

Project Final Report Form

Please complete this form including an Executive Summary and the Final project report and return by email to: research@sarf.org.uk
 SARF, PO Box 16, Birnam, Dunkeld, Perthshire PH8 0WU, Scotland

Project Details

SARF Project ID Code: SARF 041	
Project Title: Developing practical strategies for reducing the spread of harmful organisms during the transportation of live fish.	
Project: Start date 1 st April 2008	End date 31 st March 2009
Name(s) and address(s) of contractor organisation(s): University of Stirling, Stirling, FK9 4LA	
Contractor's Project Manager: James F Turnbull	
SARF Project Manager: Mark James	
Total SARF Project costs £49,928	
Total approved project expenditure £56,428	Total actual project expenditure £56,428
Total *approved staff input 0.9 years	Total *actual staff input 0.9 years
Is there any Intellectual Property arising from this project which is suitable for commercial exploitation (<i>This question requires a YES/NO answer only. All other details of any Intellectual Property must be included under the Scientific Report or in an accompanying Annex</i>). NO	
*Staff years of direct science effort	

NOTES

SARF aims to place the results of its completed research projects in the public domain wherever possible. The form is designed to capture the information on the results and outputs of SARF-funded research in a format that is easily publishable through the SARF website. This form must be completed for all SARF projects. A supplementary Final Financial Report Form must be completed where a project is paid on a monthly basis or against quarterly invoices. No Final Financial Report Form is required where payments are made at agreed milestone points.

- This form is in Word format and the boxes may be expanded or reduced, as appropriate.

ACCESS TO INFORMATION

The information collected on this form will be stored electronically and may be sent to any SARF Board Members, or to individual researchers or organisations outwith SARF for the purposes of reviewing the project. SARF may also disclose the information to any outside organisation acting as an agent authorised by SARF to process final research reports on its behalf. SARF intends to publish this form on its website, unless there are cogent reasons not to do so, which may be justified as being in line with exemptions under the Environmental Information (Scotland) Regulations or the Freedom of Information (Scotland) Act 2000. SARF may be required to release information, including personal data and commercial information, on request under the Environmental Information Regulations or the Freedom of Information Act 2000. However, SARF will not permit any unwarranted breach of confidentiality or act in contravention of its obligations under the Data Protection Act 1998.

It is SARF's intention to publish this form.

Please confirm your agreement for SARF to do so.....**YES**

(a) When preparing this and related report forms, contractors should bear in mind that SARF intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow. SARF recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (and clearly marked as "NOT TO BE PUBLISHED") so that the contents of the forms can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will SARF expect contractors to give a "No" answer. The principal reasons for withholding information should be in line with exemptions under the Environmental Information (Scotland) Regulations or the Freedom of Information (Scotland) Act 2000.

(b) If you have answered NO, please explain why the Final report should not be released into public domain

Scientific objectives

List the scientific objectives as set out in the contract. If necessary these can be expressed in abbreviated form. Indicate where amendments have been agreed with the SARF Project Manager, giving the date of amendment.

1. Recruit staff.

Completed – 1/04/08.

2. Obtain necessary licences and permissions to hold live crayfish, discharge chemicals and conduct animal experimentation.

Several licences and permissions have to be sought in order to undertake this project and ensure that practical strategies are produced. The process of obtaining these will be initiated from notification of funding to ensure work with crayfish and trout can commence at the appropriate times of year.

Completed 01/05/08.

3. Review of existing transport procedures and examine options for physical water treatment.

The majority of this activity will be undertaken in the early part of the project to allow development of potential strategies for the subsequent objectives.

Completed 01/02/09.

4. Conduct laboratory trials for efficacy and safety of potential chemical water treatments.

Efficacy against fish pathogens can be conducted early in the project. The experiments on crayfish will be mostly undertaken during May and June when juvenile animals are available, but with the capacity to replicate these with larger animals later in the year. The studies on fish will take place once chemicals effective against the crayfish and pathogens have been identified. We will consider the chemical and method of application before undertaking the appropriate testing on live fish.

Completed 01/11/08.

5. Develop and test pilot strategies on small scale fish transport.

These trials will be relatively short in duration but we may wish to replicate experiments or test alternative strategies.

Completed 01/02/09.

6. Commercial scale trial for safety of treatments.

This will be conducted at a time negotiated with the farmers involved.

Amended and presented to Project Manager 27/03/09.

7. Produce a briefing note, which will be referred to in the BTA Code of Practice, and prepare manuscripts.

Once all the information is analysed we will liaise with the BTA to produce a briefing note outlining best practice guidelines for fish transportation. This briefing note will be referred to in the BTA Code of Practice. We will also start the preparation of peer reviewed manuscripts.

Completed – 31/03/09.

Milestones

List the milestones. It is the **responsibility of the contractor** to check fully that **all** milestones have been met and to provide a detailed explanation if this has not proved possible.

Milestone		Target Date	Milestone Met	
Number	Title		In Full	On Time
1	Preparation for laboratory trials into the efficacy and safety of chemical treatments.	01/06/08 Month 3	YES	YES
2	Laboratory trials into the efficacy and safety of chemical treatments.	01/11/08 Month 8	YES	NO
3	Small and commercial scale trials to test pilot strategies.	01/03/09 Month 11	YES	NO
4	Completion of extension leaflets to disseminate project outputs to the UK trout farming industry.	31/03/09 Month 12	YES	YES

If any milestones have not been met please give an explanation below.

Minor changes were made to milestones 2 and 3. These changes were discussed at the final review meeting and found to be satisfactory.

Changes to Milestone 2.

Under scientific objective 04, we stated we would test chemical treatments on a virus, a bacteria and a protozoan parasite. A literature review identified that our candidate chemical, FAM 30, is effective against viruses and bacteria. We were unable to culture the protozoan parasite within the time frame of this project to test the efficacy of FAM 30.

Changes to Milestone 3.

Under scientific objective 06, we stated we would conduct commercial scale trials for the safety of treatments. Following development of control strategies under objective 4 and 5, we did not believe scale trials would be of benefit. Under BTA transport guidelines, fish or transport water cannot be chemically treated. FAM 30 is also widely used throughout the trout industry. Therefore, trials for the safety of treatments are not necessary. Instead of commercial trials, we consulted with trout transporters on our physical and chemical strategies for controlling the spread of crayfish. We also developed an organisational strategy through an audited labelling scheme.

Declaration

I declare that the information I have given in this form and in any associated documentation is correct to the best of my knowledge and belief.

Name: James F Turnbull

Date: 03/04/09

Position held: Project manager

Executive Summary

The executive summary must not exceed 2 sides in total of A4 (minimum font size 10) and should be understandable to the intelligent non-specialist. It should cover the main objectives, methods and research results, together with any other significant events and options for new work (the box below will expand to accommodate the Summary).

The purpose of this project was to provide the UK trout industry with practical strategies for reducing the risk of transferring potentially harmful organisms during transportation of live fish. This problem was identified as a research priority by the British Trout Association (BTA) and was highlighted in a report on signal crayfish in Scottish waters commissioned by Scottish Natural Heritage. Signal crayfish (*Pacifastacus leniusculus*) are non-native animals in Scotland and the rest of the UK with the potential to have a significant detrimental effect on ecosystems, biodiversity and the physical structure of Scotland's inland waters. They are highly invasive species and accidental translocation of this species during the movement of live fish was thought to be one means by which it can spread. Ensuring that there are no live crayfish in transport water prior to water release could significantly reduce the risk of further spread of this species.

This project was divided into three main phases, the first of which was a review of transport procedures in the UK trout industry. Seventeen transporters, including restocking farmers, farmers who grow fish for human consumption and professional live fish hauliers, were contacted by telephone and questioned on their transport procedures. The review provided us with valuable information on procedures for transportation of live fish. One of the potential risk factors identified for the spread of signal crayfish in the UK was through exchange of water by transporters during longer transportations. However, transporters stated they did not do this, claiming that water exchange during transportation was overly and unnecessarily stressful for the fish. The review identified the variation in transport procedures, and provided us with the minimum contact time for chemical treatments, which was the shortest transport reported.

The second phase was laboratory trials to test chemical water treatments. A list of chemicals used throughout the UK aquaculture industries were applied to selection criteria, such as objection to use by SEPA, if veterinary approval for use was required, human safety and cost of use. Six candidate chemicals were identified as potentially the most suitable, and pilot studies were conducted on juvenile signal crayfish to establish the most suitable chemical for this project. The most suitable chemical, for its efficacy and speed of effect, was the iodophor FAM 30. Using FAM 30, further trials were conducted under different water conditions, including hard, soft, acidic, warm and cold water, on a limited number of crayfish. There were no clear differences in time to death at manufacturer's recommended concentration between the different water conditions. A full replicated trial was conducted, with a dramatic increase in time to death from earlier experiments. It appears this was a result of using larger crayfish than the earlier experiments: after immersion in FAM 30 for one hour, the crayfish took up to 48 hours to die, compared with less than five minutes for juvenile crayfish in the same concentration of FAM 30. A further experiment was conducted where crayfish of various sizes were subjected to different periods of immersion, from 10 minutes to 60 minutes. Results indicated that in order to kill all crayfish, a minimum immersion time of 20 minutes at 2.5ml FAM 30 per litre is required, and that larger crayfish are more tolerant of FAM 30 than smaller crayfish. FAM 30 has been reported to be effective against a range of other harmful pathogens, including viruses, bacteria and the crayfish plague fungi, *Aphanomyces astaci*.

The third phase involved developing practical strategies for controlling the spread of signal crayfish. Strategies were developed to tackle the problem at the point of loading fish onto the transport tank. We propose two methods for reducing the risk of accidental spread of crayfish, depending on loading procedures. If fish are pumped or piped into the transport tank, then we propose a dewaterer device, which would separate the fish from the water, and potentially from unwanted crayfish. The water used to fill the transport tank should be filtered through a mesh net before filling. Fish would pass over a grill and into the transport tank/holding unit, and water and crayfish would fall through the grill, where crayfish would be collected in a fine mesh net. Following use, the mesh net should be soaked in FAM 30 for a minimum period of 20 minutes to kill pathogens. If fish are netted into the transport tank, then the transport tank should be filled with water filtered through a mesh net. Vigilance by the farmer during netting is required to look out for crayfish in the net. Following use, the mesh net should be immersed in FAM 30 for a minimum period of 20 minutes. A dewaterer device is the most effective method at reducing the risk of transporting signal crayfish, therefore if possible, we recommend using the dewaterer if loading with nets, or in conjunction with a sorting table (a sorting table is used by restocking farmers to identify the best fish within a batch).

