SCOTTISH NATURAL HERITAGE

C O M M I S S I O N E D   R E P O R T

Commissioned Report No. 122

Biocide trial to eradicate signal crayfish in the North Esk catchment

(Purchase Order No. 9725)

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SCOTTISH NATURAL HERITAGE

COMMISSIONED REPORT Summary

Biocide trial to eradicate signal crayfish in the North Esk catchment

Commissioned Report No. 122 (Purchase Order No. 9725)
Contractor: Stephanie Peay
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Background

Signal crayfish were illegally introduced to the North Esk catchment, Aberdeenshire between 1998 and 2003. This trial used natural pyrethrins to attempt to eradicate signal crayfish from all water bodies where they were known to be present, to minimise the risk of crayfish escaping to nearby watercourses.

Main findings

- Use of the biocide resulted in high mortality at all sites.
- Prior deoxygenation with sodium sulphite reduced the toxicity of the biocide.
- The type of substrate present in water bodies and water temperature can affect the toxicity of the biocide.
- There is wide variation in the susceptibility of individual crayfish to the treatment.
- Good estimates of the volume of water bodies are required to ensure the target dose is achieved.
- Future monitoring for crayfish will be required to establish whether the trial has been successful.

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SUMMARY

The purpose of this project was to eradicate illegally introduced signal crayfish (*Pacifastacus leniusculus*) in the North Esk catchment, Scotland. The aim was to treat all the known sites before any crayfish could escape into the river system, where there would be no means available to eradicate or control a wild population. The sites treated were an isolated gravel-pit (c.9,000m^3) with no inflow or outflow; three dammed ponds, (c.5,000m^3 in total) with a throughflow of water and a leaking, offline pond (c.7,000m^3). Crayfish were thought to be present for less than 2 years in the ponds and for up to 6 years in the gravel-pit. An abundant population had developed in the gravel pit. Although the only escape route for crayfish was overland, the proximity to a public road made the gravel pit a high risk site for unauthorised harvesting of crayfish and further illegal introductions.

The method used was treatment with a biocide, natural pyrethrum, formulated as Pyblast (Agropharm Ltd). It was chosen because of its toxicity to crayfish, low toxicity to birds and mammals and low persistence in the environment.

Toxicity tests were carried out in buckets with substrate from the sites to indicate field doses. Treatment at the gravel-pit (October 2004) comprised prior deoxygenation with sodium sulphite to stimulate emergence, then application of natural pyrethrum (Pyblast). Exposed margins were sprayed in advance with Pyblast to prevent escapes. The chemicals were applied in solution using a fire hose and fire-engine pump. Mortality of crayfish was observed in the gravel pit. Toxicity tests were run with water samples from the gravel pit, using healthy crayfish. At the chain of three ponds, throughflow was stopped, fish removed, marginal vegetation treated with Pyblast and the water sprayed with Pyblast applied by boat-mounted sprayer. Dead crayfish were seen, especially near the overflow. Water was released safely from the ponds after 1 week, by which time water beetles were already starting to recolonise the ponds. Biomonitoring showed the treatment of the ponds and release of water had no impact on aquatic invertebrates in the receiving watercourse.

Despite high mortality of crayfish at the gravel pit, one survivor was seen 4 days after treatment. The likely cause was applying Pyblast from a tank still containing sodium sulphite residue, which probably reduced the effective concentration of Pyblast below the target 0.1mg/l. Further bucket toxicity tests were carried out to test this effect. Re-treatment of the gravel pit was carried out at the end of October 2004, to a target dose of 0.15mg/l Pyblast rather than 0.1mg/l, without any deoxygenation pre-treatment. The Pyblast was applied using a boat-mounted sprayer. The water temperature was 9°C. Mortality was confirmed using caged crayfish. Although there was mortality in the first 24 hours, caged crayfish took up to 5 days for 100% mortality.

Treatment of the final pond, Castle Pond was carried out in December 2004. The treatment could only proceed once hydrological investigations confirmed where the substantial leakage from the pond was going and measures were put in place to control it. Because work had to be carried out late in the year, when the water temperature was only 4°C and crayfish were inactive, the target dose was increased to 0.2mg/l. The Pyblast remained toxic to crayfish for the first three days after treatment here. Continuous back-pumping of collected leakage was required for a 2-week recovery period. Biomonitoring was carried out using bags with freshwater shrimps (*Gammarus*) installed in the river upstream and downstream of the outfall ditch from the pond prior to treatment and inspected at frequent intervals. There was no contamination of the river during the treatment and recovery period. Toxicity tests were run with water louse *Asellus* to monitor recovery of the pond water after treatment. There was some localised impact on aquatic invertebrates when the water

was released. This may have been due to higher dose rates of Pyblast in the outfall taking longer to break down than in the pond, or to the sudden discharge of the water in the outfall ditch, which contained pollutants from two earlier incidents, involving fuel oil and then domestic sewage.

Future monitoring is required for 2–5 years to check for eradication. The target is 100% mortality and it will take time to determine whether any crayfish have survived or not. Pyblast seems to be effective, but is relatively expensive. Biocide treatment is only worthwhile before crayfish escape to watercourses. It can only be used when there is a reasonable chance of being able to treat the whole population. The natural pyrethrum breaks down quickly and harmlessly, but it will affect other aquatic invertebrates, any fish not removed in advance and potentially amphibians. This limits the use of the biocide treatment to relatively small water bodies where water can be controlled within the treatment area for the duration of the recovery period.

Signal crayfish ready to be put into cages for toxicity test in the gravel pit

1 INTRODUCTION

1.1 Aim of the project

Signal crayfish (*Pacifastacus leniusculus*) were illegally introduced into the North Esk catchment in Aberdeenshire, Scotland (Figure 1). There are no indigenous crayfish in Scotland and the stocking of crayfish into water bodies in Scotland is an offence under both UK law (Wildlife and Countryside Act 1981, as amended, Schedule 9) and Scottish law (Prohibition of Keeping of Live Fish (Crayfish) (Scotland) Order 1996 and the Nature Conservation (Scotland) Act 2004). There are concerns about the potential impact of introduced crayfish on indigenous salmonid fisheries (Griffiths *et al.* 2004), on rare aquatic species and on aquatic habitats. Despite this, there have been several recent introductions into catchments in Scotland during the late 1990s and since 2000 (P. Collen, Fisheries Research Services, *pers. comm.*)

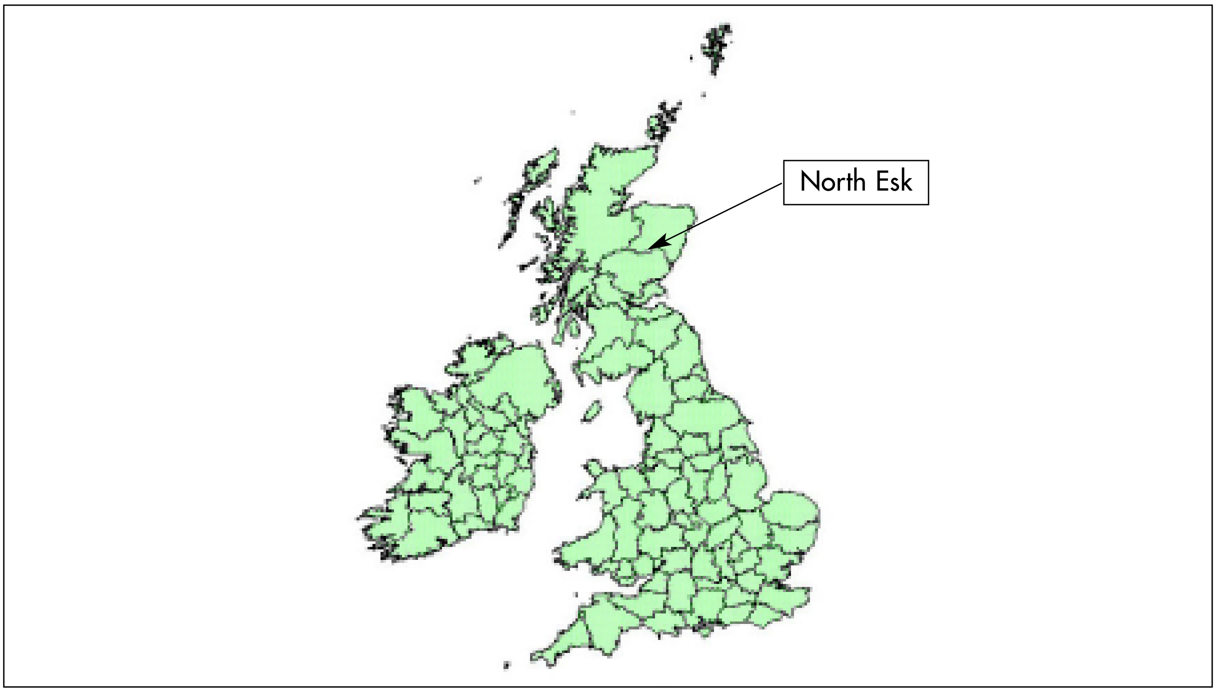
The first introduction of crayfish in the North Esk catchment was made into a recently worked gravel pit near Edzell, probably in 1998. Crayfish from this site were used to stock two separate ponds on another estate in the area, at Drumtochty, probably in spring 2003. In response to a police investigation, Fisheries Research Services confirmed the presence of crayfish in the ponds on the Drumtochty Castle Estate in 2004. It is alleged that these introductions were made by the same fisheries manager, in both cases without the knowledge or consent of the landowner.

In this case, the gravel pit is dependent on groundwater and has no connection to watercourses in the area. By contrast, the ponds on the Drumtochty Estate have a throughflow of water and although the outflow is via projecting 300mm plastic pipes, there was a high risk of crayfish escaping if the population increased to high density.

Surveys in other water bodies in the area by Fisheries Research Services did not find any other populations of crayfish.

The aim of this project was to eradicate signal crayfish from all known sites in the North Esk catchment as quickly as possible, to minimise the risk of the crayfish escaping to any watercourses. It was also to test the use of a biocide against crayfish in a range of field conditions.

Figure 1 Location of North Esk catchment



1.2 Approach

Manual removal, trapping and electrofishing had already been tried unsuccessfully in the River Clyde during 2001–2003 (Colin Bean, Scottish Natural Heritage, *pers.comm.*). Failure to eradicate crayfish has also been found in the various projects where these methods have been used in England (Peay and Hiley, 2001).

Biocides have been used against crayfish in a few studies, which are reviewed in Holdich *et al.* (1999). There are no known biocides that are selective to crayfish, or even to crustaceans, so those with potential for use against crayfish are also toxic to other aquatic invertebrates and fish.

The insecticide rotenone is occasionally used to control populations of fish. Rotenone was reported to have little effect on benthic invertebrates in Scotland (Morrison and Struthers, 1975, Morrison, 1977). In laboratory tests, Bills and Marking (1988) found they could kill rusty crayfish (*Orconectes rusticus*) in three days with rotenone at 10mg l^{-1}, compared to 0.02mg l^{-1} to kill fish. Rotenone is also affected by conditions in the field, especially plants and organic sediments and sometimes forms a foam, which reduces effectiveness (P. Hiley, *pers. obs.*). Eversole and Seller (1997) found that of 35 chemical groups reviewed, synthetic pyrethroids were the most toxic to crayfish, with a median 96-h LC_{50} value of 2.5 µg l^{-1}, compared to 350 µg l^{-1} and 352 µg l^{-1} for organochlorine and for organophosphates respectively.

There has been very little use of biocides in Europe. In one of the few examples, Laurent (1995) treated three ponds with the organophosphate insecticide fenthion and achieved 100% mortality of caged crayfish, but fenthion proved to be persistently toxic in the ponds for weeks. As Holdich *et al.* (1999) remark, statutory authorities may be concerned about the impact of biocides on non-target areas and about the persistence of some products authorised for use in agriculture, but not in water. Frutiger *et al.* (1999) describe how a decision to use a biocide against an unwanted population of non-indigenous crayfish in Switzerland was overturned in a court ruling. In the UK, the Environment Agency did not permit a synthetic pyrethroid insecticide to be trialled in a wholly enclosed water body in East Yorkshire. Reasons were not given in detail, but were mainly a response to pollution incidents in the region due to sheep-dipping with synthetic pyrethroids.

In 2000, Hiley (2003a) undertook some preliminary laboratory tests on the use of non-persistent biocides on crayfish. These were followed by some outside tank tests, in near-field conditions (Hiley and Peay, 2003 and in prep.). The most successful biocide used was Pyblast (Agropharm Ltd), consisting of 3.0% w/w pyrethrins; piperonyl butoxide, as a synergist to rapidly immobilise invertebrates, and alcohol ethoxylate. The product is approved for spraying in food handling premises and for insect control, for public hygiene or avoidance of nuisance.

The advantages of natural pyrethrum are its low toxicity to mammals and birds, its rapid breakdown in field conditions, the absence of toxic residues and its harmlessness to aquatic plants. It is, however, toxic to other aquatic crustaceans, insects, fish and probably to amphibians. Pyblast is also more expensive than modern synthetic pyrethroid insecticides. In 2004 it cost £222 for 5 l concentrate.

Preliminary work suggested that there might be some benefit in encouraging crayfish to leave their refuges and so increase their exposure to the toxicant. Deoxygenation (with sodium sulphite) was used in tank trials and was used in some of the water bodies in this project.

Summary of approach

The approach used was to:

- prevent inflow/outflow of water where applicable;
- remove fish if necessary;
- treat the margins to prevent any escapes of crayfish;
- treat the whole water body with natural pyrethrins (as Pyblast), in some instances with a pre-treatment of deoxygenation;
- keep the water contained throughout the recovery period.

Authorisations

Pyblast is not authorised for use in water, under the Control of Pesticides Regulations 1986, so permission for experimental use had to be obtained from the Biocides and Pesticides Unit of the UK Health and Safety Executive (as an Automatic Experimental Permit). Any keeping of crayfish in Scotland requires authorisation under the Prohibition of Keeping of Live Fish (Crayfish) (Scotland) Order 1996, so Fisheries Research Services obtained a permit from the Scottish Executive for crayfish to be held and transported for the project. In addition, as the treatment had potential to cause pollution, the Scottish Environment Protection Agency (SEPA) required details of the proposed work and imposed conditions for the work to proceed. The most of important of these was that the operations "must be undertaken in a manner which does not cause pollution of the nearby watercourses. Failure to do so may result in enforcement action."

1.3 Project team

Scottish Natural Heritage (SNH) provided most of the funding for the project, with some assistance from the North Esk Fisheries Board. Staff of SNH also contributed to work on site. The other statutory agencies, Fisheries Research Services and the Scottish Environment Protection Agency also provided staff time and equipment. The Environment Agency also helped by supplying crayfish for ecotoxicology tests. Table 1 shows those involved in the project, many of whom gave their time generously to the research project. The project would not have been possible without the cooperation of a range of agencies and individuals.

1.4 Report structure

The project ran from a preliminary site visit on 6th August through to the end of December 2004. Work on site was carried out in three main stages, with various tests being carried out during work on site and off-site prior to and between stages. The project was a biocide trial undertaken at large scale in the field. The detail of what was done is important to understanding of the work and for any subsequent use of the this method. Section 2 on methods includes the procedures of project work, plus an outline of some of the results, because these influenced the development of methods during the trial. The results are reported in more detail in section 3. All the tables of results are grouped together in section 5. Section 4 discusses the findings more broadly and gives recommendations on future action.

Table 1 Project team

Team members and affiliation	Involvement
Stephanie Peay Crayfish specialist	Project leader; responsible for project design, logistics, provision of miscellaneous equipment, toxicity testing, site work on all the sessions and reporting.
Isla Martin Scottish Natural Heritage	Project manager; secured funding and chemicals, plus provided help on site at gravel pit stage 1 and with biomonitoring.
Pete Hiley Crayfish specialist	Design review, and involved in site work on stages 1 and 3.
Peter Collen Fisheries Research Services	Responsible for a substantial programme of survey work prior to treatment, plus provision of equipment and site work on stage 1 and 2.
Jasper Gray Dalladies Farm, owner of gravel pit	Carried out site work in all the sessions, provided and adapted equipment on site and ensured its maintenance, plus provided help with toxicity tests and night-viewing.
Charlie Anderson Drumtochty Castle Owner of ponds	Provided accommodation, use of tools and pumps, pump fuel; investigated leakage at Castle Pond.
Dougal Lindsay Estate worker, Drumtochty	Undertook some site work, delivery of equipment and chemicals and operating pumps in stage 1. Helped with Lithium-testing. Set up dams, sump and pumps in stage 3 and operated pumps throughout recovery period (including Christmas).
Lynne Farquhar Scottish Natural Heritage	Help setting up biomonitoring for stage 3 and carried out monitoring during recovery period.
Ian Lorimer SEPA	Provided 2 days site work, plus some biomonitoring during session 1.
North Esk Fisheries Board	Funded hydrological survey at Castle Pond.
Stuart Brown SEPA	Undertook laboratory analysis of Lithium for hydrological study. A first attempt at dye-tracing was also carried out (Stuart McGowan).
Paul Bryden Field assistant	Helped with site work on stages 1 and 3, plus assisted with off-site toxicology tests and reporting.

Table 1 *(continued)*

Team members and affiliation	Involvement
Mountain Environments	Carried out contract for hydrological survey at Castle Pond.
John Littlewood and fisheries team, Environment Agency, Pickering office	Carried out two sessions of fyke-netting at a reservoir site in Yorkshire to supply test crayfish for stage 2.
Neil Winter and colleagues, Environment Agency, Hatfield office	Provided test crayfish for stage 3.
Sarah Brown and colleagues Biocides and Pesticides Unit Health and Safety Executive	Provided advice on safe use of pesticide.

