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# Surveys for the Smooth Newt (*Lissotriton vulgaris*) in south-east Melbourne

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**Appendices**

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# 1 Introduction

The Smooth Newt (*Lissotriton vulgaris*) is a cryptic, caudate amphibian native to large parts of Europe and western Asia (Arntzen et al. 2009). Until 1997 the Smooth Newt was freely available for sale in Australia as part of the aquarium pet trade, after which time it was declared a 'controlled pest'. It was subsequently declared a 'prohibited pest' in 2010. However, in 2011 the Smooth Newt was detected outside of captivity in Australia for the first time, at a site in metropolitan south-east Melbourne, Victoria. The discovery marked the establishment of an entirely new taxonomic order of amphibian in Australia, and prompted an investigation by the Victorian Government and scientists, which involved surveys to try and delimit the extent of the incursion, and an assessment into the feasibility of eradication or control. Information from these early investigations forms the extent of our knowledge base for this species in Victoria. No control efforts have been implemented to-date, and no further surveillance has been undertaken to determine the persistence of the species in the area, or its potential to spread.

Ecology Australia, on behalf of the Invasive Species Council, conducted surveys for the Smooth Newt during the spring of 2016, across an area of south-east Melbourne, to help determine the ongoing persistence of populations, and/or evidence indicating that the species may have spread. This report presents the methodology and results of surveys.

## 1.1 Background

### 1.1.1 The Smooth Newt

The Smooth Newt is a member of the amphibian order Caudata, which is not represented in the Australian fauna, and belongs to the family *Salamandridae* (newts and true salamanders); all Australian amphibians belong to the order Anura (tailless amphibians). Within its native geographic range, the Smooth Newt is one of the most widespread and common newt species, with a range that extends from Ireland, through to central Europe, southern Scandinavia and east into Ukraine and Russia (Arntzen et al. 2009). The nominate subspecies (*L. v. vulgaris*), discovered in Victoria, can be found across most of Europe.

#### Identification

Smooth Newts are small, semi-aquatic amphibians with smooth to slightly granular skin (Flood 2010). Adults measure approximately 10 cm (head to tail) and are generally olive-green to pale brown in colour with a pale throat and orange underbelly, patterned with dark, longitudinal dorsal stripes and rows of spots (see Plates 1 to 3). During the non-breeding season, females and males are similar in appearance, and can be distinguished only from subtle differences in patterning and colouration. During the breeding season, males develop a prominent red and blue wavy dorsal crest, more conspicuous spots, and a distended cloaca (see Plates 1 and 2). Juveniles (efts) resemble females in both patterning and colouration (Bowles 2009).

Newts of the *Lissotriton* genus are known to produce toxic skin secretions as an anti-predatory defence mechanism (Hanifin 2010). Smooth Newts previously captured in Victoria were also found to produce small quantities of tetrodotoxin, a potent neurotoxin which may be passed to other animals through breaks in the skin, mucous membranes or through the digestive system if newts are ingested. Juveniles and eggs are often more toxic than adults, but the toxicity of the species in Australia is not known.

### Life Cycle

Like other newt species, the Smooth Newt has a biphasic life-cycle, with aquatic and terrestrial stages. During the breeding season (typically spring and summer), newts occupy aquatic habitats, while outside of the breeding season, newts inhabit the surrounding terrestrial environment. Smooth Newts are generally nocturnal, but may be active by day and night during the breeding season. In terrestrial habitats, Smooth Newts are relatively inactive, spending most of the time in moist, terrestrial refuges (Griffiths 1984).

In the northern hemisphere, adult Smooth Newts emerge from terrestrial refuges in late-winter, to enter breeding wetlands, where they remain for the duration of the breeding season; juveniles (efts) typically spend the breeding season on land (Bell 1977, Griffiths 1984). Males generally enter breeding wetlands earlier than females, presumably to allow time to develop secondary sexual characteristics, such as the wavy dorsal crest (Griffiths 1984).

Eggs are laid in water and develop into tadpoles with external gills (Plate 4), which then metamorphose into semiaquatic juveniles, capable of breathing on land. Females lay 200 – 300 eggs per season (Arntzen et al. 2009); eggs are laid singly and wrapped in leaves of aquatic vegetation (Bell and Lawton 1975, P. Robertson, obs.), but may sometimes be laid in short strings, if there are no suitable egg-laying sites. Following metamorphosis, efts disperse onto land (Bell 1977). At the end of the breeding season, adult newts also retreat from water and return to land, to over-winter (Griffiths 1984).

The smooth newt is opportunistic and a generalist omnivore; at wetlands the diet primarily includes plant materials and aquatic invertebrates, and sometimes the eggs and larvae of fish and anuran amphibians (frogs) depending on availability (Griffiths 1986). When on land, the diet includes insects, slugs and worms (Flood 2010).

