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This report presents the results of marine and freshwater scientific work carried out by Marine Scotland Science.

A Pilot Study To Determine The Effect Of An Anti-Sea Lice Agent On The Marine Survival Of Atlantic Salmon On The West Coast Of Scotland

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Executive Summary

There are concerns that sea lice in the coastal environment are impacting on the return rates of wild Atlantic salmon on the West coast of Scotland. Studies in Norway and Ireland involving examining survival of groups of migrating salmon smolts treated with anti-sea lice medicines have shown that sea lice can adversely affect certain salmon populations. A pilot project conducted by Marine Scotland Science using portable traps determined that developing a network of experimental sites across Scotland was not likely to be feasible.

The pilot study here was to make an assessment whether the Awe catchment could be used as a West coast site that could provide an indication of impact of coastal sea lice on wild Atlantic salmon survival. This catchment was of interest as it has an automatic PIT detector located within the fish lift associated with a permanent barrage. In total 1003 salmon smolts were captured and PIT tagged on the Orchy and Strae tributaries of the Awe during spring 2017. Half of these fish (n= 454)were treated with the anti-lice compound FLUX (100 mg/ml) and the other half (n=460) treated as control fish before release to continue their migration.

In 2018 the automatic PIT detector at the Awe barrage recorded 16 returning salmon grilse from the experiment. Eleven of these were from the treated group, while the remaining 5 were from the control group. Differences between the groups was not statistically significant at an alpha level of 0.05. The total return rate was 1.6%. The numbers of returning fish detected suggests that the Awe catchment could be used for future treatment- release studies on the West coast of Scotland to assess impact of sea lice in the coastal zone.

Introduction

Sea lice is a generic term for a group of parasites that feed on the mucus and skin of fish. *Lepeophtheirus salmonis* and *Caligus elongatus* are marine species of sea louse that may negatively impact on the health of salmon *Salmo salar* in the North Atlantic (Torrison et al. 2013). *L. salmonis* in particular, is a concern to the Atlantic salmon aquaculture industry in Scotland, where infestation levels can be controlled by physical, biological or pharmacological methods (e.g. Jensen et al. 2015; Stein et al. 2016; Grontvedt et al. 2015; Murray et al. 2016).

Declines in catches of wild salmon have been steeper on the Scottish West coast than elsewhere in Scotland (Vøllestad et al. 2009). This has led to concerns that sea lice emanating from aquaculture are impacting on local populations. However, there are multiple factors that may affect salmonid survival at sea and sea lice are just one of them.

In Norway and Ireland experiments have been conducted with the aim of specifically examining the impact of sea lice on smolts migrating through aquaculture zones (Jackson et al. 2011; Vollset et al. 2016). These studies have released samples of smolts migrating out of the river and treated half of them with an anti-sea lice chemical. The other half of the fish remained untreated, as a control group. Numbers of returning fish were then monitored and compared between the groups. A meta-analysis of these studies has indicated that sea lice have an average impact of 18% on returning numbers however there is substantial variation among sites (Vollset et al. 2016).

In Scotland, a pilot project to investigate the potential of developing a network of sites was conducted using the treat/ release type methodology described above. This study was funded by SARF (Morris et al. 2019) and examined two catchments, one on the West coast (Lochy catchment) and one on the East coast (Conon catchment). In contrast to the majority of Norwegian studies wild smolts were used. Good numbers of smolts were captured on the east coast using a fixed trap in a hydropower installation across a main river. However, very low numbers of returning fish were detected on the West coast using temporary traps in tributary streams, making an assessment of impact of lice impossible.

The SARF project was a pilot and highlighted difficulties that suggested expanding the network over multiple sites on the West coast was not practical at this time. However, this conclusion does not help answer the question as to whether sea lice in the coastal environment are having an impact on west coast wild salmon stocks. In 2017 a PIT tag reader was installed into the fish lift at the Awe barrage in 2017 providing an enhanced opportunity for detecting returning salmon. Hence it became feasible to trial the treatment work on tributaries of the Awe with the expectation that a sizable proportion of the returning fish would be detected as they migrated through the main stem of the river on their return.

