Experimental infectious pancreatic necrosis infections: propagative or point-source epidemic?

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Abstract

Experimentally initiated epidemics of infectious pancreatic necrosis in rainbow-trout fry were analyzed using a modification of the standard mathematical model for a simple propagative epidemic. Contrary to expectations, the value of the transmission parameter (β) was inversely related to initial density of susceptible hosts. This anomaly can be explained if we assume that the experimental epidemics were point-source rather than propagative epidemics. The implications of this conclusion for modeling experimental and natural epidemics are discussed.
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1. Introduction

Infectious pancreatic necrosis (IPN) is a serious disease of salmonid and some non-salmonid fishes, and is the result of an acute birnavirus (IPNV) infection. Epidemics occur in rainbow-trout hatcheries worldwide, and peak mortalities occur in fish that are 30–60 days old (Wolf, 1988; McAllister, 1993; Noga, 1996). In a retrospective survey of 415 rainbow-trout hatcheries in the USA and Canada, Bebak (1996a) reported that 3% of hatcheries experienced at least one epidemic of IPN during the 2-year period covered by the survey.

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There are no effective treatments for IPN and — although a recombinant IPNV vaccine is commercially available for use in salmon in Norway (Frost and Ness, 1997; Pettersen, 1997) — there is no vaccine currently available in the US for any salmonid species. Nevertheless, it seems likely that most integrated strategies for controlling IPN in rainbow trout will eventually involve vaccination (Bootland et al., 1990, 1995; Leong et al., 1997). Accordingly, we set out to estimate what fraction of a trout population must be protected by vaccination to prevent an epidemic and to provide a means of assessing the likely consequences (in terms of fish mortality) of partial vaccine failure (McLean, 1994). Fundamental to these calculations is an estimation of the basic reproduction ratio (R_0) for IPN in trout (Anderson and May, 1992). The basic reproduction ratio is defined as the expected number of secondary cases produced by a typical infected animal during its entire period of infectiousness in a demographically stable susceptible population (Heesterbeek, 1992). If $R_0 > 1$, an epidemic will occur; if R_0 is < 1, an epidemic will not occur. A laborious (but informative) method of estimating R_0 is to fit a mathematical model of the epidemic to experimentally created epidemic episodes. The value of R_0 then can be obtained by substituting the values for model parameters estimated during the fitting process into a formula for R_0 uniquely derived from the model equations. IPNV infections are particularly suited to this kind of laboratory manipulation. Trout fry are small and easily cultured. Infection is easily initiated, dead fish are easily recognized and counted, and virus loads both in fish and in surrounding water can be detected and measured (Maheshkumar et al., 1991a,b; Bebak, 1996b; Bebak et al., 1998; McAllister and Bebak, 1997). Thus, in addition to the primary goal of estimating R_0, we undertook the experiments described in this paper to evaluate IPNV infections as a laboratory model for investigating the dynamics of propagative epidemics.

The paper begins with a description of the relevant population biology of IPNV infections in young trout and the associated model assumptions. This is followed by a description of the mathematical model used to estimate R_0. This model rests on the usual assumption that the epidemic is a propagative phenomenon. Next, we describe two experiments in which IPN epidemics were initiated and monitored. The second of these experiments has been described in detail elsewhere (Bebak et al., 1998) but is important to our interpretation of the results of the first experiment and so is briefly summarized here. After that, we show that the results of the first experiment were not consistent with the conventional assumption that we were dealing with a propagative epidemic. Finally, we present evidence that the character of the IPNV epidemics we observed in the laboratory are better explained in terms of the dynamics of a point-source epidemic.

2. The population biology of IPNV infection and model assumptions

2.1. Age-dependent mortality and the natural progression of the infection in individual fish

Clinically apparent IPN epidemics occur in rainbow trout that are <6 months old. Case mortality in these young fish can be as high as 100%. Experimental-infection trials using fingerling trout indicate that there is a latent period of about 2 days after infection before infected fish begin to shed detectable quantities of IPNV into the water and a further (less well

defined) period of 2–4 days before clinical signs become apparent (Bebak, 1996b). Accordingly, our model applies only to trout <4 months of age and begins with the explicit assumption that infected fish can be divided into three distinct classes: infected but not-yet-infectious, infectious but with no clinical signs, and infectious with clinical signs. Only fish in the last of these three classes were assumed to be at risk of death due to IPNV infection.

2.2. How the virus is introduced into a susceptible population

The water supply for a hatchery can harbor fish and invertebrates that are carriers of the IPNV. The introduction of free virus via the water supply is thought to occur, although no one has yet isolated IPNV from open or closed water supplies (Maheshkumar et al., 1991a; McAllister and Bebak, 1997).

IPNV can be introduced into a naive facility via virus-contaminated eggs, fry and fingerling life stages (Wolf, 1988; McAllister, 1993; Noga, 1996). Predaceous birds can transfer infected fish between raceways and from water supplies outside of a hatchery and IPNV has also been imported in bird feces (McAllister and Owens, 1992) and on bird bills, beaks, feathers and legs (Peters and Neukirch, 1986). In our model, we assume that the virus is introduced in fish that are in the infected but not-yet-infectious stage.

2.3. Modes of transmission within the population

Once IPNV has been introduced into a population, horizontal transmission occurs as a result of ingestion of contaminated feces, water, or other materials and as fish pass contaminated water through their buccal cavity and over their gills. Although IPNV has been isolated from reproductive products (Fijan and Giorgetti, 1978; Ahne, 1983; Mulcahy and Pascho, 1984), true vertical transmission through virus contained within oocytes and sperm has not been demonstrated (McAllister, 1993; Sadasiv, 1995). Our model begins with a population of wholly susceptible, never previously infected fish about 30 days old, and assumes that the only possible mode of transmission is horizontal.

