

# Biocide treatment of invasive signal crayfish: successes, failures and lessons learned

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## Supplementary Material

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Supplementary Table S1. Outline of stages of work likely to be required for a biocide treatment against invasive crayfish

<b>Work potentially required for a biocide treatment against invasive crayfish</b>
<p><b>Stage 1: Appraisal</b></p> <ul style="list-style-type: none"> <li>• detect invasive alien crayfish;</li> <li>• consider current status of any other alien and native crayfish in state, river basin, catchment and potential for invasion;</li> <li>• carry out rapid appraisal of technical feasibility, (scale of work likely to be required, potential benefits and potential constraints, start surveys for population distribution, considering potential limits of population now and in the next 1-2 years (in case of delays), and any effects on feasibility);</li> <li>• identify stakeholders and lead agency for next stage, start discussions;</li> <li>• start identifying resources needed and time required to obtain them;</li> <li>• make preliminary decision on benefits and feasibility, define detailed scope of feasibility if proceed (be thorough, but have regard to overall programme and the risks of delay);</li> <li>• start estimating costs;</li> <li>• obtain resources (funding and staff) for assessment stage and seek commitment in principle to funding for treatment stage (subject to feasibility assessment);</li> <li>• consider potential break points, criteria for continuing to the treatment stage or not.</li> </ul>
<p><b>Stage 2: Detailed feasibility assessment and project planning</b></p> <ul style="list-style-type: none"> <li>• appoint project leader;</li> <li>• consult with owners and occupiers, neighbours and regulatory agencies (with results of appraisal, work planned for feasibility and the work and programme if it goes to treatment); identify any stakeholder concerns or issues to be addressed in feasibility and project planning;</li> <li>• survey for extent of crayfish population, assess suitability of habitat in all areas to be treated and decide on extent of any area to be treated (beyond lower limit of detection of crayfish);</li> <li>• survey other fauna as required for impact assessment;</li> <li>• carry out detailed site survey to identify all inflows and outflows and their potential for flow management (if required) and biocide treatment, (plus additional hydrological surveys, tracer study for groundwater or leakage if required);</li> <li>• carry out bathymetric survey to calculate volume of waterbody and identify substrate type by area (sample by underwater photography, grab samples, cores or probing as required); include type, distribution and extent of aquatic plants (emergent, floating and submerged);</li> <li>• carry out toxicity tests with crayfish, water and substrate from site to determine target dosage (and/or estimate the adjustment to be made to target dosage to compensate for likely rate of environmental degradation of biocide during the treatment period, according to condition on site), take account of any likely change in water volume prior to treatment;</li> <li>• prepare a plan of technical operations, programme and quantities, including: biocide, materials, equipment, staff; any advance works, e.g. partial dewatering before treatment (benefit of storage capacity for treated water if rainfall occurs and cost reduction versus increased risk of crayfish above reduced water level), vegetation management; operations during treatment; requirement for any post-treatment management; contingency planning, including allowance for weather, equipment failure and other unplanned events; site security if required;</li> <li>• prepare health and safety risk assessment and environmental risk assessment, including for biomonitoring during treatment as required;</li> <li>• define roles and responsibilities for the operational and post-treatment stages;</li> <li>• make sure any other stakeholder issues have been addressed;</li> </ul>