We also propose an Integrated Control Strategy for the transportation of live fish in the UK, where the chemical and physical strategies are backed up with an audited labelling scheme, such as those operated by Freedom Foods, Assured Food Standards and The Soil Association Organic Standard. Members to the scheme would be designated either "Crayfish Free" or "Low Risk of Crayfish". The "Crayfish Free" label would apply if the farm and supplying water are free from signal crayfish. If the farm or supplying water has signal crayfish, then a "Low Risk of Crayfish" label would apply, providing the fish farmer takes active steps

to address the risks of accidentally transporting signal crayfish, such as the chemical and physical control methods.

This was a successful project that has produced practical chemical and physical strategies for reducing the risk of spreading the signal crayfish. We have also proposed an Integrated Control Strategy, involving an audited labelling scheme. Our proposals appear to have been well received by the trout industry and other stakeholders involved in controlling the spread of signal crayfish. The proposals were presented to fish farmers at the 2009 AGM of the British Trout Farmers Restocking Association. A briefing note, which will be referred to in the BTA Code of Practice, is in preparation in consultation with the BTA. The findings of the project have formed the basis for discussions regarding the control of the spread of crayfish via trout transportation through the Freshwater Fisheries Forum and our proposed integrated control strategy will form the basis of codes of practice for stocking.

Project Report to SARF

As a guide this report should be no longer than 20 sides of A4. This report is to provide SARF with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow SARF to publish details of the outputs. This short report to SARF does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication.

The report to SARF should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and any action resulting from the research (e.g. IP, Knowledge Transfer).

Scientific Objectives and extent to which they have been met

1. Recruit staff.

Completed – 1/04/08.

2. Obtain necessary licences and permissions to hold live crayfish, discharge chemicals and conduct animal experimentation.

Several licences and permissions have to be sought in order to undertake this project and ensure that practical strategies are produced. The process of obtaining these will be initiated from notification of funding to ensure work with crayfish and trout can commence at the appropriate times of year.

Completed 01/05/08.

3. Review of existing transport procedures and examine options for physical water treatment.

The majority of this activity will be undertaken in the early part of the project to allow development of potential strategies for the subsequent objectives.

Completed 01/02/09.

4. Conduct laboratory trials for efficacy and safety of potential chemical water treatments.

Efficacy against fish pathogens can be conducted early in the project. The experiments on crayfish will be mostly undertaken during May and June when juvenile animals are available, but with the capacity to replicate these with larger animals later in the year. The studies on fish will take place once chemicals effective against the crayfish and pathogens have been identified. We will consider the chemical and method of application before undertaking the appropriate testing on live fish.

Completed 01/11/08 – but see amendments to milestones above.

5. Develop and test pilot strategies on small scale fish transport.

These trials will be relatively short in duration but we may wish to replicate experiments or test alternative strategies.

Completed 01/02/09.

6. Commercial scale trial for safety of treatments.

This will be conducted at a time negotiated with the farmers involved.

See amendments to milestones above – 01/03/09.

7. Produce a briefing note, which will be referred to in the BTA Code of Practice, and prepare manuscripts.

Once all the information is analysed we will liaise with the BTA to produce a briefing note outlining best practice guidelines for fish transportation. This briefing note will be referred to in the BTA Code of Practice. We will also start the preparation of peer reviewed manuscripts.

Completed – 31/03/09.

Methods and Results

Scientific Objective 02 - Obtain necessary licences and permissions to hold live crayfish, discharge chemicals and conduct animal experimentation.

Following extensive discussions with the Marine Directorate of the Scottish Government, a licence to hold live signal crayfish at the Institute of Aquaculture was obtained. The licence was under the terms of the Import of Live Fish (Scotland) Act 1978 and The Prohibition of Keeping or Release of Live Fish (Specified Species) (Scotland) Order 2003.

Scientific Objective 03 - Review of existing transport procedures and examine options for physical water treatment.

Method

This scientific objective was primarily conducted as an MSc project by Ms K Bunker. To review existing transport procedures within the UK trout industry, a list of 19 live fish transporters were provided by the British Trout Association. From this list, 17 transporters agreed to participate in the study. Participants comprised 12 farmers who transport fish for restocking, three farmers who transport fish between farms for on-growing for the table market, and two professional hauliers. Participants were contacted by telephone and questioned about their transport procedures. The questionnaire is appended as Appendix 1. The questionnaire covered pre-transport, loading, during transport, unloading and post-transport procedures. To pilot the questionnaire, a meeting was held with BTA members in March 2008 to establish the content. The questionnaire was then tested on a trout farmer and alterations to the questionnaire made based on the feedback received from the meeting and test survey.

In addition to the telephone questionnaire, 11 live trout transports were observed and monitored. Five transports were by fish farmers and six were by professional haulier. The aim of monitoring the transports was to obtain information about water quality during transport, to better understand transport procedures, and to view transports to assist with development of control strategies for crayfish. We took the opportunity to collect blood samples from fish during transport to attempt to ascertain if transportation was stressful for fish through analysis of blood cortisol concentrations. We know that loading and unloading is acutely stressful (Leggatt et al. 2006, Overli et al. 2006), however we do not know if fish are stressed during the transport.

Water Quality

Four water samples were taken per complete transport: from the holding facility, the transport tank immediately post-loading, the transport tank post-transport, and the new environment. Ammonia (mg/l, Total Ammonia Nitrogen), alkalinity (mg/l as CaCO₃) and nitrite (mg/l NO₂) were measured using a Palintest 500 (Palintest, Gateshead, UK), a multiparameter photometer that measured colorimetric changes. Dissolved oxygen (DO) (mg/l) and temperature (°C) were recorded using a Hach HQ10. During the transport, DO, temperature, pH and ammonium (NH₄⁺) were recorded every 5 minutes using a multi-parameter sonde (YSI6920, Hydrodata Ltd, Herts).

Blood Samples

Blood samples were taken at 3 time points; from the holding facility before loading (n=5), after loading into the transport tanks (n=5) and after the transport, before the fish were released into the new environment (n=5). Plasma cortisol was determined by radioimmunoassay using the method described by Ellis et al. (2004) with the following alterations: cortisol antibody (rabbit anti-cortisol) was acquired from Guildhay Ltd. (Surrey, UK). Cortisol was extracted from aliquots of plasma (25-150 µl) using 1ml ethyl acetate (VWR International Ltd., UK), mixed then centrifuged (430 g for 10 minutes). 100µl aliquots of supernatant (chilled at 4°C) were evaporated under vacuum at 35°C in polypropylene tubes and then chilled prior to radioimmunoassay.

Results

Telephone Questionnaire

The questionnaire has provided us with valuable information on procedures for transportation of live fish (table 1). One of the potential risk factors identified for the spread of signal crayfish in the UK was through exchange of water by transporters during longer transportations. However, in this study, transporters stated they did not exchange water during transport. They claimed water exchange during transportation was stressful for the fish and therefore as far as the participants were concerned was not practised by any transporter. The information gathered on procedures for loading and unloading was relevant to scientific objectives 5, 6 and 7. The physical water treatment strategies were developed based on the different procedures identified for loading and unloading using either nets or pumps/pipes. The minimum duration of

transports informed the protocols for scientific objective 4. Any chemical water treatment would need to be practical, and would therefore need to apply within the timeframe of the shortest transport, i.e. around 10 minutes.

Table 1. Transport procedures for 17 live trout transporters in the UK (from a telephone questionnaire).

	Re-stocking (n=12)	On-growing (n=5)	Total (n=17)
<i>Pre-Transport</i>			
* Starvation period:			
Mean days	3 days	3 days	3 days
Mean ° days	30° days	-	30° days
Holding facility			
Concrete raceway	67%	100%	73%
Plastic lined pond	33%	0%	27%
Source of water			
River	50%	100%	60%
Borehole	25%	0%	20%
Spring	25%	0%	20%
Method of loading			
Net only	75%	0%	60%
Pump only	0%	100%	20%
Net or pump	25%	0%	20%
<i>During Transport</i>			
Duration (Hours)			
Min	0.1	0.5	0.1
Max	36	10	36
Inputs/ checks			
O ₂	100%	100%	100%
Air	16%	33%	20%
Water exchange during transport	0%	0%	0%
Visual check	16%	0%	13%
Temperature check	0%	66%	13%
<i>Post-Transport</i>			
Unloading Method			
Netting only	33%	0%	27%
Pipes only	42%	100%	53%
Netting or pipes	25%	0%	20%

* Starvation period was given by farmers in either days or ° days.

Live Trout Transports

The range of temperatures covered by the transports followed was 7°C to 16°C, with the duration of transports ranging from 35 minutes to seven hours. Mean DO concentrations ranged from 7.2 mg/l to 22.0 mg/l, the lowest of which are adequate to maintain fish health and welfare (Wedemeyer 1996).

In water, ammonia exists in two forms, as unionised ammonia (NH₃) and ionised ammonium (NH₄⁺). Total ammonia is the sum of the concentrations of the two forms. The unionised ammonia is the form which is toxic to aquatic animals. The two forms of ammonia exist in equilibrium, with the fraction of each form heavily dependent upon pH and, to a lesser extent, temperature. As pH decreases, the fraction of toxic unionised ammonia also decreases. Ammonia is produced as a metabolite by fish and excreted into the water as a waste product, it also results from bacterial decomposition of organic material. During all transports observed for this study, ionised ammonium was observed to increase (table 2), with a mean starting value of 0.36 mg/l NH₄⁺ and mean end of transport value of 3.3 mg/l NH₄⁺. However, due to changes in pH during transport, the toxic unionised ammonia concentrations did not follow the same pattern. For example, in one transport, ionised ammonium increased from 0.18 mg/l NH₄⁺ to 1.0 mg/l NH₄⁺, however, due to a fall in pH from 8.43 to 6.79 during the transport, unionised ammonia concentrations actually fell from a starting concentration of 12 µg/l NH₃ to 1.5 µg/l NH₃. Presumably the fall in pH was due to the fish

producing carbon dioxide, which acidified the water. The highest concentration of unionised ammonia recorded throughout this study was 12 µg/l NH₃. From the literature, the concentration of unionised ammonia required to kill 50% of a population in 96 hours (96h-LC₅₀) is in the range of 200-400 µg/l NH₃ (Meade 1985), therefore it does not appear that ammonia approached dangerous concentrations at any point during this study.

Table 2. Changes in water quality in transport tanks pre and post transport for 9 transports (water quality data not available for two transports).

