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Artificial egg-strip surveying for detection of pond-breeding newts: Does egg-strip substrate matter?

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Abstract. Survey methods for detecting newts (Salamandridae: Pleurodelinae) in ponds by identifying presence of eggs laid during their aquatic breeding condition are useful and often deployed across various population surveillance activities. Egg-strip surveying is an effective way of detecting newt presence in a pond by providing an egg-laying medium for newts that can be selectively inspected by a surveyor. In this study we deployed a series of different plastic substrates to investigate substrate laying preference by three species of newts; *Lissotriton helveticus*, *L. vulgaris* and *Triturus cristatus*. Our results revealed that *L. helveticus* and *L. vulgaris* significantly preferred green and longer varieties of black egg-strip substrate over a thicker less pliable plastic substrate. Contrastingly *T. cristatus* exhibited no significant preference between substrates. Results from this study indicate that if surveyors choose to use black long, black short, black, green, yellow, or red coloured substrates, or thicker black plastic substrates for egg-strip surveys, detection for *T. cristatus* remains constant but with improved detection and mild preference on more pliable plastic substrates. However, for *L. vulgaris* and *L. helveticus* the thicker gauge black egg-strips may significantly reduce the effectiveness of detection and are discriminated against compared to more pliable grades of green and longer varieties of black plastic substrate. We recommend that surveyors constructing and deploying their own home-made egg-strip substrates should carefully consider their choice of which plastic substrate material to use in constructing the equipment.

Key words. Amphibia, Caudata, egg-strip, oviposition, substrate preference, *Lissotriton helveticus*, *Lissotriton vulgaris*, *Triturus cristatus*.

Introduction

Britain has three native Pleurodelinae newts; *Triturus cristatus* (great crested newt), *Lissotriton helveticus* (palmate newt) and *Lissotriton vulgaris* (smooth newt). All three are semi-aquatic, bi-phasic amphibians, becoming aquatic to breed from March to early June (Blab 1986, Griffiths 1995, Wells 2007). The British newt species all have a similar mode of reproduction in which eggs are laid among vegetation and exotrophic tadpoles live in still water till full development into neonates (Salthe 1969, Wells 2007).

Female *T. cristatus*, *L. helveticus* and *L. vulgaris* usually oviposit their eggs on aquatic plants (Norris & Hosie 2005, Norris 2008). Although *T. cristatus*, *L. helveticus* and *L. vulgaris* do not guard their eggs during incubation their reproductive strategy can reduce embryonic mortality. British newts usually wrap their eggs among aquatic vegetation providing a higher survivorship by protecting eggs from UV radiation and reducing predation from other newts and aquatic invertebrates (Miaud 1993, Miaud 1994, Marco et al. 2001, Orizaola & Braña 2003, Pérez-Santigosa et al. 2003, Wells 2007, Tóth et al. 2011). In other species female newts may also choose an oviposition site which maximises egg survival. *Taricha granulosa* (rough-skinned newts) select oviposition sites away from their offspring's primary predator, Trichoptera larvae (caddisfly), and deposit eggs higher in the water column when caddisflies are nearby (Gall et al. 2012). Alternatively females may choose sites to maximise oviposition success. For example, *Ichthyosaura alpestris* (alpine newts) can select oviposition sites by temperature, preferring 17°C compared to lower temperatures of 12°C and higher temperatures of 22°C (Kurdíková et al. 2011).

Pleurodelinae newts are surveyed for a variety of reasons including; population surveillance, management plan related surveys and assessing the impact of land developments on the species. Therefore, it is important that techniques are accurate and effective at detecting species presence. In the United Kingdom Pleurodelinae have been successfully surveyed during their aquatic phases using a variety of techniques that include torchlight survey, funnel-trapping,

Table 1. Egg-strip substrate types and dimensions.

