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An agent-based modelling approach to estimate error in gyrodactylid population growth

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ABSTRACT

Comparative studies of gyrodactylid monogeneans on different host species or strains rely upon the observation of growth on individual fish maintained within a common environment, summarised using maximum likelihood statistical approaches. Here we describe an agent-based model of gyrodactylid population growth, which we use to evaluate errors due to stochastic reproductive variation in such experimental studies. Parameters for the model use available fecundity and mortality data derived from previously published life tables of *Gyrodactylus salaris*, and use a new data set of fecundity and mortality statistics for this species on the Neva stock of Atlantic salmon, *Salmo salar*. Mortality data were analysed using a mark-recapture analysis software package, allowing maximum-likelihood estimation of daily survivorship and mortality. We consistently found that a constant age-specific mortality schedule was most appropriate for *G. salaris* in experimental datasets, with a daily survivorship of 0.84 at 13 °C. This, however, gave unrealistically low population growth rates when used as parameters in the model, and a schedule of constantly increasing mortality was chosen as the best compromise for the model. The model also predicted a realistic age structure for the simulated populations, with 0.32 of the population not yet having given birth for the first time (pre-first birth). The model demonstrated that the population growth rate can be a useful parameter for comparing gyrodactylid populations when these are larger than 20–30 individuals, but that stochastic error rendered the parameter unusable in smaller populations. It also showed that the declining parasite population growth rate typically observed during the course of *G. salaris* infections cannot be explained through stochastic error and must therefore have a biological basis. Finally, the study showed that most gyrodactylid-host studies of this type are too small to detect subtle differences in local adaptation of gyrodactylid monogeneans between fish stocks.

1. Introduction

Gyrodactylid monogeneans have been popular model organisms to test hypotheses of host–parasite population behaviour, since the earliest computer simulations of the dynamics of these interactions (Lester and Adams, 1974a,b; Scott and Anderson, 1984). Interest in the group has also focused on the epidemiology of *Gyrodactylus salaris*, responsible for a devastating epidemic of wild salmon parr in Norway (Bakke et al., 2007). According to the paradigm (e.g. Peeler et al., 2004), the parasite was introduced into Norway in the 1970s and is highly pathogenic to East Atlantic stocks of *Salmo salar* which have no prior evolutionary exposure to this parasite (Bakke et al., 1990), and therefore fail to mount an immune response. As such, this is considered to represent an example of local adaptation, whereby geographically differentiated populations of a host species evolve different responses to a pathogen which occurs in only part of the host range (see Kawecki and Ebert, 2004). A key question concerns the extent of genetic or potential immunological resistance to *G. salaris* in Norwegian salmon populations. Despite the paradigm that there is almost none (Gilbey et al., 2006). Salte et al. (2010) demonstrated clear, heritable genetic or immunological resistance in a native Norwegian salmon stock, which could be used as the nucleus for a resistant breeding program in the future.

Most 'local adaptation' studies on susceptibility or resistance to gyrodactylids follow individually isolated fish infected with one or a few parasites, all within a common experimental environment (Bakke and Mackenzie, 1993; van Oosterhout et al., 2003; de Roij et al., 2010; Raeymaekers et al., 2011). Originally attempted for *Gyrodactylus alexanderi* on sticklebacks (Lester and Adams, 1974a,b), this approach has been used for a wide range of fish – *Gyrodactylus* interactions (Scott, 1985; Scott and Nokes, 1985; Bakke et al., 1990; others cited in Bakke et al., 2007). In such experiments, parasite burdens are enumerated at appropriate time intervals and mean worm burdens and error structures estimated for each time point. These can then be compared, either with transformed count statistics (e.g. Winger et al., 2009), or using

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Generalised Linear Modelling (GLM) approaches (van Oosterhout et al., 2003).

Local adaptation studies require measurement of the genetic variance of traits related to pathogenicity/resistance in the host population, either through following individual quantitative genetic differences, or through differences in partitioned variance for the trait based on the whole test population. There are, however, fundamental problems in the calculation of such variances for gyrodactylid monogeneans. Due to the unique viviparous reproduction of gyrodactylids, successive time points within an infection are not independent of each other. Furthermore, infections typically begin with very small founding populations in which stochasticity can have a major impact. Equally, in many gyrodactylid-host infections, an initial growth phase is followed by a decline to a small population size, either due to host response (the normal explanation, see e.g. Lester and Adams, 1974a; Scott and Anderson, 1984; Bakke et al., 2004) or to a resource-mediated density-dependent population limitation. This results in fish infected with very small burdens at the end of the infection when, again, stochasticity in reproduction and growth processes comes to the fore. It is not then possible to compare the response of two groups of fish at a particular date during infection, because the size of the gyrodactylid population on that date is critically dependent on the performance of the parasite strain earlier in the infection. This violation of the assumptions of the statistical analysis is compounded by the asymmetry in gyrodactylid birth dates. When a gyrodactylid is born, it already contains a daughter in an advanced stage of development (Wagener, 1866; Bychowsky, 1957; Braun, 1966; Harris, 1985; Cable and Harris, 2002). After the birth of this daughter, an egg cell must enter the uterus and start cleavage to form the second-born daughter, a much longer process which results in the second and subsequent births having relatively long gestations compared with the first birth (Cable and Harris, 2002). Therefore, when infections are small, stochasticity in mortality can have a massive subsequent effect on the parasite population growth rate. The resulting time-dependent 'blurring' of estimates of gyrodactylid growth at different time points makes it difficult to recognise anything but the grossest examples of parasite maladaptation to particular host strains or species (e.g. Bakke et al., 1996). To overcome this difficulty, a notation of susceptible, partially resistant and innately resistant fish has been developed (Bakke et al., 2002; Gilbey et al., 2006). A similar approach was taken by Madhavi and Anderson (1985) in trying to establish the heritability of resistance to *Gyrodactylus turnbulli* infection in guppies.