<ul style="list-style-type: none"> <li>• prepare budget for treatment based on operations plan, including for contingencies;</li> <li>• obtain approvals from relevant statutory agencies;</li> <li>• secure funding for full treatment, also for monitoring the outcome;</li> <li>• on basis of detailed feasibility study and any changes in wider conditions since initial appraisal (including status of crayfish on site and in catchment) decide whether to proceed to treatment, if so;</li> <li>• obtain confirmation of landowner acceptance and agreement on any mitigation measures, compensation provision if applicable, or legal provisions;</li> <li>• carry out procurement and contract management (if a contractor is used it may require a high level of site supervision, and commitment to quality of outcome by the contractor);</li> <li>• ensure operations staff have any prior training or qualifications required (e.g. certifications for pesticide spraying, boat handling);</li> <li>• order biocide, materials and equipment well in advance of required time;</li> <li>• plan public communications strategy, for before, during, after treatment.</li> </ul>
<p><b>Stage 3: Preparatory works on site</b></p> <ul style="list-style-type: none"> <li>• install plastic barrier fencing to prevent overland movement of crayfish, if it is to be used, plus any temporary or permanent barriers or other controls on outflows (may be done during feasibility studies);</li> <li>• remove fish and/or amphibians if required (with measures to ensure no transfer of crayfish);</li> <li>• carry out any partial dewatering well in advance, if applicable, and track changes in water level and the dryness of margins in the period prior to treatment;</li> <li>• excavate a sample of any potential refuges above water level to check for presence of crayfish (if found, raise water level prior to treatment, or dig out all areas and contain the spoil to avoid escape of crayfish);</li> <li>• manage vegetation to facilitate biocide application if necessary, e.g. mowing, herbicide, partial dredging (with biosecurity to prevent escape of crayfish);</li> <li>• test hydraulic control prior to treatment, if applicable (check for leakage, check pump functioning and the backup pumps);</li> <li>• carry out any enabling works, prepare working area/site compound, define site boundaries with signs and/or fencing to keep people away from treatment areas during application of biocide;</li> <li>• arrange for delivery of materials and equipment, e.g. material for temporary dams, biocide to a secure store, pumps, boats, sprayers, fuel, clean water for washing, materials for bioassays (may need advance collection of crayfish from site or elsewhere), emergency kit, sundry tools and spares;</li> <li>• keep following weather forecasts for before, during and after treatment and amend work programme if necessary (e.g. avoid wet period with risk of high flow if using pump control and have plenty of spare pumping capacity and/or measures to safely abort treatment if necessary).</li> </ul>
<p><b>Stage 4: Treatment</b></p> <ul style="list-style-type: none"> <li>• ensure any flow control is operating well;</li> <li>• set up biomonitoring outside treatment area as required (a check for leakage/off-site pollution);</li> <li>• prepare any biomonitoring with 'sentinel' crayfish (to monitor efficacy of biocide treatment during the works), bioassays or chemical tests to record field dosage during works and/or during the recovery period;</li> <li>• ready all equipment and materials;</li> <li>• brief all site staff on safety and all procedures; make sure roles, responsibilities, action plan and contingency procedures are understood by all;</li> <li>• have one or more people not using biocide who can deal with official visitors, media and/or</li> </ul>

<p>members of the public who come to the site;</p> <ul style="list-style-type: none"> <li>• apply biocide to the margins and to the rest of site according to depth plan, with additional application in margins if required (e.g. before dark if there is risk of crayfish emerging); apply pumped circulation, if used;</li> <li>• keep track of quantities of biocide applied in accordance with field dosing plan, note response of wild crayfish especially at the margins;</li> <li>• monitor treatment with caged crayfish, bioassays or other methods (optional, to increase confidence that all areas are adequately treated).</li> </ul>
<p><b>Stage 5: Management of post-treatment recovery</b></p> <ul style="list-style-type: none"> <li>• monitor persistence of toxicity (essential part of the operation if flow within the site is managed by diversion, pumping, temporary dams etc., optional at a fully enclosed site);</li> <li>• manage treated water, options include: no treatment, dewater to field, remove off-site, or carry out accelerated degradation of product (e.g. in tanks with sodium hypochlorite - an option if natural pyrethrum is used);</li> <li>• carry out subsequent monitoring of aquatic recovery, with or without restocking of fauna.</li> </ul>
<p><b>Stage 6: Post-treatment monitoring of outcome</b></p> <ul style="list-style-type: none"> <li>• monitor for presence of crayfish annually for 5 years or more;</li> <li>• monitor for presence of crayfish plague if site is intended for re-stocking with native crayfish, (e.g. with cages of susceptible native crayfish, or by sampling for eDNA)</li> </ul>

Supplementary Table S2. Details of projects in the UK where biocide treatment against signal crayfish was carried out