Duration (Hours)		Alkalinity (mg/l)	DO (mg/l)	Temp (°C)	pH	NH ₄ ⁺ (mg/l)	NH ₃ (µg/l)	CO ₂ (mg/l)
7	Pre	30	11.2	8.3	6.30	0.1	0.1	142
	Post		20.2	8.6	6.18	3.8	1.2	186
2	Pre	30	11.5	8.8	7.13	0.2	0.4	20
	Post		11.9	9.5	6.06	2.3	0.6	240
4.4	Pre	5	10.5	8.7	5.66	0.7	0.1	102
	Post		12.6	11.0	6.05	4.2	1.2	40
5	Pre	100	ND	10.9	7.43	0.2	1.1	33
	Post		ND	12.4	7.17	0.9	3.4	58
7	Pre	15	16.0	14.2	5.95	1.2	0.3	140
	Post		11.2	15.7	6.11	10.2	4.6	94
2	Pre	195	14.1	9.3	7.05	0.3	0.7	160
	Post		9.8	10.0	6.94	1.4	2.8	204
4.9	Pre	160	14.4	10.4	7.87	0.2	3.3	19
	Post		11.1	11.1	6.83	3.5	5.8	210
0.6	Pre	150	10.0	11.1	7.86	0.2	3.4	18
	Post		7.6	11.2	6.88	2.6	4.9	175
4.3	Pre	155	12.1	10.4	8.43	0.2	12.0	5
	Post		7.3	11.1	6.79	1.0	1.5	223

Measurements of plasma cortisol concentrations were made to attempt to find if transport is inherently stressful for fish. The stress hormone cortisol was measured at the beginning and end of transports. When fish experience a situation as stressful, typically cortisol will elevate rapidly (for up to 30 minutes) and can take one to two hours to return to normal levels (Wendelaar Bonga 1997). In view of this time lag, any transports less than two hours in length were discarded from the analysis, as any elevated cortisol levels could have been due to the initial stressor of loading and not through any stress experienced during transport. The results of analysis of cortisol concentrations were inconclusive. Of the four transports that were in excess of two hours, the mean cortisol concentrations increased for two transports and decreased for the other two. It is not possible to draw conclusions from this data, and highlights the difficulties inherent in attempting to use blood hormone parameters to assess stress in fish.

One transport, which lasted seven hours, involved a ferry crossing. During the crossing, the driver of the transporter was prohibited from attending the vehicle and monitoring DO. The probe that was monitoring DO during this period recorded a rise in DO from 21 mg/l at the start of the ferry journey to a peak of 30 mg/l (>250% saturation) at the end, when the driver was able to attend the vehicle and adjust DO levels. This raises a potential welfare issue where drivers are prevented from attending transport tanks during ferry crossing and are unable to adjust DO levels. While little is known about the effects of hyperoxia (DO >100% saturation) on fish welfare, some physiological effects have been noted (see MacIntyre et al.2008). If drivers cannot access transport tanks during ferry crossing, then it is suggested that automatic controls are installed to O₂ cylinders to prevent extremes of DO (low or high) when the vehicle is unattended.

Scientific Objective 04 - Conduct laboratory trials for efficacy and safety of potential chemical water treatments.

Selection of suitable chemicals

A list was compiled of all chemicals currently used in UK aquaculture to disinfect or treat diseases or parasites. Sources for the list included the Scottish Salmon Producers' Organisation and the British Trout

Association. Table 2 lists the chemicals under consideration.

Table 2. List of chemicals under consideration for use against signal crayfish.

Chemical	Brand	Notes
Iodophor	FAM30 (eg Evans Vanodine) Buffodine (Sterner Fish Tech) Tegodyne (DiverseyJohnson)	
Peroxy Hydrogen Peroxide	Virkon S (eg Antec International) Salartect (Brenntag) Paramove (Solvay)	Primarily Sea Lice treatment
Sodium Percarbonate Peracetic acid/ H ₂ O ₂ / Acetic acid	BioCare SPC (BioCare) Vanodox (Evans Vanodine) Proxitane (Solvay)	Same action as hydrogen peroxide
Sodium Hypochlorite Calcium Hypochlorite Chlorine Dioxide	Vortex (Proctor & Gamble) Shock Chlorine Granules (Isca Poolcare) Zydox (Seafood Directions)	
Chloramine T Formaldehyde	Halamid (Axcentive) Formalin (no brand)	User health issues, but widely used throughout industry
Quaternary Ammonium Glutaraldehyde Bronopol Antimicrobials	Cetrimide (FeF Chemicals A/S.) Vetrekil (Pharmaq Ltd) Pyceze (Novartis Animal Vaccines Ltd) Amoxicillin Potentiated sulphanamides Oxytetracycline Flofenicol Furazolidone Oxolonic Acid Sorafloxocin	Long contact time for biocidal activity required / user health issues } Prescription Only Medicines
Azamethiphos Cypermethrin Emanectin benzoate Teflubenzuron	Salmosan (Novartis Animal Vaccines Ltd) Excis (Novartis Animal Vaccines Ltd) Slice (Schering-Plough Animal Health) Calicide (Trouw Aquaculture)	

Chemicals in table 2 were subjected to the following criteria to select the most suitable candidates for crayfish control: 1) cost of use at manufacturers recommended concentrations, 2) practicality (is a licence for use required?), 3) human safety, 4) method of application (i.e. immersion, as a spray or in-feed, 5) whether SEPA would permit use and discharge of chemical at concentrations required for control of crayfish (we were in frequent contact with Mr D Sinclair from SEPA regarding this project), 6) how widespread is use throughout UK aquaculture industry? Prescription only medicines (POMs) require authorisation by a veterinary surgeon for every use. These medicines include antimicrobials (e.g. oxytetracycline, amoxicillin, potentiated sulphanamides) and some sea lice treatments (e.g. cypermethrin, azamethiphos, emamectin benzoate, teflubenzuron) and are not appropriate for this project, which is concerned with practical strategies for reducing the risk of spreading crayfish. Evidence from the literature suggests that these sea lice treatments would be highly effective against signal crayfish, however it would not be practical to obtain veterinary permission to use a chemical prior to every use. Further, the discharge of POMs is regulated by SEPA, and discharge consents would be required for each use. It was decided that POMs would not be suitable for this project, and that the project would focus on disinfectant chemicals that can be used prophylactically.

The selection process produced six potentially suitable chemicals for trials against signal crayfish; 1) Virkon S, 2) FAM 30, 3) Hydrogen Peroxide, 4) Peracetic acid/H₂O₂/Acetic acid, 5) Calcium Hypochlorite, 6) Formalin.

Six berried females were collected from a section of the River Clyde near Elvanfoot (South Lanarkshire) in July 2008. These were transported to the Institute of Aquaculture and kept under quarantine conditions, per the terms of the licence from the Marine Directorate (see objective 02 above). After the eggs hatched and juveniles had detached from the female, all juveniles were reared for one month prior to pilot studies commencing. Crayfish of varying sizes (20mm to 110mm carapace length) were collected from the River Clyde in December, January and February for latter experiments.

Experiments

Experiment 1

Preliminary trials were conducted with two methods of application, immersion and spray, for all six candidate chemicals. Five crayfish (9 mm \pm 0.8 (mean \pm SD)) were subjected to manufacturers' recommended concentrations (MRC) for up to one hour. Five minutes after an animal ceased moving it was removed from the chemical and placed in aerated freshwater. Recovery was assessed after a further hour and then the following day. Results are displayed in table 3.

Table 3. Time to death for signal crayfish in six chemicals at manufacturers' recommended concentrations.

		IMMERSION							
Chemical	Conc	Time to death (min)					Number left after 1hr	Number alive after 1hr recovery	Number alive after 24hrs
		1	2	3	4	5			
Virkon	10g in 1l	31	50	-	-	-	3	2	0
FAM 30	2.5ml in 1l	2	2	3	5	5	0	0	0
Peradox	1:50	-	-	-	-	-	5	1	0
H₂O₂	1500ppm	-	-	-	-	-	5	5	3
Formalin	170ppm	-	-	-	-	-	5	5	5
Calcium Hypochlorite	15ppm	-	-	-	-	-	5	5	2
		SPRAY							
Virkon	10g in 1l	-	-	-	-	-	5	4	0
FAM 30	2.5ml in 1l	-	-	-	-	-	5	5	5
Peradox	1:50	-	-	-	-	-	5	5	5
H₂O₂	1500ppm	-	-	-	-	-	5	5	5
Formalin	170ppm	-	-	-	-	-	5	5	5
Calcium Hypochlorite	15ppm	-	-	-	-	-	5	5	5

Experiment 2

While the pilot study was done on a small number of animals, it is clear that FAM 30 was the most effective chemical against signal crayfish, and that immersion was more effective than spray application. Further pilot studies were conducted with x2, x10 and x50 MRC. Hydrogen peroxide and calcium hypochlorite were not effective against signal crayfish, while formalin was only effective at x50 MRC. FAM 30 was selected as the most suitable chemical for this project, in view of the speed and efficacy of operation against signal crayfish.

Experiment 3

A further series of tests were conducted using FAM 30 under different water chemistries and temperatures. This was designed to simulate the range of conditions that could be anticipated to be found in UK live trout transports. The sizes of crayfish were 12mm \pm 2 (mean \pm SD). Conditions were cold water (4°C, pH 6.95, hardness 15 mg/l), warm water (20°C, pH 6.95, hardness 15 mg/l), soft, acidic water (15°C, pH 5.6, hardness 15 mg/l) and hard water (16°C, pH 7.8, hardness 170 mg/l). Results are in figure 1. While there was no clear differentiation between conditions at x1 MRC, crayfish were slowest to die at x1 MRC in cold water and quickest in warm water. Water hardness or pH, within the ranges used here, did not appear to affect time to death.

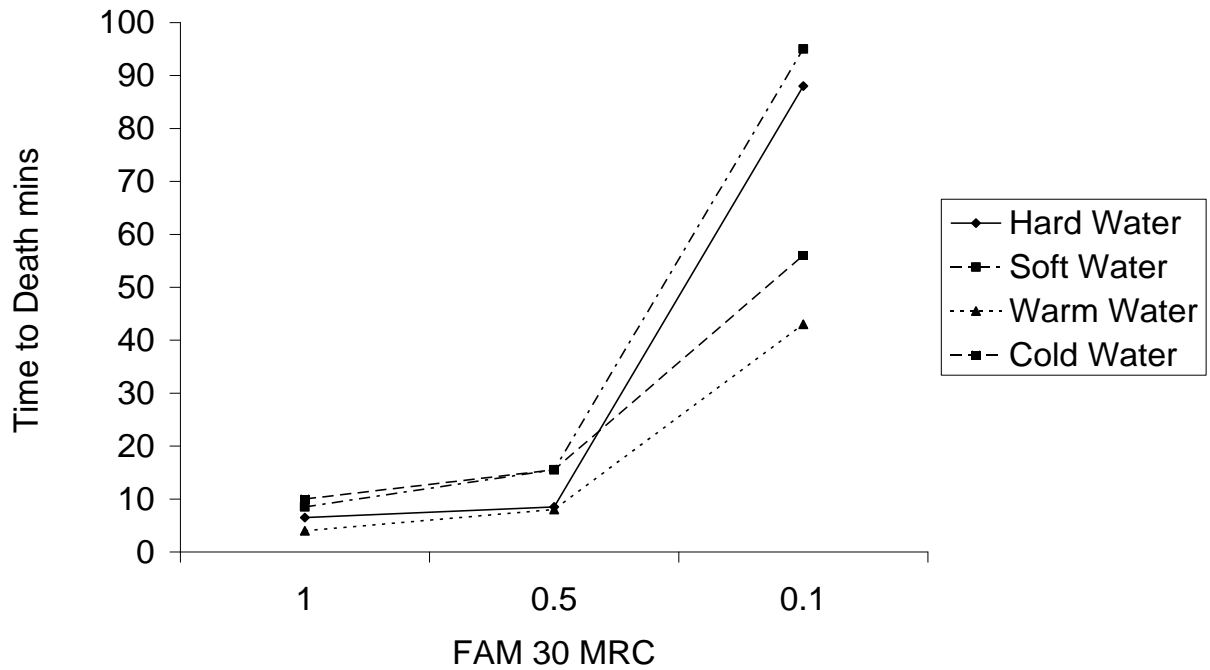


Figure 1. Efficacy of FAM 30 on signal crayfish under different environments.