### Habitats

Smooth Newts are highly adaptable and are known to occupy a wide range of habitat types supporting suitable breeding wetlands, including woodlands, meadows, and marshes, and are also known to occur in modified or artificial habitats, such as garden ponds, canals, irrigation drains and other agricultural land.  Like many other amphibian species, Smooth Newts prefer still or slow moving, shallow waterbodies, with relatively stable hydroperiods, which support abundant aquatic vegetation, used to lay eggs and provide protection for tadpoles (Bell and Lawton 1975, Ćirović et al. 2008). Rocks and fallen timber surrounding the wetland provide ideal terrestrial refuges (Griffiths 1984).

The colonisation of new habitats (i.e. wetland sites) is thought to be undertaken primarily by juvenile newts, which disperse following metamorphosis (Bell 1977).  Adults are considered to show high site fidelity, occupying relatively small and well-defined home ranges, and moving only short distances (8 - 50 m) over land, between breeding ponds and terrestrial refuges (Müllner 2001). However, dispersal distances between breeding and over-wintering sites has been found to be dependent on the landscape and surrounding habitat quality, with newts in agricultural areas moving longer distances (e.g. up to 400m) than newts in wooded habitats (Bell 1977, Schmidt et al. 2006). The modified nature of the landscape may have a similar effect on dispersal distances and population dynamics of Smooth Newts in south-east Melbourne.

### 1.1.2 Previous Surveys

The Smooth Newt was first recorded in Victoria from a building site in south-east Melbourne, captured by a member of the public who had collected a bucket of tadpoles from a pool of water. Detection of the Smooth Newt triggered a priority response by the Victorian Government which investigated the site and surrounds, capturing 15 Smooth Newts from an adjacent retarding basin between June and November 2011. A temporary fence with pit-fall traps was consequently set around the site, but was incomplete and therefore, ineffective (DPI 2013a). During spring 2012, rapid surveys were undertaken over an area of approximately 11,000 ha, in an attempt to delimit the extent of incursion (DPI 2013a). These surveys resulted in the removal of 74 smooth Newts from four locations, approximately 4 km from the initial detection site (DPI 2013b).

The spatial extent of the incursion was assessed on the basis of the known infested sites. The gross infested area was estimated to be approximately 900 ha, with a nett infested area of 8 ha (DPI 2013a). However, it was noted that the sampling was of extremely low intensity, with a focus on maximising survey area rather than intensity; many survey sites consisted of a single bait trap deployed over a single night, which conferred little confidence in the estimate (DPI 2013a).

Further trapping surveys were undertaken in 2013 by Tingley et al. (2015) to determine the geographic origin of the Smooth Newts through DNA sequencing, and to confirm the persistence of the species in the area. These surveys included four of the six initial incursion sites and three additional sites; Smooth Newts were captured from six of the seven sites, including all four of the original sites (Tingley et al. 2015). Tingley et al. (2015) noted that the highly disjunct distribution of the species across the survey area suggested that the species may have spread considerably, and was likely to be more widespread than initial surveys showed. Environmental DNA (eDNA) sampling was undertaken concurrently, and also revealed higher detection probabilities than the live trapping with bottle traps (Smart et al. 2015).

### 1.1.3 Objectives

The objectives of this survey were to:

- Determine the ongoing persistence of Smooth Newts in areas where they were initially detected (i.e. during delimitation surveys for Smooth Newts in 2012 and 2013);
- Identify any new sites occupied by Smooth Newts, which may indicate the spread of the species beyond the estimated range of initial incursion; and
- Trial additional survey techniques, to contribute to an understanding of detection of Smooth Newts in Australia, using various methods.

# 2 Survey Area

The currently known distribution of Smooth Newts in Victoria is limited to the south-eastern suburbs of Melbourne. Surveys for the Smooth Newt in 2016 were undertaken in the vicinity of known incursion sites.

Much of the survey area was covered by an extensive freshwater wetland system, forming part of a large swamp. Drainage of the swamp commenced in 1876 to facilitate settlement of the area and development of agriculture, which resulted in the construction of numerous narrow drainage lines throughout the area, which drain to Port Phillip Bay. By the 1960s the former swamp area had been largely urbanised; the area is now characterised by high-density urban development, with pockets of larger, semi-rural landholdings, intersected by a network of waterways and drains (Melbourne Water 2017).

## 2.1 Site selection

A total of 21 sites were selected for survey. Site selection commenced with a review of the locations of previous Smooth Newt detections; these areas were selected for re-survey first, to ascertain the persistence of Smooth Newts. Additional sites in the surrounding area were then selected from an assessment of potential habitat. Proximal habitat was identified using aerial imagery, with on-ground site validation used to select suitable survey sites.

A range of habitats were targeted, including slow-flowing waterways and backwaters, roadside drains, retarding basins, stormwater treatment wetlands and dams. Suitable sites were selected based on water level, expected hydrological regime and aquatic vegetation cover. In particular, ephemeral and intermittent waterbodies were targeted as these sites are less likely to support predatory fish, and therefore, may provide better quality breeding habitat (see Bell and Bell 1995, Hamer and McDonnell 2008). Consideration was also given to the proximity of more permanent waterways and wetlands.

The sites surveyed as part of this investigation are not shown to prevent illegal collection of Smooth Newts.

