Impact of a natural pyrethrin biocide on two amphibians, common toad *Bufo bufo* and palmate newt *Lissotriton helveticus*, in Highland, UK

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SUMMARY

A quarry pond in Highland, UK, was treated with PyBlast (a biocide derived from natural pyrethrin) to eradicate a population of invasive non-native signal crayfish *Pacifasticus leniusculus*. Although it was anticipated that pyrethrin application would lead to the death of all poikilothermic animals present in the quarry pond, its use was sanctioned as surveys did not reveal the presence of any protected or other scarce species. It was assumed that native fauna, including amphibians, would re-colonise from an adjacent pond which was not treated. PyBlast (0.4 mg/l) was applied from 12 to 13 June 2012. Follow-up surveys later in June, and in August and September, found no live crayfish, but established the presence of common toad *Bufo bufo* tadpoles, and both larval and adult palmate newt *Lissotriton helveticus*. All appeared developmentally and behaviourally normal. These observations suggest that common toad and palmate newt larvae are able to survive levels of Pyblast generally lethal to crustaceans, indicating that amphibian presence at a site should not necessarily halt crayfish eradication programmes.

BACKGROUND

Signal crayfish *Pacifasticus leniusculus* is an invasive non-native species, which has become established across many European and Asian countries (Gherardi *et al.* 2011). It is an omnivorous species, and has been shown to prey on amphibian eggs and larvae (Axelsson *et al.* 1997). It was first confirmed in Scotland in 1995 (Maitland 1996) and is now established there in at least 8 localities (Bean *et al.* 2006). It has been targeted for special action to halt its spread (Gladman *et al.* 2009).

ACTION

When a population of signal crayfish was discovered in July 2011 in a flooded former slate quarry (56° 40' 35" N, -5° 7' 36" W) at Ballachulish (Highland, UK), the Lochaber Fisheries Trust and the Highland Council agreed to respond before the animals could spread to other sites. Given the potential economic and environmental impacts of the crayfish, the Scottish Environmental Protection Agency (SEPA) sanctioned the use of PyBlast (Agropharm Ltd: active ingredients 3% natural pyrethrin, 15% piperonyl butoxide). PyBlast had previously been used on the first field-scale application of a biocide in the UK against signal crayfish (Peay *et al.* 2006). It is more expensive than synthetic pyrethroids but has low mammalian and avian toxicity, breaks down quickly in sunlight and does not leave toxic residues (Peay *et al.* 2006).

It was anticipated that pyrethrin application would lead to the death of all poikilothermic animals present in the quarry pond but its use was sanctioned as surveys did not reveal the presence of any protected or other scarce species. Several species of fish (eel *Anguilla anguilla*, brown trout *Salmo trutta* and stickleback *Gasterosteus aculeatus*) were known to be present, but these had been introduced by local residents and were thus not considered to be an issue for conservation. Our surveys also revealed the presence of three breeding amphibians: common frog *Rana temporaria*, common toad *Bufo bufo* and palmate newt *Lissotriton helveticus*. All of these species are common in the locality and it was assumed that they would be able to recolonise the pond. In the study by Peay *et al*. (2006) aquatic invertebrates recolonised treated ponds after 24 days. It was therefore expected that terrestrial adult amphibians would return to breed in the following spring. A survey of a nearby pond (about 50 m away) did not find any crayfish, and that pond was not treated with PyBlast.

The treated pond has an area of approximately 19,500 m^2, a median depth of 2 m and a maximum depth of 11.7 m and has no outflow. It was treated with PyBlast to reach a concentration of 0.4 mg/l from 12 to 13 June 2012, applied by boat-mounted sprayers. The pond had been bathymetrically surveyed using a plumb-line at 100 sample points and these data were used to divide the pond into zones of equal volume to ensure all parts of the pond attained concentrations of at least 0.3 mg/l Pyblast, the concentration recommended from previous eradication attempts (Peay *et al.* 2006). Mixing was encouraged by using a boat equipped with a 60 hp (44 kW) outboard motor to churn up the pond after application and by the use of three shore-mounted pumps. Deep water was reached by spraying down 6 m rigid hoses. Maximum concentrations in the pond margins, where both adult and larval amphibians were usually present, reached 1.2 mg/l. All concentrations were estimated by bioassay with the freshwater shrimp *Gammarus pulex* following the method described by Peay *et al.* (2006).

