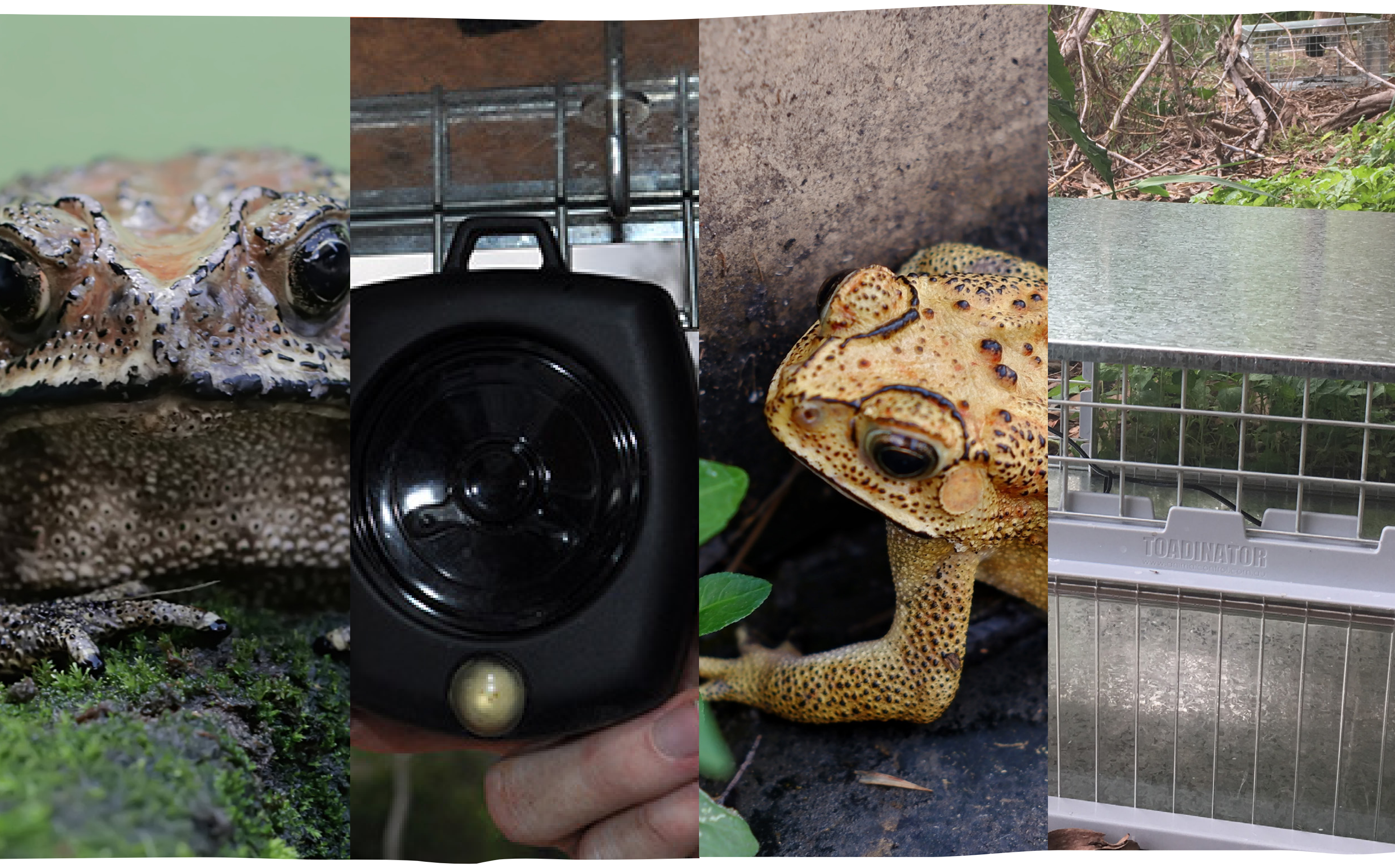
CENTRE FOR
INVASIVE SPECIES SOLUTIONS

NATIONAL PREPAREDNESS PLAN: ASIAN BLACK-SPINED TOAD

(Duttaphrynus melanostictus)

August, 2024

Version 1.0



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This National Preparedness Plan: Asian Black-spined Toad was endorsed by the Environment and Invasives Committee at Meeting 14 held on 30 August 2024.

COVER IMAGES
An Asian black-spined toad is looking for prey on a moss-covered rock. Source: Shutterstock

Attracta sound lure used in the Toadinator device. Source: ACTA

Asian black-spined toad. Source: Shutterstock

The Toadinator Cage Trap. Source: Steve Csurhes, QDAF

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Introduction

Asian black-spined toad (ABST: *Duttaphrynus melanostictus*) are known to successfully establish in new areas and have incurred into Australia on multiple occasions. This plan provides basic information and procedures that can be used to prevent, plan and respond to ABST incursions in Australia. It can be used as a reference resource for the preparation of regional/area specific emergency response plans should a toad incursion occur. A specific Response Plan template is provided at Appendix A.

Incursion Reporting

Incursion response protocols for animals (including ABST) are governed by national and State-Territory procedures, systems, plans and frameworks. Responsibilities, notifications and response procedures are detailed elsewhere and summarised in Table 1. Note that the level of expertise, capacity, training, capability and infrastructure varies between States/Territories.

Table 1. Incursion response agreements, protocols procedures, systems, plans and frameworks.

Name	Purpose
National Environmental Biosecurity Response Agreement (NEBRA 2.0)	To establish national arrangements for responses to nationally significant biosecurity incidents where there are predominately public benefits [Jan 2022]. National Environmental Biosecurity Response Agreement 2.0 - DAFF (agriculture.gov.au)
Biosecurity Incident Management System	Provides guidance in practices for the management of biosecurity incident response and

Name	Purpose
	initial recovery operations in Australia. Can be used for: • planning for response to biosecurity incidents • conducting staff training and development activities • designing and conducting exercises that focus on elements of a biosecurity response • responding to biosecurity incidents • planning and conducting evaluation of any of the above activities. **Biosecurity Emergency Management - Biosecurity Incident Management System - DAFF (agriculture.gov.au)**
National Surveillance and Diagnostics Framework	Provides an integrated approach to the funding and management surveillance and diagnostic activities to: • ensure they are supported by risk-based decision making • help prioritise the allocation of government resources and investment to areas of greatest return • maximise the use of existing capability and infrastructure. National Surveillance and Diagnostics Framework - DAFF (agriculture.gov.au)
State-Territory Response Plans	State-Territory specific plans to guide responses to high-risk invasive animal incursions.

NEBRA Reporting Requirements

In addition to State-Territory-specific reporting requirements, it is necessary to report under the National Environmental Biosecurity Response Agreement (NEBRA). Once a potential nationally significant pest or disease incursion is detected or suspected in a jurisdiction, that party must undertake the following:

- Determine whether the incursion can be managed through pre-existing cost-sharing arrangements and notify the NEBRA reporting point[1] of the incident.
    - Formal Notification of a confirmed or suspected pest must be provided to the Australian Chief Environmental Biosecurity Officer (ACEBO) – the primary NEBRA reporting point - within 24 hours.
    - Within State or Territory borders, the State or Territory officials with overall responsibility for biosecurity are responsible for notifying the NEBRA reporting point.
- Notifications for ABST should be submitted to:

    The **Australian Chief Environmental Biosecurity Officer:**

    Email: ACEBO@aff.gov.au

    Phone: (02) 6272 4623

    CC to:

    - The **Australian Chief Veterinary Officer** ACVO@aff.gov.au
- Once the reporting point is notified, an appropriate National Biosecurity Management Consultative Committee (NBMCC) may be established for the particular animal pest outbreak. The consultative committee is made up of the most appropriate experts from each jurisdiction and the Commonwealth to evaluate the incursion. An existing consultative committee such as the Consultative Committee on Environmental Biosecurity

Incidents (CCEBI) can be used for this purpose or an ad hoc NBMCC can be established.

- The NBMCC is Australia's key technical body for coordinating national responses for plant pests and diseases, weeds, ants and other terrestrial invertebrates, under the NEBRA and NEBRA-like (off-deed) arrangements. Its role is to coordinate the national technical response to nationally significant pests, including assessing the technical and cost benefit of eradication, and to make recommendations to the National Management Group (NMG) for decision. The NBMCC comprises representatives from the Australian Government, State and Territory governments and where applicable, affected industries. The NBMCC is chaired by the Australian Chief Environmental Biosecurity Officer.
- Under the NEBRA, the NBMCC is the technical committee, chaired by ACEBO. This includes undertaking assessments of the national significance of the incident, the technical feasibility and cost-benefit of eradication, and where appropriate, the proposed Preparedness Plan and making recommendations to NMG on these matters.
- The CCEBI is a proposed national consultative committee with responsibility for providing advice on the coordination of responses to environment and social amenity pest incursions under the NEBRA and NEBRA-like (off-deed) arrangements. It is proposed that the CCEBI will deal with environmental and social amenity pests that sit outside the scope of currently established committees, such as exotic reptiles, amphibians and some mammals. It is envisaged that the CCEBI will comprise of representatives from the Australian Government, State and Territory governments and scientific experts from museums and universities. Due to the diverse and

sometimes unusual nature of these types of incidents, the CCEBI may also draw on other expertise as required.

- The NMG is the decision-making body for national exotic pest and disease eradication programmes under NEBRA. The NMG is chaired by the Secretary of the Department of Agriculture, Fisheries and Forestry, but may be chaired by other Departments if required. The membership comprises senior officials as all governments and industry parties cost sharing the national response to an exotic pest or disease incursion. The NMG's role is to confirm that the incursion is nationally significant and make decisions on the technical feasibility and cost-benefit for eradicating an exotic pest or disease in accordance with a national Preparedness Plan and agreed cost shared budget.
- If the incursion does not meet the NEBRA criteria for a cost shared response, any ongoing action will fall back to the affected jurisdiction and/or affected stakeholders.
- The NMG also makes decisions on changes to the Preparedness Plan (recognising that it is a living document that may have incremental versions), expenditure limits, financial and efficiency audits of the response and cessation of the response.

Figure 1. *Duttaphrynus melanostictus.* Photo: Ian Jacobs (CC BY-NC 2.0)

General Biology

Species Classification

Duttaphrynus melanostictus (Schneider 1799)

Class:	Amphibia
Order:	Anura
Family:	Bufonidae
Genus:	Duttaphrynus
Species:	melanostictus

Common Names

Asian black-spined toad, Asian common toad, Asian spined toad, Southeast Asian toad, spectacled toad, common Indian toad, black-spined toad, common Sunda toad, black-lipped toad, keeled-nosed toad, South Asian garden toad, black-spectacled toad, house toad, Asiatic toad, Asian eyebrow-ridge toad, Asian black-spotted toad, and Javanese toad.

Identification

Asian black-spined toad (ABST*)* is a stocky, medium to large size toad, with a relatively small head and short hind limbs (Mattison 1987). It has elevated bony ridges on its head, with long dark crests that border the eyelids and down the side of the eyes to the prominent parotoid glands (Csurhes 2010). Colouration is highly variable, usually grey to red-brown, but ranging from brick-red to almost black. Colour pattern is often yellow-brown marked with dark or reddish-brown streaks and spots, and the back is covered with various sized warts which are capped with tiny dark spines (Bartlett *et al.* 2001). Small warts are also found on the soles and toes. The underside is largely whitish with fine black spots. Juveniles have a blackish band between the chin and chest, and lack warts and the conspicuous eardrum. Tadpoles are black and small, up to 15 mm long (Bartlett *et al.* 2001, Daniels 2005).

As with true toads, ABST have oval or elliptical parotid glands behind their eyes that secrete a milky, pungent substance (Bufotoxin). This powerful toxin is known to deter or kill predators.

Males have a subgular vocal sac, and when breeding, the vocal sac in the throat region becomes bright yellow/orange (Bartlett *et al.* 2001). Nuptial pads (pigmented, cornified swelling used to assist grip during copulation), develop on the inner side of the first and second fingers.

Snout-vent length of males is between 57−83 mm, and females between 65−85 mm, although females can often exceed 150 mm (Bartlett *et al.* 2001, Jørgensen *et al.* 1986).

ABST morphology is highly variable, especially the juveniles. As a result, it is often confused with co-existing species throughout its range (Daniels 2005). In Australia, it may be misidentified as a cane toad (*Rhinella marina*) and some native species.