2.4. The events following recovery

Survivors of an IPN epidemic become persistent carriers and intermittently shed virus in excretory and reproductive products (Dorson, 1982; Wolf, 1988; Bootland et al., 1991). In natural salmonid populations, intermittent shedding by carriers is thought to be a principal mechanism by which the virus ensures endemic persistence (McAllister, 1993). Maternally transmitted immunity has not been demonstrated. Given the very short time span considered by our model, we ignore the carrier state. In the model, infected fish that recover are assumed to play no further part in the epidemic. Given that maternal immunity has not been demonstrated, the model assumes that all fish are wholly susceptible to infection no matter how young.

2.5. Batch production of trout and the occurrence of IPN epidemics

Our model population is assumed to consist of a cohort of same-age fish. This approximates the situation on commercial trout farms in which batch production is

common. The total population is usually segregated into cohorts of fish that are similar in age or size, with young fish introduced into hatch house or grow-out tanks made vacant by the relocation or sale of older cohorts. These young fish can become infected at the egg, fry or fingerling stage. In our model, infection occurs at the fry stage.

3. Basic architecture of the mathematical model

The model is a development of the well-known basic epidemic model (Scott and Smith, 1994) and assumes that we begin with a cohort of fish of the same age. The host population is divided into compartments representing susceptible fish (X), fish that are infected but not yet infectious (H), infectious fish that are not yet showing clinical signs (Y_1), infectious fish that are showing clinical signs (Y_2), and fish that were infected and now are immune to further infection or have become carriers that are not currently shedding virus (Z) (Fig. 1). Contrary to usual practice, we also include a compartment (M) which represents the fish that have died as a result of the infection. This was necessary to facilitate model fitting. The fish in each compartment are represented in terms of their density per liter of water (fish/l).

Susceptible fish (X) are assumed to move into the infected but not-yet-infectious class (H) at a net rate $\beta X(Y_1 + Y_2)$, where β is the transmission parameter. Fish in class H move into the infectious class with no clinical signs (Y_1) at a constant instantaneous per capita rate (σ). Fish in infectious class (Y_1) move into the second infectious class (Y_2) at a constant instantaneous per capita rate (γ). Fish recover at a constant instantaneous per capita rate (δ). Fish in the infectious class that shows clinical signs (Y_2) that die as a result of IPNV infection do so at a constant instantaneous per capita rate (α). There are no deaths due to IPNV infection in the other infectious class (Y_1). During the course of the experiment, death due to causes other than IPNV infection was negligible and so background mortality is ignored in this model. The equations describing the rate of change in density in each compartment are

$$\frac{dX}{dt} = -\beta X(Y_1 + Y_2) \tag{1}$$

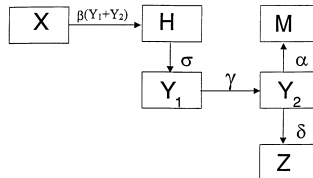$$\frac{dH}{dt} = \beta X(Y_1 + Y_2) - \sigma H \tag{2}$$

Fig. 1. Schematic representation of the compartments of the model. The rate parameters associated with each arrow represent the instantaneous, per capita rates of transition from one compartment to the next. All symbols are defined in Table 1.

Table 1
Parameter definitions for the compartmental model shown in Fig. 1 and described in the text

Symbol	Definition
X	Density of susceptible fish (fish/l)
H	Density of infected but not yet infectious fish (fish/l)
Y_1	Density of infectious fish not yet showing clinical signs (fish/l)
Y_2	Density of infectious fish that are showing clinical signs (fish/l)
M	Density of dead fish (fish/l)
Z	Density of fish that were infected and are now immune or have become carriers not currently shedding virus (fish/l)
N	Total density fish (fish/l) (N_0 = initial host density)
β	Transmission coefficient (per fish per day)
σ	Reciprocal of the time spent per fish in the infected but not yet infectious state (per fish per day)
γ	Reciprocal of the time spent per fish in compartment Y_1 (per fish per day)
α	Mortality rate of the fish in compartment Y_2 (per fish per day)
δ	Recovery rate (per fish per day)

$$\frac{dY_1}{dt} = \sigma H - \gamma Y_1 \tag{3}$$

$$\frac{dY_2}{dt} = \gamma Y_1 - \alpha Y_2 - \delta Y_2 \tag{4}$$

$$\frac{dZ}{dt} = \delta Y_2 \tag{5}$$

$$\frac{dM}{dt} = \alpha Y_2 \tag{6}$$

The model parameters are defined in Table 1.

3.1. Transmission

The transmission parameter (β) was derived based on the assumption that we were dealing with an uncomplicated propagative epidemic with homogeneous mixing of all classes of host. We began with a standard formulation for incidence: the instantaneous per capita force of infection (λ) (Smith, 1994). By definition

$$\frac{dX}{dt\,X} = -\lambda \tag{7}$$

Incidence (λ) is made up of three components: $c(N)$ is a function of the total host density (N; fish/l) and represents the rate at which any given susceptible host makes contact with any other host (susceptible, infectious or immune). $(Y_1 + Y_2)/N$ is the probability that the contact will be with an infectious host, π is the probability that the contact with an infectious host will lead to infection (De Jong et al., 1995). We assumed that the contact rate varied directly with the density of hosts (i.e. that $c(N) = \kappa N$, where κ was a constant of proportionality, representing the increase in the contact rate that accompanies every

unit increase in density). Thus

$$\frac{dX}{dt} = -\lambda X = -\pi\kappa N \frac{(Y_1 + Y_2)}{N} X \tag{8}$$

and so

$$\frac{dX}{dt} = -\pi\kappa(Y_1 + Y_2)X = -\beta(Y_1 + Y_2)X \tag{9}$$

where $\beta = \pi\kappa$, and Eq. (9) is the same as Eq. (1) of the model. Notice that changes in N due to disease-induced mortality during the course of the epidemic are irrelevant to this formulation. See Appendix A for further discussion of this representation of transmission.