2 METHODS AND PROJECT WORK

2.1 Sites description

Various sites in the North Esk catchment were assessed in summer 2004 for the necessity and feasibility of treatment. Table 2 contains descriptions of each of these ponds.

It was decided to treat:

- a gravel pit at Edzell, the site with greatest risk of unauthorised removal of crayfish;
- Castle Pond, Drumtochty;
- three of four Mains ponds, Drumtochty.

The Bison pond, the uppermost of the Mains ponds, was sufficiently far from the others to make overland colonisation unlikely in a recently established population. No crayfish were found in the pond during trapping or dewatering. It was used later as a receptor for stocked trout removed from the other ponds prior to treatment.

The stream below the Mains ponds is unsuitable for any crayfish survey. It is a steep, fast-flowing channel c. 0.5m wide, with no stones that could be searched manually and too fast and shallow for effective trapping. The outflows of the lower pond were covered with perforated plastic baskets, which would have prevented adult crayfish from exiting via the pipes, although the wire-mesh fencing surrounding the outflows was certainly not any barrier to crayfish. The perforated baskets covering the outfalls would not have prevented any washout of juveniles and indeed they have been removed on occasions during high flows. There is a risk that crayfish have already escaped, but are undetected.

The inlet stream to Castle Pond has a vertical barrier about 0.7m high, with a constructed lip forming an overhang. This would be a barrier to any upstream movement of crayfish by water, though not to overland movement. A juvenile crayfish was also found on 04/10/04 about 10m upstream of the pond, but below the step cascade. The outflow pipes were unscreened. The projecting elbow joints would discourage access by adult crayfish, but juveniles could be washed through. Old field drains found subsequently to be a major source of leakage in the pond were also potential escape routes for crayfish. Two juveniles were found in a 10m length of ditch downstream of Castle Pond on 06/10/04. Beyond that the ditch was badly polluted with fuel oil, with no benthic fauna whatever, making it unlikely that juvenile crayfish would be able to survive or colonise. The River Luther, 120m downstream of the pond, was surveyed manually, but no crayfish were found. It is possible that juveniles might have been washed out during high flows, prior to the pollution, in which case a population could be present in the River Luther, but below the threshold for detection. Nonetheless, it was decided to treat the pond, inflow and outfall ditches to remove the pond as a source for colonisation.

Table 2 Sites surveyed in North Esk catchment

Location	Size (approx.)[1]	Description	Results of crayfish trapping (Trappy® traps)
Bison Pond (Mains ponds 1, Drumtochty) NO 705 806	50x30m, est.1.0m deep, ave. (poss. up to 2m) Perimeter c. 160m. Vol. est. 1500m^3.	Drumtochty ponds all constructed about 5–6 years ago. Uppermost in a chain of ponds. Margins poached, except for bank on east side, where some undercutting, but mostly poor habitat. Bed is clay. Outfalls to pond 2, but also seepage through dam to adjacent small, steep stream. Little control of throughflow in the system of ponds. This pond stocked with carp. No known introduction of crayfish.	September 2004, 18 traps, 0 crayfish (P. Collen) Also dewatered 25/09/04, no sign of crayfish.
Small pond (Mains ponds 2 Drumtochty) NO 706 805	Triangle 20m long base 16m, ave. depth 0.5m, max 1m. Perimeter 65m Vol. est. 80m^3.	Pond well below level of bison pond and >50m away. Ungrazed banks with tussocky grass and rushes. Fringe of floating vegetation. Bank vertical, possibly undercut, clay. Immediately adjacent to pond 3 below, has elbow-joint outfall pipe. Stocked with trout. No known introduction of crayfish.	September 2004, 5 traps, 0 crayfish (P. Collen).
Triangle pond (Mains ponds 3 Drumtochty) NO 706 805	Dam length c 50m, upstream length c. 30m. Max 1.5m deep. Perimeter 100m. Vol. est. 1750m^3.	Surrounding land tussocky grassland, ungrazed. Banks with good potential for burrows. Bed clay with little stone visible. Little growth of plants in the pond. Two elbow-joint outfall pipes to lower pond, vertical outfall. Stocked with trout. No known introduction of crayfish.	September 2004, 15 traps, 0 crayfish (P. Collen).
Lower mains pond (Mains ponds 4 Drumtochty) NO 706 805	50x60m, depth. 1m average. Perimeter total 280m, plus islands c. 240m. Vol. est. 3,000m^3.	Has two inlets through dam from pond 3 above and outlet to stream. Same unmanaged grassland around it, banks vertical, c. 0.5–1m with some undercutting, good potential for burrows. Has several vegetated islands and numerous alder trees standing in water. pH6.94, alkalinity 123 μeq l^{-1}, conductivity 97 μS cm^{-1}. Two elbow-joint outfall pipes to small stream. Stocked with trout. Reputedly **stocked with about 50 crayfish in spring 2003,** although the actual stocking may have been higher, as more than 50 adult crayfish were seen before and during treatment.	July 2004, 2 crayfish from 10 traps (CPUE 0.2) (P. Collen). Found 3 on 06/08/04 in unauthorised trap left on site.
Castle Pond NO 700 749	About 70x50m, surface area calculated at 5450m^2. Depth range c. half at c. 1m, half at 1.5–2.5m, ave c. 2m. Volume calculated 6,100m^3 (at 0.35m below full, est. 8,100m^3 at overflow) Perimeter total 300m, plus 100m island.	Has inlet from River Luther, long inflow stream c. 100m. Outlets controlled at dam end by adjustable elbow joint pipes, one main one for throughflow, one high flow spill, one blocked. Substrate clay, with some gravel and cobble on surface, locally silty in shallow area. Bridge to island. Banks completely vegetated with rushes, sedges. Aquatics present including water starwort, alternate water milfoil, a few waterlilies. pH7.48, alkalinity 531 μeq l^{-1}, conductivity 116 μS cm^{-1}. Occasional use by otter and mink reported. Constructed 1998 on site of older pond. Stocked with trout. **Probably stocked with about 50 crayfish in spring 2003.**	July 2004 16 crayfish from 20 traps (CPUE 0.8). (P. Collen). One crayfish seen active by day on 06/08/04 (Peay and Collen).

Table 2 *(continued)*

Location	**Size (approx.)**[1]	**Description**	**Results of crayfish trapping** (Trappy® traps)
Gravel pit NO 632 678	Roughly 4-sided, 60m, 90m, 80m 100m, with a spur c. 10m wide projecting c. 25m into the pit from south side. Depth variable, from 1<1m to 2.5m. volume c.9,000m^3. Perimeter c. 400m.	Gravel pit with no inlet or outlet, groundwater dependent, but said to flood occasionally. Level at time of survey about 2m below bank top, with moderately steep, bare margins. Soft gravel bed and scattered cobble present, but probably patches of clay. No emergent vegetation except some *Typha latifolia* in one area near the spur. Submerged beds of *Elodea crispa* abundant, locally very dense. pH7.0, alkalinity 652 µeq l^{-1}, conductivity 114 µS cm^{-1}. Close to public road, with easy parking, though not visible from road. Several otter spraints with crayfish remains seen. Constructed c. 1997. **Stocked with crayfish, probably in 1998.**	Trapping results 150 from 10 traps 29/08/03 (P Collen). Numerous crayfish seen in margins by day 06/08/04. One seen walking out of water by day to get insects off exposed gravel (by author and other members of project team). Shoreline in places littered with crayfish remains.
Other sites			
Sawmill pond NO 634 676	About 50x10m, depth possibly 1–1.5m.	Isolated pond within working sawmill and part infilled with bark and other sawmill waste. Abundant aquatic vegetation. Unlikely to have been stocked with crayfish and little public access is likely. Some risk of colonisation overland from gravel pit, but >200m across a working timber yard.	September 2004, 12 traps, 0 crayfish (P Collen).
Loch Wee, Edzell airfield NO 626 695	About 2ha water in total and >2m deep.	Old borrowpit for construction of airfield. The loch is adjacent to a road on the north side, but entirely enclosed by high security fencing. Secure access via industrial estate. Highly unlikely to have been stocked with crayfish, no public access.	September 2004, 25 traps 0 crayfish (P Collen).

The former fishery manager, who introduced the crayfish at Drumtochty is only known to have stocked the Castle Pond and Lower Mains Pond. As a precautionary measure, it was decided to treat two of the ponds upstream of the Lower Mains Pond.

A small pond in the grounds of a sawmill near the gravel pit at Edzell was considered unlikely to have been stocked and no crayfish were found in a trapping study. The nearest water body to the north of the gravel pit was Loch Wee, but high security fencing around the site would make unauthorised access very difficult. Neither of these was thought likely to require any treatment.

Having no flowing water nearby, the gravel pit appeared to be ideal as a test site, as well as being a priority for treatment, due to the risk of any unauthorised harvesting leading to further introductions. The Mains ponds and Castle Ponds were going to be more difficult sites, because of the through-flow of water, poorer access and more marginal vegetation, but treatment looked feasible, provided the through flow could be stopped and the water adequately contained for the recovery period after treatment.

2.2 Programme of work

The programme of work on the project is summarised in Table 3 below. There were three main stages, the gravel pit and Mains ponds, re-treatment of the gravel pit, and Castle Pond. Some illustrations of the work are given in section 7.

Table 3 Programme of work

Period	**Work** (described in report section)
06/08/04 to 03/10/04	Site assessment, project planning and approvals (section 2.1)
04/09/04 to 25/09/04	Preliminary tests on recovery from Pyblast treatment (section 2.3)
03/10/04 to 10/10/04	Toxicity tests (section 2.4), treatment of gravel pit (stage 1) (section 2.5), treatment of three Mains ponds (section 2.6) and monitoring of crayfish mortality
15/10/04 to 19/10/04	Further toxicity tests (off-site) (section 2.7)
11/10/04 to 27/10/04	Project planning, preparation for re-treatment gravel pit (stage 2), including obtaining crayfish for tests (section 2.8)
28/10/04 to 03/11/04	Re-treatment of gravel pit and monitoring (stage 2) (section 2.8)
04/11/04 to 25/11/04	Project planning, preparation for treatment of Castle Pond, hydrological survey and analysis, obtaining more crayfish for tests (section 2.9 and Appendix 1)
5/12/04 to 8/12/04	Work at Castle Pond to trace and attempt blockage of drainage system
9/12/04 to 27/12/04	Set up water control at Castle Pond, treatment, containment and monitoring (section 2.9)

2.3 Preliminary recovery tests (off-site)

Preliminary tests were carried out, off-site in Yorkshire, to indicate the time required for water to recover after treatment, in field conditions. Control tanks were set up with 20 l rainwater, with or without a bed of sandy clay garden soil. The other treatments were similarly set up with and without clay. "Pyblast" (3% w/w natural pyrethrins), produced by Agropharm Ltd was used as the toxic agent. Natural pyrethrins were added to $0.1 mg\ l^{-1}$, with or without prior deoxygenation with sodium sulphite solution (20ml saturated solution in 20 l water). The tanks were kept outside, although they were not exposed to direct sunlight; except for about

2–3 hours in the afternoon, depending on weather conditions. Water temperature was about 13°C. In one treatment the tank was kept in a garage in the dark to simulate conditions in deep water.

The test animals were water louse *Asellus*, collected from a pond nearby. Batches of 20 were put into numbered sachets of netting fabric with pieces of partly decomposed leaf as a food source. Sachets were put into the tanks and the *Asellus* were inspected at intervals. Dead animals were removed and live ones returned to the tank. No sachets were used in the first 24 hours of treatment. Aeration of tanks was started 24 hours after treatment and continued for 6 days, after which the pump was turned off. Addition of new sachets and monitoring continued until *Asellus* survived in all treatments. Results are given in section 3.1 and Table 5.

2.4 Preliminary toxicity tests at Gravel Pit and Castle Pond

Crayfish population

Two sessions of overnight trapping were carried out at the gravel pit immediately prior to treatment, to indicate the relative abundance of crayfish and to obtain a sample for toxicity tests. The crayfish were stored in secure plastic crates full of plastic flower-pots and pieces of horticultural plug-tray, initially at the gravel pit and then in Castle Pond.

Preliminary toxicity tests

Hiley and Peay (2003) carried out outdoor toxicity tests on signal crayfish in August 2003, using water and substrate from a farm reservoir, when the water temperature was around 25°C. The minimum dose of natural pyrethrum (in Pyblast) required to achieve 100% mortality within 24 hours in those conditions was 0.05mg l^{-1} in the small-scale tests. On this basis, 0.1mg l^{-1} was proposed as a sensible minimum for large-scale application. Variation was expected, depending on the conditions at individual sites.

"Dirty bucket" tests were set up at the gravel pit (see Plate 1, Section 7) and Castle Pond on 4th October to establish the concentrations of Pyblast required at each site. Tests were carried out in tubs, with a layer of substrate from the water body and water, with two replicates of each treatment. Two crayfish per bucket in 10 l were used at the gravel pit and four crayfish in 30 l at Castle Pond. The treatments were controls; deoxygenation with sodium sulphite; deoxygenation and natural pyrethrins at 0.01mg l^{-1}, 0.05mg l^{-1}, 0.1mg l^{-1} and 0.2mg l^{-1}.

The condition of crayfish was assessed as shown in Table 4.

Table 4 Qualitative assessment of condition of crayfish

Ref.	Condition	Comments
SR	self-righting	normal condition, no apparent effect
SSR	slow self-righting	movement slow and often stiff, may take 1 min or more to turn over if placed on back, even in water
NSR	not self-righting	significantly affected, lying on back, but still making voluntary movements of limbs, in water or air
TAD	torpid or almost dead	no voluntary movement, lying on back, will show slight movement of limbs when touched, but in more advanced stages may only show minimal response or movement of mouthparts
D	dead	no response to touching, no eye-stalk response

Another test was set up at the gravel pit to observe the behaviour of crayfish during deoxygenation. 70 l plastic tubs were set up with 30 l water and 5 crayfish, but no substrate. A piece of netting fabric was draped over a cane to allow crayfish to climb to the surface or out of water. Behaviour was observed for about 4 hours during deoxygenation compared to control.

To test the effects of direct contact of Pyblast on crayfish, dry tests were carried out using five crayfish in tubs with about 5cm of sand and gravel substrate covering the bottom. In the controls the substrate was given a fine spray of water at the start. In the other treatments there was an initial spray with natural pyrethrin solution (see plate 2, section 7). The manufacturers standard recommendation for surface spray as an insecticide was 1 l concentrate in 9 l water, or 3g l^{-1}, applied at a rate of 1 l to 20m^2. In tests the spray was applied a few minutes before crayfish were added. They were either left on the treated surface, or were removed after 90 sec exposure and placed in a clean, dry tank. Air temperature was in the range 10–14°C.

Other preliminary work

Other preliminary work included the removal of 268 stocked trout from Castle Pond and 19 trout from the lower mains pond. The lower pond had fewer stocked fish, but it was also difficult to net, due to the presence of numerous trees standing in water and a lot of woody debris. In all, 12 adult crayfish were caught in the sweep net during the fish rescue. Great care was taken to ensure that no crayfish were left in nets and those found were killed and put into waste disposal.

Castle Pond was surveyed for crayfish by searching about 250m of the margin at night by torchlight on 4th October. Only two crayfish were seen out, with a further crayfish c. 25mm CL (carapace length) found about 10m up the inflow stream, but below the step cascade. It is not certain whether the low count was a true reflection of the relatively low abundance of the population, or was also affected by prior disturbance during netting for fish.

As a result of shutting off the inflow to Castle Pond to facilitate the fish-removal, we discovered it leaked badly. Instead of treating Castle Pond in the same week as the gravel pit and Mains ponds, we had to postpone its treatment for many weeks until the leakage was investigated further (described in section 2.8 and Appendix 1). Hence, with hindsight, it would have been better to use the night of 4th October for viewing at the gravel pit, prior to treatment on 5th October, so we had night counts of crayfish both before as well as after treatment, rather than just trapping results.

2.5 Gravel pit treatment, stage 1

The first operation at the gravel pit on 5th October was to spray a 1m wide band of the bare sand and gravel margin with Pyblast using a sprayer. A 55 l sprayer tank with an electric pump was used. This was carried in a 4-wheel drive vehicle, with a length of hose connected to a hand-held sprayer and this ensured an even distribution of the spray as an operator walked round, followed slowly by the vehicle (see plate 3, section 7).

Sodium sulphite, although soluble, tends to cake on contact with water. It was dissolved in a large metal tank using a high-pressure water-jet. The mix was then pumped out across the gravel pit from several different locations for as thorough coverage as possible. When a hire company failed to deliver a suitable pump in time, Jasper Gray, borrowed a fire-engine, which was owned by a local grain storage company. The fire-engine pump provided a powerful jet of water for mixing and applying the sulphite (plate 5, section 7). The initial intention was to apply the Pyblast from a knapsack sprayer in an inflatable boat, but the delays incurred and the limited hours of daylight necessitated application by tank-mixing and spraying it on with the fire-engine pump.