Materials and Methods

Field Work Sites And Fish Capture

The capture sites chosen were on two tributaries flowing into Loch Awe. This loch is freshwater, and is located on the river Awe in the West of Scotland. At the head of the Loch is an 18m high barrage which diverts water through a 5km tunnel to run a hydroelectric turbine. This facility is operated by SSE. Fish can bypass the barrage by a purpose built fish pass, to enter a short stretch of the River Awe that flows into the sea at Loch Etive.

Loch Awe Trapping Sites

During the spring of 2017, salmon smolts were caught on the River Strae using a fyke net (Lat. 56.4175, Long -5.01275) and on the River Orchy using two rotary screw traps (Lat. 56.4053, Long -4.97038) (Figures 1&2). The sites were chosen as they combined ease of access with substantial smolt rearing habitat in the river above the trapping stations. The location of the trapping stations were not moved during study. The traps were set daily in non-spate conditions, left overnight and fished the following day.

Figure 1. Rotary screw trap in operation on the River Orchy.

Figure 2. Fyke net being set by MSS staff on the River Strae.

Tagging And Treatment Of Migrating Salmon Smolts 2017

The fish were treated and tagged following a pre-determined Standard Operating Procedure (SOP) under Home Office Licence (PPL 70/8928). The SOP was adapted from the one developed during the SARF project. This SOP is attached to this report as Appendix 1.

Briefly salmon smolts were anaesthetised using MS-222(80 mg/l), weighed and length measured. A full duplex (FDX) PIT tag, compatible with the automatic reader installed in the Awe barrage was inserted into the fish. After recovery from the anaesthetic the fish were randomly assigned to either a control bin or a treatment bin for 1 hour before release. The treatment bin contained the anti-parasitic compound FLUX 100 mg/ml (Pharmaq- Zoetis).

All work was conducted by Marine Scotland Science (MSS) fisheries biologists. Data were recorded onto record sheets at the sites, and later transferred to the Marine Scotland FishObs database at the Freshwater Fisheries Laboratory at Faskally for secure long term storage and retrieval. Data collected was made up of site, tagging date, fish length, fish weight, treatment and PIT tag number.

Recording of returning adult salmon (2018)

An automatic PIT reader is installed into the fish lift at the Awe barrage. Tagged fish returning to the Awe site are detected by this reader as they ascend the barrage. Data was downloaded from the reader regularly throughout 2018 and was compared to the FishObs database to identify returning fish from the study. The last data download prior to this report being published was 18/12/2018. Downloads over 2019 are planned to be included as Annexes to this report.

Results

Numbers Of Salmon Smolts Tagged And Treated

In total 914 fish were caught on the River Orchy and 89 were caught on the river Strae. On the River Orchy, 454 fish were treated (460 no treatment), and on the River Strae 45 fish were treated (44 no treatment). This gave an overall of 499 treated and 504 non-treated fish. The capture rates for the two sites is given in Figures 3 and 4 with the distribution of the fish metrics in the different groups summarised in Figure 5 and Tables 1 & 2.

