage these invasive amphibians by luring them into traps or disrupting mating behaviours.

Contribution: In addition to the results on echoing in previous studies, this paper provides new observations on the near doubling of movement rate (hops/min) when a mating chorus was played. The male sexual preference for moving amplexed pairs of toads over individuals is consistent with previous literature and reflects a potential adaptive tendency for males to select larger visual targets. I included this article because it demonstrates how in cane toad populations

with male-biased operational sex ratios, males have evolved to maximize their own reproductive fitness by not only using cues elicited by reproductive females, but by male conspecifics as well.

Gray, H. M., & MacKenzie, T. R. (2016). Tactics used by cane toads, *Rhinella marina* (Linnaeus 1758) (Anura: Bufonidae), to disrupt amplexant pairs and to avoid persistent satellite males. *Herpetology Notes*, 9, 233-235.

Summary: Due to intense intrasexual competition in most anurans, it may function to maximize a male's reproductive fitness if he is able to dislodge another male from amplexus and fertilize the female's eggs himself; these peripheral individuals are known as satellite males. Here, Gray et al. (2016) present their observations of satellite males attempting to disrupt amplexant pairs in a natural population of cane toads (*Rhinella marina*) on Panama, and a discussion of these dislodging tactics. On July 28, 2015, six adult cane toads (2 amplexant pairs and 2 satellite males) were observed in a triangular-shaped pool which flowed down a rock face into a larger bottom pool. The satellite males attempted to grasp at the pairs and displace the amplexant males, and they responded with aggressive kicks and release calls. The females would also try to escape these attacks by trying to swim away from rival males with their partners on their backs. The first amplexant pair stayed in the upper pool, whereas the other pair translocated to the bottom pool and climbed the waterfall again several times without dismounting. During the 2 hours of observation, the satellite males were not successful in dislodging the amplexant males and the females did not deposit their eggs. Though the main competition tactic used by satellite males was forcing their bodies between the amplexant individuals, this behaviour may have been owed to confusion and the male being unable to distinguish if he was seeing an amplexant pair or a larger female (who produces more eggs). Overall, these observations suggest that mating behaviour in *R. marina* may employ visual tactics and additional factors than previous literature indicates. Though females tended to avoid laying their eggs in ponds with satellite males or invertebrate predators, further research is needed to understand female selection for breeding sites.

Contribution: This article demonstrates that mate choice and courtship involve other factors besides only animal size. The behaviour of the amplexant female who rolled down the waterfall and repeatedly returned to the upper pool was unexpected, as prior research suggests she should opt to conserve energy while swimming with the burden of the male on her back. Additionally, since males give a release call when mistakenly amplexed, visual recognition of conspecific sex in anurans is not entirely necessary; however, as satellite males were seen jumping onto amplexant pairs from a vantage point, visual cues may still be involved in locating mates.

Bowcock, H., Brown, G. P., & Shine, R. (2009). Beastly bondage: The costs of amplexus in cane toads (*Bufo marinus*). *Copeia*, 2009(1), 29-36. <https://doi.org/10.1643/CE-08-036>

Summary: Reproduction in organisms comes with energetic costs and risks which will differ substantially between males and females. Though the costs of other aspects of reproduction (ie. prolonged calling) have been examined in Anurans, the metabolic costs of amplexus (the posture in which a male seizes a female with his forelimbs and externally fertilizes her eggs) remain

unexplored. Bowcock et al. (2009) investigated the effects of amplexus on locomotor (sprint and swim) performance in female Cane toads (*Bufo marinus*), and if the degree of impairment depended on the male's body size. The feeding behaviour of amplexant females was also studied. An outdoor sprint track and circular pool were used to measure terrestrial and aquatic locomotor performance respectively. For the feeding experiment, the time taken for the toads in amplexus to eat crickets provided in a feeding arena was used to calculate feeding rate. The results supported the author's predictions in that both the sprinting and swimming performance of female toads was impaired due to amplexus. Amplexus decreased sprint speeds on land by about 25%, where increasing mass of the male corresponded to a decrease in the female's speed and an increase in her number of hops/meter. Swimming speeds were reduced by about 50% due to the physical burden of the male, though there were no significant differences due to the male's size, as much of his weight was supported by the water. Amplexant females consumed less crickets than did free females, which may have been due to the male's grip in front of her legs which prevented her from foraging. Overall, the results suggest that this mating position may generate sexual conflict in Cane toads and potentially other Anuran species. The authors suggest that future studies could investigate the fitness costs on amplexant females in the wild due to reduced mobility.