A dissolved oxygen meter was used from an inflatable boat to monitor deoxygenation in the gravel pit. Initial observations were made from the bank. Application of sodium sulphite started at 13:00 and a few crayfish were seen swimming actively between 13:00 and 14:00. No crayfish were seen climbing out of water by day, although one was seen trying to go head first into a burrow under stems of reedmace (*Typha latifolia*) and others were seen later, right at the edge of the water. Dosing with sodium sulphite took about 3.5 hours of continuous effort, in six tank applications. The following application of Pyblast took just over 1 hour in three tankfuls of about $2m^3$. A few healthy crayfish were allowed to walk over the margin about three hours after spraying. They soon fell over and were unable to right themselves. Conditions were mild, dry and sunny, conditions that lead to rapid breakdown of Pyblast. (Information provided by Agropharm Ltd indicated Pyblast used as a crop spray has a half life of 0.5–2 hours in direct sunlight, depending on its intensity).

The margins were given a further spray with 1 l concentrate in 9 l for the 400m perimeter, to deal with any crayfish that emerged at night. Night-viewing was carried out for nearly an hour for evidence of mortality, or any crayfish emerging. Viewing extended from the waterline in a band about 5m wide around the gravel margin.

On 6th October, the day after treatment of the gravel pit, the dissolved oxygen was monitored from a boat at various locations. Water samples were collected, 6 from near the surface and 6 from deep water. Healthy crayfish were placed in clean buckets with the water samples and their condition observed over time. Counts of dead crayfish were carried out in some $1m^2$ sections of the margins. After 17:00 the whole perimeter was sprayed again with Pyblast, this time with one of four spray-jets over water to re-dose the margin where some live crayfish were seen at night. A further 1 l of concentrate in 9 l water was applied in shallow water in the corner at or beyond the limits of the hose-jet from the fire-engine pump. Night-viewing was repeated on 6th, 7th and 9th October.

On 7th October further monitoring of dissolved oxygen was carried out, observations were made on toxicity tests and additional tests set up with crayfish in water samples taken 48 hours after treatment, these were monitored until 10th October.

2.6 Mains ponds treatment

Preparation for the treatment of the Mains ponds was carried out on 7th October. This involved diverting flow from the three ponds to a stream, shutting off the flow between ponds, measuring seepage from the lower pond and excavating a small sump to catch the seepage. The seepage was pumped back into the pond daily. On-site measurements were made to improve the volume estimates for each of the three ponds.

Kick-sampling and netting was carried out in the adjacent stream, to provide samples of mixed aquatic invertebrates for biomonitoring. These were put into mesh bags and set out upstream and about 15m downstream of the outfall from the lower mains pond, in a channel less than 0.5m wide.

Equipment was moved to site. This time, with no fire-engine available it was not possible to pre-mix and pump sodium sulphite onto the ponds and a leaf-blower was used as an alternative method. The small electric sprayer was rigged so it could be installed in an inflatable boat and powered from a 12V car-battery. The sprayer hose was connected to a 4-jet sprayer-boom attached to a pole (plate 6, section 7). Some strimming of bankside vegetation was carried out around the lower pond, but much of the marginal vegetation was inaccessible for mowing, on islands in the lower pond.

On 8th October the dry sodium sulphite was dusted onto the surface of the ponds with the leaf-blower and manually from the boat in areas of the lower pond too far from the bank. In the afternoon the Pyblast concentrate was mixed in the sprayer tank at a rate of 5 l to 45 l water and applied with the boom sprayer. Application was difficult in the lower pond, because there were many alder trees standing in water 0.5–1.2m deep; many small, marshy islands, covered by large tussocks of rushes (*Juncus effusus*) and fringed by floating grass (*Glyceria fluitans*), and fallen branches. The whole pond, overhanging tussocks and the lower parts of tree-trunks were sprayed. Where water was too shallow for the boat, the sprayer-operator waded through the muddy shallows with the spray boom and the boat-operator lifted the outboard motor and pushed the boat. The vegetation around the margin was also sprayed from the bank with a knapsack sprayer with a broad fan-jet, especially at the outfall, where crayfish were found congregating immediately underneath the plastic baskets, which covered the outfall pipes. The reason for the choice of refuge is not known, but it is possible that the movement of water provides better oxygenation, less silty substrate and perhaps food items. The smaller ponds were treated with the sodium sulphite in similar fashion to the large lower pond.

Toxicity tests of the treated pond water, using healthy crayfish, were carried out on 9th–10th October. Margins were searched for dead crayfish and a few dead trout were removed. Sampler bags in the adjacent stream were checked for survival of aquatic invertebrates, freshwater shrimps (*Gammarus* sp.), plus mayfly larvae (*Baetis*) and various caddisfly larvae (Trichoptera).

Pumping of the limited seepage from the lower pond was carried out twice a day for a week after treatment, by which time water beetles were seen swimming in both the lower mains pond and the sump. The outfall had to be opened soon afterwards, because, following heavy rain, water overflowed from the diversion channel above the uppermost (Bison) pond and flowed into the lower three ponds. As they filled to capacity, the outfalls had to be opened to relieve pressure on the earth dams. Subsequent monitoring of the sampler bags confirmed the invertebrates were still alive at the end of October, two weeks after treated water had started to be released.

2.7 Further toxicity tests (off-site)

On 10th October, five days after treatment, a single live crayfish was seen walking in the gravel pit by day, showing that 100% mortality had not been achieved. A possible reason for this was that the Pyblast was mixed in a steel tank, which was still encrusted with sodium sulphite.

Further toxicity tests were set up in Yorkshire to check the effects of sodium sulphite on toxicity of Pyblast on crayfish. Wild-caught signal crayfish were obtained locally. The tests were set up outside with 5 l rainwater in 10 l buckets with approximately 200g sandy clay subsoil; 2 crayfish in each of 2 replicates per treatment, including controls. One series involved varying the concentration of sodium sulphite as a pre-treatment;

1ml l^{-1} (the standard), 0.5, 2 or 5ml l^{-1}, followed in each case by addition of natural pyrethrins at 0.1mg l^{-1}. In the other series, deoxygenation was omitted and the natural pyrethrin dose was varied, this time to 0.05mg l^{-1}, 0.1mg l^{-1}, 0.2mg l^{-1} and 0.5mg l^{-1}. Water temperature was in the range 7–9°C. Tests were observed for four days.

2.8 Gravel pit treatment, stage 2

Further toxicity tests carried out off-site after stage 1 indicated that sodium sulphite reduced the toxicity of Pyblast and even Pyblast alone appeared to stimulate crayfish activity before immobilising them. It was decided to repeat the treatment of the gravel pit without pre-treatment with sodium sulphite and at a slightly higher dose rate of Pyblast, 0.15mg l^{-1}.

With a very large proportion of the crayfish population in the gravel pit already dead, it would be difficult to see how effective the treatment was on any remaining population. Fyke-nets were set in a farm reservoir in East Yorkshire by the Environment Agency to obtain a sample of crayfish for use in tests. These were required to supplement the remaining crayfish from the gravel pit, which were still in a submerged crate in Castle Pond. The crayfish were transported in nearly dry, chilled plastic crates full of flowerpots.

Crayfish cages were made by adding mesh lids to fine-mesh pyramid traps (used by the authors as crayfish traps in other studies) and tying the trap-lines to numbered plastic bottles as floats (see plate 7, section 7). Ten crayfish of mixed size and source were put into each of 20 cages, with some pebbles. Cages were spread out across the gravel pit. Night-viewing was carried out at the gravel pit on 28th October, in very favourable conditions, with air temperature 13°C, water 8.5°C, no wind and visibility in the gravel pit out to 3m or more from the waters edge and to more than 1m depth. No surviving crayfish were seen.

There were initial problems with the sprayer on 29th October, but once it was sorted, the gravel pit was systematically sprayed, first around the whole margin, then up and back across the water body, using marker posts on the bank to keep as even a line as possible; followed by transverse passes to complete the application. A total of 55 l Pyblast was applied, for a target concentration of 0.15mg l^{-1} in 5 tankfuls, which took 3.5 hours to apply, non-stop. The tank mixes consisted of 10 l concentrate in 45 l water, with 15 l in the last tank. The sun set before 17:30, but spraying was not finished until nearly 19:00, by which time it was fully dark and work was completed with illumination from onboard torches and the headlights of a truck.

The following day half the cages were lifted, inspected and placed back in the gravel pit, while the other half were left undisturbed. Toxicity tests were run using 5 healthy crayfish in samples of treated water.

Some time was taken inspecting the mains ponds and planning work at Castle Pond, which was given another night-viewing survey on 30th October. No crayfish were seen.

All the cages at the gravel pit were lifted on 31st October, after 2 days exposure to the treatment. The condition of each crayfish was assessed. Any that were still alive were returned to their cages and put back in the gravel pit. All cages were lifted on 3rd November, the 5th day after treatment.

2.9 Castle Pond treatment

Until the inflow and outflows of Castle Pond were shut off to facilitate removal of stocked fish on 4th October 2004, it was not known how badly the pond leaked. The loss was around 25% of total volume per day, with no reduction in the rate of loss as the level fell.

It was essential to investigate where the leakage went, so it could be intercepted before any treatment. A survey was commissioned from Mountain Environments in November, involving a bathymetric survey of the pond, spot-gauging of flows and a tracing study involving dosing of the pond with Lithium chloride. After applying the Lithium chloride, water samples were taken from the pond and at various places in the outfall ditch downstream of the pond and the main river to determine where water from the pond went and the degree to which it was diluted. The hydrological survey and Lithium tracer study showed that, with no inflow, the pond continued to lose about 25 $l\ s^{-1}$, via old field drains, into the outfall ditch. There was no direct loss to the River Luther, which skirts a field on the south side of the pond. Additional details are given in Appendix 1.

The approach was the same as for the second treatment of the gravel pit, Pyblast spray without prior deoxygenation. This time the target dose was 0.2mg l^{-1}, to allow for the treatment being undertaken in December, when crayfish would be relatively torpid and in their winter refuges, as well as allowing for the muddier conditions in this pond than at the gravel pit. The substantial leakage of water had to be controlled before any treatment could be carried out.

Crayfish for toxicity testing were obtained from the Environment Agency in the northeast Thames catchment, where an angling club is carrying out intensive trapping in the hope of reducing nuisance to anglers (Peay and Hiley, 2004).

The only barrier to the movement of escaped juvenile crayfish into the River Luther was pollution of all but the uppermost section of the outfall ditch by fuel oil, which eliminated all the aquatic invertebrates in about 100m of ditch. The source of pollution was identified and stopped, before the treatment of Castle Pond, although there was still a distinct odour of fuel oil present in the ditch before the treatment. If no action was taken, the ditch would be expected to gradually recover enough to allow escape of the crayfish to the river. It was necessary, therefore, to treat the pond and the ditch as soon as possible, despite conditions potentially being less favourable for treatment during the winter.

The main work started on Thursday 9th December, with the delivery of a 4-inch pump and arrival of the team, crayfish for tests and other equipment.

There were various delays getting the hydraulic side of the operation set up:

- a flat tyre on the excavator on Thursday;
- getting the pump to a suitable location in what had become a very muddy area, following earlier attempts by Charlie Anderson to block the field drains;
- waiting for a fitter to sort an initial problem with the main pump;
- increasing the size of sump excavated to contain the leakage;

- infilling the top and bottom ends of the outfall ditch with earth dams, with a further backup dam of sandbags at the downstream end;
- filling more sandbags ready for emergency use;
- rigging another 3-inch to pump from the outfall ditch;
- checking the main pump was running continuously.

Work was further limited by having barely 8 hours of light. Fortunately, in the whole period up to Christmas there was hardly any rain at all. Water temperature remained at 3.5–5°C. Air temperature varied rather more, with some ground frosts and temperatures down to −2°C during the main period of work, but rising on some days as high as 9°C. The temperature fell as low as −12°C during the recovery period.

Bags with freshwater shrimps (*Gammarus*) were set up in the River Luther; sachets with *Asellus* were prepared for monitoring the recovery of the pond after treatment and were stored in a small, leafy pond near Castle Pond. Crayfish were deployed in the Castle Pond in 20 numbered cages, although two of these were put in the leakage channel and sump immediately downstream of the pond.

Treatment of the outfall ditch was carried out once the dams were in place and the main pump was operating well on 11th December. The Pyblast was applied at 1 l in 9 l dilution, using a knapsack sprayer with a fan-jet nozzle. The spray was directed under each rush tussock, as well as in the water. In all, 1 l concentrate was applied to 100m of ditch and most of a further 1 l was applied upstream of the main dam to all the remaining parts of the original banking with the upper sections of outfall ditch.

As with the second treatment of the gravel pit, it was decided to omit any deoxygenation pre-treatment, due to the effect of sodium sulphite on Pyblast activity. Furthermore, the treatment with sodium sulphite at the Mains ponds showed that dry application did not provide very effective deoxygenation. The chemical needed to be dissolved in a tank before being applied to the water body and with the fire-engine not available for use at Drumtochty and pumps committed to controlling leakage, it would have been difficult to do. There was further concern about the very limited hours of daylight available (<8 hours).

The main treatment was carried out on 12th December, with the continuous pumping keeping the pond full, or nearly so. With only a small inflatable boat being available this time, the method of application had to be modified slightly. The position of the boat was controlled by a fine cord looped onto the boat fore and aft, with one person pulling the boat at a marked distance out from the perimeter of the pond and another person working the other end of the cord from the central island, while a sprayer operator knelt in the boat. To get a target concentration of 0.2mg l^{-1} natural pyrethrins a total of 46 l Pyblast was applied, in 5 tankfuls. In addition, 3 l concentrate in total was diluted and applied to the tussocky vegetation around the island, the perimeter and the inflow stream.

The outfall ditch was allowed to fill up slowly after treatment on the 11th December, from seepage from the field and a little through the sump dam, although it was partly dewatered onto the marshy field on the 13th using the small pump.

On the morning of 13th December the main pump was running, but not pumping; the sump was full to overflowing and the outfall ditch was virtually full too. Emergency procedures were carried out until the pump was fixed around midday. This involved using the small pump to dewater to the field and building small dams in the field and along the ditch bank with sandbags. A little flow did escape across grass into the river, but fortunately at a sufficiently low rate that the shrimps survived in the sampler bags upstream and 10m downstream of the confluence of the outfall ditch. As soon as the main pump was operating, we were able to pump back from the outfall ditch to the sump and back into the pond. After that incident, the leakage from the pond was successfully contained throughout the recovery period, by continuously running the main pump and by running the small pump from the outfall ditch to the sump for a period every day. Additional hose was obtained and rigged to allow use of the small pump to pump from sump to pond in any further emergency.

Toxicity tests were set up with crayfish in buckets and samples of water taken 24 hours after treatment. The margins were also checked for any dead or dying crayfish. The cages were all lifted on 14th December after 48 hours exposure and the condition of crayfish was assessed. Dead crayfish were removed and live ones returned to the pond in cages, until 15th December, when they were all lifted and transported in cooled crates to Yorkshire, where they were put into clean, aerated water. The few remaining healthy crayfish not used in tests were put in a cage on 15th December and left in the pond until 14th January, when the last cage was removed.

Recovery tests were started, using *Asellus*. The first test was carried out in buckets, but to minimise any risk of contamination a fresh set of large paper cups was used for each test thereafter and care was taken to handle *Asellus* on leaves, not by hand. *Asellus* were put in river water and in a series of dilutions of treated pond water; undiluted, 10x, 100x, 1000x and 10000x dilution. Tests were carried out on 14th, 15th, 16th, 17th, 20th and 23rd December.

The pumps were kept running until 27th December, 15 days after treatment of the pond. The dams in the ditch were then removed and water was allowed to leak into the river via the outfall ditch.

3 RESULTS

All the tables of results are given in the Tables (section 5).

3.1 Results of preliminary recovery tests (off-site)

The results of the preliminary recovery tests with *Asellus* are shown in Table 5. Water temperature was around 13°C. Pyblast treatment alone was the most persistent, with no *Asellus* surviving until 16 days after treatment and without full recovery until 21 days after treatment. The presence of clay shortened the recovery period, with 7 days to 24-hr LC_{50} (Lethal Concentration for 50% mortality in 24 hours) and full recovery somewhere between 8 and 15 days. With prior deoxygenation and Pyblast, 24-hr LC_{50} occurred just 3 days after treatment and full survival with 5 days. This suggests the sodium sulphite was reducing the toxicity of the Pyblast. Keeping conditions dark and with only natural re-aeration, 24-hr LC_{50}, for deoxygenated water with Pyblast was extended to around 15 days and complete survival to 20 days, only slightly less than for Pyblast in clean water in outdoor conditions. Not all individuals were equally susceptible. In the transition period to recovery some *Asellus* died within 24 hours of exposure, but others survived 48 hours and for many days thereafter.