Figure 2. *Duttaphrynus melanostictus.* Photo: Brian Gratwicke (CC BY 2.0)

Global Distribution

ABST is one of the most widespread and abundant species in its genus (Csurhes 2010: Figure 3). Its native distribution extends from north Pakistan through Nepal, Bangladesh, India, Sri Lanka, southern China (including Taiwan, Hong Kong and Macau), Myanmar, Lao People's Democratic Republic, Vietnam, Thailand and Cambodia to Malaysia, Singapore, and Indonesia (Sumatra, Java, Borneo, Anambas Islands and Natuna Islands) (Van Dijk *et al.* 2004).

The toad is spread and naturalised in parts of Indonesia including Bali, Sulawesi, Ambon, Manokwari, Maluku, the Guinean areas of West Papua and Papua New Guinea, and the Indian Ocean's Andaman and Nicobar Islands (Church 1960, Khan 2016, Lever 2003, Van Dijk *et al.* 2004, Whitten *et al.* 1996). It was first recoded in Madagascar in 2014 (Kolby *et al.* 2014) and has since become widespread. Further, ABST was recently discovered in East Timor (Khan 2016).

ABST has not yet established in Australia.

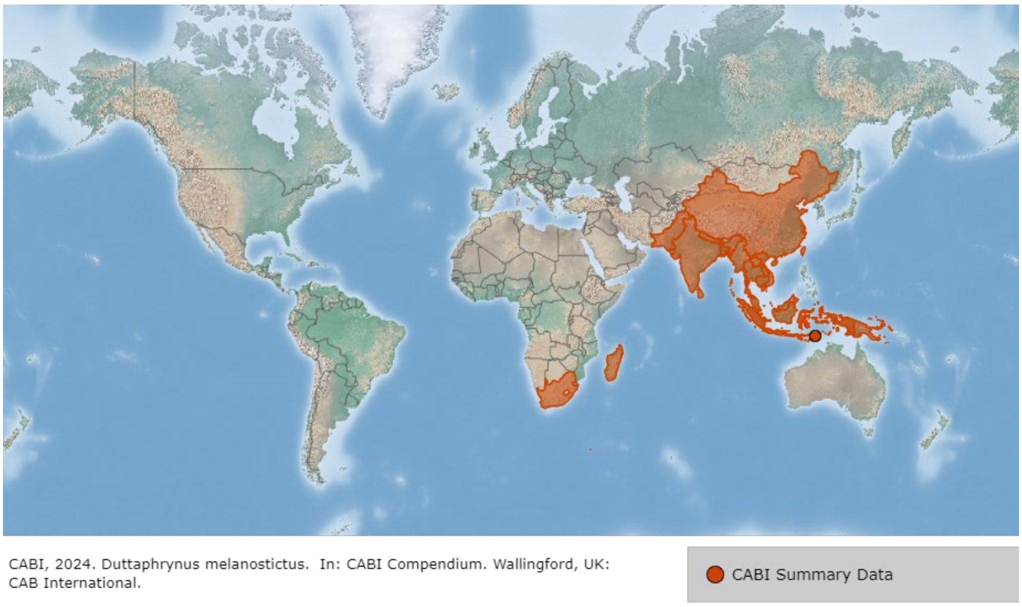

Figure 3. Map showing known distribution of *Duttaphrynus melanostictus*. Image taken from CABI Invasive Species Compendium 2024. © CABI. Map used with permission.

Behaviours and Traits

Food and Foraging

ABST has a generalist diet and will take most food items that it can fit in its mouth, typically between 5–20 mm in size (Berry and Bullock 1962). It hunts exclusively on the ground, often consuming a wide variety of invertebrates, especially ants and termites. Additional prey includes earwigs, grasshoppers, bugs, beetles, flying insects (e.g., cockroaches), spiders, and molluscs. Even invertebrates with noxious protective mechanisms such as scorpions, centipedes and millipedes are frequently taken (Berry and Bullock 1962). ABST are also known to feed on the eggs, larva and juveniles of conspecifics and other native amphibians (Gleb 2013, Mahapatra *et al.* 2017).

The tadpoles primarily eat phytoplankton (Sinha *et al.* 2001). Young toads actively feed during the night and regularly the day, most often on soft-bodied ants (Daniels 2005, Sinha *et al.* 2001).

Predation on ABST is generally low and varies depending on geography. Most common predators are snakes and birds (Gleb 2013). The lack of predators is likely due to ABST toxicity (Keomany *et al.* 2007).

Reproduction and Lifecycle

ABST breed once or twice a year (Figure 4). Breeding starts at the beginning of the wet season, but where seasons are less pronounced, breeding occurs year-round (Jørgensen *et al.* 1986). Males congregate at breeding sites including still and slow-flowing rivers ponds, puddles, and gutters where they call to females (Daniels 2005, Saidapur and Girish 2001).

Females are highly fecund and their ovaries can produce up to 40,000 eggs per clutch; these eggs can occupy a third of their body weight

(Murray *et al.* 2010, Whitten *et al.* 1996). The female lays black eggs in long strings which are enclosed in a double gelatinous capsule (Figure 5). Eggs are laid in a double jelly string around submerged vegetation (Khan 2016). The eggs develop into larvae (Figure 6) in 24−48 hours depending on water temperature (Bartlett *et al.* 2001, Jørgensen *et al.* 1986).

Figure 4. *Duttaphrynus melanostictus* in amplexus. Photo: Devesh Pandey (CC BY-SA 4.0).

Depending on habitat and food availability, hatched tadpoles reach metamorphosis within 34 to 90 days, followed by 25-30 days as metamorphs (Bartlett *et al.* 2001, Jørgensen *et al.* 1986, Saidapur and Girish 2001). ABST tadpoles emerge at different times and sizes; timing is influenced by pond size, density and presence of predators. For example, body mass of tadpoles will decrease by up to 46% and metamorphosis will occur earlier in the presence of predators.

The call of the ABST is similar to that of a telephone dial tone (creo-o-o; croro-ro-ro). The call is sustained for about 30 seconds and repeated in a chorus (example of call ABST Call). It will be heard at night and sometimes on overcast or rainy days

Figure 5. *Duttaphrynus melanostictus* spawn. Photo: Susmita Mahapatra (Mahapatra *et al*. 2017)

Figure 6. *Duttaphrynus melanostictus* tadpoles. Photos: Frogs of Borneo (CC BY-NC 3.0) and Mark O'Shea (O'Shea *et al.* 2012)

Longevity

Although longevity in the wild is unknown, ABST are known to live between 4 and 10+ years in captivity (Bartlett *et al.* 2001, Khan 2016, Mogali *et al.* 2011).

Habitat

ABST is a nocturnal, terrestrial habitat generalist, found across temperate, subtropical and tropical habitats, from sea level to 2 km elevation (Khan 2016). The species is generally detected in disturbed, lowland habitats such as secondary forests, forest margins, riparian areas and human-dominated agricultural and urban areas, while uncommon in closed forest (Rahman *et al.* 2016, Van Dijk *et al.* 2004). During the day, adults shelter under rocks, leaf-litter, logs and man-made structures such as drains, rubbish piles and houses (Figure 7). In populated areas, they often gather to feed around streetlamps at night. ABST can breed close to the ocean with tadpoles tolerating brackish water up to 1% salinity (Daniels 2005, Strahan 1957).

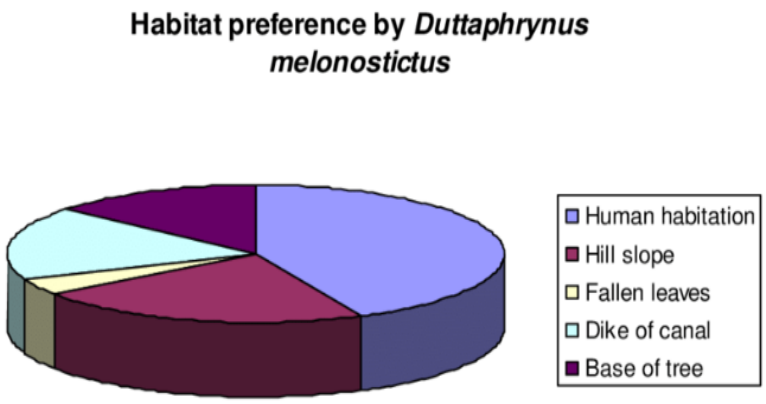

Figure 7. Research suggests that ABST prefer habitat associated with human habitation (e.g., Rahman *et al.* 2016)

Incursion Pathways

Although ABST have not yet established in Australia, future incursions are highly likely, based on the number of detections to date (Figure 8). Multiple ABST have been intercepted at Australian airports and seaports, aboard aircraft, sea vessels, and in cargo primarily originating from Asia. In addition to cargo holds, cabins and ship decks, the toads are most often detected in shipping containers, machinery and personal effects such as luggage, shoes, boxes and cartons (Table 2). A small number have been detected 'at large'; all have been single individuals and where the path of entry can be determined, shipping containers and personal luggage have been the primary incursion point.

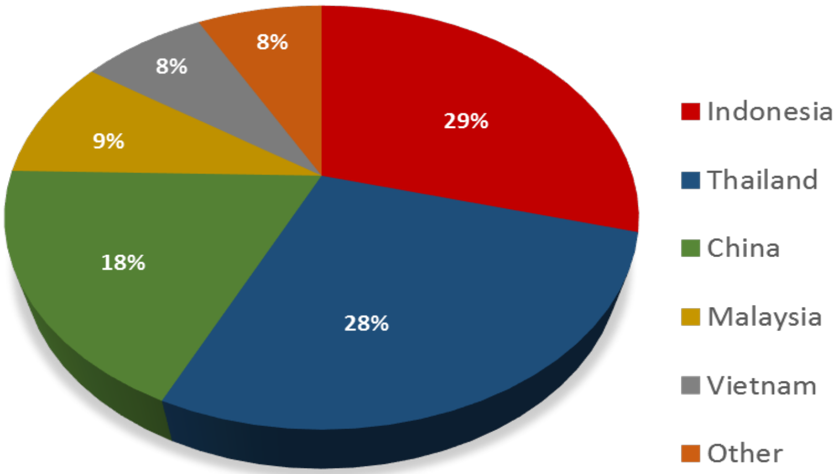

Figure 8. Incursions into Australia of ABST have originated from 10 areas; Indonesia and Thailand account for almost 60% of reported detections. 'China' includes Hong Kong and Taiwan. 'Other' includes single incidents from Christmas Island, Fiji, India, Singapore and the USA. Data supplied by Department of Agriculture, Fisheries and Forestry. *n* = 66 incidents.

Garcia-Diaz and Cassey (2014) and Henderson et al (2011) reported that 82 ABST were transported as stowaways between 1988-2012, and all were discovered in harbours and airports by Customs and Biosecurity agencies. Between 2010 and October 2019, 66 incursions from known origins were reported (Department of Agriculture, 2019). Most incidents were single individuals; one included 39 toads – 37 of which were immature. Indonesia and Thailand accounted for almost 30% each of known origin incursions (Table 2).

Table 2. Number of reported ABST incursions into Australia where country of origin is known between 2010 and 2019, and associated commodities. 'China' includes Hong Kong and Taiwan. 'Other' includes single incidents from Christmas Island, Fiji, India, Singapore and the USA. Data provided by Department of Agriculture, Fisheries and Forestry. *n* = 66 incidents.

	No Incidences			Commodities
Country of Origin	**Air**	**Sea**	**Unknown**	
Indonesia (n = 19)	5	12	2	Air: personal effects (5) Sea: container (2), container cargo (5), vessel holds/decks (5) Unknown: (2)
Thailand (n = 18)	11	4	3	Air: personal effects (10), cargo hold (1) Sea: container (1), container cargo (3) Unknown: (3)
China (n = 12)	2	10	0	Air: personal effects (1), cargo (1), Sea: container cargo (10)
Malaysia (n = 6)	2	3	1	Air: personal effects (1), cabin (1) Sea: container cargo (3) Unknown: (1)
Vietnam (n = 5)	0	3	2	Air: (0) Sea: container cargo (3) Unknown: (2)
Other* (n = 5)	1	3	1	Air: (1) Sea: container cargo (3) Unknown: (1)

Other includes Christmas Island, Fiji, India, Singapore, and USA

In terms of pathways for movement within Australia, hitch-hiking in personal effects, cargo and on transportation vehicles is the most likely path. Once established, ABST usually move within a home range size of no more than 5 km (reported in García-Díaz 2014). Brown *et al.* 2006 and Phillips *et al.* 2006 found that distances moved by cane toads at the invasion front were significantly higher than in other areas occupied. It is likely that the same pattern of movement could occur with ABST.

Depending on the frequency, routine inspections should continue to be effective at detecting new incursions. If individuals are detected during border inspections, then ABST may be prevented from establishing in Australia. However, as with cane toads, if ABST pass through the border and becomes widely established, they will be difficult, if not impossible, to eradicate.

