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Demonstrating freedom from *Gyrodactylus salaris* (Monogenea: Gyrodactylidae) in farmed rainbow trout *Oncorhynchus mykiss*

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ABSTRACT: This paper describes an approach to demonstrate freedom of individual rainbow trout farms from *Gyrodactylus salaris* Malmberg, 1957. The infection status of individual farms is relevant should *G. salaris* be introduced into a country or zone previously known to be free of the parasite. Trade from farms where *G. salaris* may have been introduced would be restricted until freedom had been demonstrated. Cage, fish and parasite sample sizes were calculated based on the minimum detectable prevalence (P*), test characteristics, population size, and Type I and II errors. Between 5 and 23 cages per farm would need to be sampled to demonstrate freedom at a cage level P* of 10%. The number of fish sampled per cage depended mainly on the test sensitivity (probability of correctly identifying an infected fish). Assuming a test sensitivity of 99% at the fish level, 59 fish per cage are needed (P* = 5%). Since *G. salaris* may exist in mixed infection with *G. derjavini*, testing a sample of gyrodactylid parasites may not result in the parasite being detected when present. Test sensitivity at the fish level depends on the number of gyrodactylids on the fish, the proportion of which are *G. salaris* and the number examined. Assuming a P* of 5% (i.e. *G. salaris* are at least 5% of the gyrodactylid population), between 20 and 73 parasites per fish would need to be sampled (depending on abundance) to maintain the Type I error at 0.01 (thus a fish level test sensitivity of 99%). This work identifies the critical information, and further research, needed to assess freedom from *G. salaris* with a known level of confidence; this is essential to provide a sound scientific basis for decision-making about disease control measures.

KEY WORDS: *Gyrodactylus salaris* · Rainbow trout · Surveys · Disease freedom · Contingency planning

INTRODUCTION

Gyrodactylus salaris, a freshwater, monogenean ectoparasite, is one of a number of gyrodactylids that infect salmonids. The parasite has a short, direct life cycle, produces live young and is highly fecund (Jansen & Bakke 1991, Harris et al. 1994). It was first described on Atlantic salmon *Salmo salar* L. in Sweden, where it did not appear to cause disease (Malmberg & Malmberg 1993). However, the pathogenicity of *G. salaris* on some strains of Atlantic salmon became clear in the early 1970s, when the parasite was introduced into Norway with juvenile Atlantic salmon imported from Sweden for aquaculture. The parasite spread to a number of wild salmon populations where it caused high levels of mortality in wild juveniles resulting in severe population decline (>90% over 5 yr) (Johnsen & Jensen 1991). Experimental investigations confirmed that strains of Atlantic salmon in Norway (Atlantic strains of Atlantic salmon) are considerably more susceptible to *G. salaris*, compared with the Baltic strain of Atlantic salmon from the river Neva (Bakke et al. 1990a); subsequently, Dalgaard et al. (2004) demonstrated variability in susceptibility between Baltic strains. *G. salaris* is able to live on a range of other species for 7 to 50 d (Bakke & Sharp

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1990, Bakke et al. 1990b, 1992a,b, Jansen & Bakke 1995, Soleng & Bakke 2001) and indefinitely in rainbow trout *Oncorhynchus mykiss* (RBT) populations (albeit at a low prevalence, low abundance and causing no signs of infection) (Bakke et al. 1991). *G. salaris* has been found on farmed RBT in Denmark, Sweden and Finland (see Bakke et al. 2007 for details). A RBT-adapted strain of *G. salaris* which is not pathogenic for salmon and can achieve infection rates of several hundred parasites per fish in RBT has been found in Denmark (Jørgensen et al. 2007). Recently, a strain of *G. salaris* has been found on Arctic charr, which has not affected Atlantic salmon in the same river. Under experimental conditions, salmon are found to be either resistant or only slightly susceptible (Olstad et al. 2007); this is the first published report of a variation in the pathogenicity of Norwegian populations of *G. salaris*.