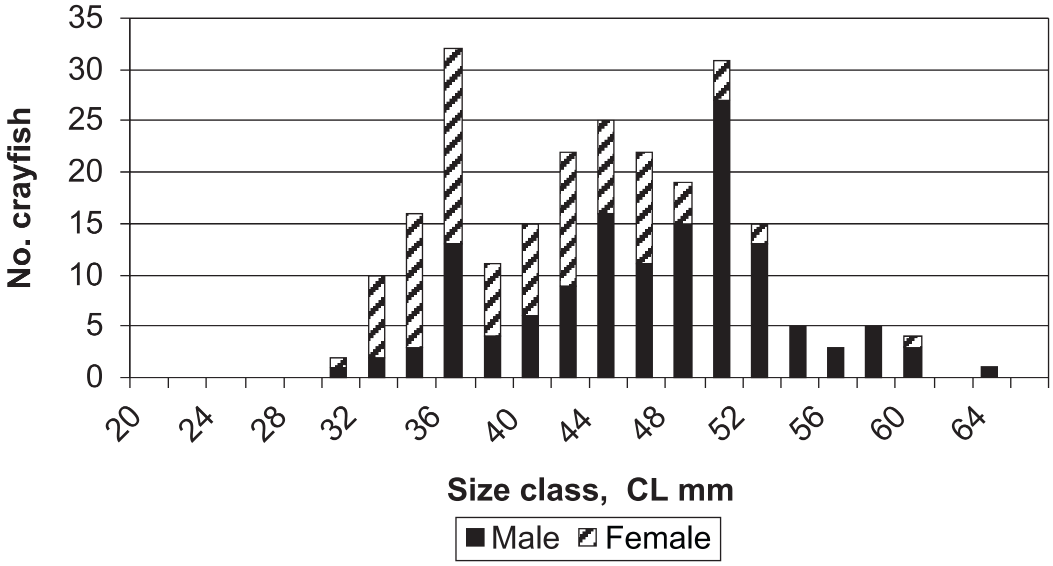
3.2 Crayfish population

The gravel pit had established an abundant population within 6 years. Table 6 shows the catch per unit effort (CPUE, no. of crayfish caught per trap) for single-night trapping sessions using Swedish Trappy traps baited with cat food. The apparent reduction from 15.1 CPUE in August 2003 to 3 CPUE in October 2004 is likely to be the effect of season, rather than any true reduction. The total catches on 2nd and 3rd October were 118 and 123 crayfish respectively from 20 traps at the same locations. This shows there was no detectable effect on the population in the vicinity of the traps from removal of the catch.

In August 2004 many crayfish were seen walking by day in the margins of the gravel pit. One was seen repeatedly emerged almost wholly out of water to grab food off the gravel shore. This suggests a population at high density. Despite this, *Elodea crispa* formed extensive beds in areas 1.5m deep or less, suggesting it may not be very palatable to the crayfish.

Figure 2 shows the size distribution of the catch. As expected, it only shows the upper tail of the population distribution, with none of the small size-classes represented in the trap catch and males representing 66% of the catch.

Figure 2 Size distribution of signal crayfish at gravel pit, Edzell, before treatment, October 2004 (from trapping)



By contrast, the recently established populations at Drumtochty had a much lower CPUE, even though they were sampled in September, when the catch would be expected to be higher than in October. CPUE was 0.8 and 0.2 at the Castle Pond and lower Mains Pond respectively.

3.3 Results of preliminary toxicity tests at the gravel pit and Castle Pond

The results of the "dirty bucket" toxicity tests on crayfish are given in Table 7 for the gravel pit and Table 8 for Castle Pond, which has very similar conditions to the Mains ponds.

At the gravel pit doses of 0.1mg l^{-1} and 0.2mg l^{-1} natural pyrethrins, preceded by deoxygenation, killed all the crayfish within 24 hours in the test. A lower dose of 0.05mg l^{-1} with deoxygenation killed half the crayfish in 24 hours, 0.01mg l^{-1} with deoxygenation, and deoxygenation alone, only killed a quarter of them.

The tests at Castle Pond were extreme, because although the pond was clear, a shovelful of very silty mud and gravel was used in each tub, which with crayfish activity and the need to net crayfish to observe condition, meant the water stayed completely opaque for the entire test. Some of the crayfish were heard doing tail flipping during the first hour of the treatment. None of the crayfish died within 24 hours in the toxicity tests at Castle Pond, although most did in tests with pyrethrins at concentrations of 0.1mg l^{-1} and 0.2mg l^{-1} within 2 days. After 39 hours, the only survivors at those concentrations were in very poor condition, with barely any limb response and eyestalk movement difficult to detect. They are unlikely to have survived another day. Some crayfish died at lower doses of Pyblast in these muddy tests, but with one still self-righting after 39 hours in the 0.05mg l^{-1} treatment, there was a risk of recovery if the low dose was used.

In the treatment with deoxygenation alone, 7 of 8 crayfish were not dead, even after 39 hours of anoxic conditions. Some of the crayfish that survived more than 24 hours at 0mg l^{-1} dissolved oxygen, without Pyblast were not self-righting when turned over, but still made movement of their limbs voluntarily.

The behaviour of the crayfish was observed in more detail in the deoxygenation test at the gravel pit, in which crayfish had the option of climbing up to the surface, as in the bucket tests carried out by Hiley and

Peay (2003). Crayfish started responding within 5 minutes of the addition of sodium sulphite, when the dissolved oxygen was falling rapidly, from 10.5 to 5mg l^{-1}. Crayfish started walking faster than in the control tank and became more aggressive. At least one undertook tail flips and several started climbing on the fabric. Even when the oxygen level was below 2mg l^{-1} crayfish were more active than in the control and it was only at 0mg l^{-1} that crayfish became torpid, 30–40 minutes after the start of deoxygenation with sodium sulphite.

The dry tests, in which crayfish were placed on sand and gravel that had been previously sprayed with Pyblast, (Table 10) showed that lightly sprayed sand and gravel had visible effects on crayfish in less than 5 minutes and they were torpid within 20 minutes. There did not appear to be much difference in the crayfish given 1.5 minutes exposure compared to those left on the dry treated surface. Even after 3–4 hours of sunshine after the spraying of the exposed banks of the gravel pit with Pyblast, the sprayed margin of the gravel pit was still toxic to crayfish (Table 11). Stiffness was apparent within 2–3 minutes. All the crayfish were dead after 28 hours.

3.4 Post-treatment site observations at the gravel pit stage 1 and Mains ponds

Gravel Pit – stage 1

Searches of the margins were carried out at night to look for live crayfish in the gravel pit after the treatment. The results are shown in Table 14. Visibility improved slightly as crayfish activity ceased and silt settled, but the increase in the number of dead crayfish from 87 on the first night to 552 on the third night reflects increased mortality rather than better clarity. It was evident that herons were taking dead crayfish, so all accessible dead ones were removed (as required by SEPA), although the toxicity of pyrethrins to birds is exceedingly low (Agropharm Ltd provided published ecotoxicology data indicating acute oral LD_{50} for mallard ducks >10,000 mg/kg).

The number of live crayfish seen decreased from 32 at night a few hours after treatment, to 2 on the second night, 1 the night after and none on the 4th night. This would have been encouraging, except for the appearance of a live crayfish on day 5.

On 6th October, nearly 24 hours after treatment, crayfish were collected from 1x1m quadrats extending from the waters edge at randomised locations along the south shore of the gravel pit. The results are summarised in Table 15. Density ranged from 1–17 crayfish m^{-2}, along an ostensibly homogeneous shoreline, with an average of 7 crayfish m^{-2}. A total of 89% mortality in the quadrats was confirmed at that stage. Survivors were put into a strong Pyblast solution and taken away for disposal.

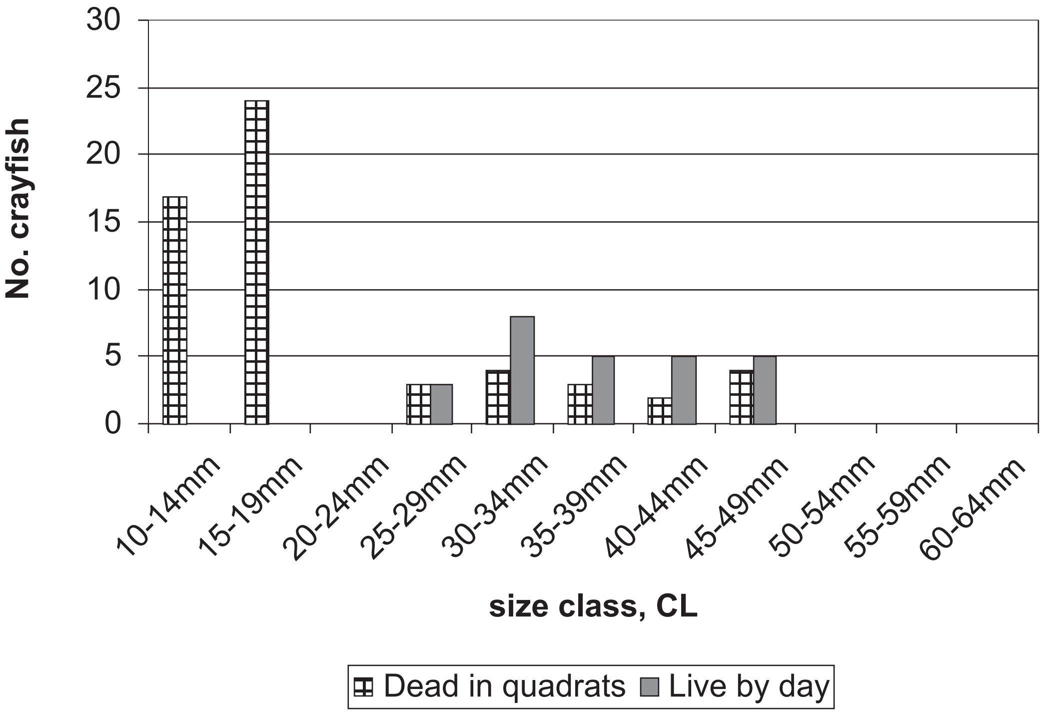
Not surprisingly, most of the crayfish seen in the unvegetated shallow margin were juveniles. There were no in-bank refuges for crayfish in this length of moderately shallow sloping shore. Indeed, the only place where there were refuges in the banks of the gravel pit was at the end of the spur, a peninsula that had been reinforced with some boulders and where there were young willow trees, with roots extending to the water level. The rest of the gravel pit margin was relatively unstable, crumbling when the steeper slopes were walked on. No burrows were seen on exposed or submerged margins. It is thought that there may be more clayey patches at the bottom. Although some crayfish were seen in the margins, with their heads out of water, none was found on the exposed gravel margins on any night.

Figure 3 shows the size distribution of crayfish seen on the afternoon of 6th October. The crayfish seen live is the total for the whole 400m perimeter. Those in the dead category are only the ones from nine 1x1m quadrats on the south side. A total of 72% of the dead crayfish in the quadrats were juveniles less than 20mm CL (carapace length), whereas there were no live juveniles under 20mm CL seen anywhere in the margins. Eighty eight per cent of live crayfish were greater than 30mm CL.

Mains ponds

At the Mains ponds, post treatment observations were much more difficult, due to the fringe of floating grass and woody debris around much of the margins of all three ponds. The ponds were treated on 8th October and although night-viewing was carried out that night, none was seen. Only three crayfish were spotted the next day, in the margin of the lower pond in a relatively clear area. Another 25 crayfish were seen on their backs, in an area enclosed by wire mesh immediately adjacent to the outfall pipes. All were dead or torpid, except for one, which was not self-righting about 18 hours after the treatment. The washings from the spent containers and spray tank were then swilled out in this area as a precautionary measure. About 30 adult crayfish had already been removed from this outfall area before the treatment.

Figure 3 Size distribution of crayfish in gravel pit margin, c. 22 hours after treatment. Live by day – all sightings, not just in quadrats.



3.5 Results of post-treatment toxicity tests at the gravel pit stage 1 and Mains ponds

Deoxygenation at the gravel pit and Mains ponds

Table 16 shows the effects of the deoxygenation treatment at the gravel pit. The day after treatment the water was markedly stratified, with water about 0.5m below the surface still well aerated, whereas water within 0.5m of the bottom was 2.2mg l^{-1} at 14:30 on the day after treatment and down to 0mg l^{-1} throughout by 18:00. Even in the margins, the dissolved oxygen remained very low for the first 5 days after treatment. Two weeks later the dissolved oxygen was still depressed in the margins, at about 5.8mg l^{-1}.

By contrast, at the Mains ponds on the day after treatment, results were highly variable (Table 17). This may be related to the problem of caking with dry application of sodium sulphite. Only at the middle pond did dissolved oxygen fall below 2mg l^{-1}. In the lower pond dissolved oxygen remained too high to stimulate any response from fish. Only when Pyblast was applied did fish respond, by running in to the margins, where they were caught and despatched on the bank. In all, 28 stocked brown trout were killed.

Toxicity at the gravel pit stage 1 and Mains ponds

Water samples were taken from various locations across the gravel pit 24 hours after treatment and healthy crayfish were added (Table 18). In samples of water taken at depth half the test crayfish died within 12 hours, nearly 60% of them within 24 hours and 75% in about 48 hours. There were some surprising results. In one sample one crayfish remained self-righting, while the other died. Samples taken across the gravel pit within 0.5m of the surface were less toxic after 24 hours. In the surface samples, no crayfish died within 12 hours, only 25% died in 24 hours and 50% in about 48 hours from the start of the test. This result may be due to a combination of the effect of Pyblast density and greater exposure to sunlight at the surface breaking down the Pyblast. There was at least partial mortality within 48 hours in all samples taken a day after treatment.

Another batch of samples was taken about 40 hours after the treatment of the gravel pit. These samples were noticeably less toxic to healthy crayfish. After more than 48 hours, there were dead crayfish in only 4 of the 7 samples. It was the not self-righting and slow self-righting survivors of this later test that showed the most improvement in condition when they were put into clean water (Table 19).

The samples taken a day after treatment of the Mains ponds suggest the middle pond had the most effective dose. In the samples from the lower pond none of the crayfish died within 24 hours or so during which the test was run. The test is inconclusive, as further deterioration of condition would have been expected if the test could have been left for another day or two.

3.6 Results of further toxicity tests (off-site)

Additional "dirty bucket" toxicity tests were carried out after the first treatment of the gravel pit, off-site in Yorkshire, to assess the effects of the deoxygenation pre-treatment on the toxicity of Pyblast to crayfish. As indicated by the more rapid recovery in the preliminary tests with *Asellus* (Table 5), the treatments with sodium sulphite were either less toxic to the crayfish, or toxic more slowly (Table 12). Once there was enough sodium sulphite added to fully deoxygenate the water, there appeared to be little difference in the adverse effect of sodium sulphite on the toxicity of Pyblast to crayfish. There was little difference in the condition of crayfish in the treatments with 1x, 2x or 5x the dose of saturated sodium sulphite required to fully deoxygenate the water. It had been expected that effective toxicity of Pyblast would be highest with no sulphite, reduced with 0.5x dose of sodium sulphite, and reduced progressively to lowest toxicity with 5x sodium sulphite, but no increased effect was seen with greater surplus of sodium sulphite.

If the Pyblast was completely degraded and deoxygenation was the only effect, the crayfish would be expected to recover completely when any survivors were transferred to clean water. With deoxygenation, if the treatment does not kill the crayfish during the period of exposure, they appear to recover fully when placed in clean, well-oxygenated water. In the treatments with sodium sulphite and Pyblast there was some improvement in condition of surviving crayfish when transferred from treatment with excess doses of sodium sulphite in addition to the Pyblast, but they did not recover fully. This suggests there was enough Pyblast, at least in the initial period, to have debilitating effects on crayfish, despite the action of the sodium sulphite.

Various doses of Pyblast were tested without prior deoxygenation, with mud/substrate. At doses of 0.2mg l^{-1} or 0.5mg l^{-1} all the crayfish died in 24–48 hours. At 0.1mg l^{-1} most crayfish died within 48 hours in the cool conditions. One survived in a torpid condition and improved somewhat when placed in clean water, but it was still not self-righting after 36 hours recovery, 4 days after the treatment. As in the "dirty bucket" toxicity test at Castle Pond, there was a high clay content in the water, although it was not as silty.

High crayfish activity was noted in some of the buckets soon after application of Pyblast, especially in the higher doses; including swimming and tail-flipping. This behaviour was also seen in some of the treatments with deoxygenation and in the gravel pit during deoxygenation, prior to application of Pyblast.

3.7 Results from the gravel pit treatment, stage 2

By the time the gravel pit was re-treated (stage 2), 24 days after the first session, there were already signs of re-colonisation by aquatic invertebrates (especially Corixidae, which were abundant). The water had re-aerated, with dissolved oxygen reading approximately 10.5mg l^{-1} at 9°C.

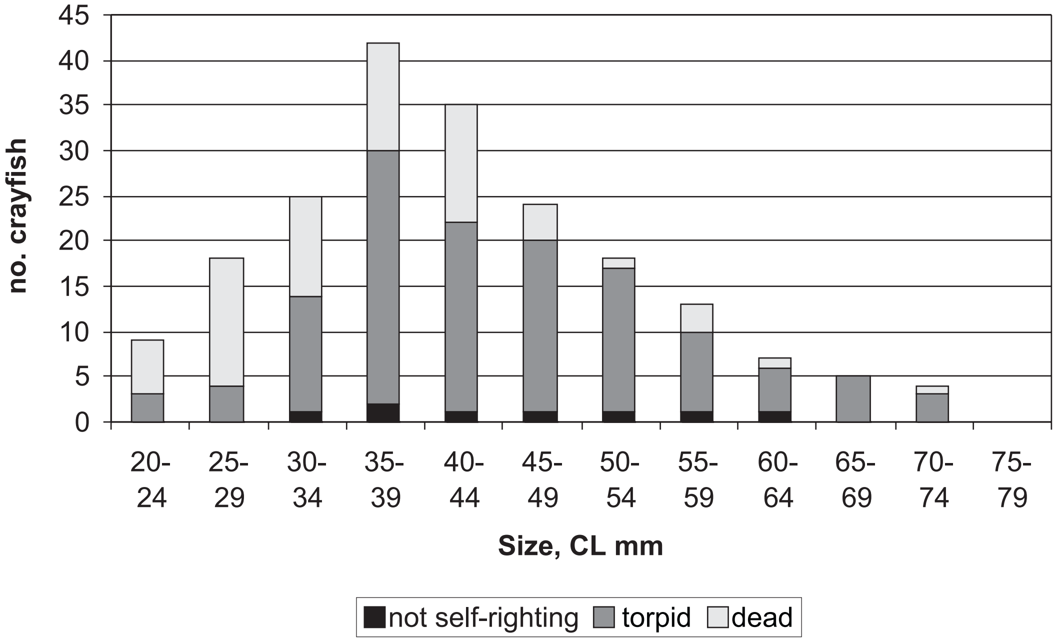
The condition of the crayfish in the test cages, placed in the pond prior to treatment, is shown in Table 21. Half the cages were lifted briefly for inspection after 1 day. The crayfish were touched or turned with a rush stem only and were left in the cages. Of these, 3 out of 10 cages had crayfish that were still self-righting. After 2 days, there was a marked deterioration in condition in the cages that had been inspected on the previous day and replaced in the gravel pit. After 2 days, only 8 crayfish in the 20 cages still showed any voluntary movement of their limbs, compared to 124 (66%) that were torpid and a further 66 (33%) confirmed as dead. After 5 days almost all survivors were dead. Only 7 showed any response and all of these were in a very poor state, with minimal or no movement of limbs, even when handled. Recovery was very unlikely, given the moribund condition. All the crayfish tested were taken for disposal.