Egg-strip substrate	Gauge	Width (cm)	Length (cm)	Number of strips	Width of strips (cm)
Black long	160	30	50	24	2.5
Black thick	500	30	25	24	2.5
Black short	160	30	12.5	24	2.5
Green	160	30	25	24	2.5
Black	160	30	25	24	2.5
Yellow	160	30	25	24	2.5
Red	160	30	25	24	2.5

netting and egg searching on natural or artificial substrates (Grayson et al. 1991, Gent & Gibson 1998, Langton et al. 2001, English Nature 2001, Sewell et al. 2013, Wilkinson 2015). All these techniques have their merits and most have been widely researched. However, egg searching using artificial egg-strips has not been so thoroughly researched to hone its effectiveness as a detection method, or achieved finer guidance on best material choices.

Pleurodelinae have been found to exhibit oviposition substrate preferences between aquatic plant species, some artificial substrates, colours and thicknesses (Miaud 1995, Araus 2007, Norris 2008). Studies have also revealed oviposition depth preferences (Miaud 1995). It is possible that a surveyor's choice in egg-strip substrate may affect a survey's efficiency. This study sampled a variety of egg-strip substrates to determine potential preferences by three British Pleurodelinae. To investigate if artificial substrates vary in their effectiveness of detection and/or species preference a range of substrate thicknesses, colours and lengths were used.

Materials and methods

Ten ponds with known newt presence were surveyed using egg-strips from March to June 2015 with egg-strips being deployed in March. Seven ponds were located in Devon and three in Dorset in the UK. Exact pond localities are not disclosed herein to protect the sensitivity of the sites. Devon ponds were coded M, P1, V1, V2 and V3 and had known presence of *T. cristatus* and *L. helveticus*. Devon ponds coded STV1 and STV2 had known presence of *L. helveticus* and *L. vulgaris*. Dorset ponds coded CR1, CR2 and GP1 had known presence of *T. cristatus*, *L. helveticus* and *L. vulgaris*.

All ponds were assessed using a modified version of Oldham et al.'s (2000) *T. cristatus* Habitat Suitability Index, as described in ARG UK (2010) advice note 5. Each were scored on their geographic location, pond area, pond permanence, water quality, shade, waterfowl impact, fish impact, pond count, terrestrial habitat and macrophyte cover. Pond temperature was also taken during each visit using a temperature probe. These components were recorded to monitor any environmental variables which may have affected egg-strip survey effectiveness and egg-strip substrate preference during the experiment.

Egg-strip substrates were constructed using black, red, yellow and green plastics. Dimensions and gauges are shown in Table 1. The black egg-strip substrate was used as a control as it is the most common material used in egg-strip surveys (Freshwater Habitats Trust 2013). The yellow, green and red egg-strip substrates were used to test for colour preference. The black thick egg-strip substrate was used to test thickness preference. Black long and black short egg-strip substrates were used to test length preference. Control lengths of plastic differed by 5 cm to the Freshwater Habitats Trust's (2013) guidelines to minimise plastic waste as maximum width of the bin liners was 50 cm.

Egg strips were cut with the fold of the bin liner intact with a 2.5 cm gap between the folded edge and start of the egg-strips to keep the plastic together. The egg-strips were unfolded and pierced in the middle, then placed on canes with 2 strips tied around the cane to prevent slippage down the cane. An elastic band was also placed coiled approximately 10 cm above the base of the cane. One strip of the control and each variable were attached to each cane to minimise any effect microhabitat may have on oviposition and egg-strip substrate preference. Strips were placed on the cane in a set order with black long at the top followed by black thick, black short, green, black, yellow and red at the bottom respectively. Figure 1 shows a schematic of a cane with egg-strip 'mops' deployed in a pond. Ten canes with egg-strip mops were placed ad-hoc in each pond in shallow areas no further than 1m from the pond margin (Fig. 2).

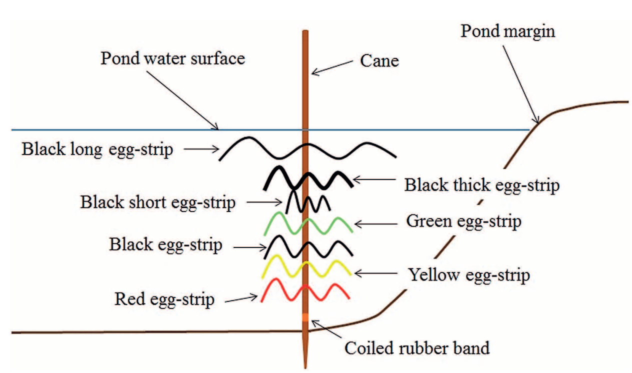

Figure 1. A schematic of a cane with egg-strip 'mops' deployed in a pond.