# 3 Methods

## 3.1 Field Surveys

Field surveys were undertaken during the expected breeding period for Smooth Newts in Victoria. Sites were surveyed between 20 September and 21 November 2016, using a variety of methods either singly or in combination. Trapping was undertaken at 17 sites, while water samples were collected for eDNA-based detection at four trapping sites, and at a further four sites. The methods used at each site are provided in Table 2. Each site was surveyed at least once; two trapping surveys were undertaken at two sites, where relatively large numbers of Smooth Newts have previously been recorded (see Table 2).

### 3.1.1 Survey techniques

Techniques used to survey for Smooth Newts within their natural range, include: searches of terrestrial refuge habitats; egg searches; spotlighting; dip-netting; and bottle traps (see Griffiths et al. 1996, Meehan 2013, Sewell et al. 2013), which target the different life-stages and habitats used by the species. Of these, trapping techniques are amongst the most reliable (Griffiths et al. 1996).

In Australia, targeting wetland habitats where newts congregate to breed during the breeding season is currently considered to provide the highest likelihood of detecting the species (DPI 2013a). This approach is also considered the most feasible, given the precise distribution of the species and their use of habitats is not known.

Previous surveys have primarily used bait traps (DPI 2013a) and bottle traps (Tingley et al. 2015) to capture Smooth Newts, with the most recent surveys also incorporating a trial of eDNA to detect their presence (see Tingley et al. 2015, Smart et al. 2015). These surveys provided an opportunity to trial additional methods, such as electrofishing and spotlighting, and to supplement traditional techniques with the use of the eDNA. However, these trials were undertaken to maximise survey efficiency and did not seek to formally assess the relative efficacy of various techniques for detection of Smooth Newts.

A combination of five different survey techniques were utilised as part of these surveys, as described below. The techniques employed at each site were dependent upon the site characteristics, including size, water depth, flow, electrical conductivity and vegetation cover. Bait trapping was employed as the standard survey technique at all 17 sites where live trapping was undertaken; all other techniques were supplementary. Detection using EDNA was used as the sole technique at four survey sites and as a supplementary technique at four trapping sites. In addition to those methods, some active searching was undertaken where potential terrestrial refuge was present (i.e. rocks, logs or artificial debris), although this was not undertaken in a standardised manner.

#### Bait trapping

Bait trapping is undertaken using rectangular, collapsible mesh traps, with a funnel entrance at each end (see Plate 5). Bait traps are most effectively deployed in shallow, still water, amongst aquatic vegetation, branches or other forms of cover. Bait trapping was undertaken at 17 sites; a total of 10 bait traps, constructed of 2 mm nylon mesh with 40 mm diameter entrance funnels, was set at each site. Site 16 was considerably larger than other sites, and so 20 bait traps were deployed at that site. Bait traps were typically spaced 5 m apart and baited with 4-inch yellow glow sticks to attract aquatic fauna. Traps were left out over a single night, and retrieved between dawn and 09:00 AEDT the following day.

### Spotlighting and active searching

Spotlight surveys can be used to detect amphibians which are active at night, either through direct encounter or reflected eye-shine. Spotlighting was undertaken at six sites, for a total of approximately 30 minutes at each site. Surveys commenced after dusk and were undertaken using a hand-held spotlight to scan the water surface, aquatic vegetation, and perimeter of the waterbody, including adjacent terrestrial habitat for adult newts, tadpoles or eggs.

### Dip netting

Dip netting involves the use of a hand-held mesh net with a long handle, to sweep through the water along areas of suitable habitat, particularly areas supporting aquatic vegetation.  Dip netting can be used to collect invertebrates, fish and tadpoles (depending on the mesh size). In heavily vegetated waterways or wetlands, dip netting may be one of the only suitable methods available. Dip netting was performed for a total of 10 minutes using a 4mm mesh net, at three sites. The net was swept through areas of suitable habitat, supporting abundant aquatic vegetation, to collect aquatic fauna.

### Electrofishing

Electrofishing is standard survey technique for aquatic species, and with appropriate settings it is particularly effective for small fish and tadpoles. Electrofishing uses a pulsed DC current to attract and temporarily immobilise aquatic species which are collected using insulated dip nets. Electrofishing was trialled at two sites, using a Smith-Root LR24 Backpack Electrofisher, for between 10 and 15 minutes elapsed time at each site. Electrofishing was used to draw aquatic fauna from dense aquatic vegetation, including macrophytes and filamentous algae, which are likely to provide cover for tadpole and adult newts. The area covered by the electrical field was subsequently dip-netted in a manner that enabled stunned aquatic fauna to be drawn towards the surface.

### Environmental DNA (eDNA)

Environmental DNA (eDNA) surveys involve the detection of fragments of species-specific DNA from the environment (i.e. water column). Collection of water samples for subsequent eDNA analysis was undertaken at eight sites. Water samples were collected in duplicate as a minimum at each site (three samples were collected from sites 16 and 20 – larger waterbodies), and filtered on-site by passing up to 500 ml of water through a 0.22 µm filter (Sterivex) using a sterile 60 ml syringe. The volumes of most samples were considerably less than 500 ml as the filters quickly clogged due to high turbidity. Clean (sterile) equipment was used at each site to avoid contamination, and care was taken to avoid the transfer of water or organic material between sites during collection of the water samples. Filters were stored on ice for a maximum of 48 hrs before DNA extraction and analysis in the laboratory.