In an earlier version of the model, each infectious class of fish (Y_1 and Y_2) was associated with a transmission parameter of different magnitude (β_1 and β_2, respectively). This was because previous work indicated that the rate at which newly infected fish shed IPNV increased to a maximum level over a span of 2–3 days (Bebak et al., 1998). We sought to provide a crude representation of this continuous process by distinguishing between the transmission parameter allotted to each class of infectious fish. In the end, we abandoned this more-complicated model because it provided no better fit to the data than one in which we assumed that the numerical value for β_1 and β_2 did not differ (see Bebak, 1996b, for a more detailed discussion).

4. Parameter estimation

Numerical model solutions and parameter estimates were obtained using the Episode algorithm and least-squares fitting procedures in the Scientist Software package (Micromath, Utah). The results for each epidemic were fitted in turn. Preliminary work suggested starting values for σ and δ (data not shown). Other plausible starting values were arrived at by trial and error.

Descriptive statistics, regression diagnostics (LVR2PLOT procedure) and all linear regression analyses (REGRESS procedure) were carried out using STATA version 4 for Windows (StataCorp, 1995). In the REGRESS procedure, the significance of the regression was tested using the F-statistic, derived from the upper-tail cumulative F-distribution with df_1 numerator and df_2 denominator degrees of freedom. For all statistical tests, an alpha-level of 0.05 was used to reject the null hypothesis.

5. Methods

5.1. Virus preparation and recovery

As described in Bebak et al. (1998), the chinook-salmon embryo-cell line (CHSE-214; Lannan et al., 1984) was used to prepare IPNV (neutralization serotype VR-299) stocks and for all infectivity assays. Stock IPNV originated from plaque-purified preparations of IPNV isolated during an IPN epidemic involving hatchery-raised rainbow trout and was

two cell-culture passages from primary isolation. The plaque assay (Wolf and Quimby, 1973), which is the gold standard for detection of IPNV, was used for all fish and water samples. All samples were assayed individually. All virus plaque counts were blinded, i.e. the tank from which the fish or water originated was unknown. Virus concentrations were expressed as plaque-forming units per milliliter (PFU/ml) for aqueous samples and as plaque-forming units per gram (PFU/g) for tissue samples. The lowest limit of detection of IPNV was 2.0×10^1 PFU/l in water and 2.0×10^2 PFU/g in fish tissue. Virus used for challenges and virus recovered from fish during the experiments was identified as IPNV by dot-blot enzyme-linked immunosorbent assay (McAllister and Schill, 1986).

Recovery of IPNV from water proceeded as described in Maheshkumar et al. (1991a,b) and McAllister and Bebak (1997), a method that results in ≥95% recovery of virus. About 5 l of water was filtered through a bilayer filter matrix. Adsorbed virus was eluted from the filter matrix by addition of two 5 ml aliquots of 3% beef extract in triple-distilled water (pH 10.0). The solution containing eluted virus was adjusted to pH 7.1–7.2 by the addition of 1N HCl. This solution was passed through a 0.45 μm low-protein-binding membrane filter and assayed for viral infectivity by the plaque method.

For fish-infectivity assays, individual fish were weighed and then homogenized with alundum. The homogenate was diluted 1:10 (w/v) in 0.1 M phosphate-buffered saline (PBS) at pH 7.2 and centrifuged at $1500 \times$ gravity (g) for 20 min at 4°C. The supernatant was passed through a 0.45 μm membrane filter, and subsequent dilutions were assayed for viral infectivity by the plaque method as described above.

5.2. Immersion challenge

Specific-pathogen-free rainbow trout were obtained from Erwin National Fish Hatchery (TN), a Class A fish-culture facility (USFWS, 1995). During the immersion challenge and experiments, fry were kept in polypropylene tanks (1.0 l culture volume; McAllister et al., 1983) supplied at about 250 ml/min with 12°C, specific-pathogen-free springwater (pH 7.1; alkalinity 160 mg/l; hardness 230 mg/l as $CaCO_3$). All fry were fed at 5% body weight per day (Zeigler Brothers, Gardners, Pennsylvania; Piper et al., 1982). A standardized immersion challenge was used to infect fish with IPNV (McAllister and Owens, 1986). Stock virus was diluted in 0.1 M phosphate buffered saline (PBS, pH 7.2) and added to a tank containing fish at a density of 1 g fish per 25 ml water to achieve a concentration of 10^5 PFU of IPNV/ml of tank water. Fish were exposed for 5 h with static conditions and aeration. At the end of the exposure, water flow was restored to 250 ml/min.

5.3. Design of experiment 1 and sampling schedule

The purpose of experiment 1 was to initiate and monitor epidemics of IPN in fish populations with a known starting density of infected and susceptible fish. In half the tanks, we used one immersion-infected fish to introduce the virus; in the rest we used three immersion-infected fish. The total intended density of infected and susceptible fish in each tank was 25, 50, 75 or 100 fish per 1 l tank (Table 2). There were three replicates at each density and infection protocol. The intended total densities (susceptible plus those

Table 2
Densities of infected and susceptible fish used in experiment 1[a]

Group	Tank number	Sham-infected fish	Infected fish	Intended density of susceptible fish (three tanks per density)			
1	1–12	0	1	24	49	74	99
2	13–24	0	3	22	47	72	97
3	25–28	1	0	24	49	74	99
4	29–32	3	0	22	47	72	97

[a] Infected fish were exposed to infectious pancreatic necrosis virus.

infected initially) were chosen to be near to, or greater than, the density of 0.5 pounds of fish per cubic feet of water recommended for culturing 1 in. rainbow trout (Piper et al., 1982). Control tanks using sham immersion-infected fish were run in parallel with the epidemic tanks. The purpose of the control tanks was to ensure that the observed mortalities could be attributed plausibly to IPNV infection and to monitor for exogenous IPNV.

