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1. CANE TOAD CHALLENGE (CTC)

1.1. *What is the Cane Toad Challenge (CTC)?* The CTC is a University of Queensland (Institute for Molecular Bioscience) community engagement and citizen science initiative, designed to fast track public access to innovative cane toad tadpole trapping technology co-invented by researchers from The University of Queensland and The University of Sydney.

1.2. *How is the CTC funded?* The CTC is actively seeking philanthropic and corporate sponsorship, research grant funding, and public donations.

1.3. *How can I join as a CTC Member?* Individuals over 18 years old may register online as CTC Members, free of charge. CTC Members will receive periodic email CTC Updates.

1.4. *How can I join as a CTC Affiliate?* CTC Affiliate membership is by invitation, and is open to a wide range of organizations. These include volunteer and not-for-profit groups, societies, philanthropic organizations and commercial businesses, as well local, regional, state and federal government agencies. Interested organisation should contact the CTC. CTC Affiliates co-sign a memorandum of understanding (MoU) with UQ, valid for 3 years, with the following benefits;

- Recognition as a supporter of the CTC community engagement and citizen science initiative.
- Recognition for advancing the protection of the Australian environment from the cane toad.
- Fast-track pathway for contributing to, and participating in CTC activities.
- Priority access to CTC technical advice and BufoTabs (subject to availability and approvals).
- Option to leverage membership in support of environment related grant applications.
- Option to leverage membership in online, on-air and in print media environment profiles.

2. CANE TOAD TADPOLE TRAPPING

2.1 How does tadpole trapping work? This technology uses natural cane toad attractant chemistry in conjunction with traps, to achieve the capture and removal of cane toad tadpoles from managed waterways (e.g. dams, ponds, streams, creeks). Tadpole trapping is an environmentally sustainable and intuitive technology, readily transferable to the public. Coordinated implementation of tadpole trapping has the potential to reduce cane toad tadpole (and adult?) populations, and alleviate the environmental impact of this toxic invasive pest.

2.2 What is the ecology behind tadpole trapping? Field observations revealed that tadpoles actively search out and consume the eggs of other female cane toads within the same body of water. Possible explanations for this behaviour include (i) providing tadpoles with a valuable source of nutrition, (ii) allowing tadpoles to recharge their toxin reserves, to help defend against potential predators, and (iii) to eliminate genetic competition. As this behaviour is successful even in low to zero visibility, it was speculated that cane toad eggs release a chemical attractant that reveals their location to cane toad tadpoles. This attractant is at the core of tadpole trapping.

2.3 What is the chemistry behind tadpole trapping? Chemical analysis identified the attractant released by eggs as very closely related to adult cane toad toxin. Female toads deposit a modified version of adult toxin to protect eggs from potential predators, unaware that this chemistry exposes the eggs to cannibalism by tadpoles. Presumably the threat posed by non-cane toad related predators exceeds that posed by cane toad tadpoles, ensuring that co-depositing of egg toxin with eggs delivers a net survival advantage. As far as we know, no other Australian aquatic species produce, or are attracted to cane toad toxin, ensuring species-specificity.

2.4 How does the CTC harvest tadpole attractant? As it is impractical to source attractant chemistry from egg masses, we harvest related chemistry from dead adult cane toads. We work with approved CTC Affiliates to redirect and recycle dead toads acquired during toad busting activities, from landfill to our laboratory. Shipments of dead adult cane toads (or excised parotoid glands) must be pre-approved and accompanied by a "Cane Toad Shipment Form". To recover attractant involves excising the parotoid glands, followed by batch-wise blending and enzymatic transformation, extraction, fractionation, purification and chemical analysis. Importantly, this process removes non-attractant chemicals, as well as all traces of animal tissue, to deliver a product that can be formulated as tadpole attractant baits.

