A deterministic model for the dynamics of furunculosis in chinook salmon

Oncorhynchus tshawytscha

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ABSTRACT: Studies were undertaken to determine the parameters of transmission of Aeromonas salmonicida in chinook salmon Oncorhynchus tshawytscha, and to develop a deterministic model of the dynamics of experimental furunculosis. For determination of disease transmission coefficient (β), disease-related mortality rate (α) and natural mortality rate (γ), fish in 70 tanks (~42 fish tank⁻¹) were each exposed to a single infectious donor fish, 7 tanks were randomly selected daily and all individuals were examined for the presence of A. salmonicida in the kidney. The proportion of susceptible (S), infected (I) and removed (R, dead) individuals were determined daily. The parameters β, α, γ, reproductive ratio (R₀) and threshold density were estimated to be 0.0214 infected ind. d⁻¹, 0.29 infected ind. d⁻¹, 0.00015 ind. d⁻¹, 3.23 and 13.56 ind., respectively. Using these parameters, a deterministic disease model of A. salmonicida infection as a cause of furunculosis was constructed. The net rate at which new individuals became infected (the incidence rate) per unit time was proportional to S × I × β. The model-produced data for S were significantly associated with experimental data (r² = 0.92). In brief, a simple SIR (susceptible-infected-removed) model was successfully utilized to simulate observed data.

KEY WORDS: Deterministic models · Disease dynamics · Furunculosis · Aeromonas salmonicida

INTRODUCTION

Microparasites (viruses, bacteria and protozoans) and macroparasites (helminths and arthropods, etc.) can regulate or limit populations of the host they infect as much as predators do, and these parasites can, in fact, be considered microbial predators (Anderson & May 1978, Mena-Lorca & Hethcote 1992). The relationship between disease and its effects on population dynamics and the dynamics of the infection process can be described mathematically (Kermarck & McKendrick 1991a–c). Extensive reviews of the subject were carried out by Anderson & May (1978), Anderson (1982), Bradley (1982) and Heesterbeek & Roberts (1995).

In the fish disease literature, effects of disease agents on their host and biological properties (e.g. genomic construction, size, shape, virulence) of various pathogens have been extensively studied, whereas their epidemiology has remained largely unexplored. In recent years, only a few limited studies on epidemiology have emerged in the area of fish diseases. The population dynamics of Ichthyophthirius multifiliis in black mollies Poecilia latipinna (Lesueur) was the first study of fish disease to determine density-dependent effects on pathogen dispersal (McCallum 1985). The doctoral dissertation of Bebek (1996) applied various epidemic parameters, such as critical threshold and basic reproductive ratio concepts, to the dynamics of infectious pancreatic...
necrosis virus, and a related report by Smith et al. (2000) discussed infectious pancreatic necrosis virus epidemics.

In the present study, *Aeromonas salmonicida*, a common bacterial fish pathogen that can cause severe disease (furunculosis) in trout and salmon hatcheries, in ornamental fish, and presumably in wild fishes, was selected to study quantitative mechanisms and efficiency of pathogen transmission leading to furunculosis disease. The objective of this study was to construct a deterministic model of the dynamics of furunculosis disease epizootics to use as an aid in understanding epidemiological aspects as the key factors for disease spread. The study focused on infection transfer and partitioning of the population into susceptible, infected and removed classes in an experimental furunculosis epizootic initiated by cohabitation of susceptible salmon with a single infectious individual.