On Day 0 of experiment 1, 32 tanks were randomly assigned, using a lottery, to four groups (Table 2). The 48 infected fish originated from a single tank of 66 fish that had been exposed to IPNV by immersion challenge about 22 h earlier. The remaining 18 fish that were not used for either Groups-1 or -2 tanks were assayed for IPNV 48 h after initial exposure. Groups-3 and -4 ran in parallel to Groups-1 and -2 but used sham immersion-challenged fish. For all groups, infected and susceptible fish were added to the tanks according to the protocol outlined in Table 2. All fish were 34 days old (0.39 g average body weight) on day 0 of the experiment. The fish were monitored for 59 days.

To ensure free-running epidemic episodes, only dead fish were removed from the tanks. Dead fish were removed, counted and recorded once a day. Beginning on day 3 of the experiment and continuing until day 46, water was sampled from sixteen of the epidemic tanks at 48–96 h intervals and processed to recover virus as described in Bebak et al. (1998). Using a lottery, the tanks to be sampled were selected at random at the beginning of the experiment. The same tanks were sampled for the duration of the experiment except for tank 18, which was lost on day 11 when its water supply was inadvertently turned off. Another tank (tank 16) with the same combination of infected and susceptible fish was substituted for tank 18. Water was sampled from two control tanks (tanks 31 and 32) on day 46. On day 59, all the fish were killed with a lethal dose of tricaine methane sulfonate (MS-222). The number of fish remaining in each tank was counted.

Between 1 and 20% of the starting density in each tank of fish from Groups-1–4 were assayed for virus (132 fish in all). Some of the dead fish were assayed immediately and some were stored at $-70°C$ until they were assayed about 2 months later.

5.4. Design of experiment 2 and sampling schedule

In experiment 1, we were interested in maintaining a free-running epidemic for the entire 50–60 day span of the epidemic. To ensure a free-running epidemic, only dead fish

were removed from the tanks. Although we were able to process a proportion of these removed dead fish for IPNV assay, we had no information on how many of the still-living fish had become infected. We entirely depended upon fish mortality as an indirect measure of the progress of the epidemic. To monitor the acquisition of virus in living fish during an IPN epidemic, we repeated the general protocol of experiment 1 but with destructive sampling of live fish. In this second experiment (experiment 2), the number of IPNV assays was kept within manageable bounds by considering only a single initial host density (25 fish/l) and focusing on the early stages of the epidemic (the first 9 days).

Experiment 2 (including all controls) has been described in greater detail elsewhere (Bebak et al., 1998). Three immersion-infected fish were placed in each of 14 treatment tanks. Twenty-two unexposed, susceptible fish from the same lot as the immersion-infected fish were added to each tank. The fish were monitored for 9 days. Dead fish were counted and removed from the tanks daily and stored at −70°C pending assay for IPNV. Beginning on day 2 after primary immersion challenge and continuing every 24–48 h, water was collected from two randomly selected treatment tanks and processed to recover IPNV. All of the fish in the sampled tanks were then killed with a lethal dose of MS-222, rinsed with pathogen-free spring water, weighed and stored at −70°C pending assay for IPNV.

6. Results

6.1. Experiment 1 (control tanks, Groups-3 and -4)

The overall cumulative incidence of death in the control tanks (numbers 25–32) was 1.9% during the day 59 of the experiment (nine fish dead out of 486). The nine control fish that died were assayed for IPNV and none was detected in any of the fish. Further, virus was not found in the water samples taken from the two control tanks (tanks 31 and 32) sampled on day 46. On a tank-by-tank basis, a slight but significant increase in overall cumulative incidence of death was associated with increasing actual fish density (using arcsine-transformed data, cumulative incidence of mortality $= 0.00036N − 0.008$; $F_{(1,6)} = 9.15$, $p = 0.02$, $r^2 = 0.6$). This background mortality was ignored when fitting model parameters.

6.2. Experiment 1 (epidemic tanks, Groups-1 and -2)

Each of the 18 immersion-infected fish not added to Groups-1 and -2 tanks was positive for IPNV. A total of 132 dead fish from the epidemic tanks were assayed by the plaque method. Of these, 126 were positive for IPNV. A subset (35) of these fish was selected by blind grab sample and the presence of IPNV was confirmed by dot-blot enzyme-linked immunosorbent assay (McAllister and Schill, 1986).

In tanks 2, 3, 4 and 6 (Group-1, one infected fish at the start, total number of fish in each tank was 25, 26, 35 and 32 fish, respectively), an epidemic did not occur. No deaths occurred in tanks 2 and 3, and only two deaths (both IPNV-positive) occurred in tanks 4 and 6. Concentrations of IPNV in the water of tanks 2, 3, 4, and 6 were <1 PFU/ml
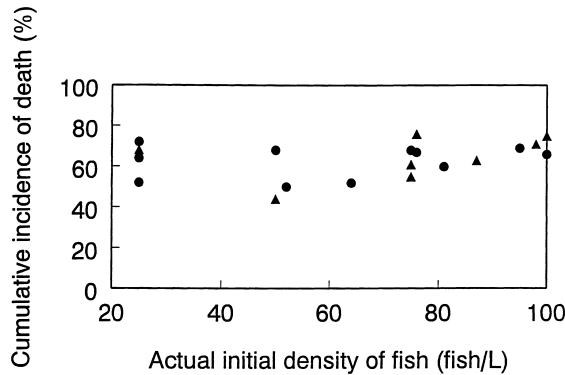
Fig. 2. Experiment 1. The total number of fish deaths during the course of epidemics of infectious pancreatic necrosis. Results from eight Group-1 tanks: (▲) one infected fish/tank at start; and 11 Group-2: (●) three infected fish per tank at start; are shown.

throughout the experiment. The data from these tanks, and from tank 18 (see above), were excluded from the analysis.

The overall cumulative incidence of death in each tank used in the analysis is shown in Fig. 2. By inspection, there was no systematic relationship between the cumulative incidence of death and actual initial fish density. The average cumulative incidence of death in the Group-1 tanks (one infected fish at the start) and the Group-2 tanks (three infected fish at the start) was 64.1% (95% confidence interval: 55.0, 73.2%) and 64.4% (95% confidence interval: 57.3, 71.5%), respectively. An example of the day-by-day cumulative mortality in Groups-1 and -2 tanks is shown in Fig. 3, and corresponding values for IPNV concentration in water of the respective tanks are shown in Fig. 4.