<b>Site name and number</b>	<b>1 Gravel pit, Edzell</b>	<b>2 Mains ponds, Auchenblae</b>	<b>3 Castle pond, Auchenblae</b>	<b>4 farm reservoir near Pocklington</b>	<b>5 Ballintuim ponds and stream</b>	<b>6 Ballachulish quarry</b>
<b>Catchment</b>	North Esk, Scotland	North Esk, Scotland	North Esk, Scotland	Yorkshire River Derwent, England	Ardle (River Tay), Scotland	Leven and Coe, Scotland
<b>Year stocked with crayfish</b>	c. 1998	c.2002/3	c.2002/3	1992	Late 1980s	2000s
<b>Who stocked crayfish and reason</b>	Fishery manager, for fish pond management	Fishery manager, for fish pond management	Fishery manager, for fish pond management	Fishery manager, for fish pond management	Owners, as ornamental stock	Children, aquarium release
<b>Year detected</b>	2004	2004	2004	2002	2003	2011
<b>Year feasibility study</b>	2004	2004	2004	2003	2005	2011
<b>Year treated</b>	2004	2004 and 2005	2004	2005	2006	2012
<b>Waterbody type</b>	Enclosed pond	3 on-line ponds	1 off-line + 100 m small watercourse (ditch)	Enclosed pond	1 offline pond, 1 online pond, 680 m small watercourse	Enclosed pond
<b>Area (ha)</b>	1.0	0.02, 0.15, 0.3	0.54	0.56	Garden pond 0.08, lower pond 0.1	2
<b>Depth, (m)</b>	1 - 2.4	0.2 - 2.0	Mainly 1.0 - 2.5	3.4	Both 0.5 - 1.8 m, stream 0.1 - 0.25, increased for treatment	0.5 - 13.0
<b>Water source</b>	Groundwater	Dammed stream	Channel from river	Pumped from river in winter, plus drainage from arable fields	Seasonal inflow, spring seepage	Catchment runoff and groundwater
<b>pH</b>	pH 7.0	pH 6.8	pH 7.0	pH 7.8 - 8.5	pH 7.0	c. pH 7

Site name and number	1 Gravel pit, Edzell	2 Mains ponds, Auchenblae	3 Castle pond, Auchenblae	4 farm reservoir near Pocklington	5 Ballintuim ponds and stream	6 Ballachulish quarry
Substrate	Gravel	Sandy clay	Sandy clay	Calcareous clay	Sandy clay (upper), sand (lower pond)	Slate
Siltation	Low	Peat and silt, up to 0.15 m silt in deep water and wetland	Thin silt layer, >0.1 m at depth	None in steep margins, moderate on flat bed	Upper pond: thin silt over butyl lining, lower pond: peat silt over sand	Low, except in deepest area
Vegetation - submerged	Moderate cover, 25%	Sparse (<5% cover), locally dense (>60% cover) at retreatment	<10%, a few patches	Sparse (<2% cover)	Sparse to moderate in garden pond	None
Vegetation - emergent	None	Locally dense floating and emergent grasses, plus standing dead trees	Scattered clumps of rushes around the margin	Tree roots from surrounding willow coppice	Reedswamp margins of <i>Typha latifolia</i> in garden pond, waterlilies and ornamental; grass/rushes by stream, wetland at lower pond	Minimal (<1%)
Water temperature at treatment, month	9 °C October	15 °C September	4 °C December	14 °C Late september	9 - 14 °C October	8 - 22 °C June
Biocide used	Natural pyrethrum (Pyblast®)	Natural pyrethrum (Pyblast®)	Natural pyrethrum (Pyblast®)	Natural pyrethrum (Pyblast®)	Natural pyrethrum (Pyblast®)	Natural pyrethrum (Pyblast®)
Target dosage (µg/l natural pyrethrins)	150	200	200	200	1000 in ponds, 2000 in stream	500
Application method	Margins: backpack sprayers, Pond: hand-held 4-jet sprayer lance from sprayer tank on boat.	1st: margins: backpack sprayers Ponds (1-3): hand-held 4-jet sprayer lance from sprayer tank on boat,	Margins: backpack sprayers, Pond: hand-held 4-jet sprayer lance from sprayer tank on boat.	Margins: backpack sprayer from boat pond: fixed front-mounted sprayer boom on boat. Large-capacity irrigation	Margins: backpack sprayers, all sites; garden pond: hand-held 4-jet sprayer from boat; pour-on biocide at intakes of	Margins: backpack sprayers; drenching margins with treated water from pond, pond: sprayer lance from backpack