**MATERIALS AND METHODS**

In order to determine $\beta$ (disease transmission coefficient), the following laboratory experiment was designed. Spring-run chinook salmon *Oncorhynchus tsawytscha*, kindly provided by the Oregon Department of Fish and Wildlife (ODFW), Willamette River Hatchery, Oregon, were held in freshwater (16 to 17°C) at the laboratory for Fish Disease Research at Hatfield Marine Science Center. The hatchery is known to be free of *Aeromonas salmonicida* in regular screening tests carried out by ODFW. Fish were held in 7.5 l aerated tanks, which were randomly assigned to treatments and were fed daily at a ratio of 1% body weight with biodiet (Bioproducts) pelleted feed. Each replicate/tank contained approximately 43 fish (mean weight 1.97 g) with a freshwater flow of 250 ml min$^{-1}$ (1.68 tank volumes h$^{-1}$). Seven replicates and 1 control were used for each treatment. *A. salmonicida*-infected donor fish were obtained by exposing spring chinook salmon to $2 \times 10^5$ *A. salmonicida* ml$^{-1}$ for 24 h at 16°C with aeration. The strain of *A. salmonicida* subsp. was originally obtained from a natural epizootic in chinook salmon held at Hatfield Marine Science Center. At the end of Day 3 post-exposure (dpe), when it was known that exposed fish were infectious (determined by previous experiments, Ogut 2001), 1 donor marked by a fin clip was released into each of the 70 treatment tanks; each control tank contained 1 fish exposed to Dulbecco's phosphate buffered saline (DPBS) in place of bacteria. For 10 d thereafter, 7 tanks were randomly selected daily and all fish in the tanks were killed by an overdose of TMS (tricaine methanesulfonate). All fish collected daily ($n = 280$) from the selected tanks were examined for signs of the disease and presence of *A. salmonicida* by streaking 10 ml of kidney onto coomassie brilliant blue agar (CBBA) (Markwardt et al. 1989) and estimating the relative number of blue colonies surrounded by a soluble brown pigment on an ordinal scale from 1 (few colonies) to 4 (confluent). One of the control tanks was also selected daily, all fish were euthanized and tested for the presence of bacteria. Mortalities in all tanks were recorded daily, and dead fish were removed from that tanks within 8 h of death, examined visually for signs of disease, and the presence of the agent in the head kidney was determined by culture as described above.

**Model construction**

It has been shown that in directly transmitted diseases, the dynamics of infection generally proceed according to the mass action theory, namely that the net rate at which hosts become infected per unit time is the product of the number of susceptible ($S$) individuals, multiplied by the number of infected ($I$) animals, multiplied by the transmission coefficient ($\beta$). The removed class ($R$) includes the cumulative number of disease-related mortalities. Natural mortality was also considered in the model. A portion of $I$ and $S$ was removed as natural mortality. Deterministic models that were used here were described by Anderson & May (1978), May & Anderson (1979), and Anderson (1982) in the following 3 equations:

$$\frac{dS}{dt} = -\beta SI - \gamma S$$  \(1\)

$$\frac{dI}{dt} = \beta SI - \alpha I - \gamma I$$  \(2\)

$$\frac{dR}{dt} = \alpha I$$  \(3\)

where $\alpha$ is the disease-specific mortality, $\gamma$ is the natural mortality rate and $t$ represents time (d).

Assumptions used in the models above include:
1. Every individual has the same chance of coming into contact with an infectious individual or the agent;
2. animals recover from infections at a rate ($v$) after which they are immune to further infection for some period of time. In this study, $v$ was not applicable since many of the infected animals died and time for development of specific immunity was too short to be effective in preventing infection ($< 10$ d); (3) $N$ (number of individuals in a population) is closed. That is, the timescale of the epidemic process of transmission and recovery (in this case 10 d) is much shorter than the timescale at which the population size would change.
due to natural births and deaths. The natural mortality rate was not significant in this study either, since few mortalities (2.7%) occurred in unexposed fish, none of which was infected with *Aeromonas salmonicida*; (4) the number of successful contacts made between susceptible and infectious individuals determines the magnitude of the disease outcome. Per unit time, a proportion of *I* produce $\beta \times I$ potentially infectious contacts. Daily incidences are described by Eq. (1).

After estimating $\beta$, $\alpha$ and $\gamma$, the reproductive ratio ($R_0$) was calculated using the formula by Anderson & May (1978):

$$ R_0 = \frac{N \times \beta}{\alpha + \gamma} \quad (4) $$

where $N$ is host population size.

The critical threshold density $N_T$ (initial number of susceptible hosts required to initiate an epizootic) was calculated using the formula:

$$ N_T = \frac{\alpha + \gamma}{\beta} \quad (5) $$

Note that the unit of infection considered throughout the study is number of fish, not weight of fish.

### Analysis and testing of models

Sensitivity analysis of the data and graphic representation of the deterministic model of furunculosis disease dynamics developed here were depicted using the Stella (Version 5.1.1) software package (High Performance Systems).

The weighted least-squares method was employed to estimate parameter values of $\beta$, $\alpha$ and $\gamma$ using the optimization technique. Statview 5.0 software package (SAS Institute) was used to carry out statistical analyses.