An aim of gyrodactylid population studies has therefore been to separate stochastic variation due to the nature of gyrodactylid reproduction, from the more interesting variation in infection outcomes which may be due to genetic or immunological differences between hosts, or even to resource-mediated density-dependent limitation of parasite population growth. Unfortunately, the high reproductive stochasticity makes it difficult to separate these two sources of variation. Here we develop an agent-based simulation model of single infections, based on described gyrodactylid life histories, to examine the error structures of gyrodactylid infections due to reproductive and survivorship stochasticity alone. We use parameters in this model from data on observations of birth and death processes in *G. salaris*, and then test it against experimental data sets available for this species.

2. Materials and methods

2.1. The model

The model was written using NetLogo 4.1.2 (Wilensky, U., 1999. NetLogo. http://ccl.northwestern.edu/netlogo/. Center for Connected Learning and Computer-Based Modeling, Northwestern University, Evanston, IL, USA). It represents the simplest possible life history of a single gyrodactylid which is able to live, reproduce and die (Fig. 1). A full description of the model, conforming to the standard protocol for publication of agent-based models of Grimm et al. (2006), is given in Supplementary data S1. The parasite is tested at each time point within the simulation as to whether it lives or dies. A single time point is considered to represent 1.5 h in the life of *G. salaris* at 13 °C. If the parasite survives, after 30 time points (45 h) its status changes to 'gravid', and then at every subsequent time point it is tested for both survival and for giving birth. Having given birth for the first time, a further 100 time points (150 h or 6.25 'days') must elapse before it becomes 'gravid' again, and it is then again tested at every time point until birth takes place. After each birth 100 time points must again elapse before the change to 'gravid', and this process repeats itself until the parasite dies. This pattern of gyrodactylid life history is based on observations on *Gyrodactylus gasterostei* (Harris, 1985, 1998) and *G. salaris* (Jansen and Bakke, 1991; Harris et al., 1994; Cable et al., 2000). For the first birth, all individuals were constrained to have given birth by the 45th time interval (77 h, or 3.25 'days'), to avoid the long 'tail' in the distribution of births generated by the binomial test function. Other births did not need to be constrained in this way. Simulations of population growth using this model were run using NetLogo, and data on individual parasites (age, age at death, age at first and all subsequent births) and on population behaviour (total population size and time elapsed) were exported into Microsoft Excel spread sheets. The data were then analysed using R software version 2.9.0 (Crawley, 2007).

2.2. Experimental data

Previous data sets (Jansen and Bakke, 1991; Cable et al., 2000) were re-analysed for this work, but additional life table data for *G. salaris* were collected from individual infections of Neva strain salmon individually isolated and examined daily for births using the approach of Braun (1966) and Harris (1998). Naïve Neva strain fish with no history of *G. salaris* infection were obtained from the Ims salmon hatchery in Western Norway, and acclimated at 13 °C in the laboratory for 6 months before use. They were individually isolated in floating cages within a larger 200 L tank, such that water was able to circulate within the cages but the fish could not escape (see Cable et al., 2000 for methodology). The infection was initiated with a single *G. salaris* Lierelva strain placed on the caudal fin of a single anaesthetised (MS222 or chlorbutanol, see Cable et al., 2000) salmon. The fish was anaesthetised every day and examined using a stereomicroscope, and the position and state of development of the parasite noted. If the parasite was not found for two consecutive days, it was considered lost, and the fish was removed from the experiment. When the parasite gave birth, the daughter or mother was transferred to a novel fish. In this way, an expanding lineage of *G. salaris* was generated (see Harris, 1998), and data collected on the dates of each birth, and the date of disappearance of the worm.

The mark-recapture software, MARK (version 6.1; Cooch and White, 2011), was used to estimate the most plausible model of age-specific mortality using the data from experimental infections of Neva fish, and that of Cable et al. (2000). Age-specific mortality and survivorship schedules were calculated using the procedure RECAPTURES ONLY within MARK (White and Burnham, 1999; Cooch and White, 2011). Survival was modelled either with the probability of both sighting the parasite (p) and of survival (φ) held constant (p_{const}, Φ_{const}), or with the probability of survival allowed to vary in a time-dependent manner (p_{const}, Φ_{t}). Mortality models obtained in this way were compared using Aikake Information Criteria (AIC) as set out in the MARK manual (Cooch and White, 2011).