A limited amount of experimental work has demonstrated that Atlantic salmon in the British Isles are susceptible to *Gyrodactylus salaris* (Bakke & Mackenzie 1993). Other populations in Europe are also highly likely to be at risk. Thus, the introduction of *G. salaris* to regions such as the British Isles might have adverse ecological and economic consequences. Recreational salmon fishing is an important source of revenue for many rural communities and the loss of salmon from an affected river may have severe localised socio-economic consequences. The ecological impact of the disappearance from a river of a species such as the Atlantic salmon is unpredictable, but potentially far-reaching. The parasite is easily controlled in farmed salmon through formalin baths, and experience in Norway has not shown the disease to be of economic significance in farmed populations. Controlling the disease in wild populations is considerably more problematic. Chemical destruction of all fish life within a river catchment is the only widely used method of control. *G. salaris* has been eradicated from 15 rivers in Norway by eliminating all fish using rotenone (a piscicide), thereby depriving the parasite of its host (survival off the host is limited to a few days) (Bakke et al. 2007). Destruction of all fish and invertebrate life forms from a river system is a drastic course of action and alternatives have been sought, including the use of aluminium sulphate (Poleo et al. 2004) which has recently been used in large scale field trials (Bakke et al. 2007). The potentially severe impact on wild salmon, a species in decline and under threat over much of its range, and the lack of environmentally acceptable control methods, make *G. salaris* probably the most important disease threat to susceptible wild salmon populations in Europe.

The UK foot-and-mouth disease epidemic of 2001 highlighted the importance of effective contingency plans for the control of exotic animal disease outbreaks (Anderson 2002). In countries such as the UK, where salmon or RBT farming is widespread, the parasite may spread with the movement of live fish (for stocking rivers, or juveniles for grow-out) before its detection (Peeler et al. 2004). Infection in RBT may be particularly important since the parasite causes few if any clinical signs and, therefore, detection may not be rapid. Following first detection of the parasite, it is crucial that the geographic distribution of the parasite is mapped so that control measures to minimise transmission can be applied to infected farms, and to allow trade in live fish to continue from parasite-free rivers. Surveillance to demonstrate freedom is, therefore, a critical element of a contingency plan. Arguably, surveys must generate a high level of confidence (>95%) that the parasite is absent (from a farm or river), because failure to detect the parasite, when present, may lead to further spread of the parasite to uninfected wild Atlantic salmon populations. Identifying *Gyrodactylus salaris* sensu stricto (i.e. the strains infecting Atlantic salmon) in farmed RBT presents a number of challenges. In this paper, we develop a survey design to demonstrate freedom from *G. salaris* at the level of a RBT farm. It was clear from the outset that much of the data required for sample size calculations were not available. Therefore, we present estimates of required sample sizes under a range of assumptions, and for different methods for identification. This work was undertaken to clearly define the key data requirements, future research, and policy decisions required to develop the farmed trout surveillance element of contingency plans for the control of *G. salaris*.

MATERIALS AND METHODS

Overview of the methodology. ***Statistical basis for demonstrating freedom:*** Surveys can only substantiate, with a certain probability, that a parasite is not present above a specified prevalence (P^*). The exact hypergeometric probability formula developed by Cameron & Baldock (1998a), that uses test characteristics (sensitivity and specificity) in the calculation of sample sizes to substantiate disease freedom, was employed. The formula has been implemented in a software programme (FreeCalc) which was used for sample size calculations (Cameron & Baldock 1998a,b).

This paper is concerned with establishing whether *Gyrodactylus salaris* is present among the population of gryodactylids living on a population of farmed rainbow trout. Thus at the parasite level, surveillance is intended to detect whether a gyrodactylid belongs to the species *G. salaris*. The target population is, therefore the population of all gyrodactylids of any species.

Gyrodactylids are unlikely to be homogenously distributed amongst their hosts; they are more likely to be over-dispersed (Scott & Anderson 1984). On a farm, RBT are kept in discrete populations in cages, ponds, raceways, tanks or other types of holding unit (which will be referred to collectively as cages). A 3-stage sampling strategy was therefore chosen, so that different P^* could be used at the level of cage, fish and parasite in the calculation of sample sizes (Cameron 2002).

Sensitivity is defined as the probability that a test correctly identifies an infected farm, cage or fish as infected and specificity is the probability that a test correctly identifies an uninfected farm, cage or fish as not infected (Dohoo et al. 2003). At the farm level the test consists of testing a sample of cages, at the cage level the test consists of examining a sample of fish and at the fish level the test consists of examining a sample of parasites. At the level of parasite, sensitivity is the probability that a *Gyrodactylus salaris* parasite is correctly identified, and the specificity is that other species of gyrodactylid parasite are not identified as *G. salaris*.