Figure 9. *Duttaphrynus melanostictus* showing distinct parotid glands. Photo: Bernard Dupont (CC BY-SA 2.0)

Eradication Potential

Based on this information coloration, size and movement patterns, ABST are likely to have low detectability at low densities e.g., (Phillips *et al.* 2006). If established, control methods developed for cane toads may be useful (Crossland *et al.* 2012, Kelehear *et al.* 2012, Pizzatto and Shine 2012a, Pizzatto and Shine 2012b, Saunders *et al.* 2010, Shine and Doody 2011, Ward-Fear *et al.* 2010). Note: no method or combination of methods and techniques have been used to successfully eradicate cane toads in Australia and this will likely be the same for ABST if the species becomes widespread.

Impacts

ABST is likely to survive throughout much of Australia, thriving primarily in coastal and northern areas.

The toad shares similar attributes, such as size, reproductive output, diet, and habitat preferences, to cane toads which have become well established in much of Australia (Van Dijk *et al.* 2004). It is reasonable to predict that if ABST become established in Australia, its impact would be comparable to that of the cane toad.

Economic

No economic impacts have been reported in ABST introduced range, although tadpoles of the family are known to clog nets, irrigation and outlet pipes and may negatively impact the aquaculture industry (Measey *et al.* 2016). Dense populations of tadpoles in commercial ponds can also consume fish and feed intended for fish (Corse and Metter 1980, Kane *et al.* 1992), and impede post-harvest sorting and grading (Roy *et al.* 2015)

ABST are used as a food source in some parts of its native range (Van Dijk *et al.* 2004), however, it is unlikely to be used as a food source in

Australia. The consumption of skin and eggs, or use of parotid gland secretions for the psychedelic properties, can cause serious illness or even death (Das *et al.* 2000, Keomany *et al.* 2007). This could have a small impact to the health care sector.

Environmental

Introduced populations of ABST are known to reproduce rapidly (Gleb 2013) and this rapid increase can result in competition and displacement of native species (Church 1960, Csurhes 2010). These toads are also known to eat the eggs, larvae and juveniles of other native amphibians, causing further environmental impacts (Gleb 2013).

Although no quantitative assessment of impacts has been carried out, the ecological impact of ABST in East Timor is considered serious (Csurhes 2010). In Indonesia, the species is known to displace the small, less active crested toad (*Ingerophrynus biporcatus*) (Iskandar 2004). (Church 1960) reported that "nothing can hinder its expansion" in Indonesia.

ABST, as do other species of the family Bufonidae, carry the chytrid fungus (*Batrachochytrium dendrobatidis*). This fungal disease is responsible for the decline and extinction of amphibians worldwide (Kilpatrick *et al.* 2010, Skerratt *et al.* 2007) including significant negative impacts on native Australian frogs e.g., (Berger *et al.* 1998, Fisher *et al.* 2009, Kilpatrick *et al.* 2010, Murray *et al.* 2010, Skerratt *et al.* 2007, Woodhams *et al.* 2006). Consequently, chytrid has been listed in Australia's National List of Reportable Diseases of Aquatic Animals (National List of Reportable Diseases) and as a key threatening process under the *Environment and Biodiversity Conservation Act 1999*. Although chytrid is established in Australia, incursions of ABST could further increase the spread and risk of chytrid infections in Australian frogs and potentially introduce strains of the pathogen not yet found in Australia. A national threat abatement plan, which identifies the research, management and other actions needed in Australia to respond to chytrid has been in place since 2006.

As with other Bufonidae, ABST produce cutaneous toxic substances that could cause harm to naive native species (Vitt and Caldwell 2013) and possibly domestic pets. However, harmful effects of poisoning of predators has not yet been reported in its introduced range (Khan 2016).

ABST can carry a number of parasites including microfilarial worms, nematodes (primarily *Oxysomatium* sp. and *Rhabdias* sp.) and trematodes (*Mesocoelium burti*) that could impact native species (Rahman *et al.* 2008) as well as pathogens such as Ranavirus, and chytrid fungus e.g., (Jancovich *et al.* 2010, Vredenburg *et al.* 2010).

Social

No social impacts have been reported in the introduced range.

ABST can pose a direct threat to human safety and there are reports that eating the skin and eggs can cause fatality (Keomany *et al.* 2007). Adult toads secrete a milky toxin with a pungent odour that contains several bioactive compounds with lethal, hypotensive, hypertensive, neurotoxic, cardiotoxic, haemolytic and sleep-inducing factors (Das *et al.* 2000, Keomany *et al.* 2007). When handled by people, the toad can cause itching in the nostrils, eyes and exposed skin (Daniels 2005). As discussed above, the consumption of skin and eggs, or use as a psychedelic drug can cause serious illness or death.

Tools and Techniques

When dealing with an ABST response, follow relevant State-Territory response planning documents and augment with the information provided here.

Design and Data Recording

Prior to the deployment of field teams, a surveillance plan should be designed and implemented. This includes:

- *Preparation*: Establish the plan of action, coordinate relevant networks, acquire/repair/develop required tools and techniques, undertake essential training, and assess and assemble necessary resources for detection and delineation actions.
- *Detection Surveillance and Delimitation*: Undertake surveys and monitoring activities to provide initial evidence on the occurrence of an ABST. A delimiting survey needs to be undertaken and then any immediate low-cost steps which have a good prospect of eliminating the incursion or controlling ABST spread must be implemented. Also provide mechanisms for reporting and verifying species identification.
- *Rapid Assessment*: Determine the distribution and abundance of the ABST and evaluate its potential risks with regard to environmental, health, and economic impacts. Identify options for rapid response based on the particular circumstances associated with the occurrence of the species. These include species type, specific location, extent of spread, and relevant authorities.
- *Rapid Response*: Establish a set of coordinated actions to eradicate the founding ABST population before it establishes and/or spreads to the extent that eradication is no longer feasible.

At a minimum, the following logs, plans and reports must be completed.

Response Activity Log - Data recording starts with a Response Activity Log (Appendix B). The log details all the activities of the response including correct identification of the organism and notification of appropriate persons/stakeholders.

Situation Report - Following the activity log, determination of the nature and scope of the incursion and any interim action to be taken is assessed and described. This starts with the completion of a situation report (Appendix C). The situation report will assist in deciding on management options, and guides the development of the Emergency Response Plan.

Incursion After Action Review – This review finalises data recording and indicates future actions, if any are required (Appendix D).

Detection and Delineation

Tools for detection and delineation of new incursions are the foundation of incursion response. Below are brief reviews of each available or potential tool that could be used for an ABST response. The list is a guide only and each State-Territory is responsible for choosing tool/s that work within their capacity and capability.

Visual Searches

Visual searches remain the primary detection method used for many amphibian species (Heyer *et al.* 2014). This method is particularly useful at habitat interfaces such as streams, rivers, roads, fences, along the base of buildings and other barriers. It is also useful to use in conjunction with other methods, or when techniques, such as trapping and toxicants, have not been developed or are ineffective. Visual searches can occur either at night, during the day or in combination. Nocturnal searches are most useful during the breeding season when

ABST are calling. Visual surveys should also include searching for larvae (tadpoles) and egg masses. Note: ABST morphology is highly variable, especially the juveniles. As a result, it may be misidentified as a cane toad (*Rhinella marina*) and some native species.

Daytime Visual Searches

Description – Can be used to delineate or control a new incursion. Visual surveillance involves trained personnel conducting directed searches for adults, metamorphs, eggs, tadpoles, or other indirect signs. Terrestrial line transects, particularly in riparian zones around waterbodies, tracks and roadsides are particularly useful. However, since the species can tolerate relatively dry conditions, they can be found away from waterbodies. In these instances, plot searches (at least 20 × 20 m) can also be used, particularly for delineation or population density estimates.

Aquatic searches should be conducted from the banks of the waterbody or from small rafts or inflatable boats. Wading is not recommended unless movement is limited as it can disturb sediment and the larvae, making them difficult to detect. Searches can be achieved unaided (naked eye); binoculars or spotting scopes are recommended for larger waterbodies as they will increase identification accuracy.

- Searches are usually conducted by foot. However, boats or terrestrial vehicles may be used to maximise the size of the searchable area.
- Focus where possible in moist areas, around waterbodies, in burrows, holes, under vegetation, base of trees, root balls of toppled trees and other refugia.
- Lift logs, rocks, debris in proximity to potential habitat to find toads. Not useful in areas of dense understorey.
- If plots are to be used, searches should be conducted in about 2-3 hrs depending on density between 0900 and 1500 hr.

- If transects are to be searched, a walk speed of >4km/hr is recommended.
- Searches are best around rain events. However, rainfall can impact visibility of spawn and tadpoles so these surveys should be conducted outside rain events.
- Often the eggs of ABST are conspicuous even when adults cannot be found. Also indicates breeding and breeding season in the absence of calling.

Timing – Daily searches for several weeks at a time is preferable (at least five survey events), although the exact number of searches will depend on season, weather, abundance, terrain and habitat. Search intensity and duration is also dependent weather/climate, the ability for ABST to move or relocate to other areas, particularly around or during rain events.

Required – Visual searching is the most widely used tool for locating many amphibians, however it can be labour intensive and therefore costly. Regardless, it is effective for detection and capturing all life stages and both sexes if the right search intensity and duration is used (Christy *et al.* 2010). Training in techniques, identification and detectability of ABST and native species are a critical component of search success (Henke 1998).

Risks – Weather, obstructing vegetation and access are three primary challenges of ABST visual surveys. Other risks include misidentification, disturbance, searcher fatigue, and health and safety of the searcher. The cryptic nature of this species can make them difficult to detect with active searches. Egg and larvae searches may have a narrow temporal window for detection. Be prepared to search over extended periods of time.

Current Feasibility of Use – Low to High depending on environment.

Nocturnal Visual Searches

Description - Can be used to delineate or control a new incursion.

Nocturnal visual searches are similar to daytime visual searches (see above) and are aided by spotlights or headlamps. Because of Australia's hot, dry climate, nocturnal searches are particularly useful during the breeding season or rain events when toads are likely on the move. Spotlighting involves shining a flashlight at an animal so the light reflects off the animal's retina, creating a noticeable eye shine (Corben and Fellers 2001). Eyeshine can be used to detect toads in water and reeds, as well as those on land.

- Optimum search conditions are in temperatures over 22°C and 80% humidity.
- At night, use transects or focus on waters' edges, riparian zones where possible.
- Larvae searches are best conducted at night when they are out in the open.
- Not recommended for spawn and larvae searches during rain.
- Recommend at least five consecutive nights of spot-lighting during breeding season (in conjunction with the use of auditory monitoring and light traps) will give a 90% confidence of absence (Parris 2009) at any one site.

Timing - See **Daytime Visual Searches** above. Temperature dependent, particularly in non-tropical areas where yearly temperature and humidity varies greatly.

Resources Required – See **Daytime Visual Searches** above.

Risks – See **Daytime Visual Searches** above.

Current Feasibility of Use – Low to High depending on environment.

Dip Netting

Description – Dip netting is the most commonly used method for sampling and collecting frog larvae. The method uses a dip net to sweep through aquatic microhabitats in areas where tadpoles are potentially active or visible. It is particularly useful when sampling while the observer remains on land, allowing minimal disturbance of the water (Shaffer *et al.* 1994).

There are two basic sampling techniques for dip netting:

- Small areas - the net is moved through the water column in a series of short sweeps with constant speed (recommended speed of 1m/sec).
- Large areas - net may be dragged along predetermined transects for a period of one to two minutes.
    - Most important to ensure constant speed of movement.

Where key identification is difficult, consideration should be given to keeping and raising larvae to metamorphosis to confirm identification.

Timing - Larvae are generally more active at night and thus more likely to be found in the water column rather than sheltering in reeds, substrate or logs. Minimum of five nights under ideal conditions. Not recommended during rain as visibility may be compromised.

Resources required – 30 cm diameter net with fine mesh and a long handle (Figure 10). Field personnel will require a larvae key, 10× or greater hand-lens or binocular microscope. A single observer should be sufficient.

Risks – Larvae detection may be difficult in waterbodies with substantial reed, logs, rocks or other substrate debris. The method does not discriminate between ABST and native larvae (Shaffer *et al.* 1994).

Identification of larvae can be difficult and may require assistance from experienced frog experts or use of larvae keys e.g., (Anstis 2007).