2.5 How does the CTC excise parotoid glands from dead toads? Dead frozen adult toads and are thawed and a sharp knife is used to cut off the parotoid glands, positioned on each front shoulder. The attached picture (Figure 1) shows the location of parotoid glands on the toad, plus dorsal and ventral views of an excised gland, and an image of a compressed gland illustrating toxin secretion. The ventral view reveals yellow vesicles inside the gland that hold the toxin. Each vesicle has an attached duct leading to the surface of the toad skin and when compressed either during a predatory attack or when handled roughly, toxin is forced out of the ducts. When thawing frozen toads the glands can leak toxin (freezing puts pressure on vesicles), and although the majority of toxin remains within the gland/vesicles, to avoid losing toxin it is important to not wash the excised glands. Thawed toads are slippery, and the toxin is quite poisonous, so it is important to use waterproof gloves under solid work gloves, and a sharp knife. When excising glands we are careful to cut deep enough to get all the vesicles intact, and to avoid skin contact. To prevent eye splashes we always wear safety glasses. Excised glands are bagged and frozen indefinitely, and can be defrosted in batches when needed to be processed into baits.

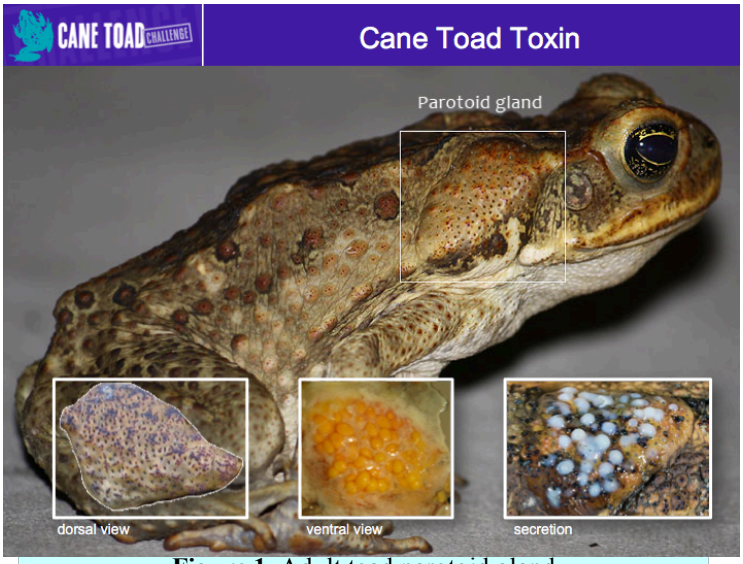

Figure 1. Adult toad parotoid gland

2.6 How does the CTC ship excised parotoid glands? Where it is necessary to transport excised parotoid glands this can either be done by hand delivery, or by post, ideally with the glands frozen. The effort and cost of mailing a frozen shipment is more or less the same regardless of the number of glands, as most of the weight is in the packaging. The strategy we use is as follows;

- Excised glands are frozen as a single block.
- Frozen glands are sealed in at least two layers of watertight ziplock bags (removing all air).
- Bagged, frozen glands are packed in a small "hard" esky with several frozen freezer packs.
- The esky lid is securely tapped shut to avoid any leakage.
- The esky is packaged in an addressed cardboard shipping box, with absorbent padding.
- The cardboard box containing the esky is shipped by overnight "cold" courier.

2.7 How does the CTC formulate tadpole attractant? To deliver attractant in a form that can be used to catch cane toad tadpoles requires a cheap, safe, environmentally sustainable, slow release formulation. This is currently achieved by absorbing a calibrated quantity of attractant on commercially available aquarium air-stones, along with green food dye. The dried, green, attractant loaded air-stones are distributed as BufoTabs.

2.8 Why does the CTC add green food dye to BufoTabs? When added to a trap the green food dye rapidly washes off, making it easier to recognize used (spent) from unused (unspent) BufoTabs.

2.9 How can I get some BufoTabs? The patent for BufoTabs has been licensed, and efforts are underway to register, manufacture and distribute these as a commercial product. As this may take 2-4 years, the CTC was established as an interim measure. The CTC is currently not in a position to release BufoTabs direct to CTC Members, however, we are working closely with CTC Affiliates to achieve a managed distribution. If you belong to or know of an organisation who may be interested in becoming a CTC Affiliate, please encourage them to visit the website and submit an expression of interest.

2.10 Are BufoTabs safe for the user? Like any pest control product the answer is a qualified yes. As the

attractant chemistry is comprised of a natural defensive toxin, BufoTabs should only be used by adults over 18 years, and users should avoid skin contact or ingestion. All end-users should read and heed advice in documentation supplied with BufoTabs, which includes a UQ Material Shipping form, and Material Safety Data Sheet (MSDS).