Egg-strips were checked six times from March to June, with a minimum of seven days between checks, to minimise disturbance. The amount of freshly-laid eggs on each different substrate were counted on each check (Fig. 2). Any eggs that had commenced developing were considered to have been counted previously. Newt eggs were

Figure 2. Egg-strips deposited in two of the ponds and *T. cristatus* eggs deposited on the egg-strip substrates. (A) Egg-strips deployed in pond; (B) egg-strips deployed in pond; (C) *T. cristatus* egg deposited on green egg-strip substrate; (D) *T. cristatus* egg deposited on red egg-strip substrate; (E) *T. cristatus* egg deposited on black egg-strip substrate.

identified as either *T. cristatus* eggs or *L. helveticus/L. vulgaris* eggs, as the eggs of *L. helveticus* and *L. vulgaris* cannot be adequately distinguished in the field. Where possible, eggs found folded into the egg-strip substrates were not unfolded to minimise disturbance and predation risk. All the egg-strips were left in the ponds until September of the study year to allow remaining eggs to develop without disturbance and ensure all eggs laid on the substrate had a chance to hatch. At the end of the breeding season in mid–late September 2015, all egg-strips were checked and removed from the pond.

An unstacked One-way ANOVA was used to test for significant differences between egg-strip substrate types. Statistical tests were completed using MINITAB™ Ver. 16.

Results

Pond environmental variables

Pond environmental variables were measured using temperature, macrophyte cover and the habitat suitability index. Table 2 shows mean environmental variables for the 6 visits to each pond and pond type.

Table 2. Mean pond environmental variables.

Pond ID	Pond type	Temperature (°C)	Macrophyte cover (%)	HSI Score (0–1)
CR1	Mature	16.08	80.00	0.98
CR2	Mature	17.23	95.00	0.78
GP1	Mature	16.65	22.50	0.84
M	New	16.37	5.00	0.71
P1	Serviced	14.28	40.00	0.76
STV1	Mature	14.90	95.00	0.75
STV2	Mature	16.07	19.17	0.86
V1	Recent	17.03	40.00	0.74
V2	Recent	17.32	10.00	0.69
V3	Recent	18.25	45.00	0.74

Egg-strip substrate preference

A total of 5957 *L. helveticus/L. vulgaris* eggs were counted across 9 ponds and a total of 2,938 *T. cristatus* eggs were counted across 7 ponds. Total numbers of *L. helveticus/L. vulgaris* and *T. cristatus* eggs in each pond is shown in Figure 3. No eggs of *L. helveticus/L. vulgaris* or *T. cristatus* were detected in pond P1. Pond P1 was omitted from further statistical tests. Total number of newt eggs laid on each egg-strip substrate and mean are shown in Table 3.

Table 3. Total N newt eggs laid on each egg-strip substrate with means in brackets.

Substrate type	Total *T. cristatus* eggs laid on each substrate, means in brackets	Total *L. vulgaris/ L. helveticus* eggs laid on each substrate, means in brackets
Red	168 (2.80)	469 (7.82)
Yellow	210 (3.50)	697 (11.62)
Green	526 (8.77)	1623 (27.05)
Black	435 (7.25)	1006 (16.77)
Black short	217 (3.62)	580 (9.67)
Black long	1362 (22.70)	1554 (25.90)
Black thick	20 (0.33)	28 (0.47)