Contribution: This study gives insight into how daily activities of cane toads (ie. feeding) are affected due to the high energy requirements of engaging in amplexus. The fact that males will even go as far as to incur short-term anorexia to avoid losing a pairing with a female demonstrates how the cost of losing a mate greatly outweighs their temporary loss in body mass. I included this article in my literature review because it also touches upon the differences in energetic costs between the sexes in species with size dimorphism, which exists in my study species (*Bufo marinus*).

Bowcock, H., Brown, G. P., & Shine, R. (2008). Sexual communication in cane toads, *Chaunus marinus*: What cues influence the duration of amplexus? *Animal Behaviour*, 74(4), 1571-1579. <https://doi.org/10.1016/j.anbehav.2007.10.011>

Summary: Complex communication across a range of sensory modalities allows reproducing animals to evaluate potential partners in ways that often differ between the sexes. Previous literature has examined female anurans' responses to advertisement calls by males, with less of a focus on the release signal produced by males (and non-reproducing females) to terminate amplexus when they have been mistakenly clasped. In *Chanus marinus*, both sexes will experience muscular flank vibrations of the body wall as part of this signal, whereas only males will give an auditory call. Bowcock et al. (2008) investigated this signal to determine if females call in a manner like males but with an inaudible frequency, and what stimuli induce males to terminate amplexus. Toad vocalizations were recorded in an anechoic chamber (a room soundproofed from external noise). The role of acoustic cues in the initiation of male dismount was studied by suppressing the vocalizations of the clasped animal with an externally fastened device that pushed against his vocal sac. Analysis of calls over a wide (4e100 000 Hz) frequency range revealed that the male's auditory call consists of a series of 'chirps' and that female toads are indeed mute. Males were less likely to dismount when a vocal toad was prevented from making a release call; however, when an artificial release call was played for a typically mute

toad, dismount did not occur. Male dismount appeared to involve synchrony between the release call and vibration of the clasped animal. This mechanism was predicted to increase the amplexant male's mating success, as hearing a release call from a nearby male-male pair could cause him to prematurely terminate his clasp with a reproductive female. Future behavioural comparisons between reproductive and non-reproductive females of vocal and mute species could test between models of sexual conflict and female choice in anurans.

Contribution: I included this article because it provides context for how cane toads can maximize their breeding success in their short mating periods of a few hours per year, while minimizing the chance that they will release their sperm onto a non-reproductive female or another male. The results confirmed that females are genuinely mute, refuting the previous idea that their auditory calls are simply inaudible by human ears. Unlike previous hypotheses that predicted that the release call alone is sufficient for amplexus termination, the authors propose that a combination of cues (auditory and mechanical stimuli) is needed for male dismount.

Najar, T., & Ferrante, L. (2020). Unusual necrophilic amplexus in *Rhinella marina* (Linnaeus, 1758). *Herpetology Notes*, 13, 1025-1026.

Summary: While some anurans show longer periods of mating and calling activity (prolonged breeders), others concentrate their reproductive activities during short time windows in the year (explosive breeders), with unusual mating situations occasionally seen in these species. In this study, Najar et al. (2020) present their observations of a case of necrophiliac amplexus involving two males and a dead female cane toad (*Rhinella marina*) on December 17th 2017. At the edge of an artificial reservoir, the males were engaged in a dispute over access to the female's body and soon began initiating amplexus with the female corpse. The specific cause of death and how long the female was diseased for were both unknown. As noted by the authors, this observation alone does not suggest whether this behaviour had come about in cane toads as an adaptive trait due to increased fitness benefits, or if it was in fact a rogue behaviour. However, there were no eggs ejected by the female's corpse either prior to her death or afterwards, meaning that the males did not achieve reproductive success through this amplexus, providing evidence for the latter prediction. The researchers hypothesized that the cause for this atypical amplexus may have been the excretion of hormones into the water by the female, which could have continued after death and attracted the males. To bolster this hypothesis, future studies would have to evaluate if female anurans that mate in aquatic environments expel pheromones to attract males.

Contribution: I included this article because the account of necrophiliac amplexus detailed by the authors is the first seen in *Rhinella marina*, suggesting that this phenomenon may be shared between other close relatives of cane toads. Unlike the functional reproductive strategy seen in the Amazonian toad *Rhinella proboscidea*, in which males promoted the release of eggs from dead females and fertilized them, male cane toads experienced no such benefits. The findings of this article advance knowledge in this field by suggesting that necrophiliac amplexus may not be entirely adaptive and could potentially be stimulated through female pheromones.