Current Feasibility of Use – High

Figure 10. Example of a dip net. Dip netting in wetlands can be an effective detection tool for ABST. Photo: Amy Hawkins (CC0 1.0)

Barriers

Description – Although barriers are often used as asset protection against already established invasive species, they can also be useful to prevent or contain a new incursion. Permanent (e.g., masonry walls) or temporary (e.g., silt) barriers can effectively block dispersal into or away from specific areas (e.g., endangered species breeding sites, cargo containment, etc.).

- Temporary barriers such as drift fences can be used to intercept moving ABST. Individuals can be captured along the fences and directed into traps (see **Trapping section** below).
    - These barriers work well during delineation phases of incursion response, or to protect a short-term event such as potential migration season, military and other

    government training exercises, and storm/fire recovery phases (Figure 11).
  - They are also useful to surround known breeding ponds to prevent emigration of breeding adults and metamorphs (Figure 12).
- Because the toads are primarily terrestrial and tend to travel along a barrier rather than climb over, a temporary barrier does not need to be high; in most instances, fences >200 mm will suffice, as long as the fencing material is pulled taunt.
  - Silt fencing, shade cloth or plastic supported by wooden sticks, posts, rebar and/or PVC pipe can work. The bottom is either buried in the ground or weighted with rocks, metal or sandbags.
  - Adding a barrier to the base of an existing permeable fence (e.g., wire or wood) is also effective.

Timing – Depends on terrain, weather, application and length of the barrier. Set up may take less than an hour to several days. Recommend checking at least once per day or as necessary. Frequent checking decreases adverse risk to non-targets.

Resources Required – Temporary barriers can be inexpensive to deploy and maintain, especially if a barrier cloth or plastic can be set up along an existing fence or structure. Silt fencing cost around $40 per 50 m. Materials will need to be stored when not in use. Construction materials are relatively inexpensive and barriers can be repositioned easily. The use of an impermeable walls where possible will also minimise required resources.

Risks – Temporary barriers may be easily breached if not set up correctly or adequately maintained. Wind, run-off and siltation could comprise the barrier. Non-targets may be diverted or trapped.

Current Feasibility of Use – Medium to High depending on area being barricaded.

Figure 11. Barrier or drift fence for amphibians. Can be used with or without pit traps Photo: Tony Clevenger (Clevenger and Huijser 2011).

Figure 12. Drift fences are effective for preventing egress and ingress to breeding areas. Photo: Rebecca Means (Means *et al.* 2013).

Trapping

Pitfall and Traps

Description – Pitfall traps are open containers buried in the ground such that the tops of the containers are level with the ground. They can be set out in a linear, grid, or multiple armed arrays (Figure 13a). They are most effective when used in conjunction with drift fencing (see Barriers). The technique has been shown to be effective when compared to other capture techniques (Case and Fisher 2001, Fisher *et al.* 2008). PVC pipe and other various containers such as buckets and coffee cans, can be used as pitfall traps for ABST (Heyer *et al.* 2014).

- Buckets or large containers like coffee cans are recommended; optimum trap type is a 20-litre bucket (Reardon *et al.* 2018). Larger pit traps should be used in hot, dry areas to provide increased insulation from heat (Heyer *et al.* 2014). Black pitfalls may create high internal temperatures when exposed to intense sunlight for long periods.
- Fitting a plastic funnel-shaped collar to the top of pitfall traps reduces escapes (Figure 13b). The collar can reduce escape and provide shade.
- Placing a wet sponge in the pitfall trap prevents amphibians from dehydrating. The sponges should be moistened daily when open. However, the use of sponges may attract ants which can kill or seriously injure small vertebrates in the traps.
- The containers should have small drain holes (approx. 5mm diameter) in the bottom to minimise flooding during rain events when traps are open. This assumes pitfalls are set up in well drained areas.
- Consider the use of slanting boards or other means of shading if trap checks are infrequent or traps are exposed to extreme heat. (Fisher *et al.* 2008) used an inverted lid set on top of the trap with wooden spacers. This design prevents most litter, sunlight, and

precipitation from entering the open bucket while allowing sufficient space for the toad to enter.

- Shelter should be provided within the pitfall traps for captured animals. Use different sized PVC pipes (approximately 150 - 200 mm long, with a 25 – 40 mm diameter). Some form of insulation should be provided in the PVC pipes if small mammals are likely to be captured as by-catch.
- To reduce mortality and increase capture probability, traps should only be opened after sunset and checked at dawn.
- Setting traps around waterbodies will increase the probability of ABST capture.
- Pit traps must be checked daily.

Figure 13. A) Example of a three-armed pitfall trap array using shadecloth and star picket drift fences, lidded buckets, and plywood shade covers. Photo: Wyoming Cooperative Fish and Wildlife Research Unit. B) An inverted funnel fitted to the top of the bucket can reduce toad escape and provide shade and a moist environment. Photo: chamownersweb.net.

Timing – Dependant on area to be trapped and ABST population density. Frequent checks decrease adverse risk to target and non-target species. Recommend checking daily (early morning) at a minimum.

Resources Required – Fabrication and materials will require modest outlay ($5-$10 per pitfall trap). Trap arrays require 1-2 personnel to setup and monitor.

Risks – Pitfall traps may not be large enough to contain a captured toad. If the trap becomes too hot or dry, the toad may die. Flooded traps may result in either mortality or escape. Since traps are not species specific, there is a high risk of non-target captures. Trapping will need to be deployed along with other detection and capture techniques and is only effective if a toad encounters the drift fence.

Current Feasibility of Use – Medium to High

Funnel Traps

Description - Minnow-type funnel traps may be an effective means of capturing larvae e.g (Griffiths 1985, Tucker 1995), however the technique has not been tested on ABST. An advantage of this technique is that traps can be checked at the surveyor's leisure, as long as the traps are set properly so larvae can access the surface and sufficient oxygen.

- Traps can be employed along transects or along the water's edge. The number of traps to be employed will be influenced by the amount of larval habitat available.
- Set traps in water deeper than the height of the funnel to ensure efficient capture of larvae. However, some access to the water surface is necessary.
- Traps do not need to be baited.
- They work most effectively when placed along banks or woody debris, which serve to concentrate the larvae into the trap (DEWHA 2010).
- Stake traps to the substrate or other suitable debris. Attaching polystyrene to the trap will ensure it stays partially submerged (Figure 14).

- A single observer is sufficient to carry out funnel trapping of larvae, but as with visual larvae sampling the observer should be proficient in larval identification techniques.

Timing – At least five consecutive nights and days of monitoring during ideal conditions depending on size of waterbody and density of larvae.

Resources Required – Commercially available and moderately priced ($15-20) minnow funnel traps, ropes and stakes, hand-lens or binocular microscope, and a larvae identification guide in addition to personnel. Flagging tape is useful in relocating traps.

Risks – Funnel traps may trap native larvae and potential predators (e.g., crayfish). These predators may kill and consume ABST larvae, possibly leading to false negative results. Identification of larvae can be difficult and will likely require assistance from experienced frog experts and/or larvae keys.

Current Feasibility of Use – Medium to high if found to be effective for ABST.

Figure 14. Using a funnel trap to capture ABST larvae can be effective. Attaching polystyrene to the trap will ensure it stays partially submerged. Photo: Taylor Edwards (Stitt *et al.* 2005).

Lure Traps (light, food and chemical cues)

Chemical

Studies in tropical Australia show that cane toad (*Bufo marinus*) larvae are strongly attracted to chemical cues from conspecific eggs released as the jelly coat breaks down (Crossland and Shine 2011). However, (Reardon *et al.* 2018) used these cues to bait ABST tadpole traps with no significant effect. At this stage, chemical attractants may not be feasible for ABST.
Timing – Must be used when tadpoles are present.
Resources Required – Unknown
Risks – Unknown
Current Feasibility of Use – low

Light

Cage traps with light a bright light source can also be used. The light attracts insects that in turn attract toads.

A trap specifically designed to capture cane toads (Toadinator®) is a compact, lightweight, portable and easy to assemble lure trap that could be used/modified for ABST (Figure 15). It attracts and traps using "finger door" technology that allows entry but not exit, and a solar powered, self-charging light and sound attractor unit (ACTA-Attracta). According to the manufacturer, the devise is effective in a range of environments including residential and high use public areas.
Timing – Dependant on area to be trapped and ABST population density. Frequent checks decrease adverse risk to target and non-target species. Recommend checking daily (early morning) at a minimum. Should not be left unchecked for longer than 4 days. When checking the trap, make sure solar panel and electronic lure are charged and working.

Resources Required – The Toadinator® with sound attractor retails for about $400 per unit. Personnel costs will depend on terrain, season, number of trapped ABST and number of traps deployed.

Risks – The trap sound attractor would need to be modified to attract ABST. At this stage, it is unknown if the ABST will enter traps. The trap will only attract breeding toads; the device is ineffective for larvae, metamorphs and sub-adults.

Current Feasibility of Use – Medium to high

Figure 15. Assembled TOADINATOR® trap. Photo: Animal Control Technologies Australia

Sound

The use of calls has long been a way to detect amphibians (Yeager *et al.* 2014). These calls are highly informative and can identify species and indicate abundance and range (Rosenthal and Ryan 2000, Wilkins *et al.* 2013). See **Passive Acoustic Monitoring** for automated methods.

Acoustic trapping can be used in a number of ways.

- Listening for frog calls and, using standard triangulation to locate calling males, is often used for delineation and capture (Heyer *et al.* 2014).

    - The method is only useful during the breeding season and for breeding adult males.
- The use of call back (playing recorded calls) to solicit males to call and potentially attracting females can also be used. See also Toadinator® above.

Timing – At least five closely-spaced surveys in suitable breed season conditions (humid, light rain, warm, little wind). May be used day or night, dusk to dawn is preferable.

Resources Required – Inexpensive recorders ($50-100) may be used for call back. Alternatively, the Toadinator® also uses calls to attract. Personnel will depend on location and size of survey area.

Risks – Quality of recording may impact efficiency of the tool. May not respond to taped calls. If not timed correctly, high risk of false negatives.

Current Feasibility of Use – Medium to high

Refugia

Artificial Refugia and Burrows

Description - Can be used to delineate, improve surveillance efforts, or control a new ABST incursion. Pipes, boards and other refugia may act as artificial burrows and shelters, and can provide moist cool microhabitats where frogs/toads congregate. Artificial refugia (also known as cover objects) are known to be successful in capturing amphibians (Christy 2001).

- Pipes of various sizes are placed in the ground with approximately 60 -80% of its length covered. Pipe length should be at least 0.2 m.

- Alternatively, cover objects made of wood, tin or other light material can be used as surface refugia. The size of cover objects should be at least 600 mm × 600 mm.
- Both may be useful in conjunction with other survey methods.
- In other species, females tend to utilise shelters more often than males, particularly during the breeding season (Christy 2001).
- Cover objects made of metals such as tin tend to be utilised more than those made of wood or ceramic.
- When using cover objects, place a rock or other object underneath to provide an adequate crawlspace underneath. The space should be no more than 30 mm.

Timing – Preferably daily inspection. Mid-morning or late afternoon.

Resources Required – Fabrication and materials will require a small outlay (~$1-$2 per cover object or artificial burrow, depending on size and material). Modest labour costs depending on area, access, ground hardness and number of refugia/burrows.

Risks – Micro-climate needs to be considered when deploying artificial refugia/burrows. Surface refugia may overheat in summer depending on the material used. Burrow entrances may also collapse, trapping the toad inside. Occupancy may take time and the probability of occupancy is low. This may necessitate the deployment of many refugia/burrows for an extended period of time. Therefore, artificial refugia/burrows will need to be deployed along with other detection and capture techniques. Likely less effective in areas of high natural refugia

Current Feasibility of Use – Medium to High

Detector Dogs

Description - Domestic dogs have been successfully used to locate a range of items such as contraband, humans, cancer, plants and animals because of their superior olfactory acuity (Browne *et al.* 2006). Their effectiveness is dependent on the scent they are searching. Where they might be indispensable is in the detection of the last few ABST in a population (i.e., at low densities).

- The dog would have to o distinguish ABST from native frogs, and potentially cane toads.
- Training of detector dogs may not be justifiable for ABST incursions unless they can be trained on multiple species/genus/Families, and can be considered a shared resource with other programs or States/Territories.
- If training was delayed until an incipient population was unequivocally established, the requisite training and testing period might be too great for the dog team to be deployed and able to successfully eradicate the species.

Timing – Ongoing. Training a new dog and handler can take between six weeks to over a year.

Resources Required – Experience with detector dogs indicates that this capability is well within a well-trained dog's abilities, but requires lengthy training. The training phase can be expensive, and maintenance costs are moderate.