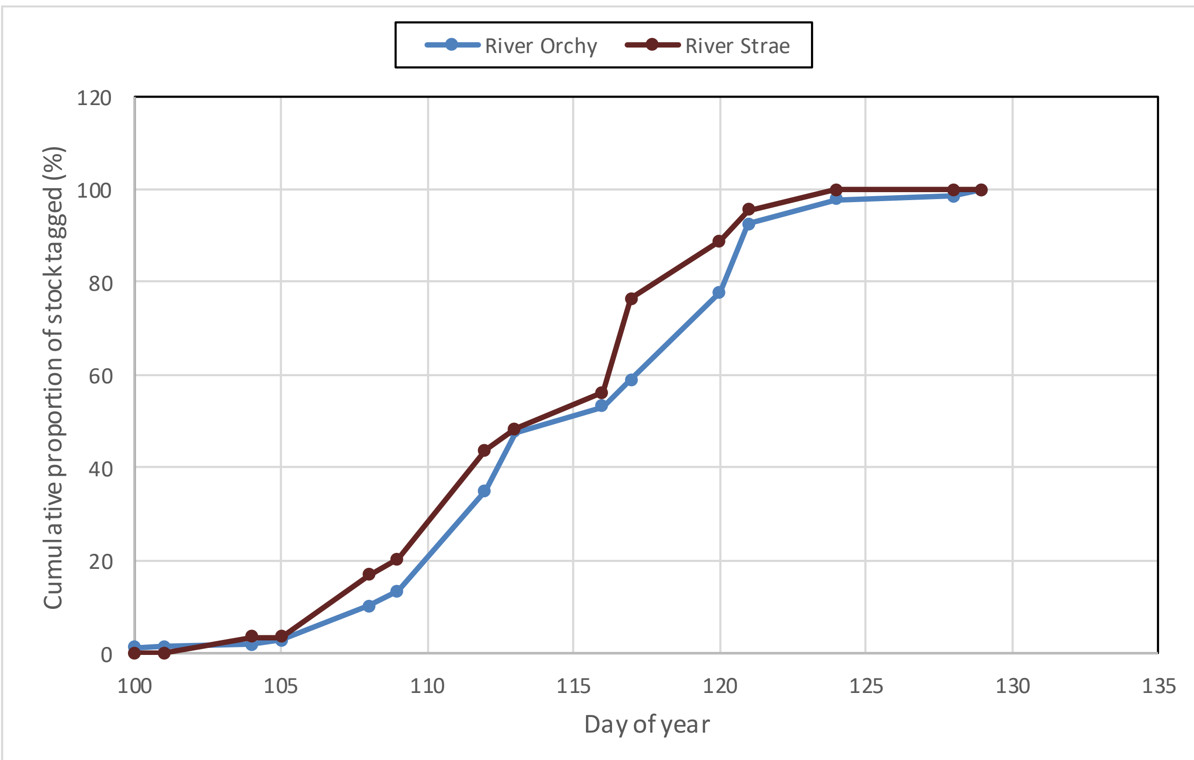

Figure 3. Cumulative proportion of fish stock tagged in each river over time.

