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Population dynamics of lymphocystis disease in estuarine flounder, *Platichthys flesus (L.)*

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The population dynamics of viral lymphocystis disease in an age-structured population of flounder, *Platichthys flesus* (Linnaeus 1758), is studied with a mathematical model. For a single cohort the model illustrates the influence of host density and acquired immunity on length-prevalence profiles. This case corresponds to a host population at demographic equilibrium. When the model is extended to several cohorts, seasonal recruitment of susceptible hosts is shown to drive seasonality in disease prevalence. In both cases, there is good qualitative agreement between model predictions and field data from the Elbe estuary, Germany.

Key words: flounder; *Platichthysflesus;* lymphocystis virus; population dynamics;

1. INTRODUCTION

Lymphocystis is a common viral infection in numerous teleost fish species. Its pathology and epidemiology have recently been reviewed by Wolf (1988) and Anders (1989).

Fish infected with lymphocystis exhibit characteristic, usually external lesions consisting of hypertrophic connective tissue cells. Virus replicates in the cytoplasm of these cells and late in the pathogenesis is released into the water when the cells disintegrate (Wolf & Carlson, 1965). Waterborne virus enters fish via some epithelium, mainly the gill surface (Wolf, 1984,1988). Gills are a privileged portal, both for their large surface area and the ventilation of water over them.

Infectiousness, with disintegrating cells releasing pathogen into the water and the lesions, is concomitant with a strong immune response (Russell, 1974; Roberts, 1975). This response is presumably instrumental in the well-documented complete recovery of farmed fish (Matsusato, 1975; Paperna *et al.*, 1982; Tanaka *et al.*, 1984; Masoero *et al.*, 1986). Antibody activity to lymphocystis has been found in apparently healthy flounder *(Platichthysflesus)* by Lorenzen & Dixon (1991), indicating that recovery is common in wild flounder and possibly goes along with acquired immunity. Wolf (1962) found evidence of protective immunity to lymphocystis in bluegill, *Lepomis macrochirus* (Rafinesque 1819).

A possible link between prevalence of lymphocystis in flounder and marine pollution has motivated a number of disease surveys (Reiersen & Fugelli, 1984; Vethaak, 1985). The field data used in our study concern a flounder nursery area, the Elbe estuary in Germany, where the disease has been studied extensively since 198 1. Sampling used a beam trawl, at several stations and at monthly to quarterly

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intervals. Details and results of the surveys during the periods September 1981-September 1982 and May 1985-May 1986 have been published in two reports (Moller, 1984, 1988) and are discussed further in Anders (1984, 1988, 1989) and Moller (1990).

The virus population is entirely dependent on its host throughout its life cycle and can thus be described by the disease status of fish. The fish population is divided into four compartments containing susceptible, infected (but not yet infectious), infectious and immune individuals.

A model predicts the spread of lymphocystis in flounders for an age-structured population of fixed size. At first, recruitment is continuous, constant and equal to death, so that the age-structure is stable. The model is used to study the influence of host density and duration of immunity on disease prevalence. The model is then extended to include seasonal recruitment, causing seasonality in disease prevalence.

II. SINGLE COHORT

The spread of lymphocystis in a single, and initially all-susceptible cohort of flounders, was followed over 10 years. This is equivalent to looking at disease status in an age cross-section of a population at dynamic equilibrium, when the age structure is stable.

MODEL

The model describes the transition of fish between disease states with increasing age of the cohort (Fig. 1). It is adapted from that of Anderson & May (1982).

Densities of susceptible, infected, infectious and immune fish are denoted X, H, Y and Z, respectively, and sum up to N, the ever decreasing density of fish in the cohort. All fish are susceptible at recruitment ($N(0)$). With increasing age a, fish either become infected at a rate $\lambda(a,\bar{Y})$, the force of infection, or leave the population at a rate $m(a)$, through death or emigration. Both rates vary with the age of the host. While mortality *per se* is assumed to be age-independent, emigration from the estuary is linked to maturation, thus mortality and emigration combined depend on age. Infected fish become infectious at a rate s, the inverse of the duration of the infected stage, or leave the population (Fig. 1). Both infected and infectious fish show gross signs of disease and are assumed to be subject to some additional disease induced mortality at a rate d. Infectious fish either recover and acquire immunity at a constant rate g, or leave the population at the rate $m(a)+d$. Immune individuals lose their immunity to be susceptible again at a rate u, the inverse of the duration of immunity. They do not show signs of disease and are only subject to non-disease mortality and emigration, at a rate $m(a)$.