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		2nd: margins: backpack sprayer; ponds: sprayer from boat, plus pour on biocide near the intake of two or more pumps on the bank; more on lower pond next day.		pump run for 6 hours for mixing after treatment. Intake and output hoses set widely apart to induce water circulation.	pumps on the bank; Stream: backpack sprayers then pumped recirculation; separate dosing of natural seepages. Lower pond: sprayer from boat, pour-on at pump intake; pump treated water to drench wetland margin.	sprayers and larger tanks on boats; pump on biocide via vertical pipe mounted on boat, in deep areas. After application, boat driven criss-cross over site. Three pumps on banks with intake and output hoses wide apart to aid mixing.
Hydraulic control by pumping required	None	Inflow diverted; intermittent return pumping from downstream sump.	Continuous return-pumping of leakage 18-25 l s <sup>-1</sup> from pond. Level reduced before treatment (unplanned). Ditch dammed, with recirculation.	None	Complex, re-circulation pumping on 5 successive (overlapped) sections of stream; dewater of ponds post-treatment	Intermittent inflows blocked above quarry face
Number of days for preparation on site and full treatment (excluding prior surveys)	3 (x2 treatments)	2 (x2 treatments)	22	3	26	6
Number of staff per day during treatment	2 - 6	2 - 7	1 - 6, + 1 at night	5	Main works, 8 - 10 by day, +1 at night, reduced in recovery phase.	4 - 20

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<b>Crayfish toxicity test – cages in pond during treatment</b> <b>Crayfish condition: SR- self-righting NSR – not self-righting T – torpid D – dead</b>  <b>Crayfish from trapping, size range c. 25 – 55 mm cl (carapace length), male ~ female</b>	20 cages of 10 crayfish (treatment 2 only). After 48 hours: 0 SR, 3% NSR, 66% T, 31% D, (all dead within 5 days).	13 cages of 25 crayfish in one pond, 2 cages in another (treatment 2). After 48 hours: 6.6% SR, 4.4% NSR, 9.8% T, 79.2% D (all d within 5 days).	20 cages of 10 crayfish. After 48 hours: 0 SR, 0.5% NSR, 63.7% T, 35.8% D (all d within 5 days).	16 cages of 20 crayfish, 2 of 25 crayfish. After 48 hours: 0 SR, 0 NSR, 2.4% T, 97.3% D; (all D within 4 days).	Garden pond: 22 cages of 15 crayfish, 5 stream sections: 5 cages of 10 each, plus 4-8 artificial burrows with 4 each). 0 SR 0 NSR recorded. After 48 hours: garden pond 17% T, 83% D; lower pond 9% T, 91% D; stream sections s1-5, %D: 100, 56, 74, 85, 71.	13 cages of 10 signal crayfish, after 24 hours from start of treatment: 8.5% SR, 4.6% NSR, 21.5% T, 65.4% D (1 cage had 10 SR). Cage with 10 SR back to pond. Other 34 survivors to clean water, mortality 100% within 48 hours since treatment. Additional application on day 2, SR survivors plus 11 new cages. New cages after 24 hours: 9.2% T, 90.8% D (plus all D all in repeat cage). All D within 48 hours.
<b>toxicity test – water samples 24 hours after dosing (5 crayfish/sample)</b> <b>Crayfish condition: SR- self-righting NSR – not self-righting</b>	4 samples; after 24 hours: 0 SR, 0 NSR, 26% T, 74% D; after 48 hours: 100% D.	6 samples; after 24 hours: 100% D (in <2 hours in 2 samples, <9 hours others).	6 samples, 3 crayfish each; after 24 hours: 0 SR, 16% NSR, 55% T, 27% D. At 48 hours: 0 SR, 11% NSR, 44.5% T, 44.5% D.	6 samples; after 24 hours: 0 SR, 0 NSR, 10% T, 90% D; after 48 hours: 100% D. Repeated test 48 hours after dosing, after 48 hours: 100% D.	Garden pond, 3 samples; after 24 hours: 0 SR, 0 NSR, 48.7% T, 51.3% D; after 48 hours: 17% T, 83% D. Repeated test 48 hours after dosing; after 24	Not run, <i>Gammarus</i> bioassays only. Samples immediately after second treatment estimate 0.75 mg/l in the deep water and 1.2 mg/l in the