6.3. Experiment 2

An average of 84% of the susceptible fish had become infected with IPNV by the end of day 9. The combined results for individual tanks are plotted in Fig. 5. The data presented in Fig. 5 does not show the steadily rising exponential pattern expected of the early stages of an epidemic — but, rather, a sudden, step-like increase in the number of infected fish over a 24 h period (between days 4 and 5). By inspection, there was no further change in the mean number of infected fish after day 5.

7. Estimated model parameters

The fitted values for each model parameter are given in Table 3. For each parameter, regressions were carried out with and without the tanks identified as outliers by the LVR2PLOT procedure. For all parameters except delta (see below), the decision to accept or reject the null hypothesis did not change when outlier tanks were removed from the regression analyses.
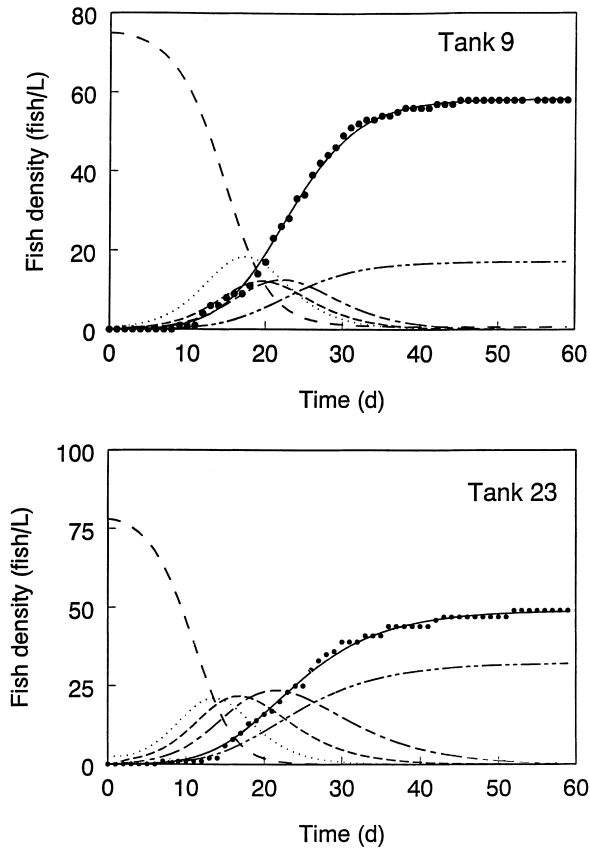
Fig. 3. Experiment 1. Typical examples of the fitted densities of fish (lines) obtained by fitting the model to the observed cumulative number of dead fish ($\bullet$). Fish were infected with infectious pancreatic necrosis virus. X (— —): density of susceptible fish (fish/l); H ($\cdot \cdot \cdot$): density of infected but not-yet-infectious fish (fish/l); Y_1 (– –): density of infectious fish not yet showing clinical signs (fish/l); Y_2 (— – – — – -): density of infectious fish that are showing clinical signs (fish/l); Z (— - - —): density of fish that were infected and are now immune or have become carriers not currently shedding virus (fish/l); M (———): density of dead fish (fish/l).

With two exceptions, the fitted values for model parameters did not change with initial host density. For Group-2 data alone (but not for Groups-1 and -2 combined), as initial host density (N) increased, there was a small increase in the value of σ (the rate at which an infected fish moved to a state in which it was shedding IPNV) ($\sigma = 0.001N + 0.17$; $F_{(1,9)} = 5.37$, $p = 0.046$, $r^2 = 0.37$). Similarly, after omitting one outlier, the analysis indicated that, as initial host density (N) increased, there was a small (but significant) decrease in the fitted value of delta (the rate at which an infected fish moved into the state in which it was no longer shedding IPNV) ($\delta = -0.0005N + 0.01$; $F_{(1,16)} = 5.18$, $p = 0.04$, $r^2 = 0.24$). We cannot account for these relationships. While it is conceivable that the rate at which an infected fish
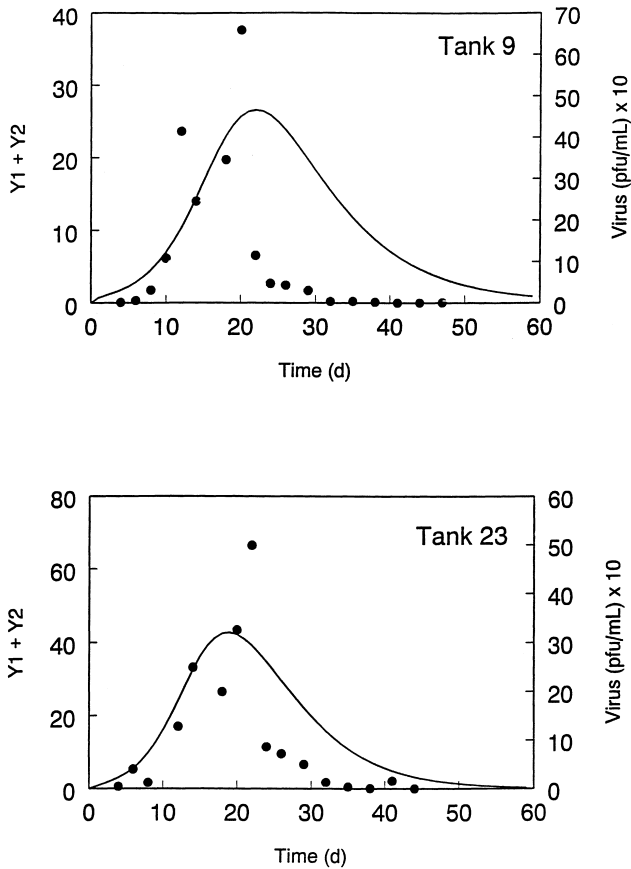
Fig. 4. Experiment 1. Typical examples of observed changes in virus concentration (●) in the tank water compared with the fitted densities of infectious fish (compartments Y_1 (density of infectious fish not yet showing clinical signs (fish/l)) and Y_2 (density of infectious fish that are showing clinical signs (fish/l)) combined, solid line). Fish were infected with infectious pancreatic necrosis virus. Notice that virus concentration does not track the fitted densities of fish shedding virus into the water.