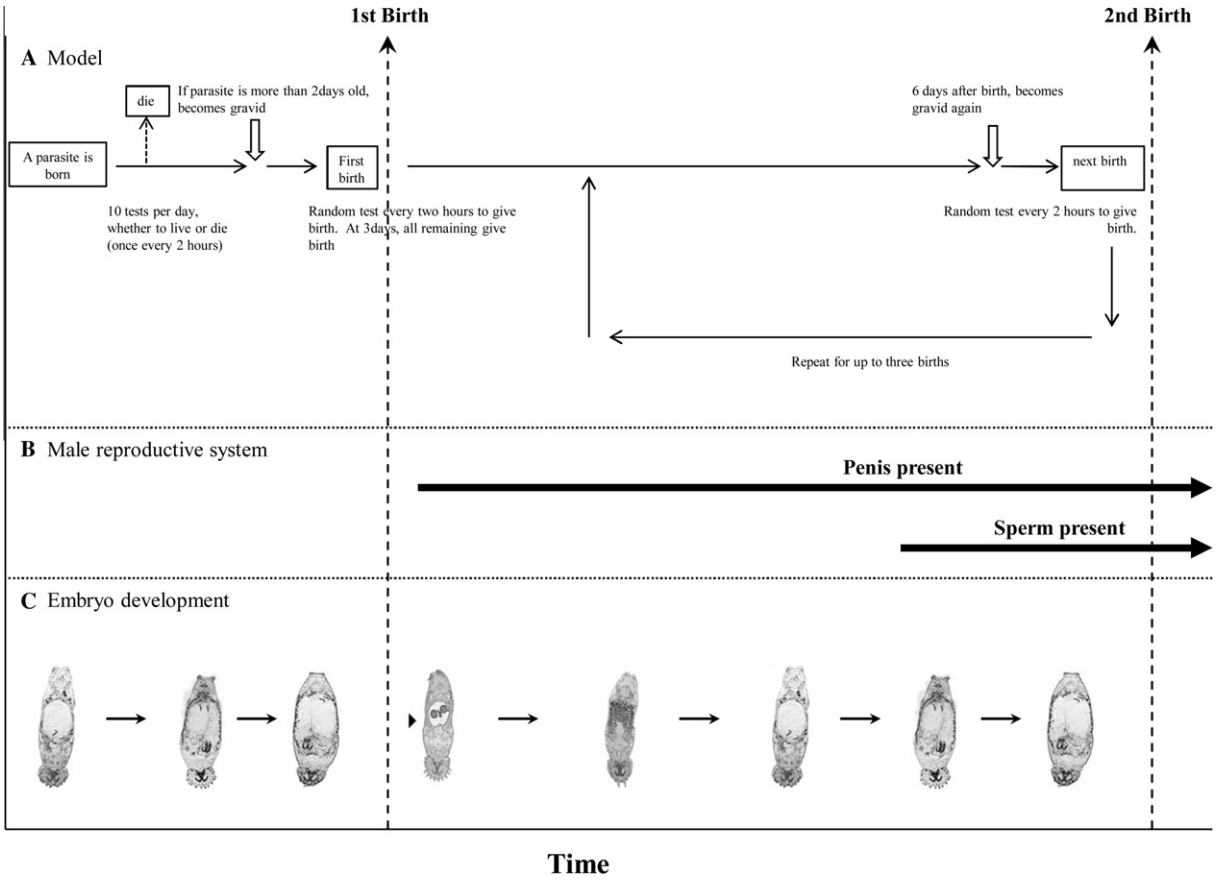

Fig. 1. Schematic representation of the agent-based model (A) relative to the development of the male reproductive system (B) and embryo (C) of *Gyrodactylus salaris*, based on Harris (1985), Jansen and Bakke (1991) and Harris et al. (1994). Note the asymmetry in the timing of births.

Simulation data sets were then modelled with both constant and increasing age-specific mortality to compare with the age-specific mortality schedule estimated from Cable et al. (2000) and the Neva data set. The proportion of pre-first birth individuals in the simulated populations was compared when they had reached a stable age structure. This parameter is reliably identified in gyrodactylid populations because pre-first birth individuals lack a penis (Harris, 1985).

3. Results

3.1. Model parameterisation

3.1.1. Age and reproduction

The model was parameterised following the observations of living worms, such that the first birth took place after approximately 2 days, similar to the gestation period observed in experimental infections at 13 °C (Jansen and Bakke, 1991; Cable et al., 2000). The interval between the first and second birth, and between all subsequent births, was 5–6 days (65–78 degree days at 13 °C), also matching previous observations (Jansen and Bakke, 1991; Cable et al., 2000) on Lierelva stock salmon (Fig. 2).

3.1.2. Life history data

The cohort of Lierelva strain *G. salaris* on Neva stock salmon generated data on 201 births, supplementing the data on 85 births on Lierelva and Alta salmon stocks collected by Cable et al. (2000). The data set included 123 first births, 53 second births, 17 third births, six fourth births and two fifth births (Table 1). The best fit mortality function for this complete data set (modelled using the procedure Recaptures Only within Mark) was with resighting probability (p) set to 1 and a constant daily survival rate (φ) of 0.84 ± 0.021 (mean ± S.E., Fig. 3A). This model had an AIC of 271, compared with the next best model (φ allowed to vary with time) which had an AIC of 310. The predicted constant survivorship predicted observed survivorship adequately (χ^2 = 11.9 with 20 degrees of freedom; $P > 0.1$). Analysis of the data of Cable et al. (2000), for the combined survivorship data (Lierelva and Alta salmon stocks) of Cable et al. (2000) was also with $p = 1$ and a constant daily survival rate (φ) of 0.84 ± 0.013 (Fig. 3A). This model had an AIC of 707 compared with a model in which φ was allowed to vary with age, which had an AIC of 715. The predicted constant survivorship gave a good fit (χ^2 = 11.9 with 20 degrees of freedom; $P > 0.1$) to the data of Cable et al. (2000).