DNA extraction and analysis was performed by cesar Pty Ltd (Parkville, Victoria). DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen Dneasy Blood and Tissue Kit). Real-time quantitative Polymerase Chain Reaction (qPCR) TaqMan® assays were used to amplify and quantify the target DNA. For each sample, three qPCRs were undertaken and all assays included negative and positive controls. A species-specific genetic probe was developed to detect smooth newt based on *L. v. vulgaris* mitochondrial cytochrome b (CytB) DNA sequence from Tingley et al. (2015), and tested for specificity against other amphibian species known to occur in the region. The probe was found to be specific to *L. v. vulgaris* (see Smart et al. 2015 for more details).  A site was regarded as positive for newt presence if at least one qPCR detected the target DNA. The amount of DNA (calculated as copies of CytB L-1 (CytB/L)) present at a site was averaged between samples (+/- st dev) (J Griffiths, cesar, pers. comm., 2017).

**Table 1 Smooth Newt survey sites, dates and survey techniques**

| SiteNo. | Survey Dates | Survey Technique | | | | |
|---|---|---|---|---|---|---|
| | | BT | DN | EF | S/AS | eDNA |
| 1 | 20/09/2016 | X | X | X | | |
| | 27/10/2016 | X | X | | X | |
| 2 | 20/09/2016 | X | | | | |
| | 5/10/2016 | X | | | | |
| | 27/10/2016 | | | | X | |
| 3 | 20/09/2016 | X | X | X | | |
| 4 | 5/10/2016 | X | | | | |
| 5 | 5/10/2016 | X | | | | |
| 6 | 19/10/2016 | X | | | | |
| 7 | 19/10/2016 | X | | | | |
| 8 | 19/10/2016 | X | | | | |
| 9 | 19/10/2016 | X | | | | |
| 10 | 19/10/2016 | X | | | | |
| | 21/11/2016 | | | | | X |
| 11 | 27/10/2016 | X | | | X | |
| 12 | 27/10/2016 | X | | | X | |
| 13 | 27/10/2016 | X | | | X | |
| 14 | 21/11/2016 | X | | | | X |
| 15 | 21/11/2016 | X | | | | X |
| 16 | 21/11/2016 | X | | | | X |
| 17 | 27/10/2016 | X | X | | X | |
| 18 | 21/11/2016 | | | | | X |
| 19 | 21/11/2016 | | | | | X |
| 20 | 21/11/2016 | | | | | X |
| 21 | 21/11/2016 | | | | | X |

BT = Bait trapping; DN = Dip Netting; EF = Electrofishing; S/AS = Spotlighting/Active Searching; ^ site is at or near a previous detection location (see Figure 2).

### 3.1.2 Species Identification

All vertebrate fauna and decapod crustaceans captured during the surveys were identified to species level, where possible. Adult Smooth Newts were easily identified by patterning and colour and the presence of a tail; tadpoles were identified by the presence of external gills behind the head, and forelimbs, which in caudate tadpoles, develop before the hindlimbs (see Plate 4).

Native species were released at the point of capture. Smooth Newts and fish species listed as 'Noxious' under the *Fisheries Act 1995* were humanely euthanased in accordance with relevant approvals and permits issued under Victorian regulations (see Section 3.2). All Smooth Newts captured were preserved in 90% ethanol. The total number of each species detected at each site was recorded separately for each method employed. Photographs, waypoints and general habitat notes were made at each site.

## 3.2 Permits

Smooth Newts are classified as a *Prohibited Pest Animal* under the Victorian *Catchment and Land Protection Act 1994* (CaLP Act). The CaLP Act prohibits the keeping, importation, sale or release of declared pest animals in Victoria without a permit. Surveys for the Smooth Newt were undertaken in accordance with Permit No. RE87 issued by the Department of Economic Development, Jobs, Transport and Resources under the CaLP Act, and relevant permits and licenses under the Victorian *Fisheries Act 1995* (RP1142), *Wildlife Act 1995* (1007806) and *Protection of Cruelty to Animals Act 1986* (SPFL 01081). Animal capture and euthanasia was undertaken in accordance with animal ethics approval (No. 11.16).

## 3.3 Limitations

The primary aim of these surveys was to establish the ongoing persistence of Smooth Newts at sites where they were first detected in south-east of Melbourne, and secondly, to identify new sites. As such, maximising the spatial coverage of the survey was a primary consideration. However, the duration of the survey was limited to the likely peak breeding period of Smooth Newts in Victoria, which limited the period during which surveys could be carried out. Surveys of each site were therefore, rapid, and in most cases, restricted to a single survey.

Most fauna species cannot be detected with certainty from a single survey, and the detection of Smooth Newts has been found to vary spatially and temporally, and with the use of different techniques (Griffiths et al. 1996, Smart et al. 2015). Given that the detection probabilities for this species in Australian habitats are not known, multiple techniques, including those used to survey Smooth Newts in its native range, and those known to be effective in capturing native amphibian species, were used to increase the probability of detection, wherever possible.