Risks – The risk of injury, particularly from parotid gland poisoning, or bites from other animals utilising the same habitat (e.g., snakes and spiders) should be considered when determining training and deployment techniques. Even a survivable poisoning may still shorten the working life of a dog if the dog becomes reluctant to hunt ABST. Since the training period can be quite lengthy, the lead time required may not warrant resource allocation unless the dog team could become a nationally shared resource. Use of detector dogs for first response may be limited if the dogs require extensive training.

Current Feasibility of Use – Medium

Forensics

Where possible, molecular tools should include voucher specimens and population genetics that can estimate effective population size and source.

Environmental DNA

Description –The use of environmental DNA (eDNA) in detection of rare and non-native species to inform management actions is increasing being used for detection (Noble *et al.* 2015). eDNA is nuclear or mitochondrial DNA that originates from cellular material shed by organisms (usually via shed skin, excrement, and gametes) into aquatic or terrestrial environments (Mahon *et al.* 2013) and frequently provides increased detection sensitivity compared to traditional detection methods (Jerde *et al.* 2011). An environmental sample is collected and the often quite small amount of DNA is then amplified by a polymerase chain reaction (PCR). By using species-specific primers that only bind to the DNA of the target species, the presence of the species can be inferred if a bar that appears on the gel on which the results are viewed. Gels are one of several methods used to detect species from eDNA; real-time PCR, or quantitative PCR (qPCR), is becoming more popular (Ginzinger 2002).

Water sampling can be successfully used to detect the eDNA of eggs and tadpoles, while soil sampling may provide an opportunity to detect metamorphosed and adult ABST. Monitoring ABST using eDNA could provide a decision support tool for long-term management strategies through the estimation of occurrence and detectability using occupancy models (Hunter *et al.* 2015). This approach can provide evidence for identifying newly colonized areas, movement corridors, and potential pathways of dispersal. eDNA tools may also assist with short- or long-term surveillance to assess the success of control or eradication efforts.

Collection of data will depend on whether water, soil or other samples (such as ABST passing by a nexus point with adhesive tape) are taken. A field test kit is used to collect eDNA samples The test kit typically contains:

- Methodology document and datasheet to record information. This will detail exactly how samples should be collected, stored and shipped.
- Sterile sampling bag (e.g., Whirl Pak™ or non-greased paper, or sealable plastic bags) for collecting samples.
- Sterile collection tubes partially filled with ethanol in which to store samples.
- Sterile ladle and clear pipette for water samples.
- Two pairs of sterile disposable rubber gloves - one to wear collecting samples, one to wear when filling collection tubes.
- Field kits may be stored in the refrigerator before use. The testing laboratory will provide specific information on transporting the samples to the lab.

For further information on the use and deployment of eDNA in the field, please contact:

Professor Dianne Gleeson

University of Canberra

https://www.ecodna.org.au/nrc

Timing – Medium to long term.

Resources Required – Moderate funding needed. Species specific genetic markers (primers) are required at an estimated cost of $500 per primer. Note that the ABST marker has been developed and is available for use. Test kits are necessary to take field samples to send to a lab for testing. The cost of test kits and analysis is currently unknown.

Risks - Imperfect detection can result in an under- or over-estimation of the distribution of the species. False positive results can also occur due to contamination. Thus, sterile field data collection and lab conditions are vital. Not all negative results mean the species is absent from the area. Low eDNA concentrations or a lack of positive detections in locations suspected of containing ABST may be due to environmental conditions (e.g., temperature, rainfall, wind, seasonality, water flow, terrain, soil etc.). Variations in sampling location, species abundance, environmental heterogeneity, assay sensitivity and detection threshold could affect the probability of detection, as will variation among samples within the same location and/or region (Furlan *et al.* 2015).

eDNA is relatively volatile and may not persist in the environment for more than 21 days (Noble *et al.* 2015). This may present a problem for new incursions because populations would be small and metamorphed ABST may be either hypermobile or so sedentary that their DNA is too scarce to detect. Infrequent shedding and defecation may also impact detectability of ABST in the wild.

eDNA will not give information on total abundance. Traditional surveillance techniques mentioned above will need to be used in addition.

Current Feasibility of Use – Medium

Passive Surveillance

Opportunistic Encounters

Description – Individual ABST are located incidentally, often in gardens or around ponds on warm, rainy nights by members of the public. While dedicated (active) searches are usually more effective per person-hour than opportunistic (passive) detections, the general public populous is much higher and better dispersed, which increases the probability of detection through opportunistic encounters. Therefore, passive detections are an important tool for incursion control.

- Wetland and herpetological groups are a great resource for passive surveillance. Engaging these groups has many advantages including expertise, enthusiasm to collaborate, networking, and their ability to interact and educate the public.
- Consideration must be given to the number of available volunteers. The pool of volunteers is limited in number and the length and intensity of their participation is partially dependent on the longevity of project.
- The key is to ensure that public reporting is used where it is most effective. Maximal efficacy is likely to occur in areas where sightings are so rare that early detection is at a premium, and too few professional searchers are available to address the need. Thus, opportunistic call-ins and volunteer searches will be most productive.
- The focus on peripheral areas and sentinel sites may conflict with volunteer desires; most prefer to participate in searches that result in many captures. Low reward rate is well known to diminish the efficacy of searchers, even trained searchers (Henke 1998).

Timing – Dependent on response/project duration and timing of media/educational material. Timing can also be impacted by the propensity of false positive detections.

Resources Required – Low cost as ABST are reported as they are incidentally located. Local pest lines or biosecurity government agencies handle such calls and need to be suitably resourced. Personnel to follow up will also be required.

Risks – Numbers of ABST detected using this method will likely be inadequate to reduce a population if one is established. However, individual incursions may be detected by opportunistic encounters. Although it is the most common tool currently in use in Australia, the method will not detect every toad. However, it can be a good indicator of a breach in a controlled area. This method could only be used in conjunction with other methods.

Current Feasibility of Use – High

Community Involvement

Description – Can be used to detect, delineate or control a new incursion. If the community is well informed of the significance of an ABST incursion, most people will be willingly to submit such information to responsible authorities.

- Opportunistic encounters are presently being reported to State-Territory or national hotlines. Hotlines of this sort have proven to be of enormous value in locating potentially invasive species e.g., (Gibble *et al.* 2014, Hawley 2007). In addition, volunteers can be encouraged to provide systematic searches in areas of special interest. Both approaches (opportunistic encounters and volunteer searches) are powerful tools that not only address a crucial information need, but also generate interest and support among the public.
- Doorknocking areas, letterbox drops, media articles and campaigns, networking with local groups are other avenues to garner community involvement.

Timing – As required and as funding allows.

Resources Required – Depends on the application but costs are usually minimal. Current platforms could be used or expanded, further cutting or sharing costs. Requires good extension material.

Risks – The use of community volunteers can potentially lead to misidentification, (Somaweera *et al.* 2010) if in areas where cane toads (*Rhinella marina*) are established, or interview methods are not well thought out and executed. The use of systems such as ADVOKATE (Bromby and Hall 2002) may mitigate risk. Irresponsible or poor handling techniques can cause injury to the species or community members. Although unlikely for this species, herpetological enthusiasts may take advantage of specimens they find and bring them into captivity. Legal liabilities need to be addressed if the community is to be actively involved.

Current Feasibility of Use – High

Automated Visual Monitoring

Description – The use of automatically triggered cameras has advanced and expanded over the years with most now utilizing digital technology triggered by active-infrared (AIR) or passive-infrared (PIR) sensors (Rovero *et al.* 2013, Swann *et al.* 2004). Under some circumstances where incursions may arise but adequate surveillance cannot be delivered, cameras may provide proof of species presence.

The design of visual monitoring is important. Understanding what the intention of camera-use and how to set up are critical factors. Recommended use may involve integrating with other tools such as trapping or barrier grids. Set up around waterbodies and movement paths is likely the most effective use. Efficacy maybe compromised for juveniles and sub-adults.

Timing – Can be used year-round, diurnally and nocturnally.

Resources Required – Cameras can be expensive ($250-$1000 per camera). The number required is dependent on the habitat and designated use of the collected information. Resources will also be needed to deploy camera, change batteries, and download and sort images.

Risks – Due to trigger limitations, the use of cameras for ABST detections may be restricted (Swann *et al.* 2004, Welbourne 2013). The size and cryptic nature of the toads may prevent the camera being triggered. Conversely, the required sensitivity may increase the number of non-targets photographed.

Current Feasibility of Use – Moderate to low

Automated Acoustic Monitoring

Acoustic monitoring utilises automated audio recorders to detect specific animal sounds. These sounds are highly informative and can identify species and indicate abundance and range (Rosenthal and Ryan 2000, Wilkins *et al.* 2013).

Acoustic sensors are quickly gaining ground in ecological research, following global trends toward automated data collection (Gibb *et al.* 2019, Sugai *et al.* 2018). Opportunities have historically been limited by technological costs and constraints, but this situation is fast improving. Although limited information exists for its use for amphibians, results to date are promising.

- Using unattended sound recording, the tool may provide long-term and cost-effective monitoring at sentinel sites, or targeted incursion areas.
- Provide almost continuous data
- Do not have to be checked daily
- Unclear how effectively current methodologies will work on ABST.

Timing – Can only be used during the breeding season.

Resources Required –Automated acoustic sensors are relatively expensive (from ~ $400 per unit). Personnel requirement dependant on accessibility and size of the area.

Risks – Standardised protocols have not been determined. Efficacy of the system requires testing on ABST. Resulting large volumes of audio datasets still present formidable logistical and analytical difficulties (Gibb *et al.* 2019).

Current Feasibility of Use – Low to Medium

Post-capture of Live ABST

Transportation of Live Specimens

General considerations and practices are required to transport live ABST within Australia. Live transportation should be avoided where possible; toads should be euthanised as soon as possible and not held for long periods of time. Information on specific transportation requirements can is described in the Commonwealth of Australia (2011) and NHMRC (2013). Note that most States/Territories have their own animal welfare legislation, which should be consulted.

- Transportation can cause distress due to confinement, movement, noise (vibration), temperature, and changes in the environment. Further, the extent of any distress may be impacted by:
    - Condition/health/temperament
    - Age and sex
    - Travel duration and type
    - Length of time without food and/or water
    - Environmental conditions, particularly extremes of temperature and humidity
    - Ability to give care during the journey
- Keep individuals in a sealed container made from non-toxic materials, with a moist sponge or paper towel on the base.
- Transport larvae and eggs in conditioned tap water.
    - Transporting in pond water should only occur if adequate protocols for pond water disposal is available and spillage is prevented.
    - Container should be sealable as to prevent spills during transport.

    - Density of larvae must not exceed more than 5 tadpoles/250 ml of water.
- Transport in the shade or an air-conditioned vehicle cab.
- Maintenance of biosecurity standards requires that only one toad be kept per container.
    - Larvae may be transported together provided they were all collected from the same location in the same waterbody.
    - This will ensure the protection of the diagnostic facility from potential pathogens.
- Any empty space around the container should be padded with shredded paper to prevent excessive movement of the container.
- A cardboard box is <u>not</u> a suitable container.

Euthanasia

This section outlines the general considerations and practices required to humanely euthanise ABST. Information has been gathered from (Leary *et al.* 2013, New *et al.* 2016) and NHMRC (2008, 2013). It follows requirements detailed in the Australian code of practice for the care and use of animals for scientific purposes (New *et al.* 2016) and NHMRC (2013), the cane toad SOP developed by (Sharp *et al.* 2011) and the Humane Euthanasia detials in the NSW DPIRD cane toads webpage NSW DPIRD (2024). Table 3 summarises each method.

Published general signs of pain or distress in ABST are not available and therefore determining negative welfare impacts on these animals can be challenging. It is difficult to use behavioural cues as the toad lacks facial expressions, can be still for long periods of time, and rarely vocalises outside mating calls. Possible signs include:

- Hunched or arching posture

- Stillness, immobility or lethargy
- Rapid respiration
- Flexing on palpation or touch
- Tightly shut eyelids
- Increase in or changes to swallowing reflex
- Dullness or colour changes
- Lameness, soreness or changes in muscle movement (e.g., spasms)
- Kicking of limbs or flicking of digits

The euthanasia of any animal must be achieved in the shortest time possible, with minimum distress. The information here is intended for remote or field situations. Where full veterinary facilities are available, there may be more appropriate alternatives (such as inhalant anaesthetics), which should be used in preference to the methods outlined here. See (Sharp *et al.* 2011, NSW DPIRD 2024) for details.