begins shedding (σ) should be higher at higher host densities (increased population stress, increased virus challenge initially), we can conceive of no biological reason why the recovery rate should increase with increasing fish density (and, presumably, increased population stress). Given the fact that the finding concerning sigma was not consistent, and that there is no clear biological explanation for a change in delta, we tentatively dismiss the relationships as artifactual. Less-easily dismissed is the decline in the value of the transmission coefficient (β) that accompanied an increase in initial host density (Fig. 6). The decline in the value of β was still highly significant even when the data were reanalyzed without the results from tanks containing <40 fish ($\beta = 0.015 - 0.0001N$; $F_{(1,13)} = 11.63$, $p = 0.0046$, $r^2 = 0.47$).
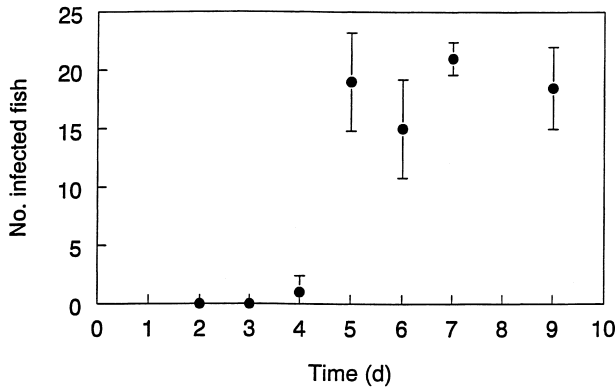
Fig. 5. Experiment 2. Average number of initially susceptible fish (±S.D.) from which infectious pancreatic necrosis virus was recovered at intervals after first exposure to fish that had been infected with the virus. Each tank contained 22 susceptible and three infectious fish at the start of the experiment.

Table 3
Estimated model parameter values for epidemics of infectious pancreatic necrosis[a]

Tank	Density of fish (fish/l)			Transmission coefficient (per fish per day)	σ	γ	α	δ
	Total	Susceptible	Infected, not yet infectious					
1	25	24	1	0.028	0.510	0.805	0.036	0.010
13	25	22	3	0.037	0.194	0.221	0.213	0.079
14	25	22	3	0.029	0.219	0.246	0.088	0.077
15	25	22	3	0.029	0.196	0.201	0.124	0.072
5	50	49	1	0.009	0.375	0.431	0.143	0.139
16	50	47	3	0.014	0.301	0.235	0.120	0.054
17	52	49	3	0.011	0.204	0.226	0.105	0.102
20	64	61	3	0.006	0.157	0.190	0.101	0.061
7	75	74	1	0.007	0.281	0.281	0.061	0.041
8	75	74	1	0.006	1.183	1.928	0.080	0.045
21	75	72	3	0.008	0.329	0.189	0.123	0.058
9	76	75	1	0.011	0.281	0.387	0.264	0.077
19	76	73	3	0.009	0.365	0.381	0.082	0.037
23	81	78	3	0.009	0.287	0.225	0.096	0.063
11	87	86	1	0.005	0.307	0.144	0.103	0.049
22	95	92	3	0.007	0.262	0.108	0.204	0.087
12	98	97	1	0.007	0.322	0.247	0.107	0.040
10	100	99	1	0.005	0.750	0.310	0.143	0.038
24	100	97	3	0.006	0.320	0.258	0.092	0.044
Mean				0.013	0.360	0.369	0.120	0.062
±95% C.I.				0.004	0.108	0.183	0.0248	0.013
$F_{(1,17)}$				54.90	1.23	0.01	0.09	1.17
P-value				<0.001	0.28	0.91	0.77	0.29

[a] Values are organized by increasing values of N_0 (initial host density). The F- and P-values refer to the linear regression of each parameter value on N (=total initial fish). Note the systematic changes in the values for the transmission parameter (β).
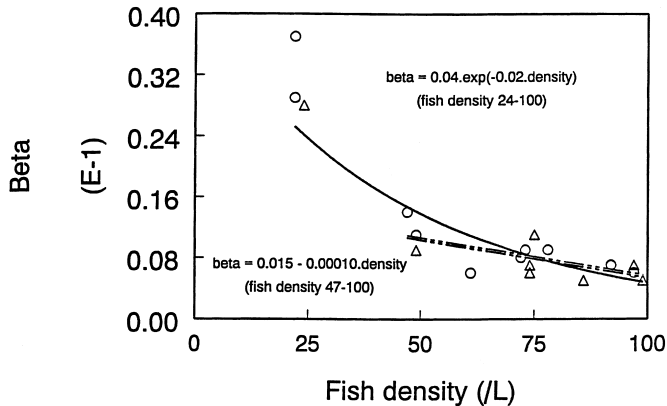
Fig. 6. Experiment 1. The relationship between the fitted value of the transmission parameter (β) and the actual initial density of fish (/l): ($\triangle$) Group-1 tanks, one infected fish/tank at start; ($\bigcirc$) Group-2 tanks, three infected fish/tank at start. Fish were exposed to infectious pancreatic necrosis virus. The figure shows the exponential function fitted to the entire data set and the linear function fitted to data corresponding to initial intended densities 50–100 fish/l. In both cases there is a significant decline in the value of β as initial fish density increases.