Firstly, the cage sample size was calculated for different sized farms (number of cages) and a range of P^* (at cage level). Secondly, the fish sample size per cage was calculated assuming an infinite population size and for a range of test sensitivities (see next section for details) and different P^* (at fish level). Thirdly, at the parasite level, sample sizes were calculated for (1) a range of Type I errors, corresponding to the sensitivity of the fish level test (= 1 – Type I error at the parasite level), (2) 3 values of P^* (parasite level) and (3) selected abundances (gyrodactylid parasites per fish) in the range from 20 to 200.

The approach taken reflects the lack of information about the minimum likely prevalence of *G. salaris* at cage, fish and parasite level.

Parameters: The convention established by Cameron & Baldock (1998a), that the null hypothesis is defined as the presence of the parasite (at or above the P^*) and the alternative hypothesis is that the parasite is absent (or below the P^*) was adopted.

The following parameters are required at each sampling stage to substantiate disease freedom (Cameron 2002): (1) population size, (2) P^*, (3) Type I error, i.e. probability that the survey incorrectly indicates the population is not infected (confidence = 1 – Type I error), (4) Type II error, i.e. probability that a population will be found to be infected when it is not (power = 1 – Type II error), (5) test sensitivity and (6) test specificity. The first-stage test sensitivity = 1 – Type I error used in second-stage sampling, and the first-stage test specificity = 1 – Type II error used in second-stage sampling (Cameron & Baldock 1998b, Cameron 2002). Similarly, stage 2 sensitivity and specificity is determined by Type I and II errors used when sampling parasites (stage 3 units). The test used to identify an individual gyrodactylid parasite as *Gyrodactylus salaris* or another species was assumed to have a sensitivity and specificity of 100% (see section on parasite sampling). Assuming a perfect test at the parasite level is used, a fish or cage may be incorrectly found to be free of *G. salaris* if only a sample of parasites and fish are examined. However, incorrectly finding the fish or cage to be infected with *G. salaris* is not possible (Type II error = 0 at all stages of sampling). The Type I error was set at 0.05 at the cage level (Table 1), and the fish sample size required to generate a Type I error of 0.05 (i.e. 95% confidence) was calculated. At the fish level, a range of sensitivities was used (50 to 99%) because the Type I error at the parasite level for an estimated P^* for *G. salaris* (the likely minimum proportion of gyrodactylids represented by *G. salaris*), depends on the number of parasites sampled per fish and the number of parasites present on the fish.

The steps in designing a 3 stage survey to substantiate freedom from *G. salaris* in a RBT farm, when a perfect test is used at the parasite level, can be summarised as follows:

(1) Define and identify first-stage (cages) and second stage (fish) assess population sizes and draw up sampling frame.

(2) Decide which method of parasite sampling is to be used at the fish-level (e.g. whole body examination or fin clipping).

(3) Determine P^* (based on minimum expected prevalence) for first-stage units (cages), second-stage units (fish) and third-stage units (parasites).

(4) Specify Type I error for first-stage unit (acceptable probability that population will be found not to be infected when it is; usually ≤5%).

(5) Specify Type I error for second- and third-stage units.

(6) Calculate sensitivity of test for second-stage units (= 1 – Type I error used at third-stage sampling).

(7) Calculate sensitivity of test for first-stage units (= 1 – Type I error used at second-stage sampling).

Table 1. Test for *Gyrodactylus salaris* infection in *Oncorhynchus mykiss*. Test characteristics and Type I and II errors used in sample size calculations. Null hypothesis is that the parasite is at greater than the minimum detectable level

Sampling unit	Sensitivity (%)	Specificity (%)	Type I error	Type II error
1. Cage	95	100	0.05	0.00
2. Fish	50–99	100	0.05	0.00
3. Parasite	100	100	0.01–0.50	0.00

Table 2. Factors influencing the likelihood of detection (= test sensitivity) of *Gyrodactylus salaris* on rainbow trout *Oncorhynchus mykiss*