Figure 4 shows the size distribution and condition of caged crayfish after 2 days exposure to treated water. The proportion of dead crayfish in size classes below 45mm CL is greater than that of 45mm CL and above.

The post-treatment toxicity test (Table 22) shows that 1 day after treatment to the target dose of 0.15mg l^{-1} the water was still toxic enough to severely debilitate all the test crayfish within 24 hours and kill them within 48 hours.

With no crayfish seen on 28th October in the night-viewing session prior to the re-treatment of the gravel pit, it was considered to be not worthwhile surveying after treatment. There was evidence, however, that the treatment had killed at least some wild-living survivors of the first treatment. Two crayfish were found recently dead in shallow water on 31/10/04, 2 days after treatment (a female, 29mm CL on the south west side near the reedmace and a male, 28mm CL, off the end of the peninsula).

Figure 4 Gravel pit stage 2, condition of caged crayfish 2 days after treatment, by size



3.8 Results from Castle Pond treatment

Two days after treatment of Castle Pond to the target concentration of 0.2mg l^{-1}, the test cages were lifted and the condition of crayfish was assessed, as shown in Table 23 and Figure 5. The proportion of crayfish dead at this stage was very similar to that at the re-treatment of the gravel pit. There were 35.7% dead, 63.7% torpid and 0.5% (1 crayfish) with some voluntary limb movement, but not self-righting. As the water temperature was only 4°C, (rather than 9°C at the gravel pit in late October), the crayfish were taken off-site for a recovery test in clean rainwater (Table 24). By the following day, after about 13 hours in clean aerated water at 6°C, there were only five crayfish that made any limb movement when touched with a rush stem. Once the water was warmed to 9°C and then 13.5°C 6 more crayfish showed a very slight limb movement when stimulated, but others that had responded earlier were dead. When the tanks were checked after 2 days in clean aerated water all the remaining crayfish were confirmed as dead.

Toxicity tests were carried out with 6 water samples taken 24 hours after the treatment of the pond (Table 25). 48 hours later eight crayfish were dead and eight were torpid. The torpid crayfish were all in poor condition, with the exception of those placed in a water sample taken immediately in front of the inflow stream. There was a small continuing inflow of river water and two of the three crayfish tested in water from this area were in noticeably better condition, on their backs but making some slight limb movements. The sample site at the inflow was very shallow and filled with soft, silty sand and gravel.

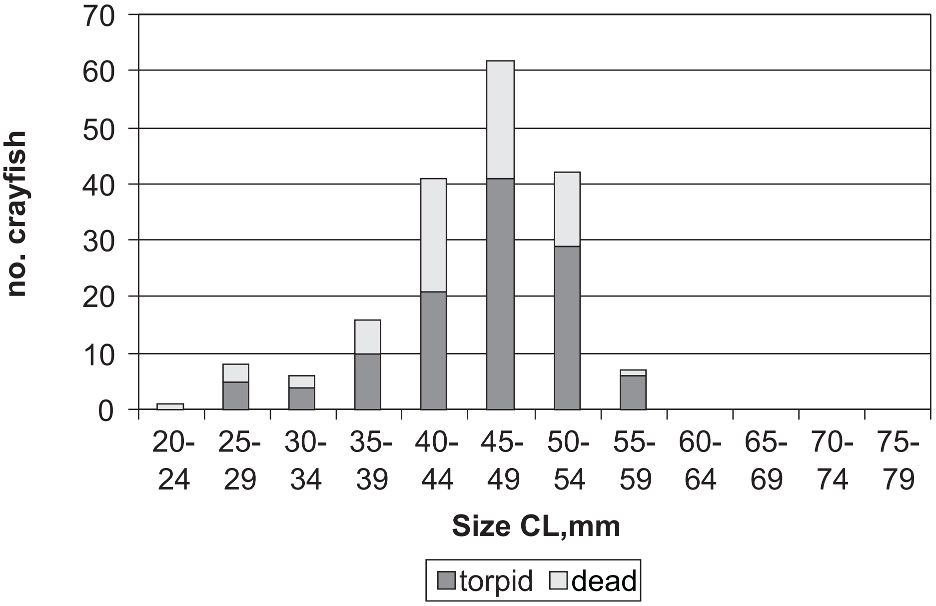
There were a few live crayfish left (10) that had not been used in tests. These were caged and put in the pond on 15/12/04, 3 days after treatment. The cage was not lifted until 14/01/05, when all the crayfish were confirmed as dead.

It was clear that treatment with Pyblast had brought at least some of the crayfish out of their refuges, as 11 were found (Table 26). This is despite overhanging vegetation and soft substrate making viewing of the shallows rather difficult, combined with the return pumping of leaked water making the pond rather turbid.

Not all of these were dead on the day after treatment, but they all died subsequently. A total of 7 dead trout was found, the few that escaped in October.

Night-viewing prior to the treatment showed no crayfish activity, which is much as expected in December. No night-viewing was carried out after the treatment, partly because of the nil return prior to treatment and also due to the increased turbidity during pumping.

Figure 5 Castle Pond, condition of caged crayfish 2 days after treatment, by size



Biomonitoring associated with Castle Pond treatment

The recovery of the Castle Pond was monitored using water louse (*Asellus*) in a dilution series. Test results are shown in Table 27. Three days after treatment of the pond the water was still toxic enough to kill *Asellus* at 100x dilution, although some *Asellus* were able to survive in water at this dilution 4 days after treatment. 11 days after treatment *Asellus* were surviving in 10x dilution and effects were starting to ameliorate in undiluted pond water.

Pumping to contain the water was maintained until 15 days after treatment. The dams in the outfall ditch were removed and water was allowed to enter the river.

The results from the preliminary recovery tests with *Asellus* (Table 5) showed it took between 2 and 5 days from partial to full survival of *Asellus*. The pump was turned off 4 days after partial survival of *Asellus* in an undiluted sample of pond water (although the test on 23rd December was observed only for a few hours after sampling and was not recorded 24 hours or more after the start of the test).

Bags of shrimps (*Gammarus*) survived in the River Luther both immediately upstream and 10m downstream of the outfall ditch from the pond, throughout the period 11th–23rd December, despite a little seepage from the treated ditch through the earth dam at the downstream end and the pump failure on 13th December, which allowed a little of the pond water to escape over grass upstream of both the biomonitoring bags. When the bags were inspected and lifted on 14/01/05, there were still live shrimps in the bag upstream of the outfall ditch, although a few had died. None was found alive in the bag downstream, indicating localised pollution after the water was released from Castle Pond. This is discussed in section 4.2.

4 DISCUSSION

4.1 Effectiveness of treatment

The treatment produced some dramatic mortality at the gravel pit in stage 1. The presence of one active crayfish showed that treatment to the target dose of 0.1mg l^{-1} was not 100% effective, at least in part due to the interference from the deoxygenation pre-treatment.

In bucket tests deoxygenation does stimulate crayfish to emerge and become active, which then increases their exposure to the Pyblast. It would be difficult to confirm this in the field, except perhaps with a series of underwater cameras. The Pyblast is adversely affected by the sodium sulphite. If it were certain that all the crayfish received a lethal dose immediately, before the Pyblast started to break down, the pre-treatment with sodium sulphite might be seen as advantageous because of the more rapid recovery of the water body compared to use of Pyblast alone. In field conditions, however, it is uncertain what dose crayfish receive if Pyblast is applied after sodium sulphite. As the Pyblast is the toxicant and is expensive, it is recommended that it is used alone.

There was some evidence that Pyblast on its own stimulated crayfish to aggression and activity, both from the dirty bucket tests and in the field. Signal crayfish show very little nocturnal activity in winter (Bubb *et al.*, 2002), so to see dead and dying crayfish on their backs around Castle Pond, when the water temperature was about 4°C shows crayfish tried to escape the toxin.

It is not certain how readily the Pyblast can diffuse into burrows at toxic rates, nor the rate at which it binds to clay or silt and becomes inactive. This was the reason for using deoxygenation as a pre-treatment, to bring crayfish into the open. At the dilution rates applied, Pyblast was denser than water, which would help it to penetrate refuges on the bottom. A test-spraying of Pyblast with a knapsack sprayer was carried out at Castle Pond over some burrows in the bed. A milky white cloud moved across the bed and then slowly dispersed. A similar effect was seen during the second treatment of the gravel pit. This density effect improves the chances of Pyblast penetrating the burrows in the early stages of treatment.

The amount of clay and silt does make a difference, as evidenced by the bucket tests. Tests carried out at the gravel pit in 0.1mg l^{-1} Pyblast with prior deoxygenation killed the crayfish within 24 hours. The same treatment in very silty clay from Castle Pond did not produce any mortality until 36 hours elapsed. Similar delayed effects were seen with sandy clay in the off-site tests. Temperature makes a difference too, with toxic effects less at low temperature. Hiley and Peay (2003) found a dose of 0.05mg l^{-1} was effective in bucket tests with clay at water temperatures around 25°C, but the gravel pit was treated at about 9°C and the Castle Pond at 4°C and the bucket tests done on site showed that higher dose rates were required. The cold temperature and low activity of crayfish prompted the use of 0.2mg l^{-1} for the treatment of Castle Pond. It was encouraging to find that water that leaked out of the pond into the sump was enough to kill all the crayfish in two cages with only 12 hours exposure. This makes it likely that any crayfish using the old field drains or other fissures would have been sufficiently exposed, especially as the follow-up toxicity test showed the water was still toxic enough to kill new crayfish three days after the treatment.

There is less certainty about the margins. Castle Pond was not full to overflowing, which meant there was a narrow zone 0.1–0.2m wide in parts of the margin where the rush stems were exposed. With relatively little stone on the bed that would offer refuges, these rush tussocks would make favourable refuges if covered.

It is not known whether any crayfish stayed in them at lower water levels. All of this area was thoroughly soused with spray, over and under the tussocks. At the application rate of 3g l^{-1}, this would certainly kill any crayfish coming in contact with it, with the effect lasting for hours, especially in a valley with no direct sunshine at all in the short winter days. If there were any burrows going deep into this vegetation, however, and crayfish were sitting in them in a state of winter torpor, they might have escaped treatment. On the other hand, they would also have been out of water and exposed to temperatures well below freezing.

Marginal vegetation was also an issue at the Mains ponds. Here, turning the elbow-joint outfalls upward allowed the ponds to be overfilled compared to usual conditions, so all of the marginal vegetation was completely inundated. Any burrows in the banks would have been submerged. Although spray from the bank may not have penetrated deeply into dense tussocks of rushes, the application from the boat was visibly effective. Angling the spray jet and the movement of the boat put a milky wash of Pyblast into the margins and bank. The effect of the sodium sulphite on the Pyblast is likely to have been less than in the first treatment at the gravel pit because the dry application led to much of it caking and falling into the mud at the bottom, rather than dissolving. Nonetheless, the lower pond in particular is silty, with a lot of leaf litter from the alder trees standing in the pond. This may explain the relatively rapid recovery time on the pond, less than a week. The aggregation of crayfish around the outfall may be an indication of less favourable conditions elsewhere in the pond.

There was quite a lot of variation in the susceptibility of individual crayfish to the treatment, even in cages, where the crayfish had the same exposure. Crayfish below 30mm CL were the most susceptible, yet there were still individuals in the 25–30mm CL that survived longer than some of the larger crayfish. The very largest animals above 60mm CL appeared to be more affected than those in the range 40–50mm CL, but the difference is not significant.

Achieving the target dose requires a reasonably good estimate of the volume of the pond. At Castle Pond an acoustic bathymetric survey was undertaken, but even so, it was necessary to make some decisions about an allowance to be made for slight inflow seepage, the turbidity from the pumping operation and how to apply the Pyblast, putting more on at the deep end and less in the shallower half. At the other ponds, estimates were based on measurements from the bank and plumbing the depth from a boat. A small allowance was made for measurement error, but there was a degree of uncertainty about whether the target dose was achieved uniformly across the pond.

The extra dosing of the banks and margins was appropriate, as during the first trial at the gravel pit some crayfish were seen walking into shallower water and a few were found with their heads or tails out of water at the edge. As these crayfish became stiff or torpid they were more vulnerable to predation by birds. There were signs that one or more herons were taking crayfish and those within reach were manually removed. No crayfish were found wholly out of water on the broad gravel margins, which had been sprayed with Pyblast as a precautionary measure, so there is very little likelihood of any having escaped this way.

4.2 Other effects

The measures put in place to prevent pollution of the River Luther were effective during the treatment period, despite the incident when the main pump failed and emergency action was needed. There was a little seepage of water through the dam at the downstream end of the ditch, but this was diluted with some additional field drainage just before it entered the river. The survival of the shrimps throughout the period up to 23rd December indicates the control was sufficient.

Nonetheless the loss of shrimps once the pond and outfall ditch was allowed to discharge suggests the release may have been made a day or two too early. Weather conditions were largely dry over Christmas and the river was at relatively low flow. Other aquatic invertebrates were found to have colonised the downstream bag, but the loss of shrimps indicates pollution had occurred, as most of those upstream survived. Residual Pyblast is the most likely reason for the impact, although the outfall ditch was also affected by other pollutants. These were naturally occurring ochre, the remains of the earlier fuel oil pollution, and a substantial overflow of domestic sewage, which occurred during a wet period between the end of October and 9th December. All of these additional pollutants were contained while the outfall ditch was dammed and kept full, but were discharged rapidly when the dams were opened. The water from the outfall ditch would have reached the river without dilution from Castle Pond when the downstream dam was removed. In addition, the outfall ditch was treated separately from the pond and because there were overhanging rushes and grasses to be treated along the length of the ditch, the resultant concentration of Pyblast in the water may have been higher than in the pond.

Ideally, additional toxicity tests would have been carried out on and after the 27th December and with water samples from the ditch as well as Castle Pond. Unfortunately, the supply of *Asellus* was finished, as the seasonal pond in which they had been stored originally was too acidic or otherwise unsuitable and significant mortality occurred before they were removed. The shady seasonal pond was used because it was well away from any possible contamination with Pyblast. The river could have been used, but there was concern about sachets being lost if the river was at high flow during December.

Gammarus was available in the river, but could only be obtained in very low numbers by the usual kick-sampling and netting. They were present in greater abundance in very shallow water under stones at the edge of the river, but as temperatures fell as low as −12°C after the treatment, the river margins were covered in thick ice and these areas could not be sampled. It was difficult even to take water samples from Castle Pond except at the pump discharge. The test with *Asellus* on 23rd December was not run for a 48-hour period, due to limited staff availability. In retrospect, it may have been better for water samples to have been sent to Yorkshire, where *Asellus* was readily available and observations could have been made for 48 hours each time. This would have introduced delay, however, during which there would have been additional cost of pump hire, fuel and local labour.

There may also be differences in the sensitivity of *Gammarus* to Pyblast, compared to *Asellus*. *Asellus* were used originally because they occur naturally in ponds and so are suitable for tests in un-aerated still water, whereas the *Gammarus* tend to be found in running water.

By contrast with Castle Pond, biomonitoring of the small stream immediately downstream of the Mains ponds confirmed the treatment of these ponds had no adverse effect on non-target areas. The insect and crustacean fauna remained alive during the treatment of the ponds and after the re-opening of the outfall. Sampling of the stream a few metres away from the lower pond, but upstream of the outflow, on the day after treatment confirmed there was no impact of any spraydrift on the stream. Terrestrial insects in the rushes and other vegetation around the margin of the pond would have been affected on the day of treatment and for some hours subsequently.

4.3 Future requirements for the project

Although there are encouraging signs that the treatments went well, the target is eradication, 100% mortality. There is no certainty of success as yet and because of this we cannot recommend wide general use of this method. There is a need for monitoring for crayfish at all the treated sites in future. The recommendation is for surveys in the first three years after treatment, year 5 and year 10; all of which need to be done with enough survey effort to give confidence in a negative result. A minimum of 20 traps per site on at least one, and preferably two or more occasions in July–September is recommended. If a single crayfish is found, it means eradication has failed.

If monitoring records any crayfish a decision would have to be made by the statutory agencies involved as to whether to make another attempt with biocide treatment, or give up and leave signal crayfish to colonise the catchment.

There is also some risk of malicious re-introduction; or accidental introduction with stocked fish, if the stock is from a supplier with signal crayfish in his fish ponds.