Experiment 4

Full replicated trials were conducted to test the efficacy of FAM 30 on signal crayfish. Crayfish were 20 mm \pm 1.6 (mean \pm SD). FAM 30 concentrations of x0.5 MRC, x1 MRC and x2 MRC were triplicated with five animals per treatment and triplicated controls. Results are described in table 4. Time to death increased dramatically from earlier pilot studies. Six crayfish survived one hour at x0.5 MRC, eight at x1 MRC and six at x2 MRC. After one hour in FAM 30, the animals were placed in aerated fresh water and survival assessed after at one hour in fresh water, 24 hours and 48 hours. Several animals showed signs of life at one hour and 24 hours post exposure, however all animals were dead 48 hours post exposure, indicating that one hour exposure to FAM 30 is fatal, although not immediately, and that the resistance of signal crayfish to FAM 30 increases with size. In view of the suggested increase in resistance to FAM 30 with size, a further experiment was proposed using a range of sizes of signal crayfish.

Table 4. Time to death of signal crayfish (n=5 per replicate per treatment) in concentrations of 0.5, 1 and 2 x manufacturer's recommended concentration (MRC) of FAM 30.

Conc (MRC)	Time to Death (mins)					Number alive after 1 hour in FAM 30	Number alive after 1 hour recovery	Number alive after 24 hours recovery	Number alive after 48 hours recovery
	1	2	3	4	5				
Control						5	5	5	5
Control						5	5	5	5
Control						5	5	5	5
0.5	28	37				3	3	1	0
0.5	27	29	58			2	3	0	0
0.5	35	38	40	51		1	3	0	0
1	24	24	30			2	1	1	0
1	30	30				3	3	1	0
1	36	36				3	1	0	0
2	24	46	46			2	2	0	0
2	21	29	29			2	2	0	0
2	20	28	46			2	2	0	0

Experiment 5

Signal crayfish were subjected to differing periods of immersion in 1x MRC of FAM 30. Triplicates of five animals per treatment were immersed for 10, 20, 30, 40, 50 and 60 minutes in FAM 30, removed into aerated fresh water for recovery, and mortality assessed immediately post immersion, one hour post immersion, 24 hours post immersion and 48 hours post immersion. Given the hypothesis that larger crayfish are more resistant to FAM 30, a range of sizes from 19mm to 68mm were placed in each treatment. Further, one large crayfish (80mm to 107mm) was subjected to each treatment in a separate tank (we were limited to one large crayfish per treatment as we were unable to collect more of the larger animals for replicates). The mean percentage mortality over the three replicates is provided in figure 2 and percent survival for different size classes for each treatment in figure 3.

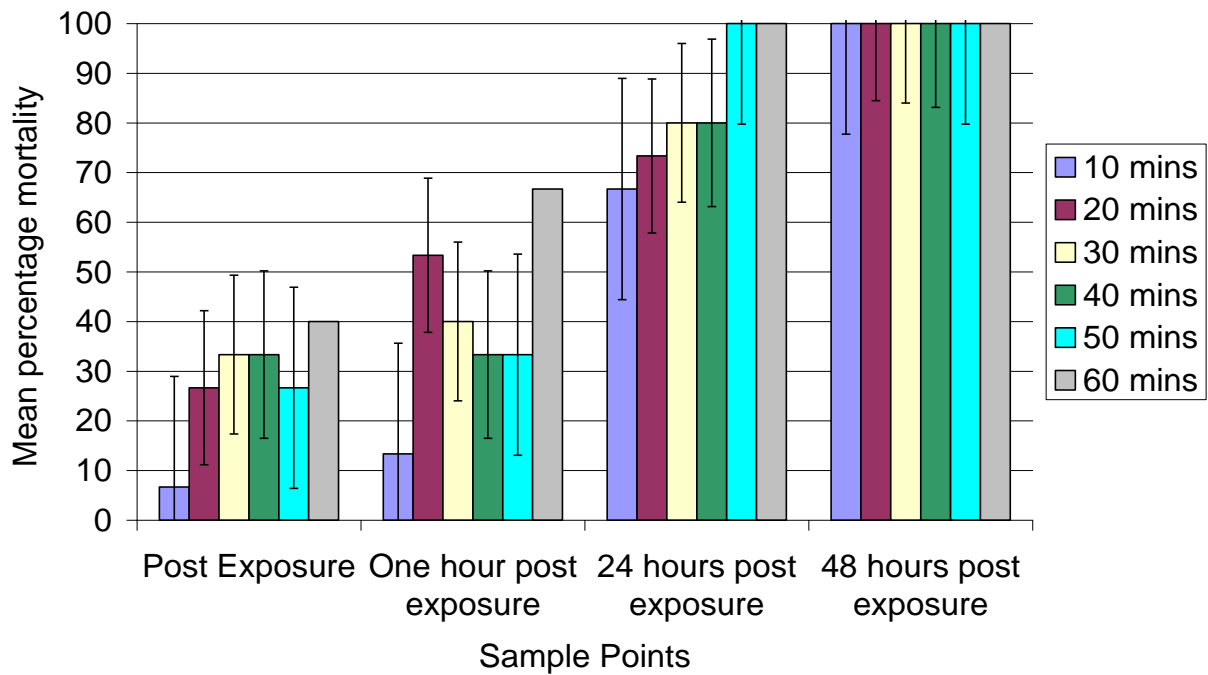
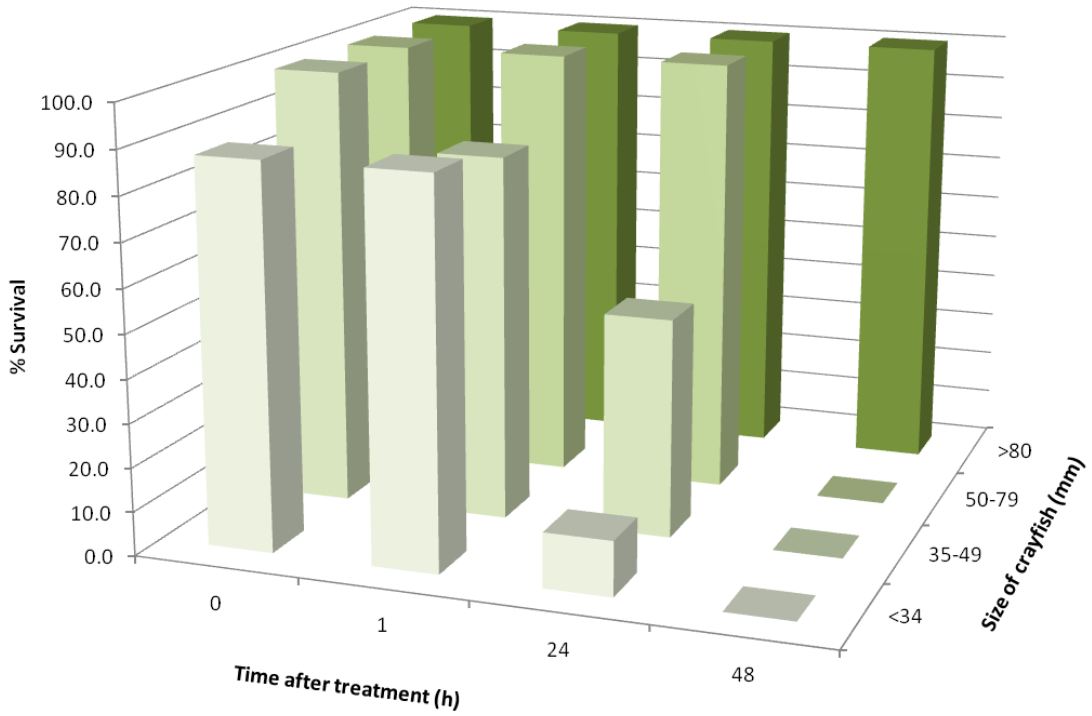
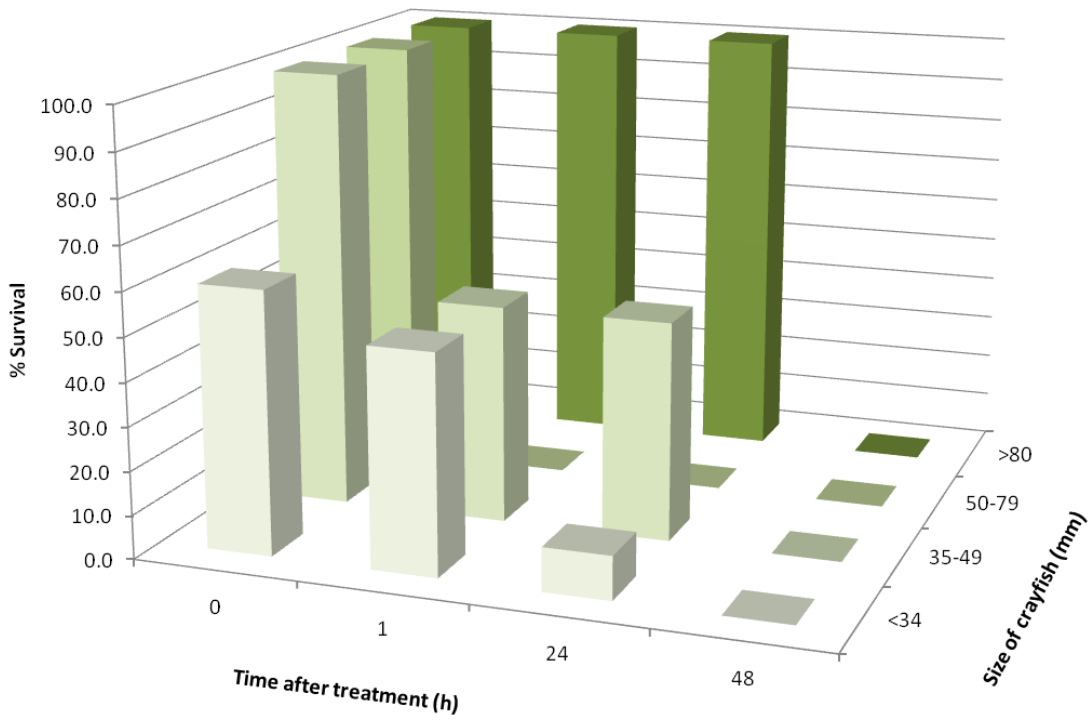