Factor	Description
Abundance (number of parasites per fish)	
Time of year	Abundance is higher during summer months
Time since last treatment for ectoparasites	Frequency of treatment will be higher in summer months when abundance is high
Loss of parasites due to sampling and processing	
Catching method	Crowding (e.g. with seine net) will result in abrasion and parasite loss (compared with using a hand net)
Time between capture and sampling	Keeping fish in a bucket or keep net before sampling will result in parasite loss due to abrasion and parasites leaving stressed fish
Euthanasia method	An anaesthetic bath may result in greater parasite loss compared to a blow on the head, depending on bath duration and density of fish in bath
Preservation method for dead fish/clipped fins	Ethanolic preservation results in a surface film of white slime that may obscure the gyrodactylids (NB samples kept in water should be examined within 72 h)
Subsample of total parasite burden examined	
Part of fish examined for parasites	When abundance is low, the probability of finding any gyrodactylids will depend on which parts of the fish are examined: (1) whole fish, (2) all fins, or (3) selected fins only (e.g. pectorals). In mixed infections, the probability of finding a *G. salaris* gyrodactylid will depend on which part of the fish is examined and the microhabitat selection of the parasite (which may vary with duration of infection)
Percentage of *G. salaris* in the gyrodactylid population	In mixed gyrodactylid infections, the sensitivity will be positively associated with the proportion of *G. salaris*
Sub-sample of identified parasites examined	In mixed gyrodactylid infections, the probability of detecting at least one *G. salaris* parasite will increase with the number of parasites examined
Diagnostic test	
PCR vs. morphometric approaches	PCR will distinguish *G. salaris* from *G. derjavini* with a very high level of probability (close to 100%)
	Sensitivity of morphometric methods depends on the skill of the parasitologist but can approach 100% in separating *G. salaris* from *G. derjavini*

(8) Calculate sample sizes for stages 1 (cages), 2 (fish) and 3 (parasites).

(9) Estimate costs of sampling and processing a fish compared with sampling a cage and determine the most cost effective combination of stage 1, 2 and 3 sample sizes through iteration.

Stage 1 sampling (cage). Rimaila-Parnanen & Wiklund (1987) and Rintamaeki-Kinnunen & Valtonen (1996) have reported the farm-level prevalence of *Gyrodactylus salaris* in Scandinavia. However, no published data exist on which to base an estimate of the minimum likely cage level prevalence (P* cage). *G. salaris* is likely to spread with the flow of water between cages; therefore, cage prevalence will be positively associated with the duration of infection and with time from last treatment for ectoparasites. Given the lack of available data, a conservative estimate of P* is needed (e.g. 10%). Sample sizes were calculated for a range of P* (5 to 75%) and for numbers of cages from 5 to 50.

Stage 2 sampling (fish). No data is available with which to estimate the minimum prevalence of *Gyrodactylus salaris* infection at the fish level (P* fish), but anecdotal evidence exists from Norway that *G. salaris* can exist on RBT at a low prevalence (<5%) (T. A. Mo pers. comm.). Prevalence is likely to be positively associated with time from introduction and water temperature. Frequent treatments for ectoparasites (more frequent in summer) will decrease prevalence. The test for *G. salaris* at the fish level is the examination of all or a sample of parasites found. If all the gyrodactylid parasites on a fish are examined using a perfect test, the fish level test sensitivity is 100%. Examining only a sample of parasites from fish with mixed infections (i.e. with *G. salaris* and *G. derjavini*) decreases the fish level test sensitivity. Sample sizes, assuming an infinite population, for P* fish ranging from 1 to 20% and test sensitivities from 50 to 99% were calculated.

Stage 3 sampling (parasite). If a large number of gyrodactylids are present on a fish, examination of a sample of parasites may be considered. A wide range of factors affects abundance and the likelihood of finding parasites on an infected fish (Table 2). The micro-

Table 3. Test for *Gyrodactylus salaris* infection in *Oncorhynchus mykiss*. Stage 1 (cage) required sample sizes (i.e. no. of cages). Assumptions: test specificity = 100%, test sensitivity = 95% (determined by number of fish sampled), Type I error = 0.05, Type II error = 0

No. of cages identified	Minimum cage-level prevalence (P*)				
	5	10	25	50	75
5[a]			5	4	2
10[b]		10	8	4	2
20	20	16	9	5	3
30	25	20	9	5	3
40	33	22	10	5	3
50	41	23	11	5	3