8. A propagative or point-source epidemic?

Our original goal was to estimate the parameters needed to estimate the basic reproduction ratio (R_0) for an IPN epidemic in rainbow trout which was initially assumed to be propagative in nature. Based on the assumptions used to construct the model, the formula for the basic reproduction ratio was

$$R_0 = \frac{\beta N}{\gamma} + \frac{\beta N}{(\delta + \alpha)} \tag{10}$$

Implicit in the formulation of the transmission parameter was that β was independent of host density — which was clearly not the case. To account for the systematic change in β with initial host density, we considered two other formulations for β that still retained the assumption that we were dealing with a propagative epidemic.

8.1. Does the contact rate saturate?

Diekmann et al. (1994) suggested that the contact rate ($c(N)$) saturates as effective host density (N) increases. This assumption is particularly pertinent when dealing with a highly contagious or sexually transmitted disease when actual contact between individuals is required for transmission. A plausible form for a saturating contact rate (Heesterbeek, 1992) is

$$c(N) = \kappa_1(1 - e^{-\kappa_2 N}) \tag{11}$$

In this case, the contact rate increases with host density to an asymptotic value (κ_1) at a

rate determined by the constant (κ_2). If this functional form is used, the transmission constant (β) becomes

$$\beta = \frac{\pi\kappa_1(1 - e^{-\kappa_2 N})}{N} \tag{12}$$

The value $(1 - \exp(-\kappa_2 N))$ increases from 0 to 1 as host density increases, while $\pi\kappa_1/N$ decreases. Given the values of N that were used in experiment 1, the value of $\pi\kappa_1/N$ will likely predominate and the value of β will decline with host density as $\pi\kappa_1/N$ decreases.

We do not believe this formulation explains our results because physical contact between fish is not required for a fish to become infected with IPNV. Fish become infected as they force water containing IPNV through the buccal cavity and over the gills. It seems likely that the concentration of virus in the water will increase with the concentration of infected fish shedding virus in their feces. Given the rapid rate of water exchange required to maintain trout fry with oxygenated water, it also seems likely that the assumption of homogeneous mixing of virus and susceptible fish is particularly appropriate. Further — and most importantly — the cumulative incidence of death in experiment 1 was the same for all densities of fish. Thus, we may assume that the attack rate was the same in all cases and that the density of infectious fish increased with initial host density. For these reasons, we believe that individual fish in experiment 1 were more likely (rather than less likely) to encounter IPNV when the initial fish density was high. This would not be predicted using a model with a saturating contact rate.

8.2. Do the fish remove substantial quantities of virus from the water?

Another possible explanation arises if we assume that infection occurs as the result of ingesting or adsorbing virus directly from the water and that this rate of virus removal is large. In that case, increasing densities of fish will proportionately reduce viral concentrations in the water and reduce the measured value of β. A model framework representing this possibility was suggested by Dr. M. Roberts (personal communication). The basic model (see Appendix A) was modified to include an equation representing virus concentration (V):

$$\frac{\mathrm{d}V}{\mathrm{d}t} = v(Y_1 + Y_2) - \xi V - \rho VN \tag{13}$$

where v is the rate at which individual fish shed IPNV, ξ the rate of virus loss due to water exchange in the tank, and ρ a constant which determines the rate at which virus is ingested or adsorbed by the fish (N). The equation for the infected but not-infectious fish (H) was modified as follows:

$$\frac{\mathrm{d}H}{\mathrm{d}t} = \tau VX - \sigma H \tag{14}$$

where τ is a constant of proportionality which determines whether or not infection occurs (see Appendix A).

When ξ is large, virus concentrations equilibrate rapidly (see Appendix A). Bebak et al. (1998) suggested that virus concentrations equilibrate within about 30 min. Thus, from Eq. (13), the equilibrium virus concentration in the water is

$$V^* = \frac{v(Y_1 + Y_2)}{\xi + \rho N} \tag{15}$$

Substituting V^* for V in the Eq. (14), we get

$$\frac{\mathrm{d}H}{\mathrm{d}t} = \frac{\tau v}{(\xi + \rho N)} X(Y_1 + Y_2) - \sigma H \tag{16}$$

and the transmission parameter (β) is given by

$$\beta = \frac{\tau v}{(\xi + \rho N)} \tag{17}$$

which clearly decreases as fish density (N) increases.

Note that this formulation explains the results of experiment 1 only if ρ is relatively large. As ρ approaches zero, the formula for the transmission coefficient approaches the standard form used in the model (i.e. β is independent of host density). Bebak et al. (1998) report that even after 2 days of virus replication, fish infected by immersion challenge had a total virus titer of only 0.06% of the total virus initially introduced into the tanks for immersion challenge. This is not indicative of a large value for the rate of ingestion or adsorption of IPNV.

8.3. A point-source epidemic?

Following the failure of each of the propagative epidemic models to account for the decline in the transmission parameter, we considered the possibility that we may have been monitoring point-source epidemics. Three results suggest that the IPN epidemics observed in experiment 1 may have been point-source rather than propagative epidemics. First, when the virus acquisition in susceptible fish was monitored directly by IPNV assay, the number of infected fish rose to a maximum value over a 24 h period (experiment 2, between days 4 and 5). There was not the exponential pattern expected of the early stages of an epidemic. Second, when only one immersion-challenged fish was used to precipitate an epidemic, there was no evidence of transmission to susceptible fish in 4 out of 12 tanks. This is consistent with the hypothesis that virus concentrations in the water had to reach a mean infective dose before IPNV could become established in the susceptible fish. Once this mean infective dose was attained, all susceptible fish became infected almost simultaneously. The mean infectious dose was always attained when three immersion-infected fish were used to precipitate the epidemic, but only 67% of the time when one immersion-infected fish was used. Third, if the epidemics in experiment 1 had been propagative rather than point-source epidemics, IPNV concentration in the water would be expected to track the predicted densities of infectious fish. This was not the case. Even when scaled to allow for differences in the units of measurement, virus concentration in the water clearly declined at a much faster rate than the predicted density of infectious fish (Fig. 4) (Bebak, 1996b).

