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Evidence Project Final Report

- **Note**
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Project identification

1. Defra Project code: FC1183
2. Project title: Susceptibility of English and Welsh salmonids to *Gyrodactylus salaris* (Platyhelminthes, Monogenea)
3. Contractor organisation(s): University of Stirling
4. Total Defra project costs (agreed fixed price): £ 89,836
5. Project: start date 01/10/2008
   end date 29/06/2012

6. It is Defra's intention to publish this form.

   Please confirm your agreement to do so..... **YES X NO** ☐

   (a) When preparing Evidence Project Final Reports contractors should bear in mind that Defra 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.

   Defra 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 In these cases, such information should be detailed in a separate annex (not to be published) so that the Evidence Project Final Report can be placed in the public domain.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 Defra expect contractors to give a "No" answer.

   In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

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

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Context of the project

Of the 430+ extant species of *Gyrodactylus*, small ectoparasitic monogenetic flukes of aquatic organisms, *Gyrodactylus salaris* is arguably the most infamous. Following the introduction of this species into Norway in the 1970s with consignments of infected salmon smolts, it has had a devastating impact on the Norwegian Atlantic salmon population decimating stocks in over 40 rivers. *Gyrodactylus salaris* is the only OIE (Office International des Epizooties) listed metazoan pathogen of fish and has been reported from 19 countries across Europe. The UK and Ireland are currently recognised as *G. salaris*-free, however, the threat that this notifiable parasite poses to each country's salmon industry is of national concern. Current British contingency plans are based on the assumption that if *G. salaris* were to be introduced, the parasite would follow similar dynamics to those on salmonid stocks from across Scandinavia, *i.e.* that Atlantic strains of Atlantic salmon, *Salmo salar*, would be highly susceptible to infection, with mortalities resulting; that brown trout, *Salmo trutta*, would be resistant and would lose their infection in a relatively short period of time; and, that grayling, *Thymallus thymallus*, would also be resistant to infection but would carry parasites, at a low level, for up to 143 days.

The aim of project FC1183

The current study set out to investigate the relative susceptibility of British salmonids to *G. salaris*, to determine whether they would follow a similar pattern of infection to their Scandinavian counterparts or whether, given their isolation since the last glaciation and potential genetic differences, would exhibit different responses. The work aimed to provide information to ensure that current contingency plans with regard to *G. salaris* in England and Wales are appropriate and robust.

Brief description of the work

All objectives were met during the project, on time as agreed with Defra.

Objective 1. To assess the susceptibility of English and Welsh salmonids to *G. salaris*.

To investigate this, *S. salar* from the Welsh River Dee, *S. trutta* from the River Tyne and *T. thymallus* from the River Nidd, raised from wild stock in Government hatcheries, were flown to a secure facility in

Norway where they were subsequently challenged with *G. salaris* (Gs). After acclimation, each fish was infected with 50 Gs (haplotype A). The dynamics on individual fish were followed against a control (Lierelva Atlantic salmon). The experiment found that the *G. salaris* on *S. salar* from the River Dee continued to rise exponentially to ~3867 Gs/fish (day 40 post-infection). These salmon were highly susceptible, more so than the Norwegian salmon control fish (~1989 Gs/fish d40 post-infection) and were unable to regulate parasite numbers. The *S. trutta* and *T. thymallus* populations, although initially susceptible, were able to mount a response after 12 (~146 Gs/fish) and 19 (~253 Gs/fish) days respectively and reduce their parasite burdens. Although the latter two hosts were able to limit their *G. salaris* numbers, both hosts carried infections for up to 110 days (*i.e.* when the experiment was terminated). The ability of *S. trutta* and *T. thymallus* to carry an infection for long periods increases the window of exposure and the potential transfer of *G. salaris* to other susceptible hosts.