[a]If only 5 cages are identified, all need to be sampled if P* ≤ 30%
[b]If only 10 cages are identified, all need to be sampled if P* ≤ 14%

habitat selection of *Gyrodactylus salaris* on RBT has been investigated (Heinecke & Buchmann 2006, Jørgensen et al. 2007). The *G. salaris* sensu stricto found in Baltic countries has a preference for the fins, whilst the Danish strain of *G. salaris* (adapted to RBT) is more likely to be found on the body of the fish, but the distribution may vary with time of infection (Heinecke & Buchmann 2006, Jørgensen et al. 2007). The microhabitat selection of *G. salaris* on Atlantic salmon may vary with abundance and with the presence of other gyrodactylid parasites. When less than 100 *G. salaris* parasites were present they were located mainly on the dorsal fin, followed by the pectoral and anal fins; as the parasite abundance increased, more were found on the caudal fin, and as the abundance reached 1000 the body became infected (Jensen & Johnsen 1992). Buchmann & Uldal (1997) found that under experimental conditions, *G. derjavini* infections on RBT were found predominately on the pectoral, caudal, pelvic and anal fins. As the population increased, peaking after 3 to 4 wk, the caudal fin became most heavily infected (Buchmann & Uldal 1997). A study of gyrodactylid parasites (*G. derjavini* and the RBT-adapted *G. salaris*) on Danish RBT farms found 100% fish level prevalence in 9 of 11 farms (10 to 15 fish examined per farm) (Nielsen & Buchmann 2001). Abundance varied from 1 to over 1500 parasites per fish, with a mean of 152 on infected fish (the distribution was overdispersed) (Nielsen & Buchmann 2001).

The number of parasites that need to be examined in order to be confident that *Gyrodactylus salaris* is not present will depend on the minimum prevalence of *G. salaris* in mixed infections (i.e. the proportion of all gyrodactylids that are *G. salaris* – P* parasite), and the total number of parasites on the fish. Nielsen & Buchmann (2001) found that on farmed RBT in Denmark, *G. salaris* was always found in mixed infections with *G. derjavini*, which predominated (77% of the total). For 3 values of P*, the sample size was calculated for a range of population sizes (total number of gyrodactylids found on the fish) and Type I errors (which determine the fish level sensitivities used in stage 2). Morphological and molecular approaches have been developed to identify *G. salaris* (reviewed by Cunningham 2002, Bakke et al. 2007), and thus discriminate it from other gyrodactylid species, and both are recognised by the OIE (OIE 2006). When used properly, both approaches have extremely high specificity and sensitivity. For the purposes of this paper, sample size calculations assumed a perfect test (100% sensitivity and specificity).

RESULTS

Sample size calculation

Stage 1 (cage level). The sample sizes are given in Table 3 for a range of numbers of cages and P*. Assuming a cage-level prevalence of 10%, the number of cages to be sampled varies from 5 to 23 depending on the number of cages (e.g. 5 to 50).

Stage 2 (individual fish). The number of fish that need to be sampled from each cage, for a range of test sensitivities and P*, to generate 95% confidence that the parasite will be detected if present, are given in Table 4. For the purposes of comparison, fish level sensitivity (i.e. probability that an infected fish will be found to be infected) of pectoral fin clipping (only examining parasites found on the pectoral fins) and whole body examination (examining all parasites present) are estimated at 50 and 99% (some parasites might be lost during handling or not detected on examination), respectively. Using these estimates and a P*

Table 4. Test for *Gyrodactylus salaris* infection in *Oncorhynchus mykiss*. Stage 2 (fish) required sample sizes (i.e. no. of fish) per cage to achieve 95% test sensitivity at the cage level for different fish level test sensitivities and design prevalences (P*). Assumptions: test specificity = 100%, population size > 10 000, Type I error = 0.05, Type II error = 0

Test sensitivity	Within-cage *G. salaris* design prevalence (P*)					
	1	2	3	5	10	20
50	598	298	199	119	59	29
60	498	248	165	99	49	24
70	427	213	142	85	42	20
80	373	186	124	74	36	18
90	332	165	110	66	32	16
99	301	150	100	59	29	14

Table 5. Test for *Gyrodactylus salaris* infection in *Oncorhynchus mykiss*. Stage 3 (parasite) required sample sizes (i.e. no. of gyrodactylids) to detect *G. salaris* in mixed gyrodactylid infections, for 3 *G. salaris* design prevalences (P*) and a range of Type I errors. Assumptions: test specificity and sensitivity = 100%, Type II error = 0