There are methods previously considered appropriate for euthanising amphibians but are now no longer acceptable. They include:

- **Rapid freezing -** Published guidelines DO NOT recommend this method it may cause pain or distress due to the formation of ice crystals on the skin and in tissues (New *et al.* 2016). Therefore, rapid freezing should only be used on deeply anaesthetised toads.
- **Benzocaine gel** - Previously reported as an effective topical euthanising agent for amphibians (Chen and Combs 1999), is no longer considered suitable for use as the effectiveness is variable and availability limited (Sharp *et al.* 2011).
- **Clove oil solution** – Clove oil, whose active ingredient is eugenol, is an effective anaesthetic and euthanising agent in fish (Ross and Ross 2009). However, recent tests on cane toads have concluded that optimum concentrations to cause death cannot be reached (Sharp *et al.* 2011).
- **Dettol®,** and **AQUI-S®** - These are not recommended due to the unacceptable distress caused to cane toads (Sharp *et al.* 2011).

Table 3. Summary of acceptable methods of euthanasia of ABST

Method	Feasibility	Cost effectiveness	Practicality
Stun/Decapitation	High	Cost effective	Must be used together, decapitation following stun. Not feasible for use on tadpoles.
Cooling followed by freezing	High	Cost effective	Placed in a suitable container with sufficient air space around each toad and then cooled at 4ºC in fridge for at least 8-12 hours. The container is then transferred to a freezer for at least 24 hours.
CO_2	High	Cost effective	Practical and cost-effective for large scale use. Exposure to 90% (or greater) concentration to CO_2 for at least 4 hrs. No more than 20 toads per 56 l bag. Not effective for tadpoles.
Hopstop® or other registered products	High	Relatively expensive	Best used for individuals or limited populations. Death is not instant; toads may suffer for 2-3 min). Two bursts of spray is necessary. Not effective for tadpoles.
Shooting	Low	Can be expensive depending on abundance and accessibility	Restriction to ricochet-safe areas and the required use of trained shooters limits use. Small body size may decrease efficiency and increase non-lethal shots. Not feasible for use on tadpoles.
Citric acid	Low/ medium	Cost effective	Has not been tested on ABST. Large scale use at 25% concentrations show promise, especially for juveniles. Not effective on tadpoles Product is non-selective and will kill non-target species including native frogs.

Method	Feasibility	Cost effectiveness	Practicality
Rotenone	Medium	Relatively expensive	Effective on tadpoles only. Has not been tested on ABST. Product is non-selective and will kill all gill-breathing non-target species
Reproductive Inhibitors	Low	Expensive	Limited work has been done in this area. Injections of melatonin has shown promise, however long-term application and efficacy has not been tested. Expensive research would be necessary.

Physical

Stun and Decapitation

Complete removal of the head, following stunning. See (Sharp *et al.* 2011) for details. Stunning must always occur prior to decapitation. This method is considered conditionally acceptable for the euthanasia of ABST when:

- it is carried out by experienced and skilled persons
- the animal is held by the back legs against a solid surface
- correct stun force and placement (head region only) using a large headed hammer or similar to stun
- the toad is promptly decapitated with a sharp knife or cleaver.

Equipment required includes:

Timing – whenever specimens are found

Resources Required – Qualified practitioner (ideally at least two), large hammer with head diameter at least 200 mm, rigid wooden or plastic chopping board or equivalent, large, sharp knife or cleaver with at least a 150 mm blade.

Risks - If carried out with insufficient force or inaccurate aim, the toad may be wounded, causing undue pain and suffering. Although a relatively simple method, it may not be cost-effective for large numbers of ABST. The process can be time and labour intensive (1-2 mins per kill) and practitioners may become fatigued.

Current Feasibility of Use – High

Shooting

Description – Shooting as a capture method for ABST is feasible but generally not used Australia. The method is a quick and effective means of humanely destroying animals, and in some situations is the only practical method available. It is most effective when the toad is large enough and readily detectable but hand capture is not possible.

Note that this section is not designed to provide specific guidance on the use of firearms as part of control programs. These are general principles only and should not be used without first consulting a firearms expert.

- Shooting should only be performed by skilled operators who have the necessary experience with firearms and who hold the appropriate licences and accreditation.
- Restriction to certain locations such as national parks, rural areas and private property for reasons of safety or visitor equanimity. There may be legal restrictions on discharging a firearm in certain areas. Police permission may be necessary.
- The accuracy and precision of firearms should be tested against inanimate targets prior to the commencement of any shooting operation.
- Head shots are preferred - correctly placed head shots cause brain function to cease and results in an instantaneous loss of consciousness. Shots must be aimed so that the projectile enters the brain, causing instant loss of consciousness.
- To maximise the impact of the shot and to minimise the risk of misdirection the range should be as short as possible (e.g. 50 - 200 mm from the head if using a rifle, 1-2 m if using a shotgun). The barrel should never be touching the toad's head.
- Ensure an effective calibre is used. Smaller calibre rifles (0.22 or 0.410 magnum) are adequate for euthanasia of ABST at short range (< 5 metres), as long as the shot is correctly positioned (Leary *et al.* 2013). Rifles are preferred to handguns because the longer barrel length ensures better control by the shooter.

Firearms are potentially hazardous and safety must be considered.

- Firearm users must strictly observe all relevant safety guidelines relating to firearm ownership, possession and use. They must only be used by fully qualified and proficient users with experience shooting amphibians.
- No-one should stand in front of the shooter, and the line of fire must prevent accidents or injury from stray bullets or ricochets.
- The line of fire must be chosen to prevent accidents or injury from stray bullets or ricochets. Most ricochets are caused by accident. While the force of the deflection decelerates the projectile, it can still retain enough energy to be deadly. The possibility of ricochet is one of the reasons for the common firearms rule - never shoot at a flat, hard surface.
- When not in use, firearms must be securely stored in a compartment that meets State legal requirements. Ammunition must be stored in a locked container separate from firearms.
- Adequate hearing protection should be worn by the shooter and others in the immediate vicinity of the shooter. Safety glasses are recommended to protect the eyes from gases, metal fragments and other particles.

Timing – As required and as funding allows.

Resources Required –Dependant on abundance of ABST, and accessibility. Minimal; cost of at least 2 personnel and equipment.

Risks – Restriction to ricochet-safe areas and the required use of trained shooters are appreciable drawbacks. ABST may be difficult to shoot as their body size offers a relatively small target.

Current Feasibility of Use – Low

Inhalants

Carbon Dioxide

Carbon dioxide (CO_2) has been used to kill large numbers of cane toads in the field, although its use may be considered controversial. ANZCCART euthanasia guidelines[1] do not recommend any inhaled agents for amphibians (Reilly 2001), a sentiment backed by (Wright 2001) as many amphibians are tolerant of hypercarbic (CO_2 retention) conditions. Conversely, the AVMA (2007) recommends CO_2 while highlighting that, although loss of consciousness develops rapidly, exposure time required for death is prolonged. (Sharp *et al.* 2011) recommends prolonged exposure time of at least 240 minutes (four hours) is required.

CO_2 is considered acceptable if:

- carbon dioxide concentration is greater than 90%
- a warming coil and/or plastic tube is used to pre-warm CO_2
- no more than 20 toads per 56-litre container/bag and
- exposure time is four hours or longer.

Timing – whenever specimens are found

Resources Required – Qualified practitioner, cylinders or compressed carbon dioxide fitted with CO_2 gas regulator, airtight container/bag to hold ABST for gassing (sealable, flow-through containers can be used). Heavy duty plastic garbage bags (approx. 56 L capacity and about 50 microns thickness) are recommended; thicker bags may not seal properly, and thinner bags are prone to puncture or diffusion of gas. Air warming coil and/or 3 m of tubing connecting regulator to the container/bag.

[1] ANZCCART guidelines are currently under review

Risks – CO_2 exposure time less than 240 mins (4 hrs) will not reliably kill toads and could lead to undue pain and suffering. Overcrowding of containers could also prolong time to death. Leaks in the system will decrease efficiency. If the CO_2 is not heated, it could cause cold shock to the toads and impact inhalation rate. If toads are not exposed to 90% CO_2 for four hours, it is possible that will survive.

Current Feasibility of Use – High

Toxicants

Hopstop®

Description - Hopstop® is an aerosol spray (developed by Pestat Pty Ltd) specifically designed to euthanise cane toads. The primary ingredients are chloroxylenol and ethanol. Chloroxylenol (the active ingredient in Dettol®) is the killing agent in the spray and the ethanol is used as an anaesthetising agent. Ethanol is an anaesthetic that reliably and rapidly induces loss of consciousness in toads in about 2 minutes. Independent trials indicate that toads are likely to experience some distress for the period post spray but prior to the ethanol taking effect (Sharp *et al.* 2011). The time to death, as indicated by cessation of heartbeat, ranges from 70 to 120 minutes. Two sprays of the product are recommended, the second after the toad stops moving.

Although chloroxylenol is one of two main ingredients in Hopstop®, using Dettol® (same active ingredient) as a substitute is not recommended as the product induces unacceptable levels of suffering and distress (Sharp *et al.* 2011).

Timing – Short term as product is fully developed and commercially available. Can be implemented immediately.

Resources Required – Minimal depending on abundance of toads. Requires at least one practitioner and a can of Hopstop®. A 300 ml can retails for around $20.

Risks – Level of pain after movement cessation is unknown and therefore toads may be exposed to suffering. Toads must be sprayed directly. Overspray onto terrestrial or aquatic habitat or onto other species may cause contamination or death of non-target species.

Current Feasibility of Use – High

Citric Acid

Description – Citric acid is a weak organic acid that occurs naturally in citrus fruits. The use of citric acid sprays is a relatively new control technique and has been successfully used to decrease coquí (*Eleutherodactylus coqui*) populations in Hawaii (Beachy *et al.* 2011). This topical toxicant was tested on ABST in Madagascar where citric acid monohydrate spray trials at concentrations of 16% and 25% were effective, especially for juveniles (Reardon *et al.* 2018). Its use is scalable without the need for individual capture or significant habitat alteration. Coupled with minimal impacts to plants and crops (Pitt and Sin 2004), citric acid shows promise for use in Australia.

Timing – Medium-term research for use in Australia is necessary. Dependant on size of area to be treated. Optimum use around the breeding season.

Resources Required – Cost of citric acid, wetting agents, backpack spray units, personnel and storage facilities. Treating one hectare with 50 kg of citric acid /using a backpack sprayer would take approximately 100 person hours and cost over $800/metric ton.

Risks – Non-targets are likely to be killed. Frogs must be sprayed directly; citric acid residue alone is not an effective control (Hara *et al.* 2008). Citric acid may only be viable to use in areas of extreme ecological degradation, and should not be used in ecologically intact environments.

Current Feasibility of Use – Medium

Rotenone

Description – Rotenone is an odourless, colourless, crystalline isoflavone used as a broad-spectrum insecticide, piscicide, and pesticide. It occurs naturally in the seeds and stems of several plants, and the roots of several members of Fabaceae. The use of rotenone has been tested and used on a variety of amphibian larvae including toads e.g., (Billman *et al.* 2011, Christy 2001, Grisak *et al.* 2007).

Concentrations of 5% rotenone applied at 1 mg/L (50 µg/L rotenone) is known to cause 100% mortality in gill-breathing tadpoles. However, rotenone is not effective on non gill-breathing metamorphs, juveniles or adults (Billman *et al.* 2012, Christy 2001).

Timing – Short term as product is fully developed and commercially available. Some testing specific to ABST tadpoles may be necessary.

Resources Required – Cost of rotenone, dispersing mechanism (e.g., backpack spray units, containers or water truck), personnel and storage facilities, tadpole collecting nets. Rotenone is relatively expensive, ranging from $20 to $35/l. Prices vary from year to year and depend on total amount purchased.

Risks – High level of non-target mortality likely to occur. Rotenone may only be viable to use in aquatic areas of extreme ecological degradation, and should not be used in ecologically intact environments as the product is non-selective.

Current Feasibility of Use – Medium

Reproductive Inhibitors

Description – Limited support exists for non-lethal reproductive inhibitor control tools for the humane treatment of ABST and avoidance of non-target deaths. Little work has been done in this area for the toad. (Chanda and Biswas 1982) found melatonin injections decrease spermatogenesis and the Leydig Cell nuclear area in ABST, but long-term application and efficacy of the method has not been tested.

Timing – Long term and slow acting.

Resources Required – Basic research for this approach is time consuming and expensive, and runs a high-risk failure.

Risks – Difficulty finding an inhibitor to impair the reproduction of ABST.

Current Feasibility of Use – Low

Sampling/Autopsy Requirements

Under some circumstances, ante-mortem and post-mortem examination, necropsy procedure, gross examination of tissues, collection and storage of samples will be required.