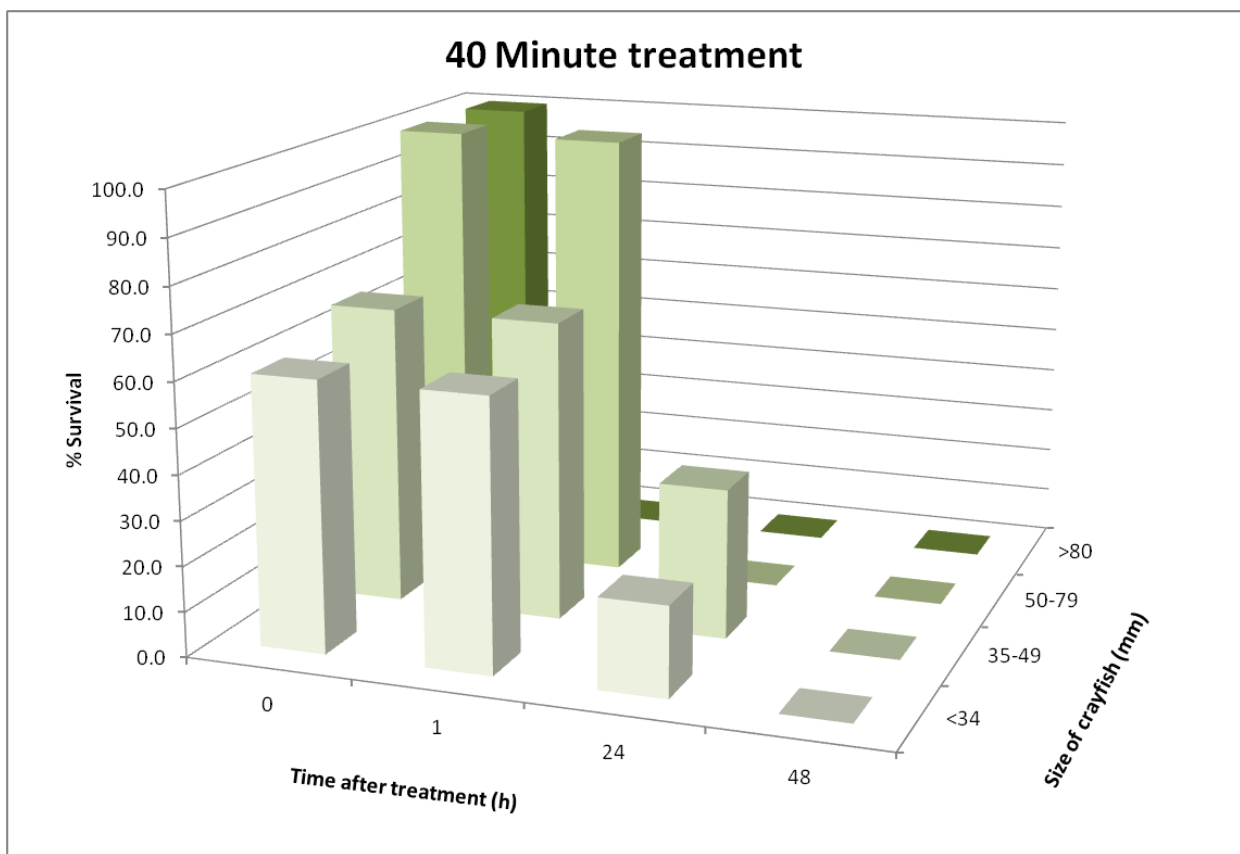
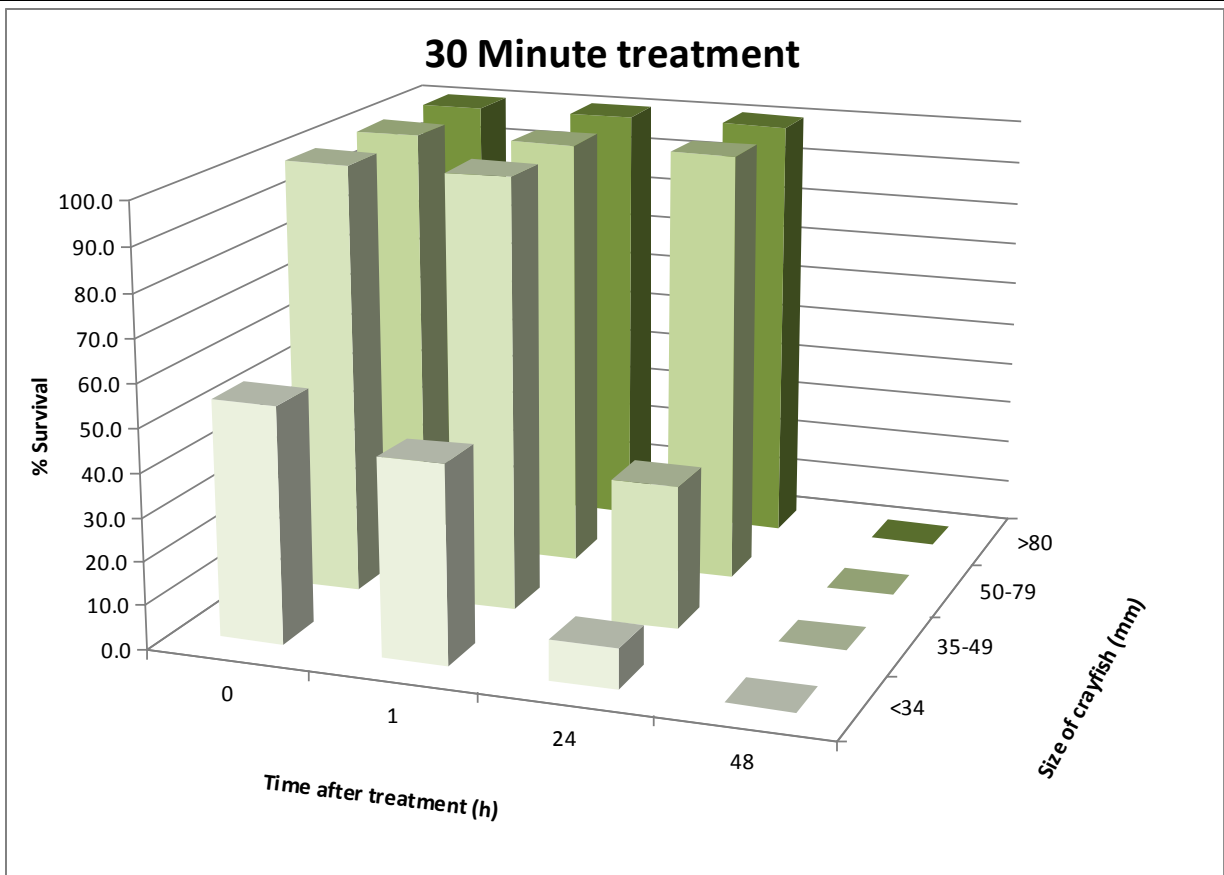
Figure 2. Mean percentage mortality (with SE bars) for signal crayfish immersed in FAM 30 for six periods, with mortality assessed at four time points.