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Table 1. Comparison between palmate newt larvae in treated and untreated ponds.

	Untreated pond	Treated pond
Length (mm)		
Snout-vent	12.6 +/- 2.8	14.7 +/- 2.6
Tail	15.4 +/- 3.8	18.2 +/- 3.7
Total	28.0 +/- 6.4	32.9 +/- 6.2
Glaesner Stage		
Range	67.0 to 69.0	67.0 to 71.0
Mean	68.7 +/- 0.8	68.6 +/- 1.1

CONSEQUENCES

Treatment led to the death of fish, anuran tadpoles (species not determined), two adult common frogs and around ten palmate newts as well as numerous invertebrates. Surveys on 18 and 25 June and from 21 to 31 August 2012 found no signal crayfish. They did however reveal small numbers of anuran tadpoles, an adult common toad (18 June) and adult palmate newt. Further surveys on 28 September 2012 found common toad tadpoles ranging from Gosner stages 30 to 46 (at which metamorphosis is completed) and palmate newt larvae ranging from Glaesner stages 60 to 71 (*i.e.* gills largely resorbed). No fish were found in any of these surveys. All amphibian larvae behaved normally (e.g. swimming, attempting to evade capture and the ability to self-right) and there were no external physical abnormalities. A later visit on 19 October 2012 found common toad tadpoles ranging from Gosner stages 31 to 33 and palmate newt larvae ranging from Glaesner stages 67 to 71 (mean: 68.6 +/- 1.1), as well as two adult palmate newts. Again there were no apparent physical or behavioural abnormalities.

Palmate newt larvae were also found during a sweep netting survey of the adjacent untreated pond on 19 October 2012. They ranged from Glaesner stages 67 to 69 (mean: 68.7 +/- 0.8). There was no significant difference between the development stages of these larvae and those captured in the treated pond on the same date ($U = 34$; $P = 0.72$); larvae from the treated pond were larger (table 1), but the differences were not significant ($U = 20.5$; $P = 0.10$).

Due to their behaviours – common toad larvae often swim near the surface and palmate newt eggs (Miaud 1995) and larvae are typically found in shallow water – both species are likely to have been exposed to PyBlast concentrations of around 0.4 mg/l. As common toads typically spawn in March in the area, it is likely that they would have been at the tadpole stage when the PyBlast was applied. Palmate newts have an extended breeding season and it is possible that the observed larvae were the result of eggs laid after the application. However, egg-laying in the area usually peaks in May and given the similarities in Glaesner stages between the treated and untreated ponds, and indeed with other ponds in the region, there is no reason to suggest that the larvae were the product of eggs laid after the application of PyBlast in June. Palmate newt larvae in the Highlands commonly overwinter, as do small numbers of anuran larvae, and so the presence of larvae this late in the season does not necessarily mean that larval growth was retarded.

DISCUSSION

A review by Palmquist *et al.* (2012) found varying effects of pyrethroids on amphibians, but in most cases some negative effects were detected. Effects in larvae included developmental abnormalities, such as tail kinking and low body mass, and behavioural abnormalities, including lack of coordination, hyperactivity, and convulsions (Palmquist *et al.* 2012). Most of the studies reviewed looked at synthetic pyrethroids, however refined natural pyrethrin has been shown to induce temporary paralysis in the coqui frog *Eleutherodactylus coqui* at concentrations of 0.2 mg/1 (Hutchinson 2003). Such paralysis would render amphibians vulnerable to predation or, if on land, to desiccation.

Not all studies have shown adverse impacts on amphibians. Exposure to permethrin led to more rapid development and increased size at metamorphosis in green frogs *Rana clamitans* (Boone 2008) and common frogs (Johansson *et al.* 2006). Boone (2008) hypothesised that the lack of competition from invertebrates had led to an increase in algal food resources. It is also possible that the elimination of invertebrate and fish predators by the pyrethrin would lead to increased larval survivorship.

This paper reports a series of unstructured observations, rather than a controlled experimental study, but suggests that at least some common toad and palmate newt larvae can survive exposure to field doses of PyBlast without developing detectable physical or behavioural abnormalities. Further study is needed to determine if there are quantifiable effects of pyrethrin application on these amphibians. Nevertheless we consider that amphibian presence at a site should not necessarily halt crayfish eradication programmes.

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