More survey effort should be made in the River Luther in late summer 2005 to see if there are any signs of a wild population near Castle Pond. Although any hitherto undetected river population is unlikely to be able to re-colonise the pond, if the crayfish are out in the river, the primary objective of prevention would be lost. "Control" of a crayfish population is not a viable option. It is not worth pursuing anything other than eradication.

The Mains ponds have too large a throughflow at times for any fine-mesh screen to be used. Better control of the inflow and diversion of runoff to the adjacent stream, away from the pond, would help, but would require design and construction, which would incur additional cost. Unless eradication is confirmed, the lower pond should be assumed to be at risk as a possible source of crayfish.

Screening of the outfall pipes at Castle Pond has been attempted, but will only work if the flow through the pond can be properly regulated. Excessive flow has already silted up half the pond to a depth of 1m or less in 5 years. The inlet pipe is oversized and although sandbags were used to provide better control of the inflow this is only a temporary measure. Ideally, there would be a permanent, adjustable inflow control structure constructed, plus a silt-trap excavated on the inflow stream to facilitate future maintenance. The major loss of water from the pond is another problem and leaves a potential escape route for crayfish if any have survived. This would require proper construction of a keyed-in clay core to completely remove and block old drains from inside the pond, or new dam, or lining. These would all incur cost to the owner and require some expertise.

Another potential problem is that if the eradication has not been successful and re-treatment is proposed, one or both landowners may have re-stocked their ponds with fish in the interim. This would either cause loss of stock, or the time and expense of a fish rescue in advance. There would be a case for re-treating both the Mains ponds and Castle Pond in summer as a precautionary measure, before any re-stocking with fish. This would incur significant additional costs and there is uncertainty as to whether the treatment is actually needed.

The high cost of Pyblast is a significant issue. The higher the dose used, the better the chance of killing all the crayfish, but the higher the cost. In addition, at higher doses the recovery period will be longer, which means at sites where water has to be controlled by pumping there are extra costs of equipment. It may also incur increased costs for biomonitoring during the recovery period for the pond.

There was no choice in the time period for the treatments in this project, as delaying the work to the following year would have had a high risk of crayfish escaping to the river system at Drumtochty. Ideally, any future treatment would be done in late July to early September, when water temperature is at its highest and crayfish are most active.

4.4 General recommendations

Dirty bucket toxicity tests with crayfish should be done prior to any treatment of a water body. Ideally, one would start a project like this in early summer, perhaps June–July, doing crayfish surveys and acquiring crayfish for tests, carrying out preliminary on-site toxicity and recovery tests, undertaking bathymetric survey, and preparing a detailed plan for any required control of water. This would allow a more accurate estimate of Pyblast requirement to be made and a judgement about dose rate versus chemical costs plus recovery time and cost. Funding would have to be secured promptly and all the resources of chemicals, equipment and staff arranged in time to ensure the treatment went ahead at the optimum time, in late July–September, ideally, during stable dry conditions in August.

The most reliable target dose is probably one that gives 100% mortality of a fairly large test sample of adult crayfish within 24 hours. This gives some allowance for variability of field conditions and for complete mortality in the water body taking 2–5 days. Both the use of caged crayfish and the post-treatment toxicity tests with crayfish were very useful. In addition, there would be some merit in checking the effective coverage of Pyblast soon after treatment using *Asellus* or *Gammarus* in diluted treated water.

Control of treated water is essential and full allowance should be made for the preparation, on-site management and cost of this. It would be preferable for different individuals to have responsibilities for the hydraulics and the biological science in any future project. In this study the need to keep the project programme as condensed as possible meant it was sometimes difficult to carry out all the physical work on site, plan the next stages and implement all the tests and monitoring at the same time.

Ponds of up to 1ha in size can be treated within a day, but advance preparation is needed and sufficient time afterwards when the water body is still toxic. It is recommended that a post-treatment period of at least three weeks is planned and more than this if a dose above 0.2mg l^{-1} is used. The actual time required may be much less, especially in warm conditions, but the resources need to be available.

The time, effort, materials and equipment required for a project like this should not be under-estimated; nor the degree of coordination and cooperation necessary, especially with a number of individuals and agencies contributing to the project in different ways. Securing the cooperation and preferably the active support of regulatory agencies is essential.

This is the first time that there has been any field scale use of a biocide against crayfish in the UK. It is also, as far as we are aware, the first use of natural pyrethrin for this purpose anywhere. The reason for this choice of biocide was the need to avoid adverse impact on non-target areas. Hence a product was chosen with low toxicity to mammals and birds and a short recovery period. The trial has shown that, with care, it is possible to carry out a treatment safely at field scale, even in ponds with a throughflow (provided it can be stopped temporarily).

The number of sites where use of biocides could be used is relatively small. It is only possible where the landowners and statutory authorities agree that a total biocide is acceptable. This means accepting the loss of any non-target fish, aquatic invertebrates and possibly amphibians in the water body to be treated.

To be worthwhile, the total extent of the crayfish population has to be treated at a high enough dose to be reasonably confident of complete mortality. If the population has already escaped into a watercourse, there will usually be no possibility of treating the whole population. The leading edge of such a population will be at a density too low to detect reliably. This means a biocide is only worth using on recently established populations where there is a reasonably good chance that it is still limited to a relatively small, treatable water body.

It is too early to be sure whether the method can achieve a complete eradication, despite promising early indications. Manual removal, electro-fishing and trapping have all been shown to be ineffective in other studies and should not be used in any attempt to eradicate or control crayfish. If use of a total biocide cannot eradicate crayfish, there are no viable alternatives currently available or in prospect.

5 TABLES

Table 5 Recovery tests with *Asellus*

Toxicity tests run at Bingley, West Yorkshire, started on 04/09/2004.

Set up: plastic tanks set up with 20 l rainwater.

Tanks with clay substrate set up using c. 3cm depth sandy clay garden subsoil.

Water temperature c.13°C.

Treatments: deoxygenation with 20ml saturated sodium sulphite.

Pyblast stock solution (3% w/w pyrethrins) made up to 10mg l^{-1} then 200ml added to give 0.1mg l^{-1}.

Aeration, was started 1 day after the treatment and continued for 6 days, except in treatment in dark.

Biomonitoring with fine netting sachets of c. 20 *Asellus*, with a piece of partly decomposed leaf.

Dead invertebrates were removed on inspection and any live ones returned to the treatment.

Additional numbered sachets were added to control tanks when fresh ones were added to the other treatments.

Date	Control	Control with clay	Pyblast	Pyblast with clay	Deoxygenation then Pyblast with clay	Deoxygenation then Pyblast with clay in dark (no aeration)
04/09/2004	Live	Live	no animals used	no animals used	no animals used	no animals used
05/09/2004	Live	Live	affected in minutes, all dead within hour	rapidly affected, all dead within 12 hours	rapidly affected, all dead within 12 hours	no animals used
06/09/2004	Live	Live	all dead in past 24 hours	all dead in past 24 hours	rapidly affected, dead within 12 hours	no animals used
07/09/2004	Live	Live	all dead in past 24 hours	all dead in past 24 hours	50% dead in past 24 hr	all dead within 12 hours
08/09/2004	Live	Live	all dead in past 24 hours	all dead in past 24 hours	survivors still live after 48 hr exposure, new batch 80% live after 24 hr	all dead within 12 hours
09/09/2004	Live (except 1)	Live	all dead in past 24 hours (<7 hours)	all dead in past 24 hours	survivors still all live after 72hr and 48hr respectively	all dead within 12 hours
10/09/2004	Live	Live (except 1)	all dead in past 24 hours	90% dead in past 24 hours	survivors live after 96, 72 and 48 hr respectively	90% dead within 12 hr, all dead in 24 hr

Table 5 *(continued)*

Date	Control	Control with clay	Pyblast	Pyblast with clay	Deoxygenation then Pyblast with clay	Deoxygenation then Pyblast with clay in dark (no aeration)
11/09/2004	Live	Live	all dead in past 24 hours	50% dead in past 24 hr	batches all live after 4, 3, 2 and 1 day respectively	all dead in past 24 hr
12/09/2004	not available	not available	not available	not available	not available	not available
13/09/2004	not available	not available	not available	not available	not available	not available
14/09/2004	not available	not available	not available	not available	not available	not available
15/09/2004	not available	not available	not available	not available	not available	not available
16/09/2004	not available	not available	not available	not available	not available	not available
17/09/2004	not available	not available	not available	not available	not available	not available
18/09/2004	not available	not available	not available	not available	not available	not available
19/09/2004	Live	Live	not available	survivors of 11/9/04 still live	survivors of 11/9/04 still live, new batch added	new batch added
20/09/2004	Live	Live	75% survival in first 12 hours	new batch 100% survive 24 hr	Live	95% live after 24 hr
21/09/2004	Live	Live	12% survival in past 24 hours	survivors 11/9/04 live, new batch 95% survive 48 hr	Live	70% dead in 48 hr, new batch added
22/09/2004	Live	Live	half earlier survivors live >48 hr, >75% new survive past 24 hr	new batch survive 24 hr, old batches survive 48 hr and 72 hr	Live except 1 in batch from 10/09/04	new batch added
23/09/2004	Live (except 1 dead)	Live	50% survive past 48 hr, 75% new survive past 24 hr	Live	Live	survivors of 19/09/04 all live, (30% total), new batches still live
24/09/2004	Live	Live	95% survive past 24 hr, survivors 48 hr and 72 hr still OK	Live	Live	
25/09/2004	Live (except 1 dead)	Live (except 1 in 2 sachets)	Live	Live	Full check of 5 batches, all live (except 3 with 1 dead in each)	3 day exposure 75% live, 4 day 66% survive, survivors of 19/09/04 still live

Table 6 Gravel pit relative abundance of crayfish

Gravel pit	29/08/2003	02/10/2004	03/10/2004
total catch	151	118	123
no. traps	10	40	40
CPUE	15.1	3.0	3.1

CPUE catch per trap (single night trapping, with Trappy® traps baited with cat food)

Table 7 Preliminary toxicity test at the gravel pit, Edzell

Set up: 10 l bucket, 5 l water from gravel pit, 1cm sand and gravel substrate, 2 crayfish (1 large, 1 med), 2 replicates.

Crayfish put in c. 2 hours in advance of sodium sulphite application. Temperature 10°C, dissolved oxygen in gravel pit 10.4mg l^{-1}l.

Deoxygenation with sodium sulphite (deoxy), saturated solution at 1ml/l, 5ml per treatment, all treatments except control.

Pyblast (Py) at variable concentration (prior dilution to 10mg l^{-1}, then further dilution to target concentration in 5 l).

Dissolved oxygen in control at 14:15 6.0mg l^{-1}, down to 4.4.3.5mg $l^{-1}$by 17:05.

All treatments with deoxygenation at 0mg l^{-1} before addition of Pyblast and remained at 0mg l^{-1}.

Date	Time (approx.)	Time elapsed hr	Control	Deoxy.	Deoxy, Py 0.01 mg l^{-1}	Deoxy, Py 0.05 mg l^{-1}	Deoxy, Py 0.1 mg l^{-1}	Deoxy, Py 0.2 mg l^{-1}
04/10/2004	14:00			start SO3-	start	start	start	start
04/10/2004	14:30				start Py	start Py	start Py	start Py
04/10/2004	15:00	1	SR	a) 2 SSR, b) 2 SSR	a)2 SSR, b) 2 SSR	a) 2 NSR b) 2 SSR	a) 2 NSR b) 2 NSR	a) 2NSR b) 2 TAD
04/10/2004	17:00	3	SR	a) 2 SSR, b) 2 SSR	a) 2 SSR, b) 2 SSR aggressive	a) 2 NSR b) 1 NSR, 1 escape swim	a) 2 TAD b) 2 TAD	a) 2 TAD b) 2 TAD
05/10/2004	09:30	19.5	SR	a) 1D, 1SSR, b) 2SSR	a) 2 SSR, b) 1NSR, 1SSR	a) 2 TAD b) 2 TAD	a) 2 TAD b) 2 TAD	a) 2 TAD b) 2 TAD
05/10/2004	12:30	22.5	SR	a) **1D**, 1SSR, b) 2SSR	a) 2 SSR, b) 1NSR, 1SSR	a) **1D**, 1 TAD b) **1D**, 1 TAD	a) **2 D** b) **2D**	a) **2 D** b) **2D**
05/10/2004	15:00	25	SR	a) **1D**, 1SSR, b) 2SSR	a)1SSR, 1SR b) 2 NSR	a) **1D**, 1 TAD b) **1D**, 1 TAD		
05/10/2004	18:00	28	SR	a) **1D**, 1SSR, b) 2NSR	a)**1D**, 1TAD b) 1TAD, 1 NSR	a) **1D**, 1 TAD b) **1D**, 1 TAD		

Key to condition of crayfish

SR self-righting, normal

SSR slow self-righting, movement impaired

NSR not self-righting, but still with voluntary movements of limbs

TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched

D dead, no eyestalk response

Table 8 Preliminary toxicity test at Castle Pond

Set up: 70 l tub, 30 l water from gravel pit, c. 1cm very silty clay substrate, 4 crayfish (2 large, 2 med), 2 replicates.

Deoxygenation with sodium sulphite, saturated solution at 1ml l^{-1}, all treatments except control.

Py= Pyblast at variable concentration (prior dilution to 10mg l^{-1}, then further dilution to target concentration).

Crayfish put in c. 1 hour in advance of sulphite application.

Note: all tanks had black silty sludge added from a backwater margin and stayed very muddy throughout.

Due to mud, crayfish had to be netted and inspected for condition on the ground.

Temperature 9.5°C.

Date	Time (approx.)	Time elapsed hr	Control	Deoxy.	Deoxy, Py 0.01 mg l^{-1}	Deoxy, Py 0.05 mg l^{-1}	Deoxy, Py 0.1 mg l^{-1}	Deoxy, Py 0.2 mg l^{-1}
04/10/2004	18:15			start sulphite	start sulphite	start sulphite	start sulphite	start sulphite
04/10/2004	18:45				start, Pyblast	crayfish active c 18:30, start, Pyblast	start, Pyblast, crayfish active in both tanks before Py	start, Pyblast
04/10/2004	22:00	3.75	SR	a) SR b) SR	a) SR b) 2 SSR, 2 SR	a) 2 SR, 2 not caught b) 4 SSR	a) SSR b) SSR	a) 4 TAD b) 4 TAD
05/10/2004	08:45	14.5	SR	a) SSR b) SSR	a) 4 SSR b) 2 SSR, 2 NSR	a) 2 NSR, 2 SSR b) 2 NSR, 2 SSR	a) 4 TAD b) 2 SSR, 2 SR	a) 4 TAD b) 4 TAD
06/10/2004	09:15	39	SR	a) **1D**, 3 TAD b) 1 TAD, 3 SSR	a) **1D**, 1 TAD, 1 NSR b) **3D**, 1 TAD	a) **2D**, 1TAD, 1SSR b) **3D**, 1TAD	a) **3D**, 1 TAD b) **3 D**, 1 TAD	a) **4D**, b) **3D**, 1TAD

Key to condition of crayfish

SR self-righting, normal

SSR slow self-righting, movement impaired

NSR not self-righting, but still with voluntary movements of limbs

TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched

D dead, no eyestalk response

Table 9 Crayfish activity during deoxygenation

Set up: 70 l tub, 30 l water from gravel pit, 5 crayfish added, mixed sizes from trap catch. Deoxygenation with saturated sodium sulphite at 1ml l^{-1}.

Climbing fabric provided, but removed at 14:45 04/10/04 when test not under close supervision.

Water temperature 10°C throughout.

		Control				Deoxygenation	
Date	**Time**	**DO mg l^{-1}**	**Observations**	**Date**	**Time**	**DO mg l^{-1}**	**Observations**
04/10/2004	12:20	10.4	5 large crayfish added	04/10/2004	12:20	10.5	5 large crayfish added
04/10/2004	13:55	9.3		04/10/2004	13:55	9.3	
04/10/2004	14:18		1 walking, 4 sitting	04/10/2004	14:11		sulphite added
04/10/2004	14:21		1 walking slowly, 4 sitting	04/10/2004	14:14	6, falling fast	
04/10/2004	14:25		4 walking slowly, 1 sitting	04/10/2004	14:16	5.2	1 tail flip, 1 climber, others walking
04/10/2004	14:27		3 walking slowly, 2 in confrontation	04/10/2004	14:20	4.5	3 in confrontation, 1 climbing, 1 walking
04/10/2004	14:27		3 still, 3 walking	04/10/2004	14:22	3.6	4 walking, 1 climbing
04/10/2004	14:35		4 sitting, 1 walking slowly	04/10/2004	14:23	3.3	3 walking, 2 in confrontation
04/10/2004	14:37		1 climbing up tail first, fell back, 4 sitting	04/10/2004	14:24	2.6	4 walking slowly, 1 walking rapidly
04/10/2004	14:40	8.9	all 5 sitting quietly	04/10/2004	14:26	1.6	all 5 walking, some aggression
05/10/2004	14:42	6.4	all 5 sitting quietly, but move normally if DO probe nearby	04/10/2004	14:29	0.4	3 walking, 1 walking rapidly, 1 climbing
05/10/2004	18:17		all 5 sitting quietly	04/10/2004	14:34	0.2	new climber, right up to surface
05/10/2004				04/10/2004	14:35	0	none moving
05/10/2004				04/10/2004	14:40	0	1 climbing other 4 not moving
				05/10/2004	14:40	0	torpid, but move limbs if touched, very slow
				05/10/2004	18:18	0	torpid

Table 10 Test of Pyblast spray on surface

Set up: 70 l tub with base covered in sand and gravel substrate from gravel pit, 5 large crayfish (>50mm CL).