P* (%)	Type I error	Abundance (gyrodactylid parasites per fish)			
		20	50	100	200
5	0.01	20	45	59	73
	0.05	19	39	45	51
	0.1	18	34	37	41
	0.2	16	26	27	30
	0.3	14	20	21	23
	0.4	12	16	17	18
	0.5	10	12	13	14
10	0.01	18	29	36	40
	0.05	16	22	25	27
	0.1	14	18	20	21
	0.2	11	14	15	15
	0.3	9	11	11	12
	0.4	8	9	9	9
	0.5	6	7	7	7
20	0.01	13	17	19	20
	0.05	10	12	13	13
	0.1	9	10	10	11
	0.2	7	7	7	8
	0.3	5	5	5	6
	0.4	4	4	4	5
	0.5	3	3	3	4

of 5% and Type I error of 5%, 119 fish per unit need to be sampled for fin clipping and 59 if whole body examination was applied. Assuming 5 cages are sampled, a total number of fish to be examined will be 595 and 295, using fin clipping or whole body examination, respectively. If RBT are infected with *Gyrodactylus derjavini*, a large number of gyrodactylid specimens may be found.

Stage 3 (parasite). The number of gryodacytlid parasites that need to be sampled from each fish, for 3 values of *Gyrodactylus salaris* P* and a range of Type I errors, are given in Table 5. Assuming a P* of 5%, between 20 and 73 parasites per fish would need to be sampled (depending on abundance) to maintain the Type I error at 0.01 (thus generating a fish level test sensitivity of 99%). If Type I error is relaxed to 50% (corresponding to a test sensitivity of 50% at fish level) between 10 and 14 parasites need to be sampled (P* = 5%) (Table 5).

Number of parasites to be examined

Sampling 59 fish from 5 cages produces a fish sample size of 295. On average 50 parasites per fish might need to be examined giving a maximum total of 14 750 gryodactylids to be examined per farm. By comparison, if 119 fish are sampled from each of 5 cages and 12 parasites sampled per fish, a total of 7140 parasites need to be examined per farm.

DISCUSSION

Demonstrating freedom from the strain of *Gyrodactylus salaris* pathogenic to Atlantic salmon in farmed RBT presents serious challenges. In the event that the parasite is discovered in a region previously free of the parasite, its distribution must be mapped so that control measures can focus on protecting free areas and minimising spread from infected areas. A wide-scale survey of wild and managed (farm or fishery) salmonid populations will be needed to establish the distribution of the parasite. Where it occurs, Arctic charr *Salvelinus alpinus* would need to be considered in surveillance plans, since it is a potential host for *G. salaris* (Bakke et al. 1996, Robertsen et al. 2007). Plans to undertake surveys to demonstrate freedom are, therefore, a critical element of outbreak contingency plans. In this paper we have discussed approaches to demonstrating freedom from *G. salaris* sensu stricto in a RBT farm and identified the data that will be needed to calculate sample sizes and assessed how parameter estimates influence sample size calculations. The same approach and calculations could be used for surveys designed to generate evidence for international recognition of disease freedom (OIE 2006). Similarly the approach can be extended to determining freedom from other pathogens and parasites of farmed fish.

Methodology

The first methods for sample size calculation to substantiate freedom assumed the use of a perfect test, an infinite population and calculated the sample size to detect at least one infected individual (Cannon & Roe 1982). A new probability formula for surveys to demonstrate freedom from disease was developed by Cameron & Baldock (1998a) and used in this paper. Their approach introduced the concept of a minimum detectable level of disease, and used estimates of test characteristics and sample sizes for finite populations, and was adopted for this paper.

In the absence of an internationally agreed design prevalence for *Gyrodactylus salaris*, and given the lack of data on which to select design prevalences, it was decided to assess sample sizes for a range of design prevalences (in part to illustrate the importance of the

design prevalence in determining sample sizes). Once better data on which to estimate sensitivity are available, a Bayesian approach using a prior distribution for estimates of test sensitivity and specificity could be employed (Johnson et al. 2004).