The question remains whether a point-source epidemic would explain the declining value for the transmission coefficient obtained given the assumption of a propagative epidemic. From experiment 2, it seems that the cumulative incidence of infection (attack risk) for IPNV in the fish populations used in experiment 2 was 84%. The cumulative incidence of death did not vary with fish density in experiment 1 and so — assuming a constant case-mortality risk for this particular host/virus combination — we can also assume that the cumulative incidence of infection did not vary with host density in experiment 1. This is what would be expected of a point-source epidemic because the proportion of hosts in which disease is expressed is a function of host phenotype and behavior rather than host density. This is not what would be expected of a propagative epidemic because in this latter case, the proportion of hosts infected depends upon the dynamics of transmission, and hence, host density. The cumulative incidence of infection (A) for a model of a simple propagative epidemic (of which our model is merely a slight modification) is given by

$$A = 1 - e^{-R_0 A} \tag{18}$$

The cumulative incidence of infection (A) in a propagative epidemic depends upon the basic reproduction ratio, R_0, which depends in turn upon the initial density of susceptible hosts (Smith, 1994). If the observed cumulative incidence of infection remains constant irrespective of initial host density, then a fit based upon the assumption of a propagative epidemic will result in parameter values that change with host density in order to maintain a constant basic reproduction ratio. Rewriting Eq. (10) for ease of reference:

$$R_0 = \frac{\beta N}{\gamma} + \frac{\beta N}{(\delta + \alpha)} \tag{19}$$

we see that if host density (N) is fixed by the experimenter, the model-fitting procedure will necessarily reduce the value of β to compensate for experimentally determined increases in host density.

8.4. What meaning should be attached to the pattern of host mortality?

If the epidemics observed in experiment 1 were indeed point-source epidemics, then we were wrong to assume that the pattern of host mortality was an indirect index of the progress of the epidemic. Rather, the cumulative mortality plotted in Fig. 3 is best viewed as a cumulative distribution function for the time to death. The sigmoidal nature of the pattern of host mortality reflects the variability in the time to death that is an inherent property of this particular host/virus combination.

9. Discussion

Our primary goal was to estimate a value for the basic reproduction ratio for IPN in rainbow trout fry. This goal was based on the premise that experimentally precipitated IPN epidemics were straightforward propagative epidemics rather than point-source epidemics. We now believe that assumption was wrong. We have presented evidence that

the experimentally precipitated epidemics had characteristics more akin to point-source outbreaks. One consequence of wrongly assuming a propagative transmission process was that the fitted value for the transmission coefficient decreased as initial host density increased. We also conclude that this IPNV/trout fry system was not a suitable experimental model for the study of propagative epidemics — despite some attractive features.

There is increasing interest in the experimental study of the transmission dynamics of microparasitic infections of fish and in the application of simple epidemic models to aid interpretation (Reno, 1998). Based upon our results, we urge caution in the choice of model.

In many infectious diseases of veterinary interest, transmission can occur by several mechanisms, including transmission via fomites or other vehicles such as food or water (e.g. salmonellosis, colibacillosis and bovine tuberculosis). In the simple epidemic model (with its fundamental assumption of homogeneous mixing of hosts or of hosts and pathogen) the rate of transmission is determined solely by host density. In reality, not all susceptible hosts are exposed to the same risk of infection — nor do all hosts experience the same probability of being infected as the result of particular modes of transmission. Although considerable progress has been made in modeling heterogeneities in transmission within a single population, all relevant models assume that the mode of transmission is the same and dependent in the same way on some aspect of host density (Anderson and May, 1992). In the case of salmonellosis, colibacillosis, bovine tuberculosis and diseases with similar transmission characteristics, only part of the total transmission will depend upon host density. The rest of the population will become infected by point-source transmission. Although it is possible to envisage mechanisms by which host density can affect the proportion of animals exposed to a point-source of pathogen (where farm animals are housed, for example), point-source transmission from the modeler's point of view will often appear to operate as a series of random — almost instantaneous — perturbations that increase the density of infected individuals. What matters is whether the proportion of the population infected in this way is large or small. As the proportion increases, the simple epidemic model becomes less and less relevant.

These conclusions would seem to conflict with the firmly established principle in aquaculture that the severity of a disease outbreak is directly affected by fish density. Indeed — in aquaculture production systems, control of mortality is often achieved through manipulation of fish density (Wedemeyer and Wood, 1974; Piper et al., 1982; Fagerlund et al., 1984; Mazur et al., 1993; LaPatra et al., 1996). When such manipulations work, they might be doing so not as a direct consequence of changes in propagative dynamics — but via some other mechanism (e.g. improved immunocompetence through alleviation of stress resulting from poor water quality conditions, competition for feed and other agonistic interactions).

Appendix A. Further discussion of $c(N)$

$c(N)$ represents the rate at which any given susceptible host makes contact with any other host. In the model described in the main text, we assumed that the rate of contact varies directly with host population density (N). That is, $c(N) = \kappa N$, where κ is a constant of proportionality. It might be objected that IPN is not a contagious disease in the sense that virus is acquired directly from the water rather than by actual contact with other fish. This objection can be dealt with as follows: suppose that each infected fish (Y) sheds virus at a constant rate (v). Suppose also that the per capita rate of change in the density of susceptible fish (X) is directly proportional to the virus concentration in the water (V). In that case, we have

$$\frac{dX}{dt} = -\tau VX \tag{A.1}$$

$$\frac{dY}{dt} = \tau VX \tag{A.2}$$

$$\frac{dV}{dt} = vY - \xi V \tag{A.3}$$

where τ is a constant of proportionality and ξ represents the rate of loss of virus from the immediate environment. When ξ is large (as it is with the fast flow of a trout raceway), the viral concentration will quickly equilibrate to some value $V^* = vY/\xi$. Substituting V^* for V in equation (X) we get

$$\frac{dX}{dt} = -\tau \frac{vY}{\xi}X = -\beta YX \tag{A.4}$$

where $\beta = \tau v/\xi$ and we have recovered an equation of the same form as Eq. (1) in the model.

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