2.11 Are BufoTabs safe for the environment? Yes. BufoTabs seek to mimic the chemical plume released by cane toad eggs. At a quantitative level, each adult toad produces sufficient attractant to prepare 5-20 BufoTabs. Put another way, a single dead toad would release 5-20 times more toxin into the environment than a single BufoTab. It's also worth noting a single BufoTab can catch as many as 1,000 to 10,000 toxic tadpoles, with a net reduction of environmental toxin load.

2.12 Is there a risk of by-catch? All experience to date is that by-catch (ie fish) is not an issue. This is in part due to the species selective nature of the pheromone attractant, further aided by small funnel entry (1 cm). It is also important to note that tadpole traps do not kill tadpoles, so in the unlikely event that by-catch is encountered there is ample opportunity to make note of and release the species involved.

2.13 Are there limitations on the use of BufoTabs? BufoTabs must be used as supplied, for the purpose of cane toad tadpole trapping, and not be modified, repurposed, sold, or distributed outside CTC Affiliate organisations. When using BufoTabs in a particular location please ensure you have authorisation, and follow all directives from landowners and/or authorities.

2.14 Do BufoTabs have a long shelf life? BufoTabs can be stored indefinitely, if kept dry and in a relatively cool (<30°C) location, away from direct sunlight. It is strongly recommended that BufoTabs be stored in the original packaging.

2.15 How long do BufoTabs work in a tadpole trap? When a BufoTab is added to a trap, the attractant is slowly released to generate an attractant plume that extends through the funnels, outside and away from the trap, attracting cane toad tadpoles back to the source (inside the trap). If the trap, BufoTab and attractant plume are left undisturbed (i.e. the trap is not removed from the water), a BufoTab should generate a sustained attractant plume for at least 24 h, depending on environmental conditions. If during this period (i.e. 2 h) the trap is successful in attracting tadpoles and is removed from the water, and drained to filter off the tadpole catch, this disturbs and negates any established plume, severely reducing the effective duration of the "partially" used BufoTab. To accommodate these issues we recommend the following sequence of events;

- Position the trap and add a new BufoTab.
- Monitor the trap over the next 2-24 h.
- Remove the trap from the water at any time (2-24 h) where there is a good catch of tadpoles.
- Drain, harvest and dispose of the tadpoles and the used BufoTab.
- If the tadpole numbers in the water body warrant, repeat (i) to (iv) in the same location.

Alternatively, if your trap design allows for it, you can avoid disturbing the attractant plume by leaving the trap (and associated attractant plume) in place and carefully netting the tadpoles directly from the trap with a small dab net. This way the trap can be left to run for the full 24 h with periodic tadpole harvests by netting. If you do use this approach be very careful to avoid accidentally killing (crushing) any tadpoles in the trap. Dead tadpoles release an alarm chemical that could negate the attractant.

2.16 How do I build a tadpole trap? Tadpole traps (funnel traps) are typically constructed by attaching plastic funnels to opposite ends of a plastic box, with the small diameter end projecting into the box. There a different methods for achieving this, with two illustrated in Figure 2. It is important that the join between funnel and box be water tight, and that the box

Figure 2. Tadpole traps

has no other holes or gaps that will allow leakage of attractant. When a bait is added to a trap the scent trail extends out through the funnels. Tadpoles sense the trail and move up the concentration gradient towards the trap. When encountering the trap the tadpoles are "funnelled" into the trap. Once in the trap the exit is small, and in the absence of a reason to exit (ie no reverse scent trail), tadpoles stay in the trap.

2.17 When should I use a tadpole trap? The best time to use tadpole traps is during periods of significant cane toad breeding, which are typically weather dependent. Traps work best when deployed in areas with high tadpole density.

2.18 Where and how should I use a tadpole trap? The range of the scent trail released by baited traps is finite, but does extend further from the trap with time. It is important to avoid competing scent trails, that could occur if multiple traps are placed in close proximity. It is also helpful to scan the area immediately around traps, and remove any dead adult toads from the water. Dead adults can release a competing scent trail. It is also important to avoid placing traps in flowing water (ie running streams) or in water bodies experiencing an inflow of water (ie rain runoff), as disturbed water will disturb the scent trail. Traps are best placed in still, shallow near shore water, sitting on the bottom, with the top 20% above water, and funnels fully submerged. Traps should be covered with a lid and shade cloth, to avoid overheating. It is important that tadpoles be captured and removed from the trap alive, as dead tadpoles release an alarm chemical that will over-ride the attractant signal.