10 Minute treatment



20 Minute treatment





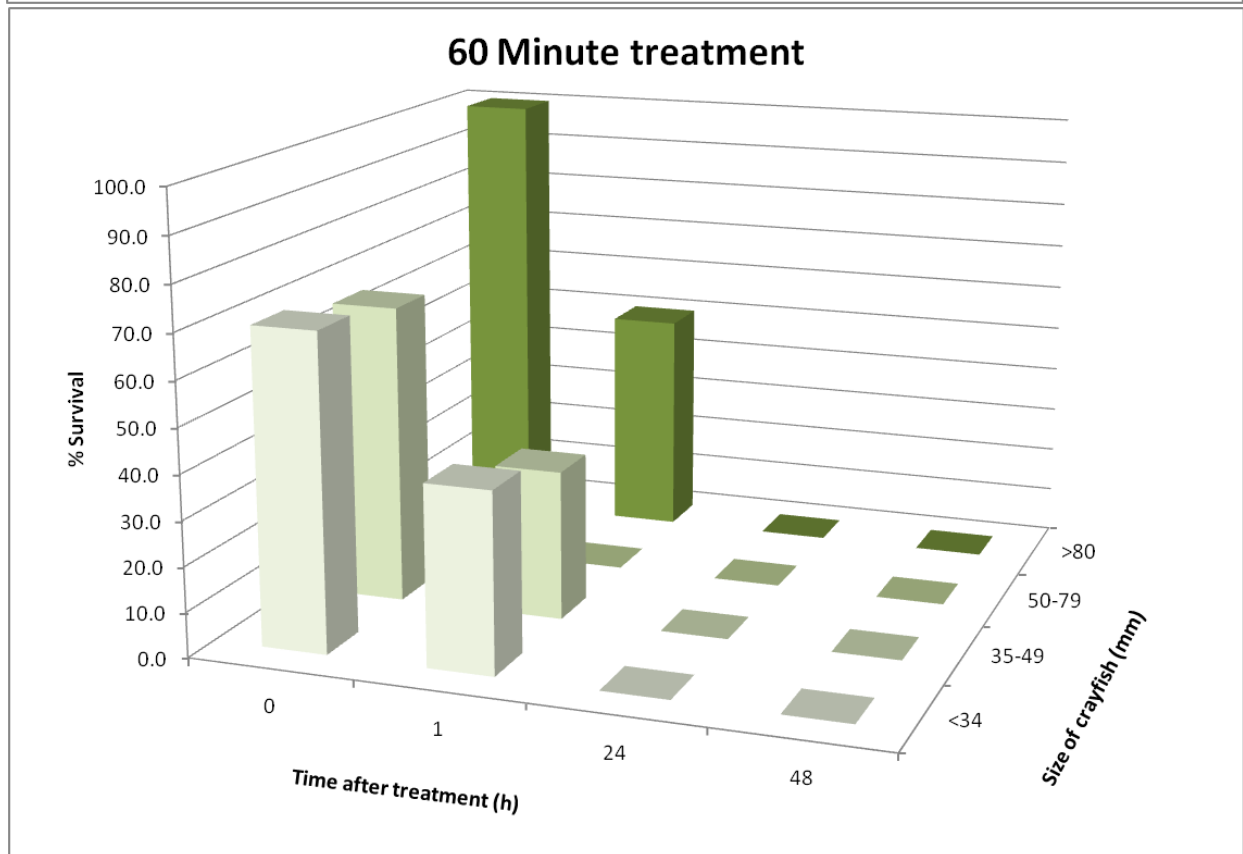
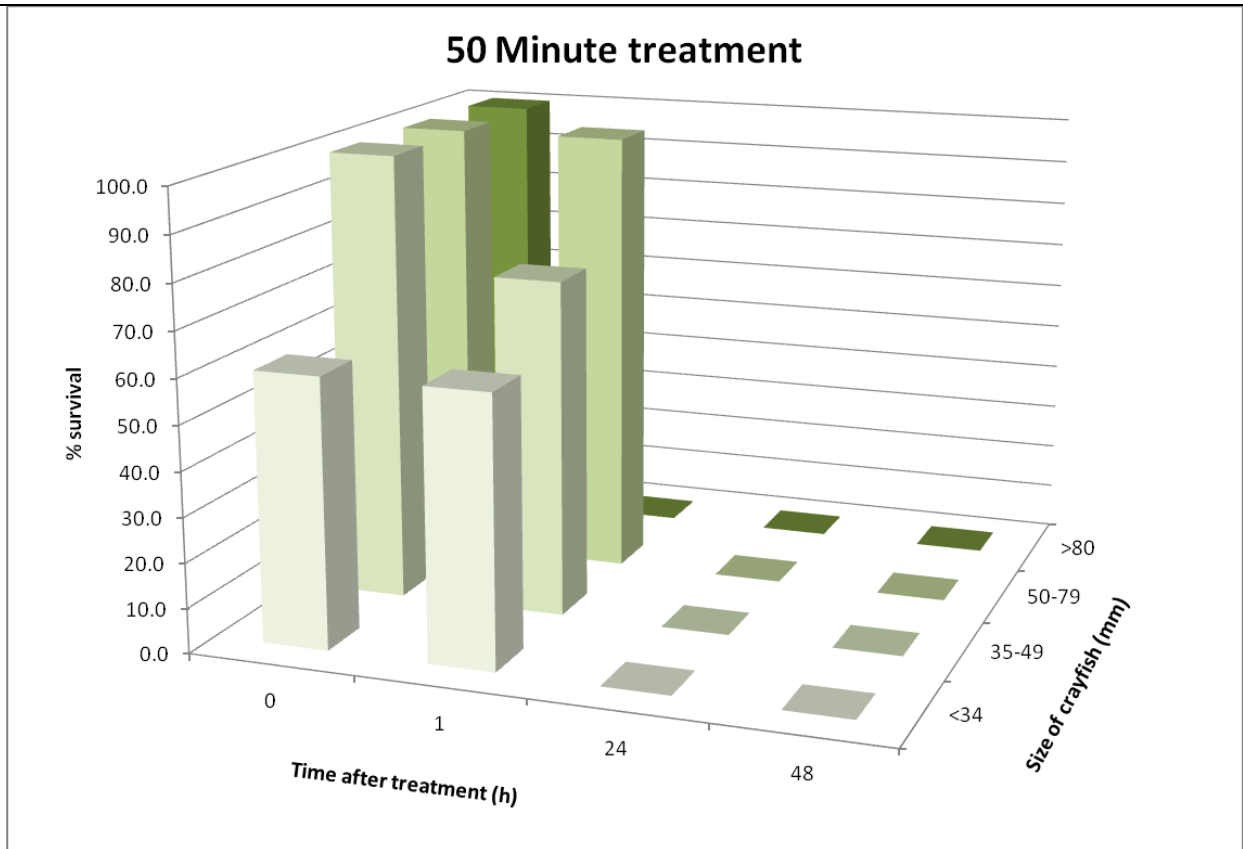


Figure 3. Percent survival of 4 size classes of signal crayfish following immersion in FAM 30 for 6 different periods (n=16 per treatment).

The results from this experiment demonstrated a treatment effect, with faster mortalities following a longer immersion in FAM 30. There was also a size effect, with larger crayfish surviving for longer following

immersion. This experiment confirmed the results from experiment 4, that immersion in FAM 30 was not immediately fatal to crayfish; however following immersion the crayfish died within 48 hours of removal from FAM 30. The exception to this was the single large crayfish in the 10 minute treatment (see figure 3), which survived until the end of the experiment at 48 hours post treatment.

Other pathogens

From a review of the literature, iodophors such as FAM 30 were recorded to be effective against Viral Haemorrhagic Septicaemia Virus (Amend & Pietsch, 1972), bacteria, including *Y. ruckeri* (Verner-Jeffreys et al. 2009) and crayfish plague, *Aphanomyces astaci* (Alderman & Polglase, 1985). There is no literature available on the efficacy of FAM 30 against the causative agent of white spot disease, *Ichthyophthirius multifiliis* (*ich*), as most white spot treatments are performed on live fish, due to the various life stages of the parasitic protozoan, and FAM is highly toxic to fish. Unfortunately, we were unable to obtain *ich* for experimentation, as there was an unusually low prevalence of white spot outbreaks on UK trout farms in 2008, which meant that *ich* could not be cultured at the university for our purposes.

Summary

Following a review of all chemicals currently used within the UK aquaculture industry, six were selected as being potentially useful for this project. Pilot studies indicated that FAM 30 was the most suitable and effective chemical for killing signal crayfish. Water chemistry did not appear to affect efficacy of FAM 30, but signal crayfish survived longer in cold water than warm water. Larger crayfish were more tolerant of FAM 30 than smaller crayfish, and a 20 minute immersion in 2.5ml/l in FAM 30 killed all sizes of crayfish within 48 hours of treatment.

Scientific Objective 05 - Develop and test pilot strategies on small scale fish transport.

Information obtained from scientific objective 3 was used to develop strategies to control the spread of signal crayfish, specifically relating to different procedures used for loading and unloading live fish. To work effectively, any strategy must be practical, feasible and simple for use within the UK trout industry, to maximise the level of uptake by the industry.

The areas of risk for spreading signal crayfish are shown in figure 4. Fish can be moved from their system on the originating farm either directly into the transport tank or into an intermediate holding unit prior to loading into the transport tank. Loading and unloading is carried out with nets or pipes (objective 3), and any strategy needs to recognise and be adaptable to the diversity of methods found within the industry.

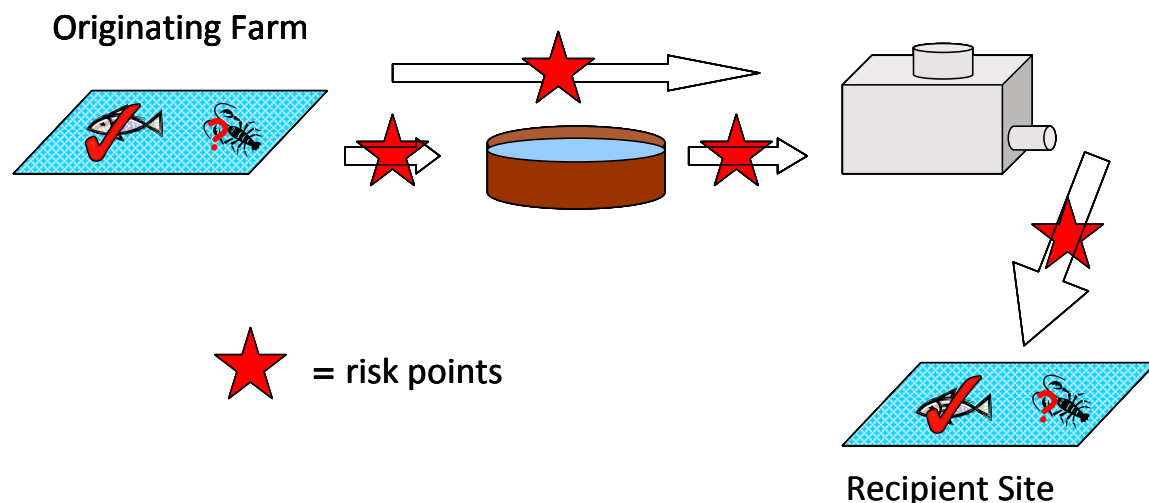


Figure 4. Areas of risk for movement of signal crayfish during live trout transport.

The main issues for minimising the risk of spreading signal crayfish are to ensure that the transport water is free of crayfish and that should any crayfish be present at the originating farm, that only fish and not crayfish are loaded onto the transport tank. The treatment of water containing fish is prohibited by British Trout Association guidelines, therefore fish and water must be separated before being chemically treated to kill pathogens. For non-BTA members who transport fish for restocking fisheries, it is unlikely they would be willing to treat water containing fish, due to possible effects of any chemical treatment on fish welfare and a withdrawal period should the fish be consumed soon after release into the fishery. It is not possible to

eliminate the risk of spreading crayfish; these strategies can only reduce the risk of accidentally spreading crayfish.

We propose two methods for reducing the risk of accidental spread of crayfish, depending on loading procedures. If fish are pumped/piped into the transport tank, then we propose a dewaterer device, which would separate the fish from the water, and potentially from unwanted crayfish. The water used to fill the transport tank should be filtered through a mesh net before filling. We suggest a net with a mesh size of 500 μ l, which will collect crayfish eggs (1-2mm diameter). Fish would pass over a grill and into the transport tank/holding unit, and water and crayfish would fall through the grill, where crayfish would be collected in a fine mesh net (figure 5). Following use, the mesh net should be soaked in FAM 30 for a minimum period of 20 minutes. The minimum immersion period of 20 minutes is recommended following the results from objective 4.