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T – torpid D - dead					hours: 46.6% T, 53.4% D. Lower pond 2 samples, all D within 48 hours.	shallow margins.
time until water not toxic repeated bioassays with Gammarus pulex) unless otherwise stated	c. 24 days, estimated from observed recolonization by invertebrates	21 - 24 days	21 days (bioassays with Asellus aquaticus)	115 - 134 days (from	23 days for garden pond; 7-11 days for stream sections and lower pond, with flushing.	34 - 60 days
Re-treatment <sup>5</sup> carried out	Yes, 24 days later. First treatment not effective due to residue from prior deoxygenation with sodium sulphite (not used subsequently). Details below are for the retreatment using pyrethrum only	Yes, 11 months later. First treatment had ineffective deoxygenation pre-treatment; details are for retreatment, using natural pyrethrum only. First treatment all areas, re-treatment two ponds, upper pond wetland area, surface spray only	No	No	No	No
Stocked with fish after treatment	No	No	Yes	No	Yes	No
Number of years of post-treatment monitoring	5	5	5	5	2 intensive, then <i>ad hoc</i> by owner	5

Site name and number	1 Gravel pit, Edzell	2 Mains ponds, Auchenblae	3 Castle pond, Auchenblae	4 farm reservoir near Pocklington	5 Ballintuim ponds and stream	6 Ballachulish quarry
<b>Annual post-treatment monitoring method and effort</b>	100 - 125 traps for 1 night, mixed types, mainly fine-mesh funnel traps (Fladen).	110 - 135 traps for 1 night, mixed types mainly Fladen.	100 traps for 1 night, mixed type for first 3 years; 60 traps for next 2 years.	100 - 107 traps for 1 night; plus 6 fyke nets (with 3m long leading net) in years 1 and 2.	150 traps for 1 night, mixed types, mainly Fladen.	15 - 17 Fladen traps checked and reset daily in summer; 195 - 267 trap nights per year.
<b>Outcome: crayfish caught in number of years after treatment</b>	No crayfish, eradication	Crayfish detected year 2 (upper and middle ponds) and year 3 (all three ponds)	Crayfish detected year 2	No crayfish, eradication	No crayfish in stream and lower pond; crayfish detected garden pond year 2 and abundant year 7	No crayfish, eradication

Table S3. Details of projects in Norway and Sweden where treatment against signal crayfish was carried out using synthetic pyrethroids

Site name	Smöjen, Sweden	Stenkyrka, Sweden	Hangvar, Sweden	Dammane ponds, Norway	Ostøya golf course ponds, Norway
<b>Catchment</b>	Gotland coastal catchment	Gotland coastal catchment	Gotland coastal catchment	Dammane, Eidanger coastal catchment	Ostøya
<b>Year stocked with crayfish</b>	C.1970	1984, in belief it was noble crayfish	Probably early 1970s	Uncertain, probably in the 1980s	Uncertain, probably in the 1990s
<b>Who stocked crayfish and reason</b>	Unknown person for harvest	Pond owner for aquaculture/harvest	Unknown person for harvest	Unknown person, but probably stocked for harvesting	Unknown person, but probably to keep vegetation at bay
<b>Year detected</b>	1985	1985	2005	2006	2009
<b>Year feasibility study</b>	2006 - 2008	2007	2006 to 2008	2007	Not done
<b>Year treated</b>	August 2008 and November 2008	August 2008	August 2009	14th and 28th May 2008	14th and 25th October 2009
<b>Waterbody type</b>	Limestone quarry with 2 separate ponds with	Pond for irrigation	Marble quarry	5 on-line ponds	6 off-line ponds, connected by water pipelines