Restrictions on site access, associated with land tenure, placed a further limitation on these surveys; most of the survey effort focused on public land (e.g. roadside drains, retarding basins and constructed wetlands). A small number of sites were located on private land, where permission was granted. However, suitable habitat for the Smooth Newt appears to be distributed across many areas of private land in this region, which have never been surveyed for the Smooth Newt.

# 4 Results

Smooth Newts were recorded at two sites:

1. Site 1; and
2. Site 14.

The largest number of Smooth Newts was captured from Site 1, where the species was first detected in 2012. A total of 90 Smooth Newts was captured over two surveys of this site (Table 3). The first survey resulted in the capture of 37 Smooth Newts, including both adults and tadpoles. A second survey was consequently undertaken, during which 53 Smooth Newt tadpoles were captured (Table 3). The detection of Smooth Newts at Site 14 represents a new location record. Bait trapping at this site resulted in the capture of two Smooth Newt tadpoles from a drain (Table 3), which was also confirmed from e-DNA samples.

A total of 18 eDNA samples were taken from the eight sites (see Appendix 1). One of two samples taken from Site 14 (i.e. where two Smooth Newt tadpoles were captured) returned a positive result for Smooth Newts, with Smooth Newt DNA detected in all three PCR replicates for that sample. The eDNA analysis also returned an equivocal result for one sample, and an inhibited result for a second sample, both of which were collected from the drain adjacent to Site 21. Analysis of the first sample – deemed equivocal – detected Smooth Newt DNA in only one of the three PCR replicates. The trace amounts of DNA detected may indicate that newts are present in low abundance, but warrants further investigation. Inhibition sometimes occurs due to a particular compound in the water, and prevents any amplification of the DNA (J Griffiths, pers. com.), thus, no analysis could be undertaken from that sample. Samples from the remaining six sites returned a negative result for the presence of Smooth Newts (see Appendix 1).

**Table 2 Smooth Newts recorded during surveys in south-east Melbourne, 2016**

| Survey Details | | Smooth Newts | | | |
|---|---|---|---|---|---|
| **Capture Method** | **Date** | **Adult Male** | **Adult Female** | **Tadpole** | **Unknown** |
| **Site 1** | | | | | |
| Electrofishing | 20/09/2016 | - | - | 14 | - |
| Bait trapping | 20/09/2016 | 2 | 7 | 10 | - |
| | 27/10/2016 | - | - | 29 | - |
| Dip netting | 20/09/2016 | - | - | 4 | - |
| | 27/10/2016 | - | - | 24 | - |
| **Site 14** | | | | | |
| Bait trapping | 21/11/2016 | - | - | 2 | - |
| eDNA | 21/11/2016 | n/a | n/a | n/a | + |

+ = positive detection

**Plate 1 Adult male Smooth Newt, in breeding condition (2016)**

**Plate 2 Adult male Smooth Newt, showing orange, spotted underbelly and distended cloaca (2016)**

**Plate 3 Adult female Smooth Newt (2016)**

**Plate 4 Smooth Newt tadpole (2016)**

Native amphibians were detected at all but four sites, each of which, were found to support large numbers of the noxious fish species Eastern Gambusia *Gambusia holbrooki*. No Eastern Gambusia were detected at either site where Smooth Newts were captured, although native frogs, including adults and tadpoles of four species, were collected in relatively good abundance.

Four species of fish were detected, all of which are exotic and three of which are declared 'Noxious' species under the Victorian *Fisheries Act 1995*. The noxious Eastern Gambusia and Oriental Weatherloach *Misgurnus anguillicaudatus* were detected at seven and five sites respectively while mature Carp *Cyprinus carpio* was observed during eDNA sampling. A single young-of-year Goldfish *Carassius auratus* was collected from a bait trap. Three decapod crustacean species were also detected (Table 4).

A summary of the species captured at each site is provided in Table 4; a breakdown of species captured at each site by survey method is given in Appendix 2.

**Table 3 Fauna species recorded from trapping surveys at sites 1 to 17 (September to November 2016)**

| Species | | Survey Site Number | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Common Name** | **Scientific Name** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** |
| Smooth Newt | *Lissotriton vulgaris* | ✓ | | | | | | | | | | | | | ✓ | | | |
| Common Eastern Froglet | *Crinia signifera* | ✓ | ✓ | | ✓ | ✓ | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ |
| Striped Marsh Frog | *Limnodynastes peronii* | ✓ | | ✓ | | | | ✓ | | | | ✓ | | ✓ | ✓ | | ✓ | ✓ |
| Spotted Marsh Frog | *Limnodynastes tasmaniensis* | ✓ | ✓ | | | | | ✓ | | | | | | | | | ✓ | ✓ |
| Southern Brown Tree Frog | *Litoria ewingii* | ✓ | | | | | | | | | | ✓ | | | ✓ | ✓ | | ✓ |
| Goldfish* | *Carassius auratus* | | | | | | | | | | | | | | | | ✓ | |
| Oriental Weatherloach* | *Misgurnus anguillicaudatus* | ✓ | | | | | | | | ✓ | | ✓ | ✓ | | | | | |
| Eastern Gambusia* | *Gambusia holbrooki* | | | | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | |
| Freshwater Shrimp | *Paratya australiensis* | | | | | | ✓ | | ✓ | | | ✓ | | | | | | |
| Common Yabby | *Cherax destructor* | | | ✓ | | ✓ | | | | ✓ | | | | | | | | ✓ |
| Granular Burrowing Crayfish | *Engaeus cunicularius* | | | ✓ | | | | | | | | | | | | | | |