Scenario tree modelling (Martin et al. 2007a,b) provides an alternative approach to developing surveillance schemes. It allows the calculation of the confidence level of a surveillance scheme using a risk based sampling approach. Risk factors, such as those presented in Table 4, could be used to identify cages or fish at greatest risk of being infected and the overall sample size may be reduced while maintaining the required confidence level. The method has been used to demonstrate disease freedom at a national level using a range of data sources (Martin et al. 2007b).

Identifying study cages and fish

Surveillance designed to demonstrate freedom, and not estimate prevalence, can use high risk (i.e. those most likely to be infected) units (e.g. cages and fish) as the study population. The prevalence of infection in the study population is fundamental in determining sample sizes. Thus, estimates are needed of the distribution of *Gyrodactylus salaris* sensu stricto in an infected farm, to establish how the prevalence of infection varies with fish age (or size) and position in farm (i.e. degree of water use), but these data are not currently available. Criteria that could be used to identify cages more likely to be infected include those which hold fish sourced from an infected farm, or which have not been treated recently for external parasites (e.g. by formalin baths). However, in many cases it may not be possible identify high risk cages. At the fish level, experience of other fish ectoparasites indicates that poorly thriving fish (i.e. dark in colour, gathered at outlet) are more likely to carry high parasite loads (though this has not been established for *G. salaris* and merits investigation).

Parasite sampling

The main gyrodactylid species found on RBT in Europe are *Gyrodactylus derjavini, G. truttae, G. salaris* and *G. teuchis* (Cunningham et al. 2001, reviewed by Bakke et al. 2007). The presence of gyrodactylid species other than *G. salaris* adds considerable complexity to the detection of the parasite. Essentially a third level of sampling is needed. The main factor determining whether a *G. salaris* parasite will be detected is the abundance of all gyrodactylid parasites, the proportion represented by *G. salaris* and the number examined. *G. salaris* can exist on RBT in very low numbers and single parasite infections have been noted (OIE 2003). Currently, no data are available for mixed infections of *G. salaris* sensu stricto with other gyrodactylid species. *G. salaris* is the only gyrodactylid species found in farmed RBT in Norway (T. A. Mo pers. comm.).

Whilst RBT exhibit a range of susceptibility to *Gyrodactylus salaris* sensu stricto, nearly all individually eliminate the parasite (Bakke et al. 1991) (though the parasite survives at a population level). It may be concluded that *G. salaris* sensu stricto will not compete effectively with *G. derjavini* in mixed infections, therefore a low P^* must be used in the absence of other evidence. Research is needed to establish the likely abundance and prevalence of *G. salaris* sensu stricto in mixed infections on RBT. Whole body examination, or inspection of fins, is recommended in the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2006) (the former is more time consuming). In single species infections, the current evidence suggests that *G. salaris* sensu stricto is found mainly on the fins (Heinecke & Buchmann 2006); however, the data on microhabitat selection in mixed infections, needed to evaluate the sensitivity of whole body versus fin examination, are not available.

The method of fin clipping has considerable logistical advantages over transporting whole fish to the laboratory. If fin clipping was used, the loss of sensitivity due to not sampling the remainder of the fish body can be compensated in 2 ways. If levels of gyrodactylid infection are high, the number of gyrodactylids investigated from the fins may be increased. If the number of gyrodactylids on the fins was low, additional fish may need to be sampled.

Diagnostic methods to identify *Gyrodactylus salaris*

The OIE recognises identification of *Gyrodactylus salaris* by morphology and morphometry of the attachment organ or DNA analysis as appropriate methods of diagnosis (OIE 2006). Identification of *G. salaris* has been thoroughly reviewed by Cunningham (2002) and Bakke et al. (2007). In this paper, a perfect test was assumed to be available for the diagnosis at the parasite level because both methods can achieve extremely high levels of sensitivity and specificity for gyrodactylid populations found on RBT (thus the choice about which method to use can be based on cost and processing time). Our key objective was to explore how factors other than method of parasite identification affected sample size calculations (e.g. parasite abundance and *G. salaris* prevalence).