Site name	Smöjen, Sweden	Stenkyrka, Sweden	Hangvar, Sweden	Dammane ponds, Norway	Ostøya golf course ponds, Norway
	interconnection				
Area (ha)	1 and 1.4	2.3	0.35	0.037 – 0.32	0.037 – 0.24
Depth (m)	3.1 - 3.8	Unknown (variable volume up to 100 000 m <sup>2</sup> )	0.5 - 5.0	0.82 – 2.27	1.8 – 3.0
Water source	Groundwater and surface runoff	Surface runoff pumped into pond from ditch	Groundwater and surface runoff	Seasonal inflow, catchment runoff	Groundwater (pumps). Surface runoff. All ponds are connected.
pH	8.4	8.3	Unknown	Unknown	Unknown
Substrate	Rock and gravel of limestone	Sandy clay	Rock and gravel	Sandy clay and gravel	Sandy clay
Siltation	Low to moderate in deeper parts	Moderate	Low	Moderate	Moderate, except for deep parts
Vegetation - submerged	Sparse to moderate with mainly stonewort	Moderate	Sparse	Sparse	Sparse in one pond, other ponds moderate to dense.
Vegetation - emergent	Moderate, with reed in some parts	Sparse, with some patches of grass, pondweed, rush and reed	Sparse	Sparse	Sparse in one pond, the other moderate to dense floating and emergent grasses
Water temperature at treatment (°C)	Treatment 1. 19.0 Treatment 2. 6.0	19.0	19.6	Treatment 1: 15-16,5 Treatment 2: 14-16,5	Treatment: 1. 5-6,5 Treatment 2: 3.4-4.5
Biocide used	Deltamethrin	Deltamethrin	Deltamethrin	Cypermethrin (BETAMAX VET®)	Cypermethrin (BETAMAX VET®)

Site name	Smöjen, Sweden	Stenkyrka, Sweden	Hangvar, Sweden	Dammane ponds, Norway	Ostøya golf course ponds, Norway
<b>Target dosage (active ingredient)</b>	0.5 µg/l (actual concentration when analysed: first treatment 0.5 and 0.6 µg/l second treatment 0.5 and 1.0 µg/l)	0.5 µg/l (actual concentration when analysed: 0.6 µg/l)	0.5 µg/l (actual concentration when analysed: 4.3 µg/l)	20 µg/l	20 µg/l
<b>Application method</b>	Diluted 1:10, spread evenly from boat; further mixed by driving boat crisscross over surface. Margins sprayed using backpack sprayer; pond water pumped onto margins and identified difficult areas, e.g. cracked cliffs and stony debris.	Diluted 1:10, spread evenly from boat; further mixed by driving boat crisscross over surface. Margins sprayed using backpack sprayer; pond water pumped onto margins and identified difficult areas, e.g. cracked cliffs and stony debris.	Deltamethrin diluted 1:10, spread evenly from boat; further mixed by driving boat crisscross over pond surface. Margins sprayed using backpack sprayer; pond water pumped onto margins and identified difficult areas, e.g. cracked cliffs and stony debris.	Pumps (boat and land based for application to ponds at 1:100 dilution), small inflows continuous dosing from drip cans (4 hours) and garden watering-cans to dose small areas and margins	Pumps (boat and land based for application to ponds), drip cans and garden watering- cans to dose small inflows and margins
<b>Hydraulic control by pumping required</b>	Continuous recirculation by pumping for 2 days	Continuous recirculation by pumping for 1 day	Continuous recirculation by pumping for 1 day	All ponds were drained after treatment in early June and late December 2008	All ponds except one, were drained after treatment, during mid-November to mid-December