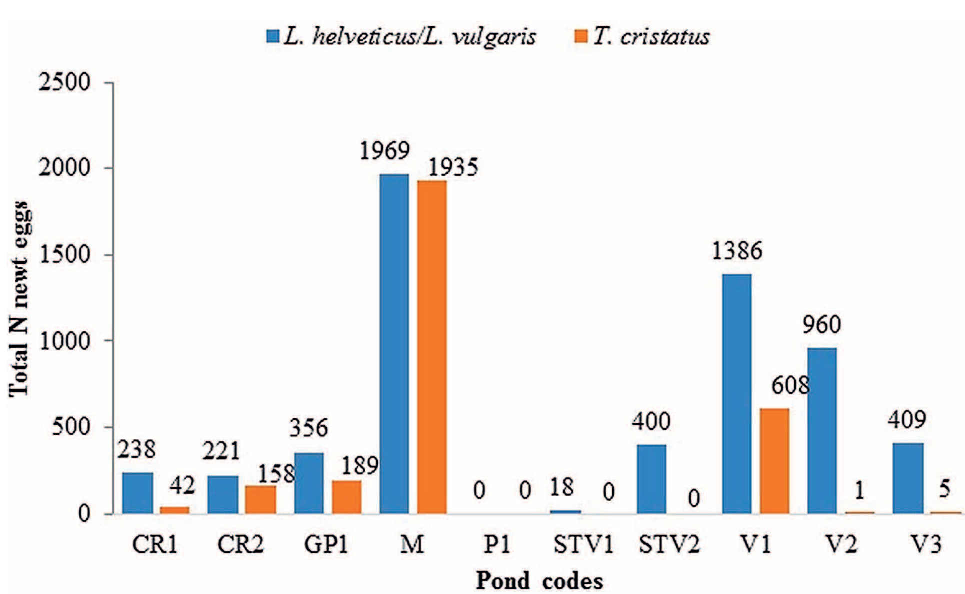

Figure 3. Total N *L. helveticus/L. vulgaris* and *T. cristatus* eggs per pond.

An unstacked one-way ANOVA with Tukey's family error post-hoc test was completed to determine any significant differences in the amount of eggs deposited on each substrate type. For *L. helveticus*/*L. vulgaris,* results from 9 ponds were used. Results from P1 were omitted, as no *L. helveticus*/*L. vulgaris* eggs were detected. There was a clear significant difference between eggs laid on each substrate ($F(6,56) = 2.79$, $p = 0.019$). A post-hoc Tukey's family error rate revealed significant differences between black long and black thick, and green and black thick egg-strip substrates (Fig. 4) with newts preferring black long and green egg-strip substrates over black thick egg-strip substrate.

Results from 7 ponds were used for *T. cristatus*. Results from P1, STV1 and STV2 were omitted as no *T. cristatus* eggs were detected. There was no significant difference between eggs laid on each substrate by *T. cristatus* ($F(6,42) = 1.39$, $p = 0.240$). Post-hoc Tukey's family error rate also showed no significant difference between eggs laid on each substrate for *T. cristatus* (Fig. 4).

Discussion

The use of egg-searching as a survey method can be useful in detection of *T. cristatus* and British *Lissotriton* spp. alongside other survey techniques (Wilkinson 2015). However, just as Griffiths et al. (1996) reported for the techniques of funnel-trapping, netting, head counts by torchlight and egg-searches in vegetation, this study found that egg-searches using egg-strips were not 100% successful in detecting presence. This was not only because eggs of *L. helveticus* and *L. vulgaris* are indistinguishable in the field, but also the method failed to identify presence of *L. helveticus* and *T. cristatus* in pond P1 where both species were known to be present.

Griffiths et al. (1996) discussed egg searching of vegetation, but did not mention egg-strip surveys using egg-strips. Wilkinson (2015) briefly summarised the concept of egg-mop use and construction but did not provide specific detail describing the techniques use or limitations.

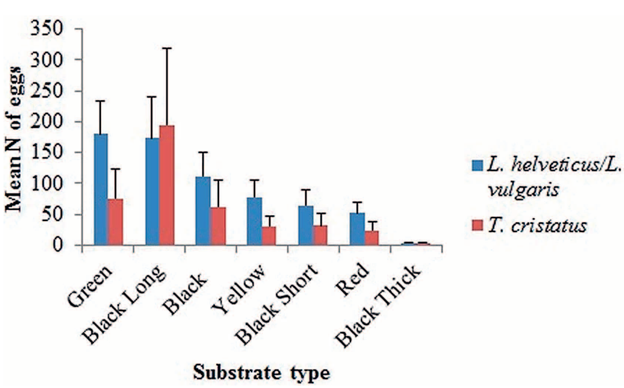

Figure 4. Results from Tukey's family error rate post-hoc test, means and standard error.