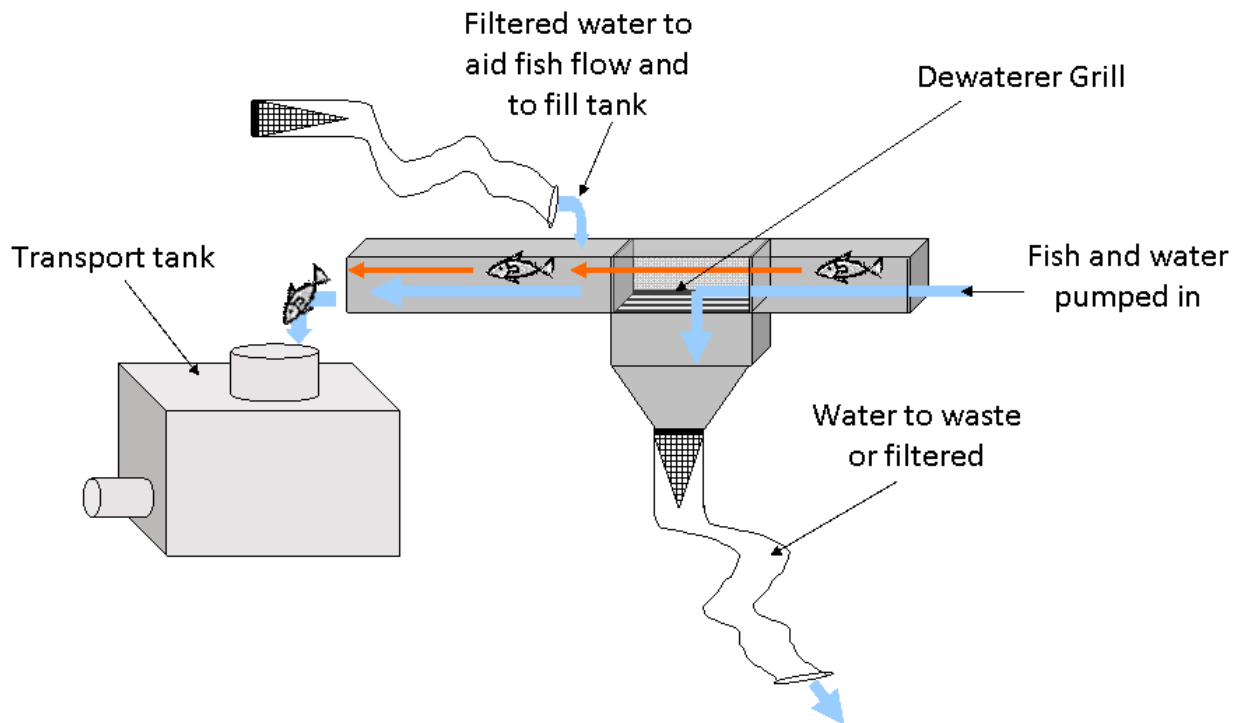


Figure 5. Suggested dewaterer operation.

If fish are netted into the transport tank, then the transport tank should be filled with water filtered through a mesh net. Vigilance by the farmer during netting is required to look out for crayfish in the net although this may not be practical. Following use, the mesh net should be immersed in FAM 30 for a minimum period of 20 minutes. If possible the dewaterer should be used with nets or in conjunction with a sorting table. (A sorting table is used by restocking farmers to identify the best fish within a batch.)

Control strategies during unloading

To attempt to deal with the risk of spreading signal crayfish at the point of unloading is neither practical nor desirable. Regardless of the actions taken to prevent the accidental release of crayfish, the risk of release at destination increases greatly if the destination is where preventative measures are taken, and is an unnecessary risk. We observed two commercial unloading methods, netting and with pipes (see figure 6). The unloading operation using pipes involved a high force of water, which actually threw several fish out of the pipe: using a dewaterer device at this point would be neither reliable nor efficient, as the force of water would mean that there would be a high chance of any crayfish present in the transport tank being released into the recipient water. Furthermore, water is required to move the fish down the pipe. If a dewaterer was used, an additional pumped source of water would be required, and this is not always available when unloading is occurring. To attempt control strategies at the point of unloading was not considered practical, feasible or desirable.



Figure 6. Unloading trout using pipes. To unload, valve on transport tank is opened, and trout flow

down pipe into receiving water.

Dewaterer

A trial dewaterer was constructed using foam PVC, with dimensions of width 22cm, height 25cm, length 100cm and length of grill 50cm (figure 7). The size of crayfish that the dewaterer could deal with will depend upon the size of the spaces between the bars of the grill. This was tested with grill sizes of 2mm between bars, 4mm, 6mm, 8mm, 10mm, 12mm, 14mm, 20mm and 25mm. Five groups of sizes of dead crayfish were used (<20mm, n=20; 20-35mm, n=20; 35-50mm, n=20; 50-80mm, n=19; 80+mm, n=7). A constant flow of water was passed over the dewaterer and the groups of crayfish introduced. The results are presented in figure 8.

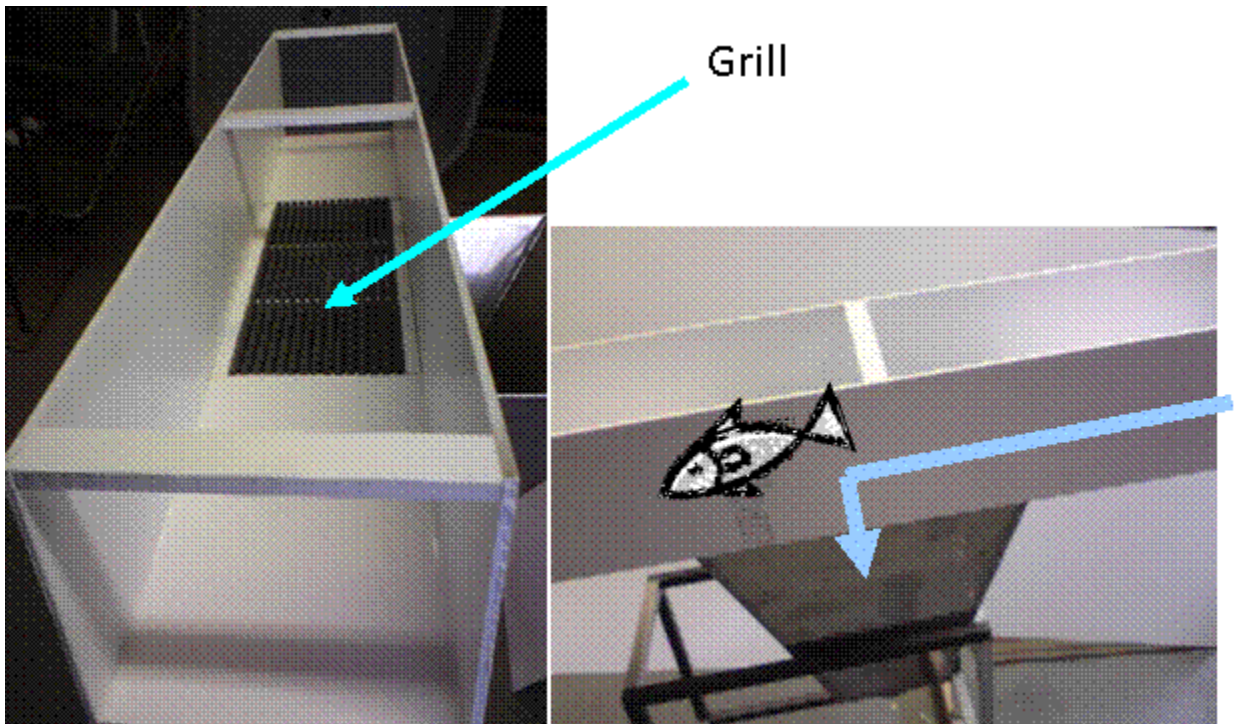


Figure 7. Trial dewaterer. Potentially infected water falls through grill, where it can be filtered through a mesh net.

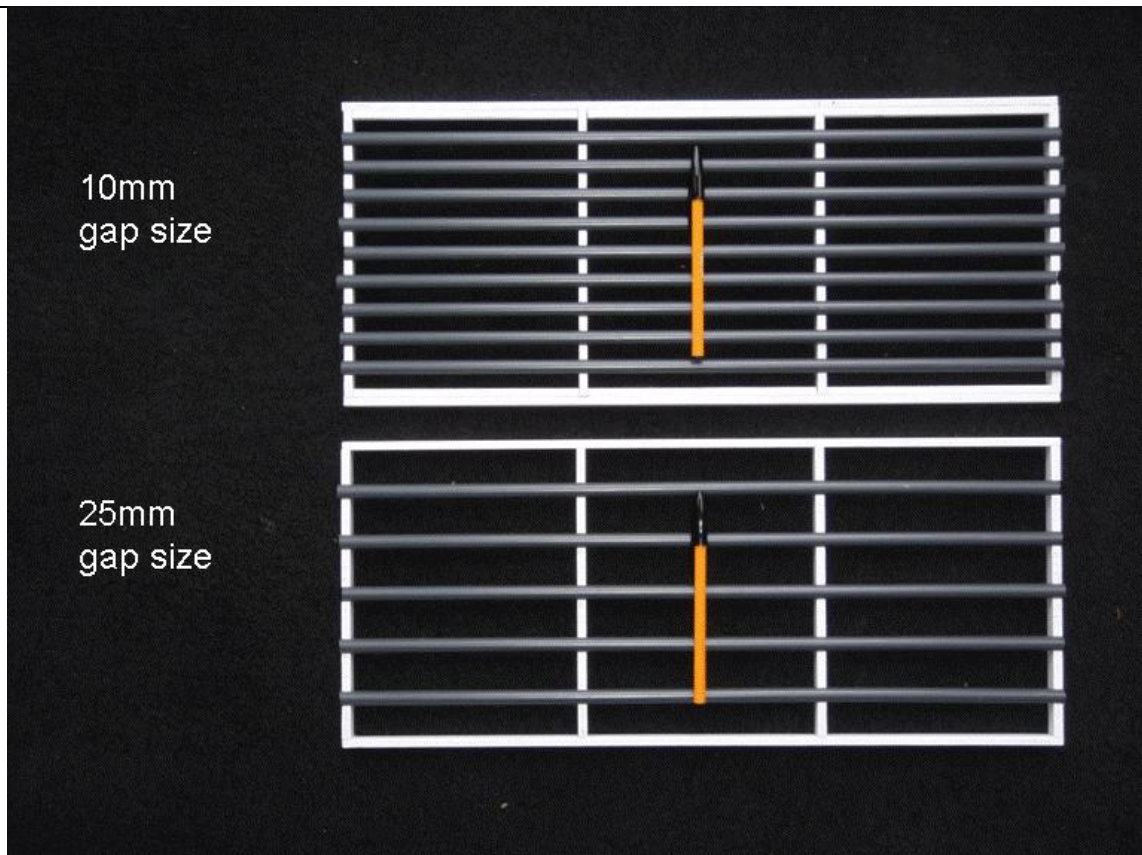


Figure 8. Grills for trial dewaterer. Examples shown are 10mm and 25mm grills.