### RESULTS

During the 10 d of the experiment, no control fish that died ($n = 12$) showed evidence of *Aeromonas salmonicida* in the kidney ($n = 440$). Of 69 donor fish used in all experimental tanks, 64 that died during the experiment were infected with *A. salmonicida* (93%). There were 5 surviving donor fish after 10 d, 3 of which were infected with *A. salmonicida*. The tanks in which the 2 negative donors were placed were harvested on Days 1 and 2, and no fish in either tank were found to be infected. Seventy-six percent of the donor fish had died from furunculosis within 72 h of being placed in the tank with recipient fish. Thus, recipient fish were exposed to infectious fish for less than 3 d. Raw data for numbers of fish in the $S$, $I$ and $R$ classes are presented in Table 1. All of the fish ($n = 2928$) from 69 treatment tanks (1 replicate tank at Day 7 was not used due to technical problems) were assayed for the presence of *A. salmonicida* on CBB (Coomassie Brilliant Blue) agar. Of all exposed fish, 689 (23.5%) were found to be infected with *A. salmonicida* during the 10 d of the experiment. Approximately half (355 fish) of the infected salmon died of furunculosis (51% of infected, 12.1% of all exposed) by 10 d post-exposure to infectious fish. The 7 replicate tanks from the Day 10 treatment were observed over the longest time, 10 d. Of the 298 fish accumulatively sampled on Day 10, 132 (~45%) survived the epizootic, with the rest (54%) dying of furunculosis-related disease. Sixty-seven percent ($n = 88$) of survivors at Day 10 were infected with *A. salmonicida*, with only 1 having died of natural causes (bacteria not detected). Furunculosis-related mortality among 7 replicate tanks ranged between 66 and 95.1% on Day 10 post-exposure.

**Parameters used to estimate the dynamics of furunculosis**

Dynamics of the experimental furunculosis epizootic were successfully described and simulated by simple mathematical modeling techniques described in the ‘Materials and methods’ section. The partitioning of chinook salmon among $S$, $I$, and $R$ classes during the furunculosis epizootic is displayed in Figs. 1, 2 & 3, along with data produced from the models (Eqs. 1, 2 & 3). The $R$ class did not consider immune fish because of the short duration of the experiment relative to the development of the acquired immune response of salmon and the high rate of mortality among infected fish.

$\alpha$, $\beta$ and $\gamma$ were estimated as follows: from Eqs. (1), (2) & (3), the calculated, daily predicted values for $S$, $I$, $\alpha$ and $\gamma$ were obtained using Euler’s method at a time step of 0.25 d, e.g.:

$$ S_{t+1} = S_t + \frac{dS}{dt} \times 0.25 \quad (6) $$

Then, parameter values were estimated from weighted least-squares by minimizing the residual sums of squares (RSS) from all compartments:

$$ RSS = \sum \left( \frac{S_i - \hat{S}_i}{\sigma S_i} \right)^2 + \sum \left( \frac{I_i - \hat{I}_i}{\sigma I_i} \right)^2 + \sum \left( \frac{Q_i - \hat{Q}_i}{\sigma Q_i} \right)^2 + \sum \left( \frac{M_i - \hat{M}_i}{\sigma M_i} \right)^2 \quad (7) $$

where $\sigma$ is the variance obtained from 7 replicate tanks per treatment (day), $i$ is time (d), $M$ is disease related mortality (numbers) and $Q$ is natural mortality (num-
bers). Since daily data were available, cumulative predicted and observed values of each compartment were compared. For the first 2 d no disease-related mortalities were found. Therefore, expected values were adjusted to 0 for the first 2 d. The model described 92% of the variation obtained from the experimental data.

\[ \beta = 0.0214 \text{ infected ind. d}^{-1} \]

\[ \gamma = 0.287 \text{ infected ind. d}^{-1} \]

Utilizing the population at risk \( (N = 42) \) in each tank and the parameters above, as well as the natural mortality rate \( \gamma \), the basic reproductive rate \( (R_0) \) of Aeromonas salmonicida was calculated to be 3.23.

The critical threshold density \( (N_T) \) is the population value below which the incidence of infection begins to decline. In the small-scale experiments carried out here, with \( N_0 = 42 \) and a volume of 7.5 l, the calculated \( N_T \) (Eq. 5) was approximately 14 (13.6). This value was calculated from Eq. (5). Graphically, it can also be demonstrated as shown in Fig. 4. Thus, only when ca. \( 2/3 \) of the fish had entered either the infected or removed classes did the incidence begin to decline with the resultant abrogation of the epizootic.
DISCUSSION

Models developed to describe dynamics of microparasitic diseases (caused by viruses and bacteria) are widely used in human and veterinary epidemiology (Bailey 1975, MacKenzie & Bishop 2001). Little work, however, has been done on similar modeling of the dynamics of disease in fish. Due to the assumptions (population is closed and mixing is homogenous), these types of models could efficiently be utilized in fish disease management. These studies were carried out to construct basic models of the dynamics of furunculosis as a base for epidemiological studies to evaluate various risk factors for disease occurrence.