Pyblast at spray dilution of 1 l concentrate in 9 l water (3g l^{-1}). Treatments: control sprayed once with water before addition of crayfish.

Pyblast spray, sprayed once prior to addition crayfish, complete cover but droplets not coalescing.

Pyblast 1.5 min, crayfish moved to clean, dry tub after 1.5 min exposure.

Air temperature in range 10–13°C by day, falling to 9–11°C at night.

Control			Pyblast spray			Pyblast spray 1.5 min		
Date	**Time**	**Observation**	**Date**	**Time**	**Observation**	**Date**	**Time**	**Observation**
04/10/2004			04/10/2004	13:05	spray application	04/10/2004	13:05	spray application
04/10/2004	13:08	crayfish added	04/10/2004	13:08	crayfish added	04/10/2004	13:13	5 crayfish added
04/10/2004	13:15	crayfish active	04/10/2004	13:12	crayfish very active cf. control	04/10/2004	13:14	5 crayfish removed to clean tank
04/10/2004	13:20	crayfish sitting, not moving, but some raise chelae if see observer	04/10/2004	13:19	crayfish live, but not SR if turned over	04/10/2004	13:28	crayfish on sides, still moving, 1 doing tail flipping
04/10/2004	13:40	crayfish sitting quietly, well spaced	04/10/2004	13:28	1 TAD, 4 on sides, limited movement, NSR	04/10/2004	13:36	all 5 on bank or side, no movement of legs unless touched
04/10/2004	14:03	4 crayfish quiet, 1 threat display, then walking	04/10/2004	14:05	all on backs, NSR, limited movement in tail or legs when touched	04/10/2004	14:07	as with crayfish still on treated substrate, all on backs, minimal occasional movement of pleopods
04/10/2004	14:38	4 quiet, 1 walking	04/10/2004	14:38	all on backs, NSR, still some small movements pleopods	04/10/2004	14:39	all on backs, NSR, only very slight movement
05/10/2004	09:43	all quiet until see observers, then 2 making threat display	04/10/2004	18:30	5 NSR/TAD	04/10/2004	18:30	TAD
05/10/2004	14:45	4 quiet, 1 making threat display	05/10/2004	09:45	5 NSR/TAD	05/10/2004	09:45	TAD, but some limb movement if touched

Table 10 *(continued)*

Control			Pyblast spray			Pyblast spray 1.5 min		
Date	**Time**	**Observation**	**Date**	**Time**	**Observation**	**Date**	**Time**	**Observation**
05/10/2004	18:15	All 5 quiet, mobile if tub disturbed	05/10/2004	14:49	**1 D**, 4 TAD	05/10/2004	14:47	**1 D**, 4 TAD
			05/10/2004	18:16	**2 D**, 3 TAD	05/10/2004	18:17	**3 D**, 2 TAD

Key to condition of crayfish

SR self-righting, normal

SSR slow self-righting, movement impaired

NSR not self-righting, but still with voluntary movements of limbs

TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched

D dead, no eyestalk response

Table 11 Test of effect of sprayed margin, 3–4 hours after treatment

Set up: gravel pit margin sprayed with Pyblast (1 l in 9 l concentration, applied to 200m^2) at about 12:00 04/10/04, sunny conditions.

Observations to see if any crayfish emerging on to banks; found a few in very shallow water in margins, partly exposed on w side of spur.

Moved these crayfish individually to top side sprayed margin and allowed them to walk towards water.

Date	Time	Observations
05/10/2004	15:42	crayfish allowed to walk over c. 1.2 m sprayed margin
05/10/2004	15:44	crayfish moved to untreated ground, movement slow, stiff, difficulty righting
05/10/2004	15:51	crayfish not self-righting
05/10/2004	15:49	next crayfish exposed, 2 minutes
05/10/2004	15:54	3 more crayfish exposed, took 2- 2.25 minutes to walk 1m to just short of water (recaptured)
05/10/2004	16:00	all crayfish put in clean bucket
05/10/2004	18:00	still live, but NSR, some still with limb movement, others hardly any
06/10/2004	20:20	crayfish all dead

Table 12 Additional toxicity tests – effects of different doses of sodium sulphite with Pyblast

Set up: 10 l buckets with sandy clay loam subsoil, 5 l rainwater, 4 crayfish. Deoxygenation with saturated solution sodium sulphite, calculated full dose 1ml/l.

Pyblast diluted to stock solution 10mg l^{-1} (1m l concentrate in 3 l water), then diluted to give dose of 0.1mg l^{-1} in tests.

Note: water from rain butt not uniform dissolved oxygen, higher at top after recent rain. Fresh rain in open 10.4mg l^{-1}, water temperature 7.5–9°C.

Note: water muddy from first introduction of crayfish, so all crayfish netted for inspection and tested for condition on ground.

		Individual crayfish used in treatment replicates a and b, by sex (M/F) and sizes, CL mm													
Date	**Time**		a) F34, F30 b) M30, M30		a) M39, M56 b) M48, M44		a) M48, M53 b) M48, F34		a) M40, M58 b) M43, M48		a) M45, M36 b) M51, M45		a) M50, M42 b) 46, F54	
		Treatment and observations of dissolved oxygen (DO) concentration													
		Time elapsed	**Control**	**DO** mg l^{-1}	**no sulphite**	**DO** mg l^{-1}	**0.5x sulphite**	**DO** mg l^{-1}	**1x sulphite**	**DO** mg l^{-1}	**2x sulphite**	**DO** mg l^{-1}	**5x sulphite**	**DO** mg l^{-1}
15/10/2004	16:00	0		7.2/ 7.2		7.8/ 7.6	sulphite added	7.7/ 7.9	sulphite added	7.7/ 8.0	sulphite added	8.0/ 8.1	sulphite added	8.0/ 7.9
	16:10							2.7/ 4.4		1.3/ 0.1		0.1/ 0.1		0/0
15/10/2004	16:30	0.5		6.4/ 6.4	Pyblast added	7.2/ 7.2	Pyblast added	0.2/ 0.1	Pyblast added	0/0	Pyblast added	0/0	Pyblast added	0/0
					activity, tail flip in b 16:45		activity, tail flip in b 16:48, 1 tail up 17:18		activity, antennae out of water in a and b				activity, claws up in b 16:45	
15/10/2004	17:30	1.5	a) 2 SR b) 2 SR	6.3/ 6.3	a) 1TAD, 1 NSR, b) 1TAD, 1 NSR	6.3/ 6.3	a) 2 NSR, b) 2 NSR		a) 1 NSR, 1 SR b) 2 NSR		a) 1 NSR, 1 SSR b) 1 SSR, 1 SR		a) 2 NSR b) 1 NSR, 1 SSR?	
15/10/2004	20:30	4.5	a) 2 SR b) 2 SR		a) 1TAD, 1 NSR, b) **1 D**, 1 NSR		a) 2 NSR, b) 1 TAD, 1 NSR		a) 1 TAD, 1 NSR, b) 2 NSR		a) 2 NSR, b) 2NSR		a) 1 TAD, 1 NSR b) 2 NSR	

Table 12 *(continued)*

		Individual crayfish used in treatment replicates a and b, by sex (M/F) and sizes, CL mm												
Date	**Time**		a) F34, F30 b) M30, M30		a) M39, M56 b) M48, M44		a) M48, M53 b) M48, F34		a) M40, M58 b) M43, M48		a) M45, M36 b) M51, M45		a) M50, M42 b) 46, F54	
		Treatment and observations of dissolved oxygen (DO) concentration												
		Time elapsed	**Control**	**DO** mg l^{-1}	**no sulphite**	**DO** mg l^{-1}	**0.5x sulphite**	**DO** mg l^{-1}	**1x sulphite**	**DO** mg l^{-1}	**2x sulphite**	**DO** mg l^{-1}	**5x sulphite**	**DO** mg l^{-1}
16/10/2004	08:00	16	a) 2 SR b) 2 SR	4.8/ 5.1	a) 2 NSR, b) **1 D**, 1 NSR	4.8/ 5.1	a) 2 NSR/ SSR?, b) 1 TAD, 1 NSR	0.8/ 0.9	a) 2 NSR, b) **1 D**, 1 NSR	0.1/0	a) 2 NSR, b) 2 NSR	0/0	a) **1 D**, 1 TAD b) 2 NSR	0/0
16/10/2004	17:00	25	a) 2 SR b) 2 SR	3.4/ 3.4	a) 2 TAD, b) **2 D**	3.4/ 3.4	a) 1 NSR, 1 SSR, b) 1 TAD, 1 NSR	0.8/ 1.1	a) **1 D**, 1 NSR b) **1D**, 1 TAD	0/0	a) 2 NSR/ TAD b) **1D**, 1 TAD	0/0	a) **1 D**, 1 TAD b) **1 D**, 1 NSR	0/0
17/10/2004	10:00	42	a) 2 SR b) 1 SR, 1 damaged	4.8/ 3.8	a) **2 D**, b) **2 D**	4.8/ 3.8	a) 1NSR, 1 SSR, b) **1 D**, 1 NSR	2.4/ 2.4	a) **1 D**, 1 NSR b) **1 D**, 1 NSR	0.9/ 0.1	a) **1 D**, 1 TAD b) **2 D**	0.6/ 0.2	a) **1 D**, 1 TAD b) **1 D**, 1 NSR	0.1/ 0
			survivors moved to clean water		no survivors		3 survivors moved to clean water		2 survivors moved to clean water		1 survivor moved to clean water		2 survivors moved to clean water	
17/10/2004	18:30	50.5	a) 2 SR, b) 1 SR, 1 dead predation				1 NSR, 1 SSR, 1 SR		1 NSR, 1 SSR		**1 D**		1 TAD, 1 NSR	
19/10/2004	20:00	100	a) 2 SR, b) 1 SR, 1 dead predation				**1 D**, 2 SR (M45, M48)		1 SSR (M58), 1 SR (M43)				1 NSR (M42), 1 SSR (F54 berried)	

Key to condition of crayfish

SR self-righting, normal

SSR slow self-righting, movement impaired

NSR not self-righting, but still with voluntary movements of limbs

TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched

D dead, no eyestalk response

Table 13 Additional toxicity tests – Pyblast doses, without deoxygenation

Set up: 10 l buckets with sandy clay loam subsoil, 5 l rainwater, 4 crayfish. Pyblast diluted to stock solution 10mg l^{-1} (1ml concentrate in 3 l water), then further diluted to give dose in tests.

Water temperature 7.5–9°C.

Date	Time	Time elapsed	Control	DO mg l^{-1}	**Py 0.05** mg l^{-1}	**DO** mg l^{-1}	**Py 0.1** mg l^{-1}	**DO** mg l^{-1}	**Py 0.2** mg l^{-1}	**DO** mg l^{-1}	**Py 0.5** mg l^{-1}	**DO** mg l^{-1}
15/10/2004	16:30	0			Pyblast added	8.5	Pyblast added	8.3	Pyblast added	8.4/8.2	Pyblast added	8
15/10/2004	16:40-17:30						crayfish activity		lots activity		lots activity	
15/10/2004	17:30	1	All SR		a) 2 SR, b) 2 NSR		a) 2 NSR, b) 2 NSR/TAD		a) 2 NSR b) 2 NSR		a) 2 TAD b) 2 TAD	
15/10/2004	20:30	4	All SR		a) 2 NSR, b) 1 TAD, 1 NSR		a) 1 TAD, 1 NSR, b) 2 TAD		a) 2 TAD b) 2 TAD		a) 2 TAD b) 2 TAD	
16/10/2004	08:30	16	All SR		a) **1D**, 1 TAD, b) **1D**, 1 NSR	4.4/4.2	a) **1D**, 1 TAD b) **2D**	7.9/7.5	a) **1D**, 1 TAD b) **1D**, 1 TAD	7.7/7.4	a) **1D**, 1 TAD b) **1D**, 1 TAD	7.9/8.4
16/10/2004	17:30	25	All SR		a) **1D**, 1 TAD, b) **1D**, 1 NSR	3.4/3.2	a) 2 NSR/TAD, b) **2D**	5.2/6.0	a) **2D**, b) **2D**	6.6/6.6	a) **2D**, b) **1D**, 1 TAD	6.8/6.6
17/10/2004	10:30	42	All SR		a) **2D**, b) **1D**, 1 TAD	4.7/2.3	a) **1D**, 1 NSR/TAD, b) **2D**	3.8/6.8	a) **2D**, b) **2D**	6.8/7.6	a) **2D**, b) **2D**	6.3/6.7
					1 survivor to clean water		1 survivor to clean water		no survivors		no survivors	
17/10/2004	18:30	50	All SR		1 NSR		1 NSR					
19/10/2004	20:30	100	All SR		1 NSR/SSR		1 NSR					

Key to condition of crayfish

SR self-righting, normal
SSR slow self-righting, movement impaired
NSR not self-righting, but still with voluntary movements of limbs
TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched
D dead, no eyestalk response

Table 14 Observations in shallow margins of gravel pit after treatment (stage 1)

Date	Time start	Time finish	Time elapsed since treatment, hr	Observations	No. observed dead	No. observed live
05/10/2004	20:45	21:30	4	3 observers night-viewing, conditions dry and clear covered all 400m perimeter, could see 1–2m, up to 0.5m deep. 3 live in v. shallow water, backs exposed. Variable density, NW corner had highest live:dead ratio c. 50%, including a few juveniles. Saw large adults dead, as well as juveniles down to young of year.	87	32
				Searched at least 3m wide exposed gravel margin	0	0
06/10/2004	15:45	c.16:30		Concentrated on W and N sides, but whole margin checked	NA	22
06/10/2004	20:25	21:21	28	2 observers night-viewing, conditions dry and clear covered whole perimeter, concentrating search for live crayfish. Better visibility, at least 2m, very still, settled. Many more dead than last night, lots more medium sized and large juveniles, no very big adults seen.	NA	2
				Searched at least 3 m wide exposed gravel margin.	0	0
07/10/2004	10:30			2 live crayfish put in bucket last night, now dead.		
07/10/2004	20:45	22:00	53	3 observers night-viewing, conditions dry, visibility good to 2–3m, clear water, settled silt. 1 live caught and put in bucket.	552	1
09/10/2004	20:10			live crayfish caught yesterday now TAD. Any accessible dead crayfish cleared out.		
09/10/2004	20:30	21:15	100	2 observers night-viewing, viewed all margins. Visibility in water very good c. 2–3m. Very still.	NA	0
10/10/2004	14:30	15:30	118	**1 live crayfish**, c. 35mm CL seen walking slowly in open margin in c. 0.5m water by day, c. half way between bank and band of *Elodea crispa*. Escaped.	NA	1
28/10/04	19:35	20:05	23 days	Night-viewing, excellent visibility, to 3m or more of shallows and >1m depth. Substrate all settled. Water 8.5°C., air 13°C, very still.	0	0

Key to condition of crayfish

SR self-righting, normal
SSR slow self-righting, movement impaired
NSR not self-righting, but still with voluntary movements of limbs
TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched
D dead, no eyestalk response

Table 15 Crayfish found lying in the margins of the gravel pit on 06/10/04 (stage 1)

Quadrat no. (1x1 m)	No. male	No. female	total no.
1	1	0	1
2	5	3	8
3	7	9	15
4	1	2	3
5	1	1	2
6	3	2	5
7	3	4	7
8	0	5	5
9	6	11	17
total	**27**	**37**	**63**
average no. m^{-2}	7		
SD	5.590		
median	5		
Condition c. 22 hour after treatment			
SR	1		
SSR	2		
NSR	2		
TAD	2		
D	56		

Table 16 Post-treatment dissolved oxygen and locations of water samples at the gravel pit, Edzell (stage 1)

Set up: dosing of gravel pit with sodium sulphite solution started at 13:00 05/10/04, completed 16:30, water temperature 9–10°C.

Clean water stored in open drums remained at dissolved oxygen c.11.4mg l^{-1}.

			Dissolved oxygen, mg l^{-1},							
Date	**time**	**depth**	**Inlet to W of spur**	**W side N of spur**	**NW corner**	**NE corner**	**mid water N and and E of spur end**	**E side**	**SE corner**	**S side and E of spur**
05/10/2004	10:45	margin	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8
05/10/2004	14:30	surface	8.5	8.9	8.8	9.6			9.2	8.7
		at depth	2.2	2.2	2.2	2.2			2.2	2.2
05/10/2004	18:00	surface			9.4		8	8.3	8.3	8.9
		at depth			0		0	0	0	0.2
		margin								8.7
06/10/2004	15:00	surface	2.2, in weed		1.4	0.9	0.9	1.2	1.1	
		depth	0		0	0	0	0.2	1	
06/10/2004	16:30	surface								
		at depth	0, sample D5)		0, sample D4, S4	0, sample D3, S3	0, sample D6, S6		0, sample D2, S2	3.5, sample D1, S1
07/10/2004	11:00	surface			0		1.4	0	0.7	
		at depth			0, sample D3x		0, sample D4x	0, sample D2x	0.9, sample D1x	
		margin	sample S3x, *Typha*					sample S2x	sample S1x	
07/10/2004	21:00	margin	2.4	2.2	0.5	0		1	0.6	3
		margin	2.2	2.1	0.7	0.5				3
		margin	2.3							1.4
10/10/2004	15:00	margin	1.4	2.9	0.9	0.3		0.1	0.7*	1.3
		margin			1.4			0.8		
19/10/2004	day	margin	6		5.9	5.8		5.8		5.2

Table 17 Post-treatment dissolved oxygen at the Mains ponds

Note: air temperature 9°C, water 9°C.