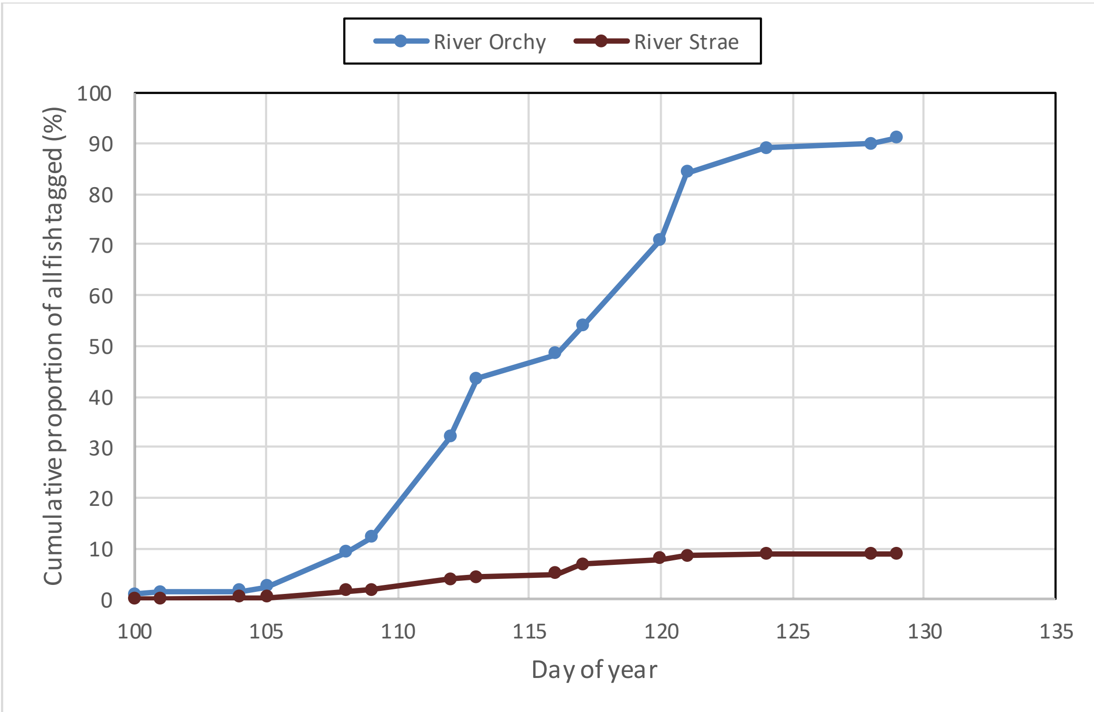

Figure 4. Cumulative proportion of fish tagged over time.

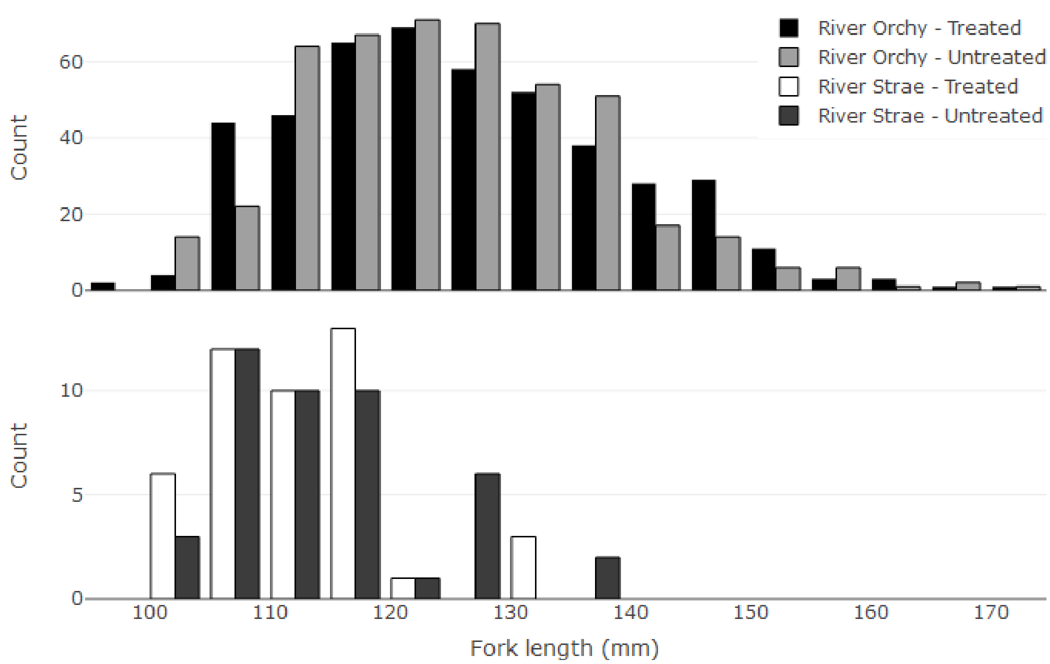

Figure 5. Length distribution of treated and untreated fish on the River Orchy and River Strae respectively.

2017	Weight (g)								
	Control fish			Treated fish			Total fish		
	min	max	Mean ±SD	min	max	Mean ±SD	min	max	Mean ±SD
Strae	9.3	25.2	14.20 ±3.78	9.0	22.4	13.39 ±3.00	9	25.2	13.79 ±3.39
Orchy	7.5	47.5	18.58 ±5.16	8.5	47.5	19.01 ±6.18	7.5	47.5	18.80 ±5.97

Table 1. Wet weight of wild salmon smolts caught, PIT tagged and treated.