3.1.3. Mortality

Three potential age-specific mortality patterns have been associated with gyrodactylid monogeneans. The death rate may be constant throughout life; it may increase through life (Anderson et al., 1977; Cable et al., 2000); alternatively the pattern of mortality may be more complex, with peaks of mortality following every birth (Cable et al., 2000). The most appropriate mortality function identified using Mark (see Section 3.1.2) was for a constant daily mortality of 0.84. Using this constant death function however, it was difficult to achieve realistic patterns of population reproductive growth. This suggests that mortality should not be constant but should increase in an age-dependent fashion as suggested by Scott and Anderson (1984). On the other hand, all analyses of experimental data using Mark to model age-dependent mortality failed to detect any evidence of age-dependence. A range of constant

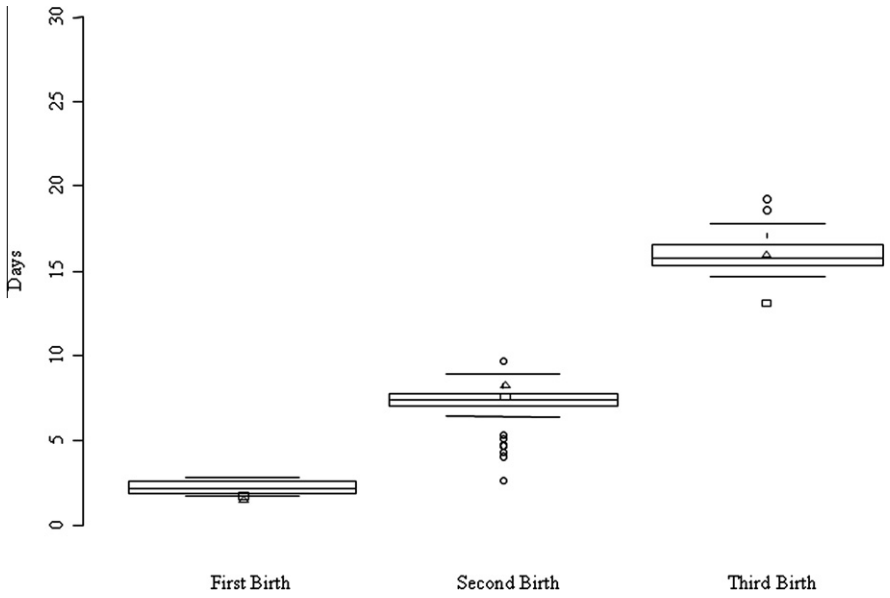

Fig. 2. Distribution of the first, second and third births of *Gyrodactylus salaris* on Atlantic Salmon (*Salmo salar*). Boxplots show data obtained using the agent-based simulation model. Outliers are shown as circles. Triangles represent the mean number of days obtained for each birth by Cable et al. (2000) on Alta salmon stock. Gestation periods as observed by Jansen and Bakke (1991) on Lone and Drammenselva Salmon stocks are shown as squares.

Table 1
Birth distribution for the Lierelva strain of *Gyrodactylus salaris* cohort on Neva salmon.

Births	Mean (days)	Range (days)	Sample size
First birth	2	1.5–4	123
Second birth	8.5	6–11	53
Third birth	15.5	13–17	17
Fourth birth	25	23–28	6
Fifth birth	31.5	29–34	2

and increasing age-specific mortality schedules was therefore evaluated using simulation (Table 2), calculating age-specific survivorship using MARK by allowing φ to vary, and then constructing a linear regression based on the daily survivorship estimates (Fig. 3B). The increasing age-specific mortality function was then fitted to simulation data ($\chi^2 = 2.676$, $P < 0.95$ with 20 degrees of freedom for results from a typical simulation). A compromise mortality schedule, in which mortality increased but in a constant, age-dependent manner, was chosen for the final simulations used in this work (Model 4 in Table 2). This model had a realistic (if slightly low) population growth rate and a stable age structure with a proportion of pre-first birth monogeneans equal to 0.33, similar to the value observed in natural *G. salaris* populations by Harris et al. (1994). This compromise model was therefore chosen as the starting point for all subsequent analyses.

3.2. Stochastic error in individual parasite populations

Multiple simulations of the model under identical conditions can be considered as equivalent to multiple replicates of a gyrodactylid infection on genetically identical hosts with identical ontogenetic histories maintained under identical environmental conditions. Using the terminology of Dennis et al. (2006), the model allows estimation of process noise (especially demographic stochasticity) under conditions where environmental noise and observation error are eliminated. A small consistent error was associated with exponential population growth in the absence of mortality in the model, due to demographic noise associated with the age distribution of each birth (Fig. 4A). Incorporating mortality

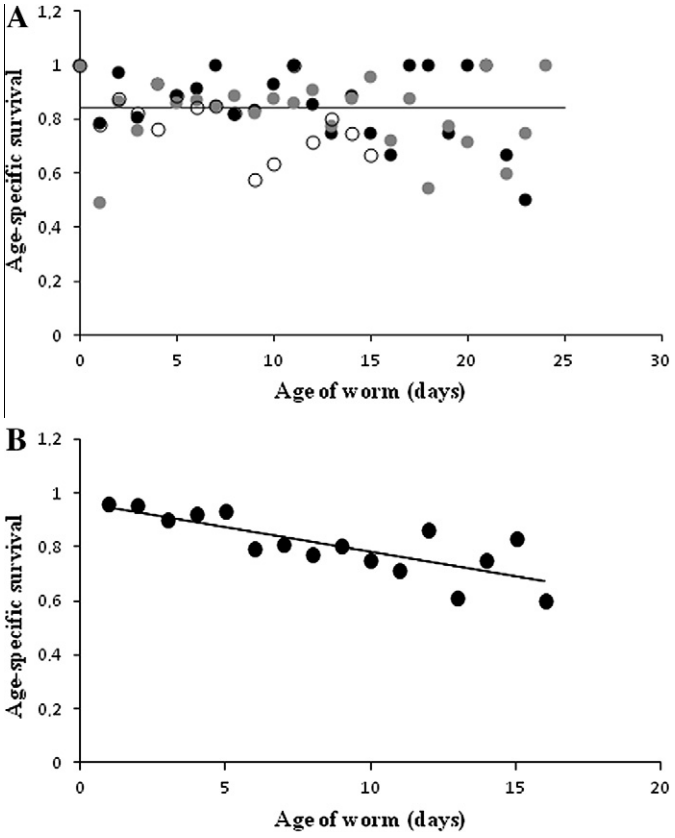

Fig. 3. Age-specific survivorship data, derived from the RECAPTURES ONLY procedure within MARK. (A) Circles indicate daily estimates of parasite survivorship for experimental studies. Data for Alta (open circles) and Lierelva (closed circles) salmon from Cable et al. (2000). Neva salmon (grey circles) are from the present study. The line represents constant survivorship of 0.84 per day estimated from MARK. (B) Simulation data from model 4 (Table 2). Circles represent daily survivorship, the solid line is age-decreasing survivorship of 0.96 – (0.02/day) derived from MARK.

however, greatly increases stochastic variation, with variance increasing as mortality increased (Fig. 4B). As mortality was

Table 2
Effect of different mortality models on population parameters in the NetLogo parasite growth model, including population growth rate (R_0).

Model No.	Mortality model	Age-specific survival	R_0	Proportion of pre-first births
Model 1	Constant	0.92	1.02	0.33
Model 2	Constant	0.89	1.02	0.33
Model 3	Constant	0.87	1.01	0.34
Model 4	Increasing with age	0.96 – (0.02/day)	1.02	0.35

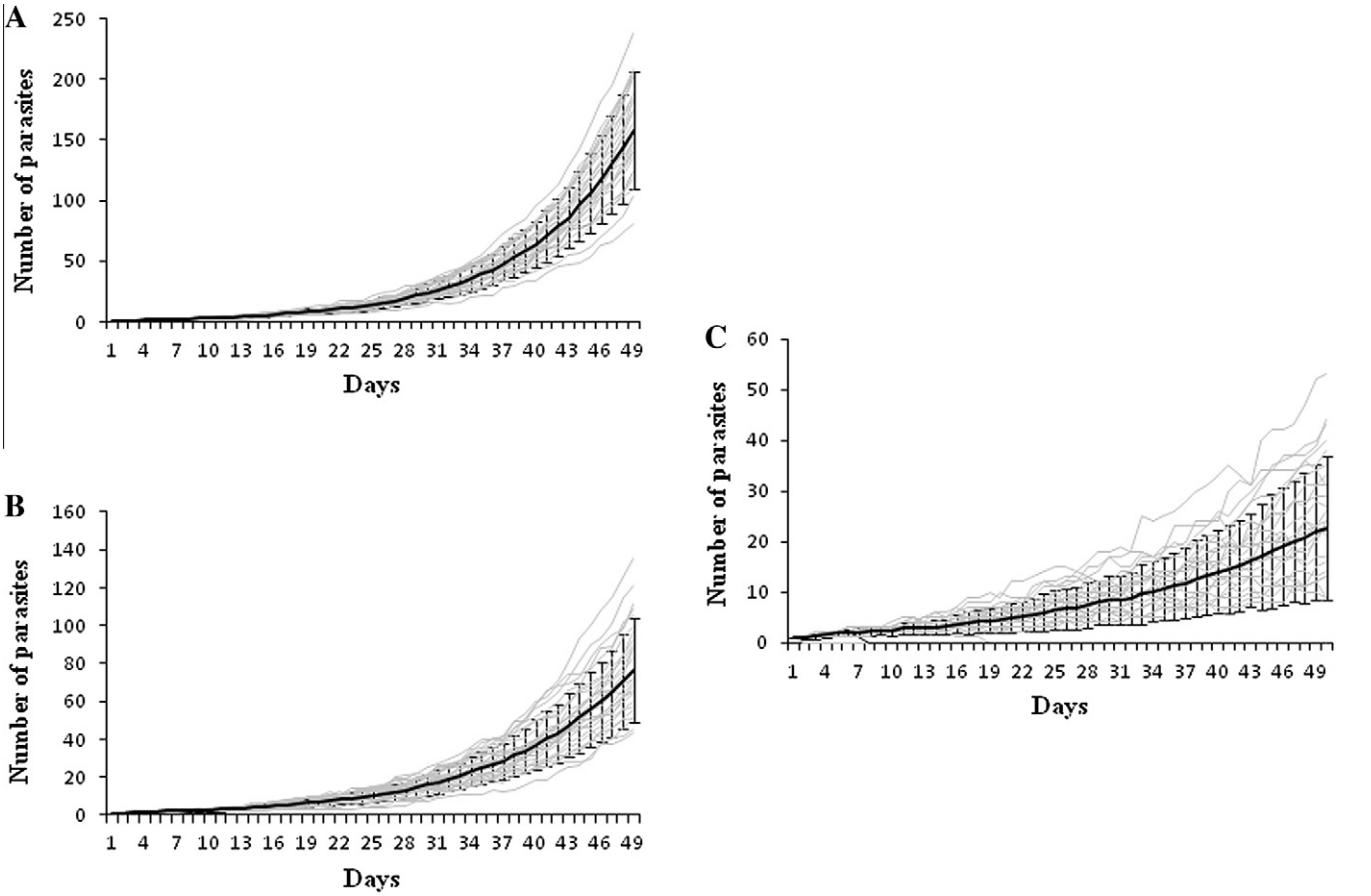

Fig. 4. Simulated survivorship curves for *Gyrodactylus salaris*, under conditions of no mortality (A), moderate mortality increasing throughout life (B) and high mortality increasing throughout life (C). Pale grey lines represent individual population trajectories, the heavy black line is the mean, bars represent standard errors. As mortality increases, the mean becomes associated more with the lower one-third of growth rates and is a less adequate descriptor of population behaviour. Note the difference in scales regarding parasite numbers.

allowed to increase, the utility of mean infection size as a measure to capture the pattern of population growth declined (Fig. 4B and C).

3.3. Error in population growth rates

A key parameter in comparing gyrodactylid performance on different hosts, apart from mean population sizes, has been population growth rate, r (e.g. van Oosterhout et al., 2003). This is defined as

$$r = (\ln N_t - \ln N_{t-1})/t$$

where N_t = the parasite population at time point t, N_{t-1} = the parasite population at the previous time point, and t = the time period in days between the two time points. This population growth rate is easy to compute for each fish infected but there is no theoretical basis for estimating the error of this statistic.

The distribution of population growth rates approached normality as the parasite population size increased. For $N > 20$, agreement with normality, as tested with the Anderson Darling Normality test, implemented through package nortest (version 1.0, http://cran.r-project.org/web/packages/nortest) within *R*, is supported ($P = 0.5295$). Analysis of simulations showed that below a population size of 20, population growth is distributed non-normally and cannot be analysed reliably. For populations with normally distributed population growth rates (i.e. $N > 20$) the variance declined with sample size (Fig. 5). These properties of the population growth rate statistic are such that it is possible to distinguish parasite populations with significantly different growth rates using Student's t test when the growth rate differs by more than 0.02, when population size is between 20 and 100 parasites per fish.

The population growth parameter r has been shown to change systematically during *G. salaris* infections (Bakke et al., 2002, 2004), declining steadily throughout the infection, even on susceptible salmon stocks. In the absence of an error structure for the growth rate, it was impossible to establish the importance of this observation. However, during exponential growth of the simulation models, mean population growth rate and the

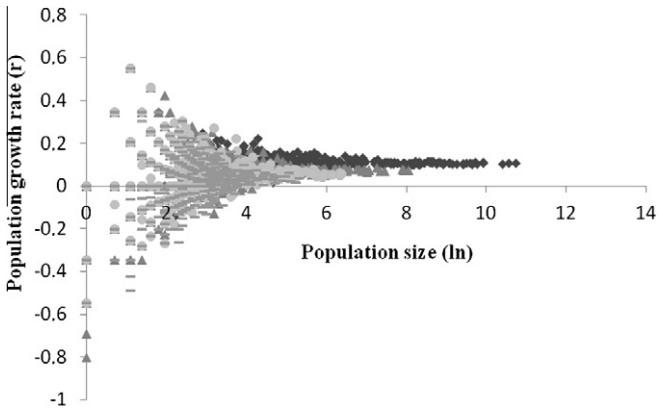

Fig. 5. Convergence of *Gyrodactylus salaris* population growth rates (r) as population size increases under the five mortality schedules for the simulation listed in Table 2.

associated error did not change once the population had achieved a size of 30–50 worms. The observed decline in growth rates noted in experimental studies must therefore represent a time (Fig. 6A) or density (Fig. 6B) dependent (e.g. immunological) response to the *G. salaris* population.

4. Discussion

Gyrodactylid monogeneans have a mode of reproduction which is unique in the animal kingdom; mature embryos develop in utero, so when a gyrodactylid is born, it already contains a near-term daughter embryo, which in turn contains an embryo within, on which the marginal hooks and tips of the hamulus points can be seen (Bakke et al., 2007). They give birth to individual daughters, so population growth resembles the binary reproduction of bacteria or protists. However, the gestation period of the first-born daughter is only one-quarter that of all the other daughters, due to the developmental advantage gained by this embryo while its parent is still an embryo (Harris, 1985; Fig. 1). As a result of this binary reproduction, and the skewing in birth dates caused by development in utero, error structures for population growth of gyrodactylid monogeneans have always been poorly understood. The agent-based model presented in this work mimics the unique gyrodactylid reproductive biology; it resembles the Gyro-Scope model of van Oosterhout et al. (2008), but is restricted to an exploration of parasite population growth subject to random stochastic variation, when all hosts are considered genetically and phenotypically equivalent and environmental conditions are held constant. It therefore allows an exploration of the error structures which might be expected as a result of random stochasticity in birth and death processes, making it possible in turn to identify phenomena in gyrodactylid population dynamics which may have biological

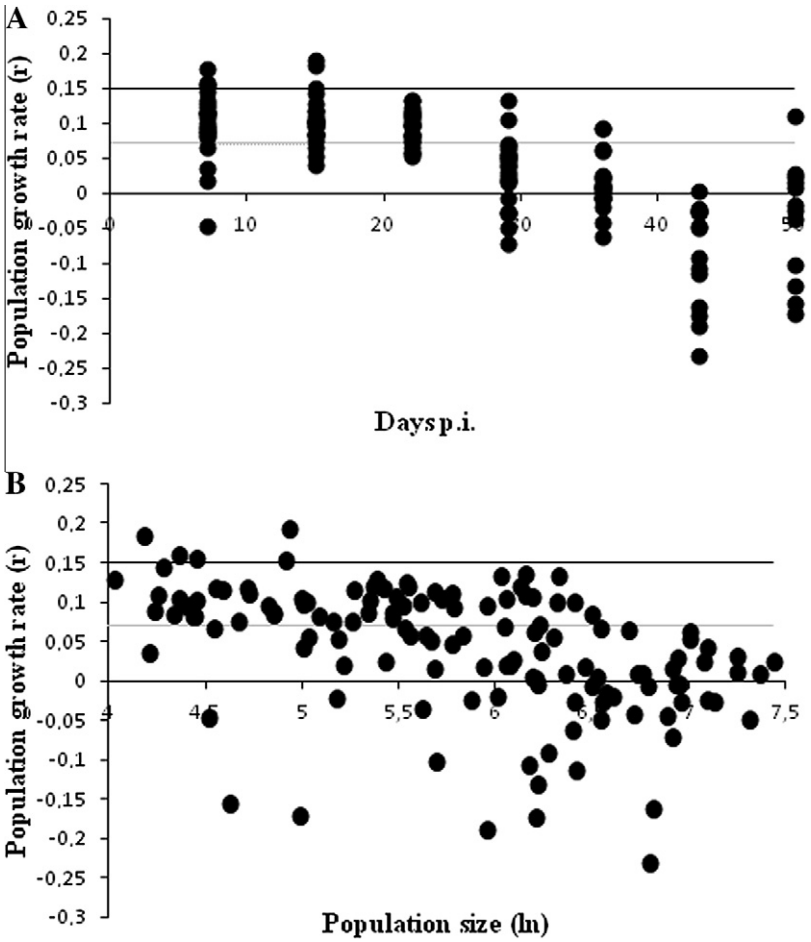

Fig. 6. Comparison between model simulations and experimental data from the Lierelva data set of Bakke et al. (2004). Black circles represent growth rates from individual fishes throughout the 50 day experiment, either in relation to age of infection (A) or the size of the parasite population in the week preceding the measurement of growth rate (B). The black line is the growth rate at the start of the experiment, as predicted on the basis of exponential population growth throughout; the pale grey line represents the lower 95% confidence limit for this growth rate, as estimated using the simulation model data presented in Fig. 5.

significance. The use of the mark-recapture software MARK (Cooch and White, 2011) has also allowed a more sophisticated analysis of gyrodactylid mortality patterns. This software was designed for analysis of free-living wild vertebrate populations; however, the methodology of estimating gyrodactylid fecundity and mortality by daily observation (Braun, 1966; Harris, 1998; Cable et al., 2000) is conceptually similar to a mark-recapture experiment, where failure to observe a parasite is equivalent to failure to recapture or resight a marked animal. The software could therefore be directly applied to the cohort data collected in the present work, bringing the power of the maximum likelihood algorithms for mortality analysis embedded within MARK to a parasitological problem. This approach also allows comparison of survivorship and mortality on different hosts; we intend to return to this in a future report.

The model presented has used parameters from known life history variables for *G. salaris*, an important pathogen of salmon which has been the subject of exhaustive study by Bakke and co-workers (e.g. Bakke et al., 2002, 2007). At 13 °C, this parasite gives birth for the first time at ca. 2 days, and then subsequently every 5 days, with a maximum fecundity of five daughters (Jansen and Bakke, 1991; Cable et al., 2000; present data). Given a constant temperature of 13 °C, the fifth daughter would be born after 27 days. *Gyrodactylus salaris* at this temperature has a maximal population growth rate r of 0.15 (Jansen and Bakke, 1991). In the model, parasites are assumed to be lost from the fish throughout life in a random fashion. The model was tested using both a constant death function (probability of death in any one time interval is constant) and an increasing death function (probability of death in any one time interval increased with age). The former was preferred by Cable et al. (2000) as a more simple explanation of survivorship data; however, Scott and Anderson (1984) used an increasing mortality function as more theoretically appropriate (Anderson et al., 1978). In the current work, both the new data set and that of Cable et al. (2000) were fitted using constant and increasing age-specific mortality functions within the mark-recapture analysis software MARK, which had not been available at the time of the previous study (Cable et al., 2000). In the case of both data sets from experimental observations of three strains of salmon conducted by different workers 10 years apart, the unambiguous conclusion is that a constant mortality function is most appropriate, with remarkably similar daily survivorship estimates of 0.84. The data set of Cable et al. (2000) included 89 worms on Alta strain salmon, of which 30 survived beyond the second birth (8 days); the present work utilised observations on 200 parasites on Neva stock salmon, so the failure to detect increasing age-specific mortality is unlikely to be due to a small sample size. It could however be unrepresentative of the situation in natural infections due to the effects of daily handling and observation. This is also amenable to analysis with MARK and will be the subject of a future report.

This simulation model has provided important insights into the error structure of population growth rate, and the behaviour of this parameter in 'infections' which vary only as a result of stochastic variation. This parameter is derived either as the difference between the natural logarithms of population size on different days of the infection, or by solving the Euler equation based on estimated mortality and fecundity schedules. Estimates of the error structures of population growth rates are not well understood (Elvarez-Buylla and Slatkin, 1991), but the current simulation studies also indicate that they are approximately normally distributed (Meyer et al., 1986), especially when populations contain more than 20 parasites (Fig. 5). For infections of less than 20 worms, the error in population growth rate is disproportionately large, and we do not recommend the use of this parameter to compare gyrodactylid population growth on different hosts when the peak parasite population is only c. 20 parasites per fish. The distribution for all infections (including those of less than 20 individuals) is complex, especially because populations which perform badly due to stochastic fluctuations in mortality or fecundity at the beginning of the infection tend to become extinct and are lost from the sample set. This becomes a particular problem in the identification of resistant or susceptible hosts; extinction of the parasite population tends to remove resistant hosts from the sample set, leading to over-representation of susceptible hosts as the infection progresses. This is very clear, for example, in Harris et al. (2011), where resistant charr hosts would have ceased to be included in population growth rates after 3 weeks, due to the extinction of their parasite infra-populations. This loss of resistant hosts from data sets makes it harder to understand the observed decline in parasite population reproductive rates seen throughout *G. salaris* infections (e.g. Bakke et al., 2004). This is a consistent feature of *G. salaris* infections of both susceptible and resistant fish, and yet would not be predicted by any model of purely random variation; under models of random variation, the population growth rate settles after an initial period of fluctuation to a constant value, with the duration of the period of fluctuation determined by generation time. As noted above, the loss of infections from resistant fishes would lead to an increase in the apparent population growth rate across the fish population. Loss of susceptible hosts through parasite-induced host mortality could lead to an apparent decline in population growth rates; however this does not usually become apparent for several weeks in experiments such as that described by Bakke et al. (2004). The only other possible explanation of this observation is density- or time-dependence in parasite population growth, either through a crowding effect in the parasite population, or due to a host immune response. In general, the decline in population size of gyrodactylid monogeneans in the later stages of an infection is usually attributed to immunity (e.g. Scott and Anderson, 1984; Bakke et al., 2004), although the latter is commonly not supposed to occur in susceptible Norwegian salmon populations (Peeler et al., 2004). In a few experiments with challenge infections (e.g. Scott and Robinson, 1984; Lindenstrøm et al., 2003) the presence of memory, and the identification of immune-relevant molecules in the epidermis (Lindenstrøm et al., 2003) are strongly suggestive of an immune response. Nevertheless, gyrodactylid infections are unusual in that the immunologically relevant organ, the host epidermis, is also the main dietary component of the parasites, raising the possibility that the cause of the decline in gyrodactylid abundance is multifactorial. The immune response may be involved, but at the same time, gyrodactylid grazing may prevent epidermal cell maturation and expression of a normal host response. This was clearly shown by Lindenstrøm and Buchmann (2000) where an initial increase in mucus cell density is replaced by significant depletion at the end of the study. Gyrodactylid monogeneans are assumed to have complex nutritional requirements derived from the host epidermis, and their growth performance on epidermal surfaces where mucus cells fail to differentiate, or in which significant thinning of the epidermal surface occurs (Sterud et al., 1998) is unclear, although effects of such resource depletion on parasite population growth might be expected. The situation is further complicated by the impact of stress-related hormones such as cortisol or dexamethasone which immunosuppress and lead to an increase in gyrodactylid population growth (Lindenstrøm and Buchmann, 1998; Harris et al., 2000). Since gyrodactylids are themselves stressors, they may influence their own population growth rate through stress-mediated modulation of the host immune response. Given the complexity of these interactions between dietary quality, immunity and host stress, the decline in the population growth rate of *G. salaris* on Lierelva salmon (Bakke and Mackenzie, 1993; Bakke et al., 2004) deserves further attention.

A final point which is clear from this study is that in general, attempts to identify ecologically relevant local adaptation in gyrodactylid-fish populations have used insufficiently large samples of hosts. Unlike studies with mice (Behnke et al., 2006), the fish populations used in gyrodactylid susceptibility/resistance experiments are either derived at no more than one or two generations removed from the wild (e.g. de Roij et al., 2010), or are based on ill-defined 'stocks' of hosts determined by the parentage of fish used to set up the initial culture. In no case can the stock of salmon used in experimental studies be considered truly representative of the genotypes present in the river of origin of the stock. In the large study by Salte et al. (2010), up to 10% of families of salmon from a susceptible population (Drammen) exhibited heritable resistance to *G. salaris*, but this would be difficult to detect in smaller studies. To take a more optimistic example from other case studies of disease resistance in salmon, an 'unusually reproducible' Quantitative Trait Locus (QTL) mapping with Infectious Pancreatic Necrosis (IPN) susceptibility in salmon was found in seven out of 20 tested fish and the high resistance allele was estimated to be present in 30% of fish (Moen et al., 2009). Bakke et al. (1990) utilised four populations of 50 salmon to show susceptibility/resistance of *G. salaris*, but only followed individual infections in 20 fish. De Roij et al. (2010) followed individual infections on 30 fish in five populations. Such studies can determine gross differences in susceptibility/resistance, such as those between Baltic and Norwegian Atlantic salmon (Bakke et al., 1990), but there is a high probability that a susceptibility locus present in 10% or 30% of the fish will not be detected when a substantial proportion of infections become extinct through simple stochasticity. The limit to such studies is the number of fish which can be processed by the investigator and, for example, de Roij et al. (2010) with five populations of 30 fish was close to the upper limit for a simultaneous monitoring effort by a single individual. This suggests that detection of subtle or low-frequency polymorphisms in host susceptibility to *G. salaris*, which are likely to be more relevant in an evolutionary context, require alternative study methods to the basic individual infection experiment with parametric statistical analysis as simulated in this report.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j. ijpara.2012.05.012.

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