* Introduced species

**A**

**C**

**B**

**D**

**Plate 5 Smooth Newt habitats in south-east Melbourne**

**A – Site 1, roadside drain showing where tadpoles were captured using electrofishing (2016);**

**B – Site 1, roadside drain showing capture location of adult Smooth Newts using bait trapping (2016);**

**C – Site 14, new site record for Smooth Newts, roadside drain showing capture location of tadpoles using bait traps (2016);**

**D – Site 2, ephemeral wetland where Smooth Newts were initially captured in 2012.**

# 5 Discussion

The results of these surveys confirm the persistence of Smooth Newts in south-east Melbourne, five years after the species was first detected living outside of captivity in Australia, with the capture of Smooth Newts at one of the previous detection sites and at a newly documented site (see Figure 1). Both sites offer suitable breeding habitat, as indicated by the presence of Smooth Newt tadpoles. Both sites were shallow (<0.5 m), supported a dense cover of emergent macrophytes and mats of floating filamentous algae, are subject to periodic inundation and drying, and are consistent with documented habitat requirements for the Smooth Newt within in its native range (e.g. see Griffiths 1984).

A total of 90 individuals were captured from the drainage line at Site 1, including nine adult and 81 tadpole newts, the largest number collected from any site to-date; 48 newts were captured from around this site during earlier surveys (DPI unpubl. data). The persistence of newts at this site, and the large numbers of tadpoles captured, suggests that this site provides high quality breeding habitat for the species.

Smooth Newt tadpoles were also captured at Site 14, where the species has not been recorded previously. The detection of Smooth Newts at this site is significant as it not only represents a new location, but is situated east of a large waterway, where newts have not been previously recorded. All known infestation sites are located along drainage channels of an adjacent waterway, on the western side of the creek. Trapping was undertaken to the east of this creek in 2012, between c. 500 m and 1.25 km south of Site 14, but failed to detect any newts (DPI unpubl. data).

The results of this survey suggest that the species may have crossed the lower reaches of the large creek, which is a larger than the waterway system in which they were previously known. Despite such habitats reportedly not being favoured by the species, this would indicate that larger, flowing waterways do not necessarily represent a physical barrier to the dispersal of Smooth Newts. Alternately, it has been suggested previously, that Smooth Newts may have been deliberately 'seeded' at multiple sites (DPI 2013a), rather than having dispersed from a single site, based on the distribution of a small number of sites which are separated by relatively large distances.

The original source, timing and the spatial extent of the incursion remains unknown. However, home range, dispersal patterns and movements of recently naturalised species are typically more erratic and larger than in their native habitats (see DPI 2013). Dispersal distances of the Smooth Newt are also likely to be dependent on the quality of surrounding habitat, with immigration distances of up to 400 m recorded in agricultural areas of Europe (Blab 1986 in DPI 2013, Müllner 2001). Given the modified hydrology of this region south-east of Melbourne, it is likely that the potential for widespread dispersal of Smooth Newts is high and that localised flooding and water re-direction would further facilitate dispersal (DPI 2013). Therefore, it would be prudent to presume that detections from multiple sites are the likely result of dispersal, until there is evidence to the contrary.

The detection of Smooth Newts east of the large is also concerning for the potential threat it represents to populations of the threatened Dwarf Galaxias *Galaxiella pusilla* fish which are known to near site 14. Although, the NEBRA national significance assessment (ABARES 2013) suggested that Smooth Newts would not occupy Dwarf Galaxias habitats, that assessment was based on a single paper, demonstrating negative impacts of an invasive North American Crayfish (*Procambarus clarkia*) on Smooth Newts, and by extension, concluded that Smooth Newts would avoid Dwarf Galaxias habitats also occupied by crayfish species.

Interestingly, no Smooth Newts were detected at Site 10, where 15 newts were initially captured in 2011, from either trapping surveys or eDNA. Similarly, no newts were captured at the wetland at Site 2, where 68 smooth newts were captured during early delimitation surveys (DPI 2013b), despite repeated survey effort, and the use of multiple survey techniques. Drainage works have been undertaken in the region since 2012, and it is also possible that local changes to hydrology have altered the suitability of habitat and, possibly, the distribution or detection of Smooth Newts. Detection probabilities for Smooth Newts have been found to vary spatially and temporally, as well as with survey technique and site characteristics (Griffiths et al. 1996, Smart et al. 2015).