We believe describing such method could better aid surveyor's choice of techniques. Griffiths et al. (1996) discouraged use of egg-searching of vegetation in ponds with little or no vegetation, but did not suggest that in place of this method egg-strips could be used. This study used a variety of ponds with varying macrophyte cover, in order to better understand the natural in-situ variation typical of many newt ponds in the UK. Pond M possessed the lowest vegetation cover, but had the highest number of eggs of both *L. helveticus*/*L. vulgaris* and *T. cristatus*. In the absence of aquatic vegetation, it is possible that provision of egg-strip mops are not only a useful survey technique, but might also assist breeding populations. In newly developing ponds, such as those made for conservation of the species and/or mitigation for development, the provision of artificial egg-strips could discourage newts from laying eggs on debris on the pond floor by providing a more suitable egg-laying substrate. Eggs laid on the pond floor may be subject to potentially lower O_2, which in turn may limit respiratory metabolism during embryonic development, and their risk of predation by Trichoptera may be higher (Hopkins & Hanford 1943, Miaud 1995). Also provision of egg-strips where a pond lacks suitable oviposition sites may prevent females from leaving the pond without ovipositing, or from laying strings of eggs which can potentially suffer from UV degradation and/or higher predation risks (Bell & Lawton 1975, Miaud 1993, Miaud 1994, Marco et al. 2001, Orizaola & Braña 2003, Pérez-Santigosa et al. 2003).

Pond P1 was excluded from statistical tests as no eggs of *L. helveticus*/*L. vulgaris* or *T. cristatus* were detected on the egg-strips for the whole survey period. However, torchlight surveys conducted for monitoring of this refurbished pond confirmed presence of *L. helveticus* and *T. cristatus* (R. Griffin & T. Lewis pers. obs.). It would appear in this pond that newts may prefer natural vegetation over egg-strip substrates. Although population abundance is not presented herein for any of the ponds, it is possible that pond P1 may have had fewer newts attending it, and therefore reduced numbers of eggs laid, because it was excluded by newt fencing as part of a nearby development project.

The results of this study suggest that *L. helveticus*/*L. vulgaris* has a preference for green egg-strip substrate and black long egg-strip substrate over black thick egg-strip substrate. Therefore for *L. vulgaris*/*L. helveticus* using black thick egg-strips may significantly affect the efficiency of a survey compared to deploying green and black long egg-strips. However, preferences between the two species cannot be made as their eggs are virtually indistinguishable in the field.

Our results may be skewed towards *L. helveticus,* which was known to be present in all 10 ponds, whereas *L. vulgaris* was only previously known to be present in 5 of the ponds. This substrate preference may be partially explained by results from Miaud (1995) that found *L. helveticus* preferred thin substrates of 100 gauge plastic over 200 gauge and 400 gauge plastics. The black thick egg-strip substrate had the least amount of *L. helveticus*/*L. vulgaris* eggs laid on it, however, there was no significant difference between the amount of eggs laid on the 160 gauge red, yellow, black and black short egg strips when compared to the 500 gauge black thick egg-strips. Therefore there could be other variables affecting egg-strip substrate preference. Miaud (1995) also found that *L. helveticus* had a depth preference, preferring to lay eggs 0–10 cm below the water surface. In this study it is likely that black long egg-strips were within this depth range of 0–10 cm and therefore this might account for a preference toward this substrate. Alternatively as the black long egg-strips were closer to the surface, it is also possible some strips were floating directly on the water surface and therefore gained higher temperatures than fully-submerged egg-strips and those which were further below the water surface. Female newts can have a temperature preference for oviposition, as well as higher temperatures increasing embryonic developmental rates, which may have led to a preference for black long egg-strip substrate (Kurdíková et al. 2011). One possibility for the preference of both black long and green substrate could also be due to presence of predators. Black long egg-strip substrate was installed closest to the water surface followed by black thick and green egg-strip substrates respectively. Gall et al. (2012) found when predatory Trichoptera were nearby female newts, the females moved egg-laying behaviour higher up in the water column to avoid Trichoptera as they are largely benthic. Presence of Trichoptera and Trichoptera larvae was detected at all 10 ponds and this, although unproven, may potentially have influenced higher egg laying on both black long and green egg-strips. The black thick egg-strips may not have been affected in this way due to *L. helveticus* and *L. vulgaris* general preference toward thinner substrates (Miaud 1995, Norris 2008). However, there were no significant differences between the black egg-strip substrate used as the control and the green, black long, black short, black thick, yellow and red egg-strip substrates.