2017	Fork length (mm)								
	Control fish			Treated fish			Total fish		
	min	max	Mean ±SD	min	max	Mean ±SD	min	max	Mean ±SD
Strae	102	137	114.57 ±8.65	100	132	112.86 ±7.41	100	137	113.45 ±8.08
Orchy	100	170	124.85 ±12.22	95	171	125.75 ±13.26	95	171	125.30 ±12.75

Table 2. Length of wild salmon smolts caught, PIT tagged and treated.

Returning Salmon Tags Detected In Fish Lift (2018)

Sixteen tags were detected by the automatic PIT tag reader at the barrage. Of these 11 were from treated groups and 5 were from the non-treated groups. Details of these fish are given in table 3.

PIT tag code	Date tagged	Weight when tagged (g)	Length when tagged (mm)	Anti-lice Treatment (Y/N)	Detection date / time
DC0057380F	23/04/2017	24.1	137	N	04/06/2018 08:17
DC00574EB1	18/04/2017	12.2	112	Y	17/08/2018 16:14
DC00576251	01/05/2017	13.9	115	Y	26/08/2018 15:59
DC005732FE	01/05/2017	17.5	126	Y	08/09/2018 16:46
DC0057408E	27/04/2017	27.2	141	Y	10/09/2018 16:19
DC00571E49	22/04/2017	24.9	139	Y	10/09/2018 16:30
DC005736A6	18/04/2017	22.5	136	N	11/09/2018 08:08
DC00573242	23/04/2017	29.5	147	Y	11/09/2018 12:16
DC00573A2C	01/05/2017	21.6	130	Y	11/09/2018 12:27
DC00571EE4	26/04/2017	22.9	143	Y	11/09/2018 16:24
DC00573E78	22/04/2017	21.9	133	N	12/09/2018 12:22
DC00575006	01/05/2017	14.2	115	Y	12/09/2018 12:43
DC005710BE	23/04/2017	16.5	123	Y	29/09/2018 12:47
DC00574B3F	01/05/2017	17.6	121	N	07/10/2018 16:43
DC00576492*	26/04/2017	14.1	115	N	16/10/2018 16:35
DC00575FC1	01/05/2017	15.5	117	Y	24/10/2018 12:04

Table 3. Returning fish detections. All fish originated from the river Orchy except for (*) which was caught on the Strae.

The percentage of fish returning in the treated group was 2.02% and in the non-treated group 0.99%. Using a one-sided fishers exact test (R base package), with the significance level set at $\alpha=0.05$, the difference between groups was non-significant ($p=0.104$).

Discussion

The aim of this pilot study was to investigate the possibility of using the Awe catchment as a site for quantifying the impact that coastal sea lice have on returning salmon numbers.

A related study, conducted on the Lochy river system (tagging smolts during 2015-2016) concluded that developing a network of river sites, using temporary trap installations on the West coast, when coupled with low numbers of returning fish, was not a realistic strategy for determining impacts of sea lice on wild salmon in Scotland (Morris et al. 2019). Therefore it is notable that the proportion of detected fish returning was substantially higher at the Awe site than during the Lochy study (1.6% compared to 0.05%). The most obvious difference between the sites was the design and siting of the Awe trap.

Adult salmon entering the Awe were detected during assisted ascent into a large freshwater Loch prior to their entry into the final spawning tributaries. In contrast the trapping/ detection sites used during the Lochy study were both situated in tributaries feeding into the main stem. The PIT detector at the Awe was protected by the barrage and operated continuously. This is in contrast to those on the Lochy, during which the efficacy was compromised when they were overtopped and/or damaged in high flows/ spate and required frequent remedial work to maintain their operation and efficiency. Because of the relatively low numbers of returning fish detected, any differences present between the two experimental sites could exaggerate the variance in total numbers of fish detected between the studies, and therefore further inferences comparing total returning numbers to these rivers cannot be made.