Cost - The cost of these procedures varies between States/Territories and providers, estimated at around $130 for specimens up to 5 kg, and $200 for specimens between 5 – 60 kg (Wisniewski 2012a, Wisniewski 2012b).

Considerations - Cost includes euthanasia via lethal injection. Cost for analysis of necropsy samples is additional and dependant on the number of samples.

Voucher Specimens

Voucher specimens provide verifiable and permanent records of wildlife and environmental (Clemann *et al.* 2014). Where possible and practical, voucher specimens should be collected and lodged at one of Australia's State-Territory museums. A voucher specimen is usually (but not always) a whole animal that is killed humanely, preserved and retained in an accessible collection museum. It serves as a basis of study and is retained as a reference.

- The museum should be contacted as soon as possible to determine if a voucher specimen is necessary. The museum contact may guide the process.
- It is the responsibility of the senior investigator/incursion manager to ensure that the specimen becomes part of a publicly accessible scientific reference collection.
- To be optimally useful, voucher specimens should be lodged with a museum that can properly house and curate them, and ensure available for further study.
- This is dependent upon specimens being properly stored or prepared after collecting, or maintained in live condition, before delivery to such institutions.
- Proper documentation of the specimens is essential. Data should be maintained with the specimen.
- Consultation with the institution before collecting will ensure that there is an understanding of the proper preservation and holding techniques, necessary equipment and essential data required.
- Arrangements should also be made to ensure voucher specimens can be accepted by the institution.
- The senior investigator/incursion manager is responsible for ensuring competency regarding the collection of voucher specimens.
- Animal Ethics Committee approval may be required for the taking of some voucher specimens under certain circumstances.

Disposal Methods

The primary objective of disposal of carcasses, animal products, materials and waste is to prevent the spread of infection. This process is therefore an essential part of an animal disease eradication program.

Disposal should be completed as soon as possible after destruction to minimise opportunities for infectious material to disperse. As part of preparedness planning, potential stakeholders should be identified and engaged in the process of identifying appropriate disposal methods. Please refer to AHA (2015) for specific methods and techniques.

If voucher specimens are not required, toad carcasses can be disposed of by either incineration or deep burial. Carcasses should be disposed of properly and in accordance with acceptable practices as required by local councils and applicable State-Territory or Commonwealth regulations at a Federal quarantine or suitably accredited facility.

Incineration - Remains are destroyed in a high temperature incinerator at a cost of around 4 - 5 $/kg.

Deep Burial - Remains are buried to reduce transmission and spread of disease at a minor cost. This option can only apply for burial in a Council approved animal pit.

Disease, Parasite, Infection Risks

The ABST can carry a number of parasites including microfilarial worms, nematodes, ticks and leeches in addition to pathogens and fungi (Rahman *et al.* 2008, Rebelo and Measey 2017).

Below is a summary table (Table 4) describing some of the common disease risks, parasites and fungal infections that could be transmitted from ABST. This summary is not comprehensive and is designed to highlight potential disease issues that may result from an ABST incursion. Each of Australia's States and Territories has a Wildlife Health Australia (WHA) Coordinator appointed by the corresponding Chief Veterinary Officers. If there has been an incursion and there is concern regarding disease risks, the relevant State-Territory WHA Coordinator should be contacted.

WHA has fact sheets for amphibians including comprehensive fact sheets for chytrid fungus and Ranavirus. Additional information can be found on the Amphibian Disease Knowledgebase and published literature (Berger and Green 2012, Berger *et al.* 1998, Kilpatrick *et al.* 2010, Murray *et al.* 2010, Reed *et al.* 2000, Vredenburg *et al.* 2010).

Table 4. Common disease risks, parasites and fungal infections potentially effecting ABST. WHS (work health and safety) considerations listed here are in addition to those discussed below.

Disease	Disease Agent	Impact and Transmission Route	Additional WHS Considerations
Chytridiomycosis	*Batrachochytrium dendrobatidis*	Zoospore from infected skin, primarily spread through water	Wear nitrile or latex gloves when handling and wash hands frequently
Ranaviruses	*Bohle iridovirus* (BIV), North American/UK Ranaviruses, Venezuelan Guatopo virus	Direct contact, necrophagy, and indirectly via water and fomites	Wear nitrile or latex gloves when handling and wash hands frequently
Salmonellosis	*Salmonella* spp. bacterium	Faecal-oral transmission through direct or indirect ingestion. Captivity appears to predispose ABST to excrete Salmonella which could then be ingested	Wear nitrile or latex gloves when handling and wash hands frequently
Mycobacteriosis	*Mycobacterium* spp.	Exposure of wounded skin to contaminated animals, soil or water	As above plus protective mask and eye protection if splash hazard present
Flavobacteriosis	*Flavobacterium* spp.	Primarily occurs in captive colonies	As above plus protective mask and eye protection if splash hazard present
Chlamydiosis	*Chlamydia pneumonia*	Direct contact, respiratory route of faecal-oral route	As above plus respiratory protection

Disease	Disease Agent	Impact and Transmission Route	Additional WHS Considerations
Aeromoniasis	Aeromonas hydrophila – "red-leg syndrome" or amphibian septicaemia	Ingestion of contaminated water	As above plus protective mask and eye protection if splash hazard present
Saprophytic fungi	Zygomycosis, Phycomycosis, Mucormycosis	Inhalation, ingestion or inoculation with spores	As above plus respiratory protection
Gastroenteritis	*Escheria coli*	Wound contamination or ingestion of contaminated water	As above plus protective mask and eye protection if splash hazard present
Sparganosis	Cestodes, genus *Spirometra* (tapeworms)	Ingestion of undercooked frog meat, skin penetration or contaminated water	Gloves and wash hands frequently
Gnathostomiasis	Nematode larvae (e.g., *Aplectana macintoshii*)	Handling/ingestion of contaminated water	As above plus protective mask and eye protection if splash hazard present
External parasites	Mites, leaches (e.g., *Tritetrabdella taiwana*	Direct contact with animal	Gloves

Workplace Health and Safety (WHS) Considerations

Below is a summary table (Table 5) describing some treatment specific WHS considerations associated with handling ABST. Note, this section and the previous section may be used to develop a Job/Activity Hazard Analysis (JHA) that includes ABST detection and capture. Hygiene and quarantine protocols for the control of disease in amphibians is available online.

Table 5. Work Health and Safety (WHS) concerns and recommended treatment in addition to those listed in **Disease, Parasite and Infection Risk** section above.

Hazard	Control
Accidental contact	Be alert at all times for ABST Do not reach into dark cracks, crevices, or holes. Do not place hands or feet in places out of view. Use caution when moving, sitting on, or stepping over rocks or logs. Use leather gloves or tools when turning or moving potential snake refugia. Use caution when walking through tall grass or heavy brush, particularly off-trail. Carefully move and shake out boots and equipment before putting them on or away in the morning.
Spread of viral, bacteria and other disease	Ensure proper cleaning, disinfecting and /or sterilising of equipment and footwear when moving between sites. Wear single-use latex or nitrile gloves when handling toads. Appropriate hand hygiene techniques may negate to use of latex gloves. ABST obtained at different sites are kept isolated from each other.

Hazard	Control
Potential Injury caused by toxins	Single-use latex or nitrile gloves should be worn when handling to decrease the risk of handling and parotid gland excretion. ABST should be euthanized immediately in the field wherever possible When stunning an ABST, the blow may rupture and spray toxin. Operators should use a full-face mask/visor. If poisoning occurs, contact a doctor or the Poisons Information Centre (Tel: 131 126).
Injury caused by methods	Personal protective equipment including gloves and appropriate eye/face protection should be worm during application. Ensure when stunning ABST, to avoid fingers being struck by the hammers. Working with sharp knives can be dangerous. Operators should use a cut resistant glove to protect the hand that is restraining the toad. When working with CO_2, open bags in a well-ventilated area. Care must be taken when lifting and transporting cylinders as they are heavy. If CO_2 is inhaled, remove patient from the contaminated area to allow them to breathe in fresh air. If patient is not breathing, keep airway clear and resuscitate. HopStop® is supplied in a pressurised dispenser, and should be treated like any other aerosol product. Do not incinerate, puncture, light or smoke while using. Use outdoor only. Store in cool place. Avoid contact of products with skin, eyes and clothes. Wear suitable clothing, and gloves when using products or handling toads. In case of accidental skin contact, wash the affected area immediately with soap and water. In case of eye contact, wash eyes immediately with water. Ensure sprays are directed away from yourself or any other person. If poisoning occurs, contact a doctor or the Poisons Information Centre (Tel: 131 126).

Regulatory Requirements

Consideration to national and State-Territory regulatory requirements is necessary. Table 6 lists some legislation, regulation and permits that will need to be considered when responding to an incursion. Please note that the list does not contain all regulatory requirements as these can differ between States and Territories.

Table 6. List of legislation and permits that may be required when responding to an incursion. Table also includes the rationale behind the need and what legislation the requirement is based.

Permits and Training	Why Required	Legislation
Animal Ethics Approval	To trap, sample and euthanize toads. AEC approval is only required for research and teaching purposes under the NHMRC Code	Varies by State-Territory
Health Licence	To possess and use controlled substances such as barbiturates for euthanasia	Varies by State-Territory
Local Land Manager (e.g. Council, Parks or Water authority	Seek appropriate approvals to access land or facilities	Varies by region/municipality
Private property owners	Seek appropriate approvals to access land or facilities	Varies by owner
Pest Animal – Approved Collections Permit	Permits are required for any captured or seized animals. They allow pest animals to be kept in captivity for longer than 12 hours. Animals retained for communications and engagement activities must be kept under permit	Varies by State-Territory
Scientific Permit	To carry out collection (including inadvertent), live capture and release of wildlife. This permit is	Varies by State-Territory

Permits and Training	Why Required	Legislation
	necessary for potential non-targets	
Firearm Permit	Required if firearms are used. Also includes special circumstances such as use in a populous (urban) environment, or use of a silencer	National Firearms Agreement (1996) Varies by State-Territory

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Appendix A: Response Plan Template

Response Plan: Asian Black-Spined Toad (*Duttaphrynus melanostictus*)

VERSION	DATE	AUTHOR(s)	APPROVER
1.0	July 2024	J. Billing (EBO) & T. Buckmaster (CISS)	

Background (Copied from ABST Plan – NEBRA reporting requirements pp 6-8)

In addition to State-Territory-specific reporting requirements, it is necessary to report under the National Environmental Biosecurity Response Agreement (NEBRA). Once a potential nationally significant pest or disease incursion is detected or suspected in a jurisdiction, that party must undertake the following:

- Determine whether the incursion can be managed through pre-existing cost-sharing arrangements and notify the NEBRA reporting point[2] of the incident.
    - Formal Notification of a confirmed or suspected pest must be provided to the Australian Chief Environmental Biosecurity Officer (ACEBO) – the primary NEBRA reporting point - within 24 hours.

[2] Regardless of the cost sharing, the NEBRA secretariat/reporting point should be notified of the incursion within the first 24 hrs of discovery.

    - Within State or Territory borders, the State or Territory officials with overall responsibility for biosecurity are responsible for notifying the NEBRA reporting point.
- Notifications for ABST should be submitted to:

    The **Australian Chief Environmental Biosecurity Officer:**

    Email: ACEBO@aff.gov.au

    Phone: (02) 6272 4623

    CC to:

    - The **Australian Chief Veterinary Officer** ACVO@aff.gov.au

- Once the reporting point is notified, an appropriate National Biosecurity Management Consultative Committee (NBMCC) may be established for the particular animal pest outbreak. The consultative committee is made up of the most appropriate experts from each jurisdiction and the Commonwealth to evaluate the incursion. An existing consultative committee such as the Consultative Committee on Environmental Biosecurity Incidents (CCEBI) can be used for this purpose or an ad hoc NBMCC can be established.
    - The NBMCC is Australia's key technical body for coordinating national responses for plant pests and diseases, weeds, ants and other terrestrial invertebrates, under the NEBRA and NEBRA-like (off-deed) arrangements. Its role is to coordinate the national technical response to nationally significant pests, including assessing the technical and cost benefit of eradication, and to make recommendations to the National Management Group (NMG) for decision. The NBMCC comprises representatives from the Australian Government, State and Territory governments and where

applicable, affected industries. The NBMCC is chaired by the Australian Chief Environmental Biosecurity Officer.