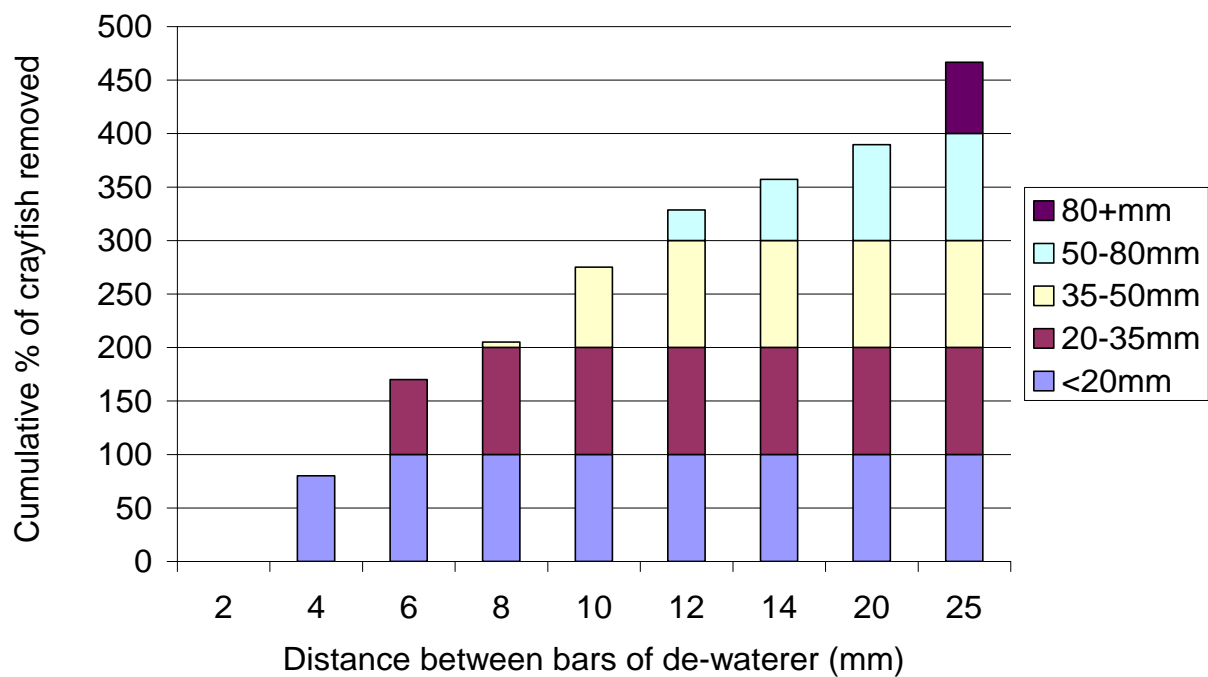


Figure 9. Percentage of signal crayfish that passed through bars of dewaterer.

No crayfish fell between bars with a 2mm gap, while 100% of the smallest crayfish fell through the grill bars at 6mm. The 20-35mm and 35-50mm groups all fell through 8mm and 12mm gaps respectively, and the 50-80mm group all fell through the 25mm gap. Not all crayfish larger than 80mm fell through the largest 25mm gap. Only crayfish that fell through the bars into a collection bin were counted: many crayfish were caught in the grill bars and did not fall through or pass over the dewaterer. It is worth noting that this experiment was

conducted with dead crayfish. Under the terms of our licence (objective 2), we could not remove live crayfish from quarantine conditions to conduct this experiment. Should the experiment be repeated with live crayfish, then it is possible that different results would be obtained, although it would be only supposition to estimate if more or fewer crayfish would fall through the gaps: live crayfish could either propel themselves over the grill or wriggle through the gaps. Dead fish were also passed down the dewaterer, demonstrating that it is suitable for use on a trout farm.

Summary

Our strategy for reducing the risk of accidentally spreading signal crayfish through live trout transports involved a combination of chemical and physical treatments. Treatments should be administered before transport. Water used to fill transport tanks should be filtered using a mesh net. Following use, mesh nets should be soaked in FAM 30 disinfectant for a minimum of 20 minutes. If fish are loaded onto transport tanks using nets, the farmer should be extra vigilant for signal crayfish. If fish are pumped or pipes used to load onto transport tanks, a dewaterer should be used to separate fish from potentially contaminated water. The size of grill used on the dewaterer will depend upon the size of fish to be transported, and will affect the size of crayfish that fall through the gaps.

Scientific Objective 06 - Commercial scale trial for safety of treatments.

Our experiences and observations on live trout transports have shown that there is great variability in loading and unloading throughout the industry. This not only applies to procedures but to local conditions on farm, which influence how and where a dewaterer would be sited. Any trials conducted would have been limited, as we would be unable to use live crayfish. Trials would also not have provided us with any additional information on control strategies for crayfish. Rather, we opted to expend effort consulting with the industry on our proposals and see if they were acceptable and feasible, and if there were any objections.

In February 2009, the concepts and control strategies proposed in objective 5 were presented to restocking farmers, many of whom transport their own fish, at the British Trout Farmers Restocking Association AGM in Exmoor. No objections were raised to the proposals following the presentation. A local farmer and transporter of fish was also consulted on our proposals. The farmer felt that our proposals were practical and feasible. It was agreed that control strategies at the point of unloading were not practical. Reportedly, dewaterer devices are common throughout the salmon industry, and therefore the farmer we interviewed does not believe that widespread use of dewaterers within the trout industry would be a major issue. It does appear to be accepted within the industry that signal crayfish pose a significant concern.

In addition to our proposed chemical and physical strategies, there is the need for an organisational strategy to control the spread of signal crayfish through live fish transports. We propose an Integrated Control Strategy, where the chemical and physical strategies are backed up with an audited labelling scheme, such as those operated by Freedom Foods, Assured Food Standards and The Soil Association Organic Standard. Members to the scheme would be designated either "Crayfish Free" or "Low Risk of Crayfish". The "Crayfish Free" label would apply if the farm and supplying water are free from signal crayfish. If the farm or supplying water have signal crayfish, then a "Low Risk of Crayfish" label would apply, providing the fish farmer takes active steps to address the risks of accidentally transporting signal crayfish, such as the chemical and physical methods outlined in objective 5. A further stipulation of such a scheme could be that the transport of fish from a crayfish infected source to a crayfish free source is forbidden. This proposal would require a UK wide sampling programme to identify the signal crayfish status of every farm that signs up to the scheme, and the scheme would require auditing to maintain its integrity and effectiveness. This proposal is suggested as a commercial option for people selling and transporting trout with clients concerned about the threat from signal crayfish, and will only be implemented if the people purchasing trout demand it, although initial indications are that this will be the case.

Scientific Objective 07 - Produce a briefing note, which will be referred to in the BTA Code of Practice, and prepare manuscripts.

A briefing note outlining best practice for reducing the risk of spreading signal crayfish through live fish transportation was prepared for the BTA and is attached as appendix 2. We contacted an aquaculture engineering firm (Scanbio Scotland Ltd, Corpach, Fort William) to obtain an indication of price for a dewaterer.

Discussion of results and reliability

The review of transport procedures in objective 3 involved structured discussions with many live trout transporters, including the two main professional hauliers servicing the industry. We are confident that the

data is a reliable and thorough representation of transport procedures in the industry. The criteria used to select the most suitable chemical were appropriate for this project (objective 4). The range of environmental conditions tested was designed to simulate conditions likely to be found in fish transports in the UK. We tested a range of chemical concentrations and a range of crayfish sizes and are confident that the results are robust and our method thorough. The strategies for objective 5 were built upon the results of objective 3 and were developed following identification of the main areas of risk for spreading crayfish. The grill size: crayfish size dewaterer test (objective 5) was performed with dead crayfish; it is likely that results would have been different had live crayfish been used. The results from this experiment should be used as a guide only.

Implications of findings

This project established the range of procedures used to transport live trout within the UK (objective 3). Prior to the project, it was suggested that the spread of signal crayfish might have been facilitated through water exchange during transport, however, the project established that no trout transporters exchanged water during transport. Criteria were applied to all chemicals currently used within the UK aquaculture industry to find the most suitable chemical for this project (objective 4) and, following pilot studies, the iodophor FAM 30 was identified as the most suitable with its speed and efficacy for killing signal crayfish. FAM 30 is already widely used throughout the aquaculture industries, and is effective against other pathogens, including viruses, bacteria and fungi, including that responsible for crayfish plague, *Aphanomyces astaci*. Larger crayfish survived for longer in FAM 30 than small crayfish, however, following a minimum immersion time of 20 minutes in FAM 30, all crayfish died within 48 hours (objective 4). This finding contributed to the development of practical strategies (objective 5) as it provided a benchmark for the minimum contact time to disinfect using FAM 30. We have developed two practical strategies for reducing the risk of accidentally spreading signal crayfish through live trout transports, combining physical and chemical treatments. Should fish be netted into transport tanks, then the water which fills the tank should be filtered through a fine mesh net, with a mesh size of 500µl, which should then be disinfected using FAM 30. Fish that are pumped or piped into transport tanks should first pass over a dewaterer before loading; to separate fish from potentially crayfish infected water. The dewaterer may also be used with fish that are netted. The size of the dewaterer grills determine the size of crayfish that pass through the grill. Our strategies were presented to the industry (objective 6) and no objections were received. A briefing note was prepared for the industry (objective 7) setting our proposed integrated control strategy to reduce the risk of spreading signal crayfish through live trout transport.

It is impractical to eliminate the risk of spreading signal crayfish through the transportation of live fish, however, we have proposed an integrated control strategy with physical, chemical and audited labelling scheme of "crayfish free" and "low risk of crayfish". All the indications from the BTA and farmers with whom we had contact would suggest these suggestions will be well received and applied. If these recommendations are implemented the risk of spreading crayfish in trout transportations, which was already considered to be low, would be reduced to a practical minimum.

Possible future work

This project has identified practical physical and chemical strategies for reducing the risk of spreading signal crayfish through live trout transport. Future work would be to develop organisational strategies e.g. the audited labelling scheme ("crayfish free" and "low risk of crayfish") to complement the strategies developed under this project, outlined in objective 6

In December 2008, Defra released their aquatic animal health research requirements for 2009/10, which included a call for tenders to research the control of crayfish plague, principally through controlling the spread of signal crayfish. The University of Stirling has joined a research consortium led by CEFAS, and including the Environment Agency and Universities of Portsmouth and Newcastle, to submit an application for funding for this project. If funded the results from this project would be used to inform recommendations for reducing the spread of crayfish and crayfish plague nationally and in other industries in addition to trout.

Knowledge Transfer

The briefing note prepared for objective 7 will be referred to in the BTA Code of Practice. It is anticipated that an article will be prepared for Finfish News, to include the briefing note, which will reach BTA non-members. The findings of the project have formed the basis for discussions regarding the control of the spread of crayfish via trout transportation through the Freshwater Fisheries Forum and our proposed integrated control strategy will form the basis of codes of practice for stocking.

In addition, there are the following outputs and proposed outputs.

Bunker, K. (2008) Trout transportation in the UK; methods and changes in water quality. M.Sc thesis. University of Stirling.

Publications (In prep.):

Bunker, K., Berrill, I. K., MacIntyre, C. M., Turnbull, J. F. A review of transportation methods used in the UK trout farming industry.

Berrill, I. K., MacIntyre, C. M., Turnbull, J. F. The efficacy of chemicals in the control of signal crayfish.

Presentations:

Berrill, I. K. Developing practical strategies for reducing the spread of harmful organisms during the transportation of live fish. British Trout Farmers Restocking Association conference, Somerset, UK. February 2009.

Turnbull, J.F. Research at University of Stirling affecting the trout industry. British Trout Association AGM, Lechlade, August 2009.

References to published material

This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project (the box below will expand).

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