In comparing different river systems and the effect of the sea lice treatment the most pertinent statistic is the proportion of returners between groups. In the Awe study there appears to be a bias towards treated fish returning. This would be expected if coastal sea lice are impacting on returning adult numbers. However, this is in contrast to the results of the SARF project where results at the Conon site on the East coast had lower numbers of treated fish returning in comparison to the control group (Morris et al. 2019) suggesting a possible effect of the treatment chemical. In interpreting the results from these types of experiments it is desirable to conduct them across multiple years to ascertain the level of variability both between years and the two sites to determine whether the differences can be considered significant or the result of natural variability within the system.

A potential issue regarding West coast sites, including the Awe catchment, is obtaining sufficient smolts across multiple years to use in the treatment and control groups for the returning fish numbers to be interpreted correctly. The fish used in the Awe study were from two tributaries and if this work were to continue more tributaries would likely need to be included to ensure sufficient numbers of returning adults to include in the analysis.

In conclusion, this pilot study was to examine the utility of the Awe catchment as a site to conduct treatment-release experiments to quantify the potential impact of sea lice in the coastal environment. The number of grilse returning to the site after capture and treatment as smolts indicates that this catchment, would be a suitable site to conduct this type of experiment.

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Appendix 1: Tagging And Treatment SOP (After Morris Et Al. 2019)

Standard Operating Procedure (SOP) For Treatment And PIT Tagging, Of Wild Salmon Smolts - Awe 2017

General notes on procedure

- Staff undertaking the work should have previous experience and be aware of all related Risk and COSHH assessments.
- Tagging should be undertaken near to the trapping site and a supply of fresh running water.
- A covered tagging shelter protected from the weather is desirable. However, depending on numbers of fish / location of trap, alternatives such as working from the back of a vehicle or working on a bench / table are possible.
- In all cases, equipment should be set up at a working height appropriate for those conducting the tagging.
- All hand written recording sheets are photocopied at the end of each day and stored securely (i.e. each batch stored in a different location e.g. one batch in Trust's office, one batch in accommodation).
- As soon as practically possible after tagging, the contents of all hand written recording sheets are entered into the FishObs data preparation utility (DPU) and exported to Excel for error checking at a later date.

Treatment And PIT Tagging protocol

- PIT tags are stored (at least overnight) in 70 % ethanol or industrial methylated spirit and washed in sterile saline before inserting into fish. A sieve, petri dishes and large plastic screw top containers are provided for this purpose.

1. Set traps and leave to fish overnight.

2. Visit (first) site the following morning. Remove all fish from the trap and place in an in-river holding box* [or *in situ* holding box in the case of a rotary screw trap (RST)]. Make sure trap is no longer deployed to catch fish.

* *Depending on the numbers of fish caught, it may be possible to avoid the need for an in-river holding box and transfer fish directly from the trap to a bin of fresh aerated river water prior to processing*.

3. Screen smolts and retain large fish (those estimated to be ≥ 135 mm fork length) in the in-river holding box for possible acoustic tagging.

4. Set up three bins on level ground. Approximately half-fill one of these bins with fresh river water and add an aerator. This is the “stock bin” used to hold fish from the in-river holding box prior to processing. Fill the other two bins with 25 L of fresh river water and add an aerator to each. Clearly mark one of these bins with a “1” (this is “bin 1”) and one of these bins with a “2” (this is “bin 2”). Set up an anaesthetic bath (MS222, 80 ppm: equivalent to 0.4 g of MS222 dissolved in 5 L of water).

5. Transfer a batch of fish from the in-river holding box to the “stock bin”. Transfer a few fish at a time (typically ≤ 5) from the “stock bin” into the anaesthetic bath.

6. <u>PIT tagging of wild salmon smolts* (i.e. typically those ≥ 100 mm fork length and / or with a silvery appearance and blackening fins that are not obviously of hatchery origin):</u>

* *Should be quite obvious in April / May. If in doubt, do not tag*.

Aseptic procedures are followed during tagging to minimise any possible infection / cross-contamination. Disposable gloves are to be worn.

A sterile scalpel / blade should be used at start of tagging and changed regularly (at least every 10th fish). Blades should be cleaned with an alcohol wipe and rinsed in sterile saline between fish.