Site name	Smöjen, Sweden	Stenkyrka, Sweden	Hangvar, Sweden	Dammane ponds, Norway	Ostøya golf course ponds, Norway
Number of days for preparation on site and full treatment (excluding prior surveys)	3	2	2	2	2
Number of staff per day during treatment	Up to 5	5	4	4 for each treatment	3 for each treatment
Crayfish toxicity test	5 cages each with 3 noble crayfish placed in each pond, checked repeated during treatment. All crayfish dead within 24 hours.	5 cages each with 3 noble crayfish placed in each pond, checked repeated during treatment. All crayfish dead within 24 hours.	4 cages each with 3 noble crayfish placed in each pond, checked repeated during treatment. All crayfish dead within 24 hours.	4 cages of 3 signal crayfish in one pond, during each treatment. All dead within 18 hours.	4 cages of 3 signal crayfish in one pond, during each treatment.
Days until water not toxic	1 month after first treatment: 0.009 and 0.0 µg/l in the two ponds. No further death in caged noble crayfish 27 days post treatment. 3.5 month after second treatment: 0.008 and 0.003 µg/l. Slow breakdown probably due to low temperature.	1 month after treatment: 0.01 µg/l. No further death in caged noble crayfish 16 days post treatment	1 month after treatment: 0.033 µg/l. 2 months after treatment: 0.002 µg/l. No deaths in caged noble crayfish introduced into pond two months and three days post treatment	Not known	Not known
Re-treatment carried out	Yes, second treatment after 2.5	No	No	Each locality treated twice (see above)	Each locality treated twice (see above)

Site name	Smöjen, Sweden	Stenkyrka, Sweden	Hangvar, Sweden	Dammane ponds, Norway	Ostøya golf course ponds, Norway
	months				
<b>Stocked with fish or indigenous crayfish after treatment</b>	Stocked with noble crayfish in year after treatment	Stocked with noble crayfish 2 months after treatment	Stocked with noble crayfish by unknown, probably landowner	No (not stocked with noble crayfish)	Yes, with fish (not stocked with noble crayfish)
<b>Number of years of post-treatment monitoring</b>	7	7	6	6	6
<b>Annual post-treatment monitoring method and effort</b>	Visual check for signal crayfish the same year as treated. Later check on stocked noble crayfish, both visual and with traps.	Visual check for signal crayfish the same year as treated. Later check on stocked noble crayfish, both visual and with traps.	Visual check for signal crayfish the same year as treated. Later check on stocked noble crayfish, both visual and with traps.	Caged living noble crayfish and water samples for detection of crayfish plague. Trap-fishing.	Caged living noble crayfish and water samples for detection of crayfish plague. Trap-fishing.
<b>Outcome, crayfish caught in number of years after treatment</b>	No signal crayfish detected, Eradication Only noble crayfish found, ongoing 7+ years after stocking	No signal crayfish detected, Eradication Only noble crayfish found, ongoing 7+ years after stocking	No signal crayfish detected Eradication Only noble crayfish found, ongoing 6+ years after stocking	No signal crayfish detected, Eradication	No signal crayfish detected, Eradication

Table S4. Summary of projects in the UK in which feasibility of biocide treatment against signal crayfish was assessed, but treatment was not carried out