Although the true spatial distribution and extent of the Smooth Newt incursion cannot be determined from the previous or current surveys, results of these surveys suggest that the infested area is likely to be larger than originally estimated. All surveys to-date, have been rapid and limited with regard to survey intensity, and the use of multiple survey techniques. Further survey work is required to determine the distribution of Smooth Newts in the south-eastern region of Melbourne. Future surveys should concentrate on habitats east of the large creek at Site 14 and on the wetland habitats to the north. Multiple survey techniques should also be used.

The current surveys trialled the use of electrofishing to capture Smooth Newts for the first time. The technique was found to be successful and captured the most individuals for least survey effort (time), although no adults were captured using this method. It is possible that habitat use by adult newts differs diurnally and nocturnally; with adult newts possibly only entering the water at night. Electrofishing may be an effective and rapid surveillance method; however further trials are warranted, including diurnal and nocturnal comparisons.

This study appears to provide further confirmation of the high degree of spatial and temporal variability of Smooth Newt distribution, habitat use, and/or detectability in the incursion area, in addition to the potential for ongoing dispersal and spread. Although rapid, single surveys offer the most cost-effective approach to maximise detections across a large area (i.e. by maximising the number of sites surveyed), Smart et al. (2015) showed that detection probabilities associated with traditional capture methods and eDNA varies substantially across days and months, and that both methods can give independent information on distribution, particularly when comparing single surveys (Smart et al. 2015). Considering that newly established species are typically patchily distributed, integration of eDNA with traditional survey techniques is likely to increase the probability of detection, particularly if the species is present in low abundance.

There is an urgent need to re-evaluate the extent of incursion and potential for control; both 2016 detection sites are small and appear conducive to control through water level manipulation (draining) and/or physical removal of Smooth Newts. It is also imperative to re-consider potential long-term impacts and costs. The cost of delineating the true extent of incursion and the subsequent control will have increased in the five years since the first detection and will continue to grow over time, until a threshold is reached where the cost of control is prohibitive. We believe there is insufficient evidence to suggest that this threshold has already been crossed.

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# 7 Glossary

| | |
|---|---|
| Anuran | Without a tail. Specifically, tailless amphibian species belonging to the taxonomic order Anura, including frogs and toads. |
| Bioregion | Defined geographical regions of Australia with similar climatic and geophysical characteristics, and which generally contain a suite of distinct ecosystems and species |
| CaLP Act | Victorian *Catchment and Land Protection Act 1994* |
| Caudate | Latin for tail. Specifically, amphibian species which possess a tail belonging to the taxonomic order Caudata, including salamanders and newts. |
| Cloaca | A Posterior ventral opening of the shared alimentary canal, urinary tract and reproductive tract. Cloacal swelling in caudate amphibians occurs due to the enlargement of cloacal glands. |
| Dispersal | Movement away from an existing population or area of habitat to another |
| Dorsal | Referring to the back or uppermost surface |
| Eft | A juvenile newt at the stage of development where it has become fully terrestrial |
| Exotic | Plants, animals, fungi and other organisms that have been introduced (deliberately or accidentally) to Australia or a given area after European settlement |
| Home range | The geographic area in which an animal normally ranges as part of its daily activities |
| Hydroperiod | The period of time during which a wetland or waterway holds water |
| Introduced | Deliberately or accidentally brought to Australia or part of Australia, usually by human agency |
| Macrophyte | Large aquatic plant that with an emergent, submergent or floating life-form |
| Metamorphose | Post embryonic developmental change which involve structural and physiological and behavioural change. |
| Neurotoxin | A toxin which acts upon the nervous system and affects the function of nerve tissue |
| Nocturnal | Most active at night, or during the period of darkness |
| Tadpole | Larval phase of amphibian species, which is wholly aquatic |
| Taxonomic order | A taxonomic rank, used in the classification of organisms, below that of Kingdom, Phylum and Class. |

## Appendix 1 Results of eDNA surveys 2016

| Site | Sample Code | Volume | No. Positive PCRs (of 3) | Result |
|---|---|---|---|---|
| 10 | RB1 | 170 | 0 | negative |
| | RB2 | 250 | 0 | negative |
| | RB3 | 250 | 0 | negative |
| 14 | BG10 | 185 | 3 | **positive** |
| | BG11 | 190 | 0 | negative |
| 15 | BGW12 | 125 | 0 | negative |
| | BGW13 | 80 | 0 | negative |
| 16 | BP14 | 125 | 0 | negative |
| | BP15 | 150 | 0 | negative |
| | BP16 | 100 | 0 | negative |
| 18 | BB6 | 170 | 0 | negative |
| | BB7 | 150 | 0 | negative |
| 19 | WB8 | 135 | 0 | negative |
| | WB9 | 80 | 0 | negative |
| 20 | MD17 | 95 | 0 | Negative |
| | MD18 | 115 | 0 | negative |
| 21 | SC4 | 160 | 1 | **equivocal** |
| | SC5 | 80 | 0 | **inhibited** |