The model consists of four ordinary differential equations:

$$\frac{dX}{da} = -\{m(a) + \lambda(a, \bar{Y})\} X(a) + u Z(a) \quad (1)$$

$$\frac{dH}{da} = \lambda(a, \bar{Y}) X(a) - \{m(a) + d + s\} H(a) \quad (2)$$

$$\frac{dY}{da} = s H(a) - \{m(a) + d + g\} Y(a) \quad (3)$$

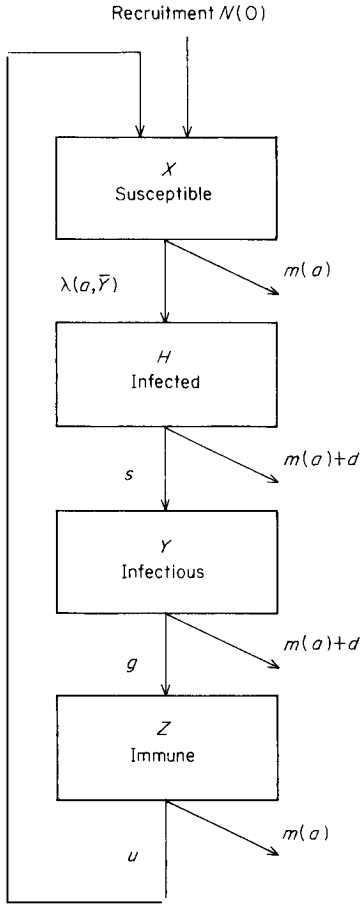

FIG. 1. Flowchart of the model, showing the possible transitions between disease states and the corresponding rates.

$$\frac{dZ}{da} = g\,Y(a) - \{m(a) + u\}\,Z(a). \tag{4}$$

These equations combine gain and loss terms for the four compartments illustrated in Fig. 1. In equation (1), for example, $\{m(a)+\lambda(a, \bar{Y})\}X(a)$ susceptible fish die or become infected, and $u\,Z(a)$ immune fish become susceptible again. In a situation of dynamic equilibrium, the total density of infectious individuals of all ages is constant over time, and is given by integrating $Y(a)$ over age:

$$\bar{Y} = \int_{a=0}^{\infty} Y(a)da. \tag{5}$$

The force of infection $\lambda(a, \bar{Y})$ describes exposure of susceptible fish to the pathogen. It depends on the density of waterborne virus, which is itself proportional to the total density of infectious fish $\bar{Y}$. Exposure also depends on the portal available to the virus to enter susceptible fish, the gill surface. Gill surface area increases with fish metabolic rate, i.e. approximately with fish weight raised to

TABLE I. Parameter values

$N(0)$ $N(0)i$	Initial all-susceptible population densities	1000 fish
$1/s$	Duration of the infected phase	2 months
$1/g$	Duration of the infectious phase	5 months
$1/u$	Duration of acquired immunity	2 years
$m(a)$	Mortality and emigration	0·4 per year for $a<2{\cdot}2$ years 1·4 per year for $a>=2{\cdot}2$ years
d	Disease induced mortality	0·2 per year
k	VBGF k	0·3 per year
L_∞	VBGF L_∞	40·0 cm
a_0	VBGF a_0	−0·3 years
β	Transmission coefficient ($cm^{-2{\cdot}6}$ per fish per year)	
	Single cohort	$5{\cdot}05 \times 10^{-9}$
	Multi-cohort	$5{\cdot}90 \times 10^{-9}$

the power 0·86 (Brett & Groves, 1979). Weight is itself proportional to the third power of length, thus making gill size proportional to fish length to the power 2·6. Fish length is derived from age via the von Bertalanffy growth function (VBGF). The force of infection, for a susceptible fish of age a, among $\bar{Y}$ infectious fish becomes:

$$\lambda(a, \bar{Y}) = \beta \bar{Y}\{L_\infty(1-e^{-k(a-a_0)})\}^{2{\cdot}6} \quad (6)$$

with β the transmission coefficient, and L_∞, k and a_0, the von Bertalannfy growth parameters.

The system of equations is solved numerically, with the initial condition that all fish are susceptible at recruitment and given the constraints defined by equations (5) and (6). All parameter values are listed in Table I. They were chosen on the basis of laboratory experiments (Anders, 1984) and unpublished field data on the population biology of flounder in the Elbe. Parameter values cannot be statistically estimated from existing data, and approximate values are used. Model predictions are thus intended to provide qualitative rather than quantitative insight.

MODEL PREDICTIONS

In Fig. 2, infected and infectious fish are pooled to give disease prevalence. All fish are susceptible at recruitment, and hardly develop disease in their first year. Exposure rises quickly with the rapid growth of fish in the first 4 years, resulting in a fast increase in $\lambda(a, \bar{Y})$, the rate at which new infections are acquired. In 2-year-old flounders, disease is present but immunity, concomitant with a recovery from infectiousness, is still rare. In older age groups the frequency of immune fish exceeds that of diseased ones and increases to about 40% in 10-year-olds. The counteracting effects of increasing exposure and a decreasing proportion of susceptibles stabilizes prevalence around 10% after 5 years.

Immune fish are expected to show specific antibody activity to lymphocystis in serological tests. Predicted immunity profiles could be compared to serological surveys, such as by Lorenzen & Dixon (1991).