Contingency planning

The European fish health directive (2006/88/EC) makes clear that Member States should 'ensure the necessary level of preparedness to effectively tackle … emergency situations related to … outbreaks of serious exotic … diseases'. Resource planning is critical to guaranteeing that contingency plans for disease outbreaks can be effectively implemented. Estimates of the number of samples that might need to be processed under the worst-case scenario for an outbreak are needed. In this paper estimates are based on the available data. The method used allows different combinations of cage, fish and parasite sample sizes that generate the same degree of confidence (to detect a farm as being infected or free from infection) to be calculated (Cameron & Baldock 1998b). Controlling an outbreak of *G. salaris* will be particularly problematic in countries with a geographically widespread RBT population in farms and fisheries, such as the UK. The prevalence and abundance of *Gyrodactylus derjavini* in RBT farms (currently not known) will largely determine the number of samples that need to be processed and the demands on diagnostic facilities when undertaking surveys to map the distribution of *G. salaris*. Based on this paper, guidelines for sampling and sample sizes can be devised. However, better estimates of prevalence and abundance of *G. derjavini* infections and the likely prevalence of *G. salaris* (within and between cages) are needed to develop sampling strategies with more accurate levels of confidence. The results of this work indicated that resource planning, based on the fin sampling, should allow for approximately 300 fish and 15 000 parasites to be sampled to establish the status of the individual farm.

In the event of a *Gyrodactylus salaris* introduction, a ban on all movements of susceptible species may be necessary to minimise the risk of spread. This would have serious economic implications since most RBT farms rely on either buying or selling live fish. It would therefore be essential to allow farms in parasite-free river catchments to recommence trading as soon as possible. Nevertheless, surveys to demonstrate freedom should generate a high level of confidence (>95%) that the parasite is absent, because failure to detect the parasite may lead to further spread. Control measures will operate at a river catchment level (i.e. if *G. salaris* is present the movement of fish from all farms on the catchment will be regulated). The level of confidence that a catchment is free of *G. salaris* can be determined by combining the results from surveys of all farmed, fishery and wild salmonid populations in the catchment (Cannon 2002). Ultimately, the required level of confidence that the parasite is absent before allowing movement of live fish is a political decision, which must weigh the costs of surveillance and movement restrictions against the consequences of incorrectly concluding a farm or river is free of *G. salaris*. Currently, lack of information about key parameter estimates means the confidence generated by a survey to establish freedom from *G. salaris* in farmed RBT cannot be estimated with any certainty.

It is open to question whether either morphology or the currently available molecular tools can be used to process, in an acceptable time frame, the large number of samples that are likely to be generated by a survey to establish the nationwide distribution. The future development of automated image analysis or molecular techniques will reduce processing time (which may be further decreased for molecular analysis by pooling samples). Nevertheless, alternatives should be considered and one option is the use of sentinel Atlantic salmon on RBT farms. Juvenile Atlantic salmon are highly susceptible to *Gyrodactylus salaris*, relatively less susceptible to *G. derjavini* (Buchmann et al. 2004), and are likely to become infected with high abundance over a period of days (Bakke et al. 1999). The logistical problems of sourcing juvenile Atlantic salmon at all times of the year and transporting them to farms will need to be considered. Placing Atlantic salmon in a potentially infected farm will increase infection pressure and, therefore, the risk of downstream spread. This approach will require validation as a method of identification.

CONCLUSION

In this paper we have attempted to develop the scientific basis for the design of surveys to substantiate freedom from *Gyrodactylus salaris* in a RBT farm—a vital element of emergency planning for exotic diseases. Estimates of sample sizes have been calculated based on the best available data. It is essential that surveys to substantiate freedom produce a result with a known degree of confidence. An assessment of the required sample sizes is critical to the resources that would be required to deal with the introduction of *G. salaris*. We have identified the information required to calculate sample sizes and confidence levels, much of which is currently unavailable. Research is needed to address these data gaps and thus improve the basis for the design of surveys. *G. salaris* can exist at low prevalences and at low abundances, and, therefore, the sample size required to generate an acceptable level of confidence of freedom will be large. In addition, the presence of *G. derjavini* may result in very large numbers of specimens to be analysed. The logistical problems and resources required to collect and process a sufficient number of samples to map the distribution of

G. salaris in a short period of time are considerable. Alternative approaches, such as sentinel Atlantic salmon, should be considered. Some aspects of disease control are political in so far as the competing interests of different groups (e.g. farmers and those representing the interests of wild fish) must be weighed. The transparent and science based approach to surveillance for freedom from *G. salaris* outlined in this paper provides the evidence base for policy decisions.

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