## Appendix 2 Fauna species captured at each survey site, by survey method

| Site Number | Species | | BT | DN | EF | S/AS |
|---|---|---|---|---|---|---|
| 1 | Common Eastern Froglet | *Crinia signifera* | ✓ | ✓ | ✓ | ✓ |
| | Striped Marsh Frog | *Limnodynastes peronii* | ✓ | ✓ | ✓ | ✓ |
| | Spotted Marsh Frog | *Limnodynastes tasmaniensis* | ✓ | | | ✓ |
| | Smooth Newt | *Lissotriton vulgaris* | ✓ | ✓ | ✓ | |
| | Oriental Weatherloach | *Misgurnus anguillicaudatus* | ✓ | | ✓ | ✓ |
| 2 | Common Eastern Froglet | *Crinia signifera* | | | | ✓ |
| | Spotted Marsh Frog | *Limnodynastes tasmaniensis* | | | | ✓ |
| 3 | Granular Burrowing Crayfish | *Engaeus cunicularius* | ✓ | | ✓ | |
| | Common Yabby | *Cherax destructor* | | | ✓ | |
| | Striped Marsh Frog | *Limnodynastes peronii* | ✓ | ✓ | ✓ | |
| 4 | Eastern Gambusia | *Gambusia holbrooki* | ✓ | | | |
| | Common Eastern Froglet | *Crinia signifera* | ✓ | | | |
| 5 | Common Yabby | *Cherax destructor* | ✓ | | | |
| | Common Eastern Froglet | *Crinia signifera* | ✓ | | | |
| | Southern Brown Tree Frog | *Litoria ewingii* | ✓ | | | |
| 6 | Eastern Gambusia | *Gambusia holbrooki* | ✓ | | | |
| | Freshwater Shrimp | *Paratya australiensis* | ✓ | | | |
| 7 | Striped Marsh Frog | *Limnodynastes peronii* | ✓ | | | |
| | Spotted Marsh Frog | *Limnodynastes tasmaniensis* | ✓ | | | |
| | Common Eastern Froglet | *Crinia signifera* | ✓ | | | |
| 8 | Eastern Gambusia | *Gambusia holbrooki* | ✓ | | | |
| | Freshwater Shrimp | *Paratya australiensis* | ✓ | | | |
| | Common Yabby | *Cherax destructor* | ✓ | | | |
| 9 | Eastern Gambusia | *Gambusia holbrooki* | ✓ | | | |
| | Southern Brown Tree Frog | *Litoria ewingii* | ✓ | | | |
| | Oriental Weatherloach | *Misgurnus anguillicaudatus* | ✓ | | | |
| 10 | Eastern Gambusia | *Gambusia holbrooki* | ✓ | | | |
| 11 | Common Eastern Froglet | *Crinia signifera* | ✓ | | | ✓ |
| | Eastern Gambusia | *Gambusia holbrooki* | ✓ | | | ✓ |
| | Oriental Weatherloach | *Misgurnus anguillicaudatus* | ✓ | | | ✓ |
| | Freshwater Shrimp | *Paratya australiensis* | ✓ | | | |
| 12 | Eastern Gambusia | *Gambusia holbrooki* | ✓ | | | ✓ |
| | Oriental Weatherloach | *Misgurnus anguillicaudatus* | ✓ | | | ✓ |
| | Common Eastern Froglet | *Crinia signifera* | ✓ | | | ✓ |

| Site Number | Species | | BT | DN | EF | S/AS |
|---|---|---|---|---|---|---|
| 13 | Common Eastern Froglet | *Crinia signifera* | ✓ | | | ✓ |
| | Striped Marsh Frog | *Limnodynastes peronii* | | | | ✓ |
| 14 | Common Eastern Froglet | *Crinia signifera* | ✓ | | | ✓ |
| | Striped Marsh Frog | *Limnodynastes peronii* | ✓ | | | |
| | Smooth Newt | *Lissotriton vulgaris* | ✓ | | | |
| | Southern Brown Tree Frog | *Litoria ewingii* | ✓ | | | |
| 15 | Common Eastern Froglet | *Crinia signifera* | ✓ | | | |
| | Southern Brown Tree Frog | *Litoria ewingii* | ✓ | | | |
| 16 | Goldfish | *Carassius auratus* | ✓ | | | |
| | Striped Marsh Frog | *Limnodynastes peronii* | ✓ | | | |
| | Spotted Marsh Frog | *Limnodynastes tasmaniensis* | ✓ | | | |
| 17 | Common Yabby | *Cherax destructor* | ✓ | | | |
| | Spotted Marsh Frog | *Limnodynastes tasmaniensis* | ✓ | ✓ | | |
| | Oriental Weatherloach | *Misgurnus anguillicaudatus* | ✓ | | | |
| | Common Eastern Froglet | *Crinia signifera* | | ✓ | | ✓ |
| | Spotted Marsh Frog | *Limnodynastes tasmaniensis* | | | | ✓ |
| | Southern Brown Tree Frog | *Litoria ewingii* | | | | ✓ |

BT = Bait trapping; DN = Dip Netting; EF = Electrofishing; S/AS = Spotlighting/Active Searching