Objective 2. Investigate the population growth of *G. salaris* under field and laboratory conditions.
Current contingency plans, however, are based on an understanding of *G. salaris* population dynamics largely derived from a single experimental protocol carried out using a common domestic water supply in Oslo. It is known though that water composition can affect the course of gyrodactylid infection. To explore this, the water chemistry at *Gyrodactylus* positive sites within England Wales were investigated, which found that pH is an important risk factor in their establishment. Given the importance of pH, the influence of different tannins and humic substances affecting water pH on the survival of gyrodactylids was investigated. This natural organic material is produced from decaying vegetation and their concentration as dissolved organic carbon (DOC) in river systems can vary markedly. The effect of one compound, tannic acid, on the survival of *Gyrodactylus* was studied. The trial found that the 1 hour LC50 was <100 ppm tannic acid although lower doses may have impacts on the parasite population.

Objective 3. To conduct further *G. salaris*-based experimentation in support of UK contingency planning.
Two sets of experiments were conducted under this object: 1) to explore alternative means of controlling *G. salaris* using chemicals; and, 2) to explore the *G. salaris* infection process on Atlantic salmon using microarray.

Gyrodactylus salaris infections in Norway are currently managed through the use of the biocide rotenone and / or aluminium sulphate. There are, however, human health concerns (*i.e.* Parkinson's and Alzheimer's) regarding the use of both compounds and so an alternative treatment agent is sought. Trials with bronopol (2-bromo-2-nitropropane-1,3-diol) a broad-spectrum disinfectant were conducted. The LC50 for *G. salaris* was determined to ~50 ppm bronopol for 4 h whilst the LC50 for *G. arcuatus*, a species found on 3-spine sticklebacks, was considerably higher at ~375 ppm for 5 h. Although while these results demonstrate that bronopol could be used to control infections of *G. salaris* in confined aquaria, this does not mean that this advocates its use in river systems as there are a plethora of logistic, economic and environmental considerations to consider. This does, however, take important steps towards investigating alternative control agents for use in the event of an outbreak.

The microarray trial set out to determine which host genes and processes are being up or down regulated at four time points (0, 7, 14 and 28 days post-infection) during a *G. salaris* infection. Identifying these processes may provide critical insights into the host-parasite interaction, how the host responds throughout the infection and how *G. salaris* possibly circumvents its host's responses. The experimental work has finished and while it is evident that a number of systems are being up and down regulated, the data are complex require some interpretation. By examining these we hope to gain a clearer understanding of the host-parasite interaction, and use the information to develop novel control measures to either protect the host or reduce the impact that this pathogen has, thereby contributing to national contingency plans and management strategies in the event of its introduction into the country.

Impact statement
The study provides critical data on the relative susceptibility of British salmonids to *G. salaris.* These findings suggest national contingency plans require modifying to account for prolonged infection by *G. salaris* on brown trout, which could cause infections to persist in areas where current contingency plan would not expect them to.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the 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 Exchange).

Please see the 20 page attached report.

References to published material

9. 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.

Two publications incorporating aspects of the work done under FC1183 were published during the tenure of the grant. These are:

Paladini, G., Gustinelli, A., Fioravanti, M.L., Hansen, H. & Shinn, A.P. (2009) The first report of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes, Monogenea) on Italian cultured stocks of rainbow trout (*Oncorhynchus mykiss*). *Veterinary Parasitology,* **165**, 290-297.

Shinn, A.P., Bron, J.E., Collins, C., García-Vásquez, A., Snow, M., Paladini, G., Lindenstrøm, T., Longshaw, M., Matějusová, I., Stone, D.M., Turnbull, J.F., Picon-Camacho, S., Vázquez Rivera, C., Duguid, R.A., Mo, T.A., Hansen, H., Olstad, K., Cable, J., Harris, P.D., Kerr, R., Graham, D., Buchmann, K., Raynard, R. & Irving, S. (2010) Multi-centre testing and validation of current protocols for *Gyrodactylus salaris* (Monogenea) identification. *International Journal of Parasitology*. **40**, 1455-1467.

In addition, 5 manuscripts are in preparation. Two of these cover major findings of the work *i.e.* the susceptibility of English and Welsh salmonids to *G. salaris* (1), and the microarray analysis (2). In addition, one other major manuscript has evolved from the work which looks at the geographic distribution and impact of *G. salaris* across Europe. The two other papers in preparation cover, (1) bronopol treatment of *G. salaris*, and, (2) alternative *G. salaris* treatments including tannic acid.