  - Under the NEBRA, the NBMCC is the technical committee, chaired by ACEBO. This includes undertaking assessments of the national significance of the incident, the technical feasibility and cost-benefit of eradication, and where appropriate, the proposed Preparedness Plan and making recommendations to NMG on these matters.
  - The CCEBI is a proposed national consultative committee with responsibility for providing advice on the coordination of responses to environment and social amenity pest incursions under the NEBRA and NEBRA-like (off-deed) arrangements. It is proposed that the CCEBI will deal with environmental and social amenity pests that sit outside the scope of currently established committees, such as exotic reptiles, amphibians and some mammals. It is envisaged that the CCEBI will comprise of representatives from the Australian Government, State and Territory governments and scientific experts from museums and universities. Due to the diverse and sometimes unusual nature of these types of incidents, the CCEBI may also draw on other expertise as required.

- The NMG is the decision-making body for national exotic pest and disease eradication programmes under NEBRA established in 2021. The NMG is chaired by the Secretary of the Department of Agriculture, Fisheries and Forestry. The membership comprises senior officials as all governments and industry parties cost sharing the national response to an exotic pest or disease incursion. The NMG's role is to make decisions on the technical feasibility and cost-benefit for eradicating an exotic pest or disease in accordance with a national Preparedness Plan and agreed cost shared budget.

- If the NMG decides that a national response is not required, any ongoing action will fall back to the affected jurisdiction and/or affected stakeholders.
- The NMG also makes decisions on changes to the Preparedness Plan (recognising that it is a living document that may have incremental versions), expenditure limits, financial and efficiency audits of the response and cessation of the response.

1. Aim and objectives of the Response Plan

1.1 Aim

1.2 Objectives

2. Current status of the Incident

2.1 EPP details

2.2 Affected host(s)

2.3 Other known hosts in the affected area

2.3 Diagnostic details

2.4 Description and affect

2.5 Extent of Incident

2.6 Additional impacts

3. Feasibility of eradication

3.1 Technical feasibility of eradication

3.2 Economic feasibility of eradication

4. Response activities for Emergency Containment and eradication

4.1 Overall response strategy

4.2 Zoning, quarantine and movement controls

4.3 Diagnostics and scientific support

4.4 Trace forward and traceback

4.5 Surveillance and monitoring

4.6 Decontamination, destruction and disposal

4.7 Host free/fallow period

4.8 Owner Reimbursement Costs

5. Proof of freedom activities

6. Public relations and communications activities

7. Social support mechanisms

8. Response Plan management and governance

9. Information systems and services

10. Reporting

10.1 NBMCC and NMG reporting

10.2 International notifications

11. Response Plan review

11.1 Key performance indicators/program milestones

11.2 Trigger points to review the Response Plan

11.3 Efficiency audit

12. Financial management of Response Plan

12.1 Indicative budget

12.1.2 Salary and wages

12.1.3 Operational costs

12.1.4 Capital items

12.1.5 Owner Reimbursement Costs

12.2 Accounting and reporting expenditure

12.2.1 Response Plan expenditure report

12.2.2 Accounting and cost claims processes

12.3 Financial audit

13. Appendices

Appendix B: Activity Log Template

Incursion Event Name		Incursion/Detection Event No	
Project Sponsor/Agency		Sheet Number	

Activity Number	Date	Time	Action (e.g., phone call, conversation, email, activity)	Follow up action required	Name/signature

Appendix C: Situation Report Template

Note: Report structure based on Animal Health Australia (AHA) Australian Veterinary Emergency Plan (AUSVETPLAN)

To:

From:

Date:

Subject: Biosecurity Incursion (Species name)

WHAT WE KNOW AND DON'T KNOW

Information available at present

What has happened?

- A short factual summary about the current status of the incursion.
- What is the organism?
  - Description, distribution, habitat, basic biology and tolerances, known impacts.
  - Name and contact details of identification expert
- Is it a suspected incursion or has it been confirmed?
- When was it discovered? How? By whom?
- Where is the incident? How widespread? Area of incursion and delineation
- Clinical symptoms, diagnosis, how it has spread, human health implications.

How did it happen?

- Relevant history in Australia (new to Australia or record of previous incursion/s) and overseas
- Pathway, activity and/or vectors of incursion (suspected or known)

Risk of establishment

- Species risk assessment
- Likelihood of spread

Implications

- Environmental and/or agricultural repercussions
- Environmental/Human health and/or food safety implications
- Trade implications/restrictions
- How long will it take to resolve the incursion (either complete or hand over to ongoing control operations)?

WHAT WE ARE DOING

What actions are currently underway to manage and resolve the incursion?

- Containment, tracing and eradication activities (activated incursion plans and procedures)
- Delineate incursion, quarantine areas and movement restrictions
- Trace forwards/trace backs (determine pathways and point/s of incursion/s)
- Surveillance and monitoring
- Testing/diagnostics
- Border security measures
- Product recalls, bans, food/product safety info (if relevant)
- Market access protocols/negotiations
- Meetings/taskforces
- Cost-sharing arrangements and/or financial assistance
- Current public information
- Ongoing management issues

WHAT WE WANT TO/NEED TO DO

- Additional options for immediate response
- Options for medium- and long-term response
- Movement restrictions (e.g., transportation modes, livestock, vegetation etc.)

- Implement and maintain good biosecurity practices
- Immediately report any signs of incursion species
- Follow instructions given by biosecurity authorities

INFORMATION SOURCES

List sources:

- For further information visit website, social media sites, phone hotline number.

Suspected Pathways and Vector

Life Cycle Implications

- Time of Year, Weather Implications

Habitat in the Area

Hydrography in the Area

RISK

Likelihood of Spread

Pest Status in Australia and Overseas

MANAGEMENT

Options for Immediate Treatment

Options for Medium to Long Term Treatment

RECOMMENDATIONS

Appendix D: Review and After-Action Review

The Post-Incursion After Action Review document is the final document produced for a particular incursion. It should be used to assess the success of the response, inform future incursion events, 'tidy up' any loose ends and formally close the response. Specifically, it should seek to accomplish three things:

- Confirm all work has been performed and documented in line with requirements;
- Provides a summary of what happened and where to find relevant information; and
- Ensures that any lessons learnt are passed on.

<table>
<tr><th>Report Name</th><td colspan="3"></td></tr>
<tr><th>Report Date</th><td></td><th>Detection Event No</th><td></td></tr>
<tr><th>Author/s</th><td colspan="3"></td></tr>
<tr><th>Project Sponsor/Agency</th><td colspan="3"></td></tr>
</table>

Revision History

Version Number	Date	Reason/Comments	Reviewed by

Distribution List

Name	Agency	Involvement in Incursion

Name	Agency	Involvement in Incursion

Citation

This report should be cited as:

(Author/s). (Year). (Species Name) Incursion Response - Review and Closeout Report. (Lead Agency). State-Territory).

SUMMARY INCURSION INFORMATION

To reduce repetitive work, this information may be derived from the Annual Vertebrate Detection database, or vice versa.

Organism	common and scientific name
New or persisting Incursion	Is this the first record of the organism in the area or is it a persisting incursion?
Initial Detection Date/Time	
Number and type of Individuals Detected	Specify whether numbers are actual or estimated. Include sex, age (if possible), size, and any other traits if known or can be estimated
Initial Detection Location	Towns and postcode, GPS latitude and longitude, and/or hyperlink/reference to a map
Brief Description of What Happened and Area	e.g., Member of public phoned to report a boa constrictor in a suburban bushland near the Spit, Gold Coast. Bushland approx. 10 ha and surrounded by mowed grass, shrubs, and trees in residential area
Suspected Source Location	e.g., Central America, Brazil, illegal pet trade, unknown
Suspected Vector	e.g., aircraft, shipping container, luggage, illegal pet trade, unknown
Pest Status	Summary of national and international status if known. For international status, check http://www.issg.org/database/welcome/
Outcome	e.g., Individual found/not found, captured, euthanized, unknown What is the fate of the organism/s?

Incident Documentation	Include hyperlinks or citations all documents associated with this incursion

INCURSION RESPONSE – AFTER ACTION SUMMARY

Planned Activity	Supporting Documents	Did Activity go to plan?	Deviation from Activity Plan
Describe the primary tasks within each Activity	e.g., Communication Plan, Incursion Plan, methodology paper, etc. Include citation or hyperlink	Yes/No	If activity deviated from what was planned, record the difference/s here
Management and Incursion Team			
Detection			
Tracking or Tracing (monitoring of location and status of species)			
Surveillance			
Control			
Communication (including media)			
Reporting			
Resourcing			

SUCCESSES

From the table above, list all aspects of the incursion response that you deem successful.

1.1

1.2

1.3

CHALLENGES

From the table above, list all aspects of the incursion response that you consider were a challenge and could be improved.

2.1

2.2

2.3

HANDOVER TO OPERATIONS

If eradication is not feasible, describe how the incursion response will be handed over to operations or activities terminated.

RECOMMENDATIONS

Based on the incursion response experience, we recommend the following:

- [refer to number above (1.1) etc]
- [refer to number above (2.1) etc]
- [refer to number above (2.3) etc]

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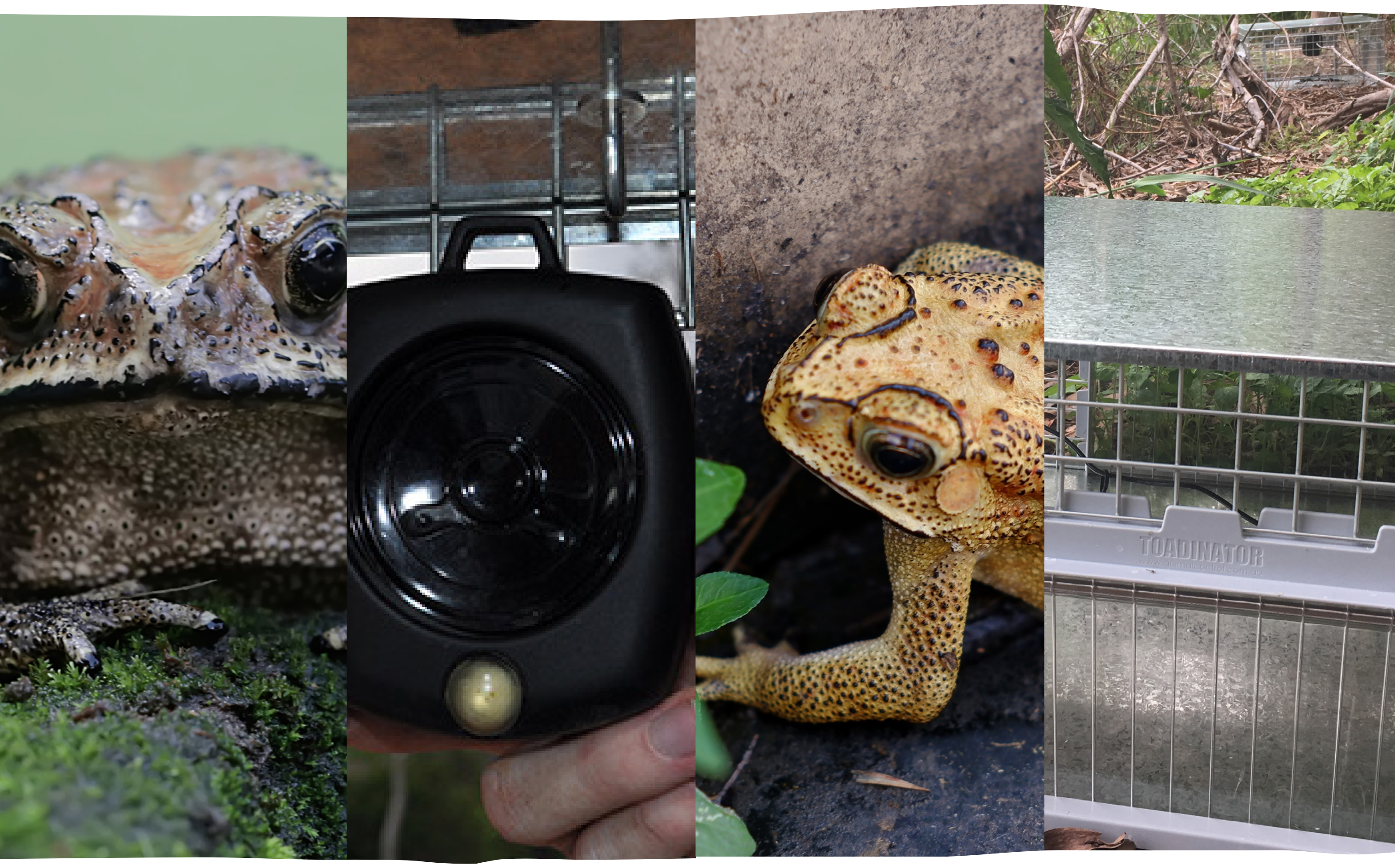

Australian Government