Site name	7 ponds near Holt	8 ponds near Painscastle	9 Ribble tributary	10 ponds near Llyswen	11 quarry pond	12 north Cumbria ponds + river	13 south Cumbria ponds
Catchment	Glaven, England	Bachawy, Wales	Ribble, England	Wye, Wales	Tweed, Scotland	Eden, England	confidential
Year stocked with crayfish	Early 1980s	1990s	Late 1990s	After 1996	Unknown	Unknown	Unknown
Stocked by	Owners, as ornamental	Owners, as ornamental	Individuals, for wild harvest	Without owner's knowledge, probably angler	Unknown	Owners, as ornamental	Unknown
Year detected	2007	2005	2002	2012	2009	2012	2011
Feasibility study	2009	2009	2005	2012	2010	2012	2011
Year of work	2009-2010, surveys and detailed planning	2010, planning; 2011-2012, planning, barrier fencing, amphibian survey	2006-2007 planning, funding, detailed design, surveys; 2008 groundwater tracing, tests on hydraulic control	2012 crayfish survey, cost benefit assessment; experimental dewatering	2011, some planning, 2012 test of partial dewater, re-costed	2012 site appraisal only, considering ark site options for indigenous crayfish	2012 funding, delay, test of partial dewater carried out
Waterbody type	Series of small interconnected garden ponds+mill leat	6 ponds (3 linked) dug by river	Small headwater stream	2 online ponds plus ditches and culverts	Former quarry	Garden pond, small outfall stream, plus section of river	1 site with 2 small ponds, 1 site large
Area (ha)	0.09, 0.18, + 800m	0.01-0.3	2.5 km	0.5, 1.0	c. 2	<0.3, + watercourses	<0.5 - c. 3
Water source	Catchment runoff/ spring seepages	Catchment runoff/ spring seepages	Catchment runoff+baseflow	Mill leat and land drains	Groundwater	Unknown	Catchment runoff/ spring seepages
Stage terminated	3 (preparations)	3 (preparations)	3 (preparations)	1 (appraisal)	2 (detailed feasibility)	1 (appraisal)	1 (appraisal)

Supplementary Table S5. Annual monitoring results of crayfish trapping at sites treated with natural pyrethrum

waterbody (ha)	years since treatment	0 (before treatment )	1	2	3	4	5	average trap area (m <sup>2</sup> )
Gravel pit (site 1) (1.0)	crayfish Traps (CPUE <sup>1</sup> )	241 80 (3.0)	0 100 (0)	0 111 (0)	0 125 (0)	0 115 (0)	0 108 (0)	89.9
Mains ponds (site 2)								
lower pond (0.3)	crayfish traps	2 10 (0.2)	3* 25 (0.12)	0 90 (0)	0 75 (0)	3 75 (0.4)	0 75 (0)	54.7
middle pond (0.15)	crayfish Traps (CPUE)	0 5 (0)	0* 10 (0)	0 12 (0)	<b>1</b> 45 (0.02)	5 30 (0.17)	11 30 (0.37)	81.7
upper pond (0.02)	crayfish traps	0 5 (0)	ns ns	0 8 (0)	<b>1</b> 15 (0.07)	2 8 (0.25)	0 15 (0)	19.2
Castle pond (site 3) (0.54)	crayfish Traps (CPUE)	16 20 (0.8)	0 45 (0)	<b>1</b> 100 (0.01)	0 100 (0)	13 60 (0.22)	5 60 (0.08)	81.6
Farm reservoir (site 4) 0.56	crayfish Traps (CPUE)	185 98 (1.9)	0 88 (0)	0 100 (0)	0 107 (0)	0 100 (0)	0 100 (0)	56.8
	crayfish fyke nets (CPUE)	208 6 (34.6)	0 6 (0)	ns 6	ns ns	ns ns	ns ns	
Ballintuim (site 5)								
garden pond (0.54)	crayfish Traps (CPUE)	109 40 (2.4)	0 96 (0)	0 100 (0)	<b>1</b> 100 (0.01)	ns ns	ns ns	54.8
stream	crayfish	0 (4 by hand)	0	0	0	ns	ns	
	traps	10 (0)	10 (0)	10 (0)	10 (0)	ns	ns	
lower pond (0.18)	crayfish Traps (CPUE)	0 40 (0)	0 40 (0)	0 40 (0)	0 40 (0)	ns ns	ns ns	45.0
Ballachulish (site 6) (2.3)	crayfish Total trap nights (CPUE)	14 36 (0.39)	0 200 (0)	0 200 (0)	0 195 (0)	0 200 (0)	0 267 (0)	

1 CPUE = Catch Per Unit Crayfish (crayfish/number of traps)

\* Mains ponds re-treatment one year after first treatment, year numbering from first treatment

Crayfish catches in bold are those which showed failure to fully eradicate the population.