No significant difference was found in the amount of eggs laid on any of the seven different egg-strip substrates for *T. cristatus*. This may be due to their larger body size in comparison to *L. helveticus* and *L. vulgaris,* which may allow them to exploit a larger range of substrate thicknesses (Miaud 1995, Norris 2008). Miaud (1995) found *T. cristatus* preferred to oviposit on the 200 gauge plastic followed by the 400 gauge plastic and 100 gauge plastic with an overall significant preference towards thicker substrates. The majority of the current studies plastics were 160 gauge substrates which varied in colour and length, however, this thickness is close to the 200 gauge intermediate substrate used by Miaud (1995) which was found to be preferred over the 100 and 400 substrate with 67% of eggs laid on the 200 gauge substrate followed by 23% on the 400 gauge substrate and 10% on the 100 gauge substrate. Although there was no significant difference in the amount of eggs laid between the different egg-strip types, the results suggest use of the black long egg-strips, green or black egg-strip substrates may slightly increase the amount of *T. cristatus* eggs laid on them compared to the other substrates. However, the increase in numbers of eggs detected on the black long egg-strips may be due to the increased surface area in comparison to the other egg-strip types. The results also suggest use of the black thick egg-strips may decrease the amount of *T. cristatus* eggs laid on them compared to the other substrates.

The results for *T. cristatus* and *L. helveticus*/*L. vulgaris* contradict the findings of Araus (2007), who found that *I. alpestris* preferred lighter-coloured egg-strip substrates over dark-coloured egg-strip substrates whereas the current study found no such preference between darker and lighter coloured egg-strip substrates. This could be due to the difference in the colour of substrates provided. Araus (2007) used yellow, white, transparent, blue and red egg-strip substrates whereas the current study used black, green, yellow and red. Alternatively there may be differences in colour preference between species.

Results in this study are also in contrast to Miaud's (1995) study which included choice of natural oviposition substrate. Miaud (1995) found *T. cristatus* to have a stronger preference towards ovipositing on certain plant species within ponds, whereas *L. helveticus* did not exhibit such strong preferences toward certain plant species. In this study *T. cristatus* had no significant preferences towards certain substrates and *L. helveticus*/*L. vulgaris* had a strong preference to green and black long substrates over black thick substrates. Such differences in results may also be affected by aquatic plants providing more olfactory environmental cues and tactile cues than the egg-strips, which may affect substrate choice (Norris 2008). Egg-strips have a smooth surface, which may affect adhesion of the egg to the egg-strip, and this too may be a factor in oviposition substrate selection (Kampf & Nussinovitch 1999).

The sample sizes in this study were relatively small, with different newt assemblages in each pond. The study also only focused on ponds in southwest English counties of Devon and Dorset in the UK. A repeat study with a larger sample size, consistent Urodele newt assemblage and more widespread distribution of ponds may increase the reliability of results. Further studies which vary where each substrate is located in the water column, record the depth of each substrate, and presence of predators, may enhance determination of whether preference is caused by colour, thickness, presence of predators, or substrate depth.

In conclusion this study found that egg searching using egg-strip mops is a useful technique in newt surveys and particularly useful in ponds with little or no submerged vegetation. However, like other newt survey techniques, is not 100% successful in detecting species presence. Provision of egg-strips may also be useful as a conservation tool in newly created ponds or those with little or no submerged vegetation to promote breeding. This study found that *L. helveticus* and *L. vulgaris* may prefer certain egg-strip substrates, but *T. cristatus* does not appear to be exhibit such preferences.

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