2.19 I've caught tadpoles, so what should I do with them? Cane toad tadpoles should be harvested live from traps by either filtering through a cloth or sieve, or by a small hand held net. Once harvested, live cane toad tadpoles should be transferred with a minimum volume of water to a plastic bag or another suitable sealed container. In an adaptation of the cool-freeze method approved for euthanizing adult toads, the sealed bag/container of cane toad tadpoles should be cooled (e.g. on ice) until tadpoles are non-motile, after which it can be transferred to a freezer and frozen solid. Be aware that dead cane toad tadpoles are toxic and if left in the environment may be scavenged and poison native predators. Be sure to bag and bin dead cane toad tadpoles along with used BufoTabs.

2.20 What do I do with a used BufoTab? Bag and bin along with the dead tadpoles.

2.21 Do frog tadpoles get attracted to traps? All evidence to date suggests that BufoTabs only attract and trap cane toad tadpoles. This does not mean that other species cannot randomly wander into the trap, but there is currently no evidence of significant accidental trapping of frog tadpoles. Nevertheless, it is always good practice to monitor traps for unwanted by-catch, and in the unlikely event that by-catch is encountered take note and release (alive).

2.22 Will tadpole trapping deliver an environmental benefit? This is a good question, and the jury is still out. The answer will likely only become evident after we roll out the technology for a sustained period of time, and assess impact. That said, we are confident tadpole trapping can remove very large numbers of toxic tadpoles from the environment, and this will undoubtedly reduce toxin load. In some ecosystems the removal of competing cane toad tadpoles will also release habitat and resources, to the benefit of other aquatic species. We are hopeful that the large-scale removal of tadpoles, coordinated with hand capture and culling of adult toads, will significantly reduce local cane toad populations. This will most likely be evident in and around managed water bodies such as farm dams and public water features (i.e. streams, lakes, ponds, golf courses, wet lands etc…).

2.23 Will tadpole trapping eradicate cane toads in Australia? Cane toad tadpole trapping is a valuable tool, but it alone will not eradicate cane toads from Australia. It can be used to remove tadpoles, and hopefully reduce juvenile and adult cane toad populations, in and around accessible water features – delivering localized environmental returns. As local as these returns may be, they are still wins in the war against the toad. By its very nature, tadpole trapping is labour intensive (albeit less so than adult capture) and cannot be comprehensively rolled out over a continent as vast as Australia. It has a role to play, but we need to continue the research, to find additional approaches to achieve a nation wide solution to the Cane Toad Challenge.

3. ADULT CANE TOADS

3.1 What is the most humane way to kill adult toads? The safest and most humane way for the public to kill adult toads is the cool/freeze method. This method involves bagging toads and cooling them in a fridge until comatose (i.e. not moving), followed by storing in a freezer until frozen solid. For further information on this method download and read "Is cooling then freezing" a humane way to kill amphibians and reptiles?"

3.2 Are there methods for killing adult toads that are not recommended? Any method that is inhumane in not acceptable. This includes golf clubs, mallets and other bashing implements. We also caution against the use of chemicals (i.e. Dettol, Hopstop, salt, bleach…) as these are indiscriminate and will kill species other than cane toads. Equally problematic, if dispersed in the environment some chemicals can seriously degrade the viability of aquatic ecosystems.

3.3 Does the CTC need dead toads? Yes. The CTC extracts cane toad tadpole attractant from dead adult cane toads. To do this we need a supply of dead toads.

3.4 Where does the CTC source its dead toads? We can only receive toads that have been collected and killed humanely by approved CTC Affiliates, as part of ongoing cane toad control activities - allowing us to divert dead toads from landfill, to produce attractant. CTC Affiliates that would like to be approved to supply dead toads should contact the CTC to confirm that their protocols are humane, and to receive advice on processing and shipping. The CTC cannot accept dead toads from members of the public, or from unapproved CTC Affiliates.