Date	Time	DO mg l^{-1}	Location	Date	Time	DO mg l^{-1}	Location
09/10/2004	11:00–12:00		lower pond, left from outlet	10/10/2004	10:30 –11:00	4.3	lower pond near outlet
09/10/2004	11:00–12:00	4.2		10/10/2004	10:30 –11:00	6.7	lower pond near inlet
09/10/2004	11:00–12:00	5		10/10/2004	10:30 –11:00	6.8	lower pond W side
09/10/2004	11:00–12:00	4.7		10/10/2004	10:30 –11:00	5.7	lower pond near access point
09/10/2004	11:00–12:00	5.9		10/10/2004	10:30 –11:00	1.2	triangle/middle pond, W side
09/10/2004	11:00–12:00	5	lower pond at inlet from triangle pond	10/10/2004	10:30 –11:00	5.2	small pond from dam
09/10/2004	11:00–12:00	7.9	lower pond W side	19/10/2004	day	4.8	lower pond near outlet, left
09/10/2004	11:00–12:00	7		19/10/2004	day	4.4	
09/10/2004	11:00–12:00	8.7		19/10/2004	day	4.9	
09/10/2004	11:00–12:00	10.7		19/10/2004	day	5.3	
09/10/2004	11:00–12:00	10.2		19/10/2004	day	4.9	middle pond
09/10/2004	11:00–12:00	6.9	lower pond near access point	19/10/2004	day	5.2	
09/10/2004	11:00–12:00	5.5		19/10/2004	day	5	
09/10/2004	11:00–12:00	4.8		19/10/2004	day	5.7	small pond
09/10/2004	11:00–12:00	0.7	middle pond, left from outlet				
09/10/2004	11:00–12:00	1.3					
09/10/2004	11:00–12:00	1.7					
09/10/2004	11:00–12:00	0.7	middle pond, near inlet				
09/10/2004	11:00–12:00	0					
09/10/2004	11:00–12:00	5.9	small pond, left from dam				
09/10/2004	11:00–12:00	6.1					
09/10/2004	11:00–12:00	0.6	small pond near inlet (stopped off)				
09/10/2004	11:00–12:00	4.3					

Table 18 Post-treatment toxicity tests at the gravel pit (stage 1)

Set up: 10 l buckets, approx 5 l treated water from various location in gravel pit, 2 crayfish added. Dx samples at depth, Sx samples near surface.

			Observations of condition of crayfish put into samples of treated water						
Date	**Time**	**hr since treatment**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	
06/10/2004	16:30	23	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	
07/10/2004	10:30	39.5	**1 D**, 1 TAD	2 SSR	**1 D**, 1 SSR	**1 D**, 1 SSR	**1 D**, 1 SSR	**2 D**	
07/10/2004	22:00	51	**1 D**, 1 TAD	2 NSR	**2 D**	**1 D**, 1NSR	**1 D**, 1 SR	**2 D**	
09/10/2004	20:00	100	**2 D**	**1 D**, 1 NSR	**2 D**	**1 D**, 1 NSR	**1 D**, 1 SR	**2 D**	
Date	**Time**	**hrs since treatment**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	
06/10/2004	15:00	21	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	
07/10/2004	10:30	39.5	1 NSR, 1 SSR	1 NSR, 1 SR	1 TAD, 1 SSR	2 NSR	1 TAD, 1 SSR	2 NSR	
07/10/2004	22:00	51	2 TAD	**1 D**, 1 SSR	**1 D**, 1 NSR	**1 D** 1 NSR	1 TAD, 1 SSR	2 TAD	
09/10/2004	20:00	100	**1 D**, 1 NSR/TAD	**1 D**, 1 SSR	**1 D**, 1 NSR	**1 D**, 1 NSR	**1 D**, 1 SSR	**1 D**, 1 TAD	
Date	**Time**	**hrs since treatment**	**D1x**	**D2x**	**D3x**	**D4x**	**S1x**	**S2x**	**S3x**
07/10/2004	10:30	39.5	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*	*crayfish added*
07/10/2004	22:00	51	2 SR	1 NSR, 1 SR	2 SR	1 SSR, 1 SR	**1 D**, 1 TAD	2 SR	2 TAD
09/10/2004	20:00	100	1 NSR, 1 SR	2 NSR	2 SSR	**2 D**	**2 D**	**1 D**, 1 SSR	**1 D**, 1 TAD

Table 19 Recovery after toxicity tests at gravel pit (stage 1)

Set up: 3 no. 10 l buckets, with 5 l clean untreated water (from gravel pit, stored in drums prior to treatment of the gravel pit with deoxygenation and Pyblast). Added the survivors from (Table 18) toxicity tests with treated water. Hours since treatment is from treatment of the gravel pit. Total time during this test is 18 hours, with one final observation.

Date	time	hr since treatment	Condition (recovery period in clean water)		
			Crayfish at start 20:30 assessed as TAD	Crayfish at start assessed as NSR	Crayfish at start assessed as SSR/SR
09/10/2004	20:30	100.5	4 TAD	4 NSR	9 SSR/SR
10/10/2004	14:30	118.5	2 NSR, 2 SSR	2 NSR, 2 SR	**1D**, 1 TAD, 3 SSR, 4 SR

Table 20 Post-treatment toxicity test at Mains ponds

Set up: 10 l bucket, approx 5 l treated water from surface in margins of 3 ponds, 2 crayfish added

Date	Time	hr since treatment	M1 lower near outlet	M2 lower pond SE near access	M3 lower pond, W side opp. Island	M4 triangle/middle pond at dam	M5 small/upper pond at dam
09/10/2004	16:45	24	crayfish added	crayfish added	crayfish added	crayfish added	crayfish added
10/10/2004	10:00	40	1 NSR, stiff, but can grip, 1 SR aggressive	1 TAD, 1NSR	2 NSR	**2 D**	**1 D**, 1 SR aggressive

Key to condition of crayfish

SR self-righting, normal
SSR slow self-righting, movement impaired
NSR not self-righting, but still with voluntary movements of limbs
TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched
D dead, no eyestalk response

Table 21 Gravel pit re-treatment (stage 2), cage tests

Set up: 20 cages of 10 (9 in cages 11 and 15) crayfish set out across the bed of the gravel pit, prior to treatment to target dose 0.15mg l^{-1} natural pyrethrins.

na = not available, as only half the cages were inspected briefly after 1 day exposure.

All dead crayfish removed after inspection at 2 days.

	Condition after 1 day treatment					Condition after 2 days treatment			Condition after 5 days treatment	
cage no.	**SR**	**SSR**	**NSR**	**TAD**	**D**	**NSR**	**TAD**	**D**	**TAD**	**D**
1	na	na	na	na	na	0	6	4	0	6
2	na	na	na	na	na	0	5	5	0	5
3	na	na	na	na	na	0	7	3	0	7
4	none	none	none	about half	about half	0	4	6	0	4
5	none	none	all on backs	none	none	0	4	6	0	4
6	na	na	na	na	na	0	8	2	0	8
7	1 aggressive	1	most	some	none	0	8	2	2	6
8	none	none	most	some	none	0	9	1	0	9
9	none	none	none	some	rest	0	4	6	0	4
10	na	na	na	na	na	0	5	5	0	5
11	none	none	none	most	4?	0	6	3	0	6
12	none	none	none	most	3	0	5	5	0	5
13	most	some	none	none	none	0	9	1	0	9
14	na	na	na	na	na	0	7	3	3	4
15	none	none	some	most	3	0	6	3	0	6
16	na	na	na	na	na	6	3	1	1	8
17	na	na	na	na	na	0	6	4	1	5
18	na	na	na	na	na	0	8	2	0	8
19	na	na	na	na	na	0	6	2	0	8
20	all but 1	1	none	none	none	0	8	2	0	8

Table 22 Post-treatment toxicity tests at gravel pit, re-treatment (stage 2)

Set up: 70 l tubs, approx 20 l treated water from various location in gravel pit, 5 crayfish added, 6 in sample 1.

Samples all taken from margins on 30/10/04, 1 day after treatment.

Water temperature c. 9°C.

Condition 31/10/04 after 24 hours exposed to treated water				
Sample	**SR**	**NSR**	**TAD**	**D**
1	0	0	2	4
2	0	0	1	5
3	0	0	1	5
4	0	0	2	3
Condition 03/11/04 after 4 days exposure to treated water				
				All dead

Key to condition of crayfish

SR self-righting, normal

SSR slow self-righting, movement impaired

NSR not self-righting, but still with voluntary movements of limbs

TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched

D dead, no eyestalk response

Table 23 Condition of crayfish at Castle Pond, 14/12/04, 2 days after treatment with Pyblast

20 crayfish cages set up. Used fine-mesh (4mm) straight-entry pyramid traps, modified with a mesh lid, secured by ties.

Cages attached to lines with plastic bottle floats, set out across pond, with cages on the bed.

Total 9 crayfish in each, with a range of sizes.

Set out 10/12/04, 2 days before treatment to target dose 0.2mg l^{-1} natural pyrethrins. Water temperature 4.5°C.

Cage 8 in outflow stream below pond, cage 18 in the sump catching leakage for pumping back to pond, only had 16 hr exposure.

Cage no.	SR	SSR	NSR	TAD	D
1	0	0	0	5	4
2	0	0	0	6	3
3	0	0	0	5	4
4	0	0	0	6	3
5	0	0	0	9	1
6	0	0	0	6	3
7	0	0	0	5	4
8	0	0	0	9	1
9	0	0	0	6	3
10	0	0	0	4	5
11	0	0	0	6	3
12	0	0	0	7	2
13	0	0	0	7	2
14	0	0	0	4	5
15	0	0	0	5	4
16	0	0	0	4	5
17	0	0	0	7	2
18	0	0	1	6	2
19	0	0	0	5	4
20	0	0	0	4	5

Table 24 Recovery test with caged crayfish from Castle Pond

Crayfish transported to West Yorkshire in chilled crates, 15/12/04, 3 days after treatment, stayed at 5°C.

Crayfish put in tanks with rainwater, aerated, water temperature 6°C initially.

Date	Time	Treatment	Condition
16/12/2004	08:45		no voluntary movement, 5 with slight movement when touched
16/12/2004	09:15	increased temperature to 9°C	
16/12/2004	11:15		no voluntary movement, total 8 with slight movement when touched
16/12/2004		increased temperature to 13°C	
16/12/2004	11:30		3 more crayfish with minimal response
16/12/2004	13:30		7 with minimal response, 4 now confirmed dead
17/12/2004	20:00	7.5°C	all 117 confirmed dead, 5 days after treatment

Key to condition of crayfish

SR self-righting, normal
SSR slow self-righting, movement impaired
NSR not self-righting, but still with voluntary movements of limbs
TAD torpid, or almost dead, minimal movement, eg mouthparts only, slight movement of limbs when touched
D dead, no eyestalk response

Table 25 Post-treatment toxicity test at Castle Pond

Test 1

Set up: 10 l buckets with treated water from Castle Pond, c. 5 l taken 13/12/04, 12:00, c. 24 hours after treatment to target dose 0.2mg l^{-1} natural pyrethrins.
3 healthy crayfish in each bucket.

	Sample	1	2	3	4	5	6
Date	**Time**	nr inflow	S side 1/2 way	nr pump discharge	N side island	sump	outflow ditch u/s pump
13/12/2004	14:00	2SR, 1SSR	1SR, 2NSR	1SSR, 2SR	2SR, 1TAD	2NSR,1TAD	2SR, 1NSR
13/12/2004	16:00	2SR, 1NSR	3TAD	1NSR, 1D	2TAD, 1D	1NSR, 1TAD, 1D	2SR, 1NSR
14/12/2004	12:00	1SSR, 2NSR	2TAD, 1D	3TAD	2TAD, 1D	1TAD, 2D	2TAD,1D
15/12/2004	11:30	2NSR, 1TAD	1TAD, 2D	2TAD, 1D	2TAD, 1D	3D	2TAD,1D

Test 2

Set up: 8 healthy crayfish re-caged and put into Castle Pond, 15/12/04, 3 days after treatment of pond.
inspected 14/01/05, all dead.

Table 26 Post-treatment observations at Castle Pond

Wild crayfish found dead in shallow margin 1 day after treatment, 6 round island and 5 round edge, but limited visibility. Collected 7 for inspection.

Date	Time	TAD	D
13/12/2004	12:30	7	0
14/12/2004	11:45	5	2
15/12/2004	11:15	2	5
16/12/2004	08:45	0	7

Table 27 Recovery of Castle Pond, tests with *Asellus*

Set up: sample of treated pond water, diluted with river water in large clean paper cup, 10 *Asellus* added.

Date	Time	Samples					
15/12/2004	10:15	**pond**	**x10**	**x100**	**x1000**	**x10000**	**river water**
15/12/2004	11:45	dead	dead	2 dead, 8 live	live	live	live
16/12/2004		dead	dead	live and dead	live	live	live
16/12/2004	new sample	dead	dead	live	na	na	live
17/12/2004		dead	dead	live	na	na	live
17/12/2004	new sample	dead	dead	live	na	na	live
20/12/2004		dead	dead	live	na	na	live
20/12/2004	new sample	dead	dead slowly	live	na	na	live
23/12/2004	new sample	dead slowly	live	live	na	na	live

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7 PLATES

Plate 1 Preliminary toxicity tests at the gravel pit

Plate 2 Dry toxicity tests, crayfish affected on substrate sprayed with Pyblast

Plate 3   Spraying margins at the gravel pit with Pyblast

Plate 4   Mixing sodium sulphite for application at the gravel pit (stage 1)

Plate 5 Applying Pyblast with fire hose at the gravel pit (stage 1)

Plate 6 Spraying Pyblast on the lower Mains Pond

Plate 7 Preparing cages for toxicity testing at gravel pit treatment, (stage 2)

Plate 8 Dead signal crayfish at the gravel pit (stage 1).

8 APPENDICES

Appendix 1 Castle Pond survey

A hydrological survey was undertaken on 11–13th November, 2004 by Mountain Environments and was reported separately to SNH. Funding was provided by the North Esk Fisheries Board. Laboratory analysis was by SEPA. The results are summarised below.

Appendix Table A1.1 Water samples after application of Lithium chloride tracer

Date time sample	**12/11/2004 12:15**	**Date time sample**	**12/11/2004 14:15**
Sample No.	**Lithium $\mu g\ l^{-1}$**	**Sample No.**	**Lithium $\mu g\ l^{-1}$**
1/1	5.36	2/1	4.39
1/2	4.67	2/2	4.37
1/3	4.48	2/3	4.39
1/4	4.66	2/4	4.33
1/5	4.43	2/5	4.33
1/6	8.89	2/6	8.13
1/7	115.1	2/7	171.5
1/8	139.4	2/8	185.1
1/9	187.5	2/9	201.9
1/10	130.6	2/10	178.9
1/11	3.74	2/11	3.61
1/12	104.3	2/12	191.6
1/13	38.98	2/13	89
Date time sample	**13/11/2004 08:40**	**Date time sample**	**13/11/2004 10:15**
Sample No	**Lithium $\mu g\ l^{-1}$**	**Sample No**	**Lithium $\mu g\ l^{-1}$**
3/1	1.86	4/1	1.9
3/2	1.73	4/2	1.71
3/3	1.9	4/3	1.66
3/4	1.75	4/4	1.73
3/5	1.71	4/5	1.67
3/6	1.73	4/6	2.32
3/7	104.9	4/7	95.54
3/8	110.05	4/8	98.99
3/9	112.05	4/9	108.06
3/10	112.1	4/10	109.89
3/11	122.9	4/11	118.3
3/12	114.2	4/12	113.85
3/13	123.5	4/13	118.45

Lithium chloride applied 12/11/2004 10:15

Locations (see Appendix Figure 1)

1	R Luther u/s/ inlet ditch
2	R Luther next to Castle Pond a
3	R Luther next to Castle Pond b
4	R Luther d/s Castle Pond
5	R Luther u/s confluence outfall ditch
6	R Luther d/s confluence outfall ditch
7	Outfall ditch u/s confluence R Luther
8	Outfall ditch midway
9	Field drain
10	Outfall ditch u/s field drain
11	Outfall ditch d/s outfall 2
12	Outfall ditch d/s outfall 1
13	Castle Pond

The results from the flow measurement survey show that water is lost very rapidly from the pond. With the inlet stopped off the flow in the outfall ditch was measured at approximately 24 l s^{-1} (12/11/04). Of this approximately 9 l s^{-1} was entering an arm of the outfall ditch immediately downstream of the pond, either by seepage through the dam or old field drainage. By subtraction about 15 l s^{-1} was entering from a field drain. Any other drainage from the field to the outfall ditch was negligible at the time of the survey.

The Lithium tracer test showed the whole outfall ditch was running with water coming directly from the pond within 2 hours of application. There was no dilution within the ditch system. The lower concentration in the pond in the first two samples may be due to a lag in the complete mixing of the Lithium within the pond. The indications are that the Lithium started leaking out of the bottom of the pond almost immediately.

The values of Lithium in the river upstream of the outfall ditch are probably trace contamination of samples. The reason for the low level of Lithium downstream of the confluence is less clear. Results suggest dilution in the Luther was in the order of 10–50 fold, but flow measurements on 12/11/04 indicate a 5-fold dilution available (129 l s^{-1} in the River Luther upstream of confluence with the ditch).

There was no evidence of any loss of pond water southward directly to the River Luther. All flow appeared to be to the outfall ditch.

Appendix Figure 1 Location of sampling points at Castle Pond