When a fish is fully immobilised in the anaesthetic, remove the fish from the anaesthetic and measure fork length (to the nearest mm), and record wet weight (to the nearest 0.1 g).

The fish is held by hand, belly up and a small incision (approx. 3 mm) with a No. 11 scalpel is made anterior to the pelvic fins on the mid-ventral surface.

A PIT tag is scanned and the code noted by the assistant before the PIT tag is inserted into the body cavity through the incision.

The fish is scanned with a hand held scanner and the code is checked against that noted already.

Place alternate fish into either “bin 1” or “bin 2” and allow to recover. Record a “1” or a “2” on the recording sheet as appropriate**. <u>For any other fish, record fork length only, allow to recover in a bucket of fresh river water and return to the site as soon as fully recovered.</u>

*** Once the number of PIT tagged fish reaches 80 (40 in "bin 1" and 40 in "bin 2"), if there are more fish to PIT tag it will be necessary to set up an additional pair of bins. To do this, set up a further two bins on level ground. Fill both bins with 25 L of fresh river water and add an aerator to each. Clearly mark one of these bins with a "3" (this is "bin 3") and one of these bins with a "4" (this is "bin 4"). Continue by recording a "3" or a "4" on the recording sheet as appropriate.*

7. Once either 160 fish, or all fish, have been processed (whichever happens first), set up a further set of bins on level ground (2 bins will be required for up to 80 fish; 4 bins will be required for between 81 and 160 fish). Fill these bins with an appropriate volume of fresh river water (i.e. depending upon the number of fish to be placed in each - see Table 1) and add an aerator to each bin. Clearly mark half of these bins with a "C" ("control bin(s)") and clearly mark the other half of these bins with a "T" ("treatment bin(s)"). Use "C1" and "C2" to differentiate between control bins and "T1" and "T2" to differentiate between treatment bins.

8. Pour the appropriate amount of the treatment compound from the vial into the "treatment bin(s)" - see Table 1) and thoroughly rinse out the vial in the treatment bin water. Ensure thorough mixing of the compound in the "treatment bin(s)" by stirring with a wooden pole.

9. Flip a coin:

If the coin shows "heads":

Transfer the fish from "bin 1" (and "bin 3") to the "control bin(s)" "C1" (and "C2"), and transfer the fish from "bin 2" (and "bin 4") to the "treatment bin(s)" "T1" (and "T2").

If the coin shows "tails":

Transfer the fish from "bin 1" (and "bin 3") to the "treatment bin(s)" "T1" (and "T2"), and transfer the fish from "bin 2" (and "bin 4") to the "control bin(s)" "C1" (and "C2").

Record a "C1", "C2", "T1" or "T2" on the recording sheet as appropriate.

10. Leave the fish in the "control bin(s)" and "treatment bin(s)" for 1 hour. Bins to be covered with lids during this time.

11. Collect all fish using designated hand nets and buckets (designated nets and buckets for the treated fish to be clearly marked with a "T" and designated nets and buckets for the control fish to be clearly marked with a "C"), and place in fresh bags with aerated water for transport to site of release. Bags are sealed and transported to below Awe barrage. The bags are introduced into the river water and opened, releasing fish.

12. Using designated buckets (clearly marked with a “T”), bale water from the “treatment bin(s)” into designated waste water containers (20 L plastic jerry cans with screw lids - to be used for no other purpose). Use a funnel to minimise risk of spillage, and carry out the baling procedure with the waste water containers sitting in a large tray. Water from all other bins (i.e. any not containing the treatment compound) should be poured away to the environment.

13. Repeat Steps 4 to 12 as required, depending on the number of fish caught.

14. Transport the sealed waste water containers to the designated waste water holding facility and decant to the designated 1000 L IBC storage tanks.

15. Leave the (first) site and proceed to the second site if appropriate.

Table 1.

Number of fish	**Volume of water (L)**	**Volume of treatment compound (ml)**
1-10	25	0.5
11-20	50	1.0
21-30	75	1.5
31-40	100	2.0

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