Estimation of parameters for disease dynamics

The laboratory experiments undertaken here allowed us to describe a dynamic model for the initial stages of furunculosis disease in salmonids. The rate parameters describing the movement of segments of the population at risk from susceptible (S) to infected (I) and subsequently to removed (R) classes were calculated from the data and used to construct the dynamic model of the infection and disease.

The transmission coefficient is the sum of effects related to the host, pathogen, and environment. The value determines daily incidences. Moreover, peracute, acute, chronic situations are also determined with the transmission coefficient (Fig. 5). The time to peak number of infected and dead individuals is also changed by the value of the transmission coefficient.

The basic reproductive ratio ($R_0$) is defined as the number of susceptible animals which, on average, become infected by a single infectious individual during its entire period of infectiousness. There are numerous reports on theoretical aspects of $R_0$ (e.g. Anderson 1981, Fine et al. 1982). The value of $R_0$ for an infection within a population can be estimated from knowledge of the biological characteristics of the disease, such as host density, and rates of infection, recovery and host mortality. Some factors determining the magnitude of $R_0$ are specific to the parasite itself (e.g. mechanisms of transfer and stability in the environment), whereas others are...
specific to the host (e.g. host density, size, species, behavior) or environment (temperature, flow rate, water quality). Effects of all parameters related to the disease-causing agent and the host, under the controlled conditions provided, are summarized under the parameter transmission coefficient, $\beta$, which is essential in the calculation of $R_0$. The condition $R_0 = 1$, indicating an enzootic situation, defines a transmission threshold below which the disease agent is unable to maintain itself within the host population. Any reduction in the value of $R_0$ below unity will act to reduce disease prevalence and incidence to a level at which infection cannot be sustained and the pathogen will be eliminated from the population (Fig. 6).

In the studies reported here, for these experimental conditions, $R_0$ was calculated to be 3.25, indicating that for each day 1 infectious fish would be able to successfully transmit the pathogen to more than 3 susceptible individuals (primary infection). Each of these, in turn, could become infectious approximately 3 d postexposure (Ogut, 2001) and infect 3 more fish, leading to a rapid epizootic. This, in fact, did occur in the experiment. Using the calculated value for $R_0$ of 3.25, approximately 3 rounds of infection (primary, secondary and tertiary) would have occurred within the 10 d of the experiment, leading to a prediction of approximately 80% of the susceptible fish (34/42) infected by Day 10. Sampling for the presence of the bacteria in the kidney of fish that had died from furunculosis by Day 10, as well as those that had survived the 10 d experimental period, showed that on average for 7 tanks, 84.8% (244/290) of the fish were infected with *Aeromonas salmonicida*. The $R_0$ value obtained from these experiments is generally lower than those published for human infectious diseases, which range between 4 and 18 (Anderson & May, 1991).

### Estimation of critical threshold

It should be emphasized that, as the data suggest, the value of $N_T$ is dynamic according to factors related to host, pathogen and environment. If conditions are more severe, fewer than 14 animals could be required to initiate an epizootic, or vice versa. In brief, the value of $N_T$ is important and could serve as a starting point optimal for chinook salmon under the defined conditions.
CONCLUSIONS

The data gathered from the experiments, and the models of infection dynamics constructed from those data, indicate that *Aeromonas salmonicida* is a very efficient pathogen under conditions that are similar to those that might be operative for a typical production facility for Chinook salmon, as well as other salmonids. The dynamic model describing the initiation and extent of a furunculosis epizootic closely followed the actual results.

These types of deterministic models of the dynamics of diseases such as furunculosis can be valuable in aquaculture because they can be utilized to help predict the likelihood of the development of disease and whether its nature will be enzootic disease, epizootic disease, or an infectious carrier state with no evidence of clinical disease. Further refinement of models constructed here will be needed, utilizing factors that are known to affect disease outcomes such as fish age, density, temperature and other stress-inducing factors to predict the disease outcome. Further refinement will occur with the incorporation of stochastic (probabilistic) parameters into the models, and utilization of these types of models for other infectious diseases of fishes.

The model described here correlates closely with the observed data because this is how the parameters were estimated. Before the model can be used more widely with confidence, it needs to be validated against naturally occurring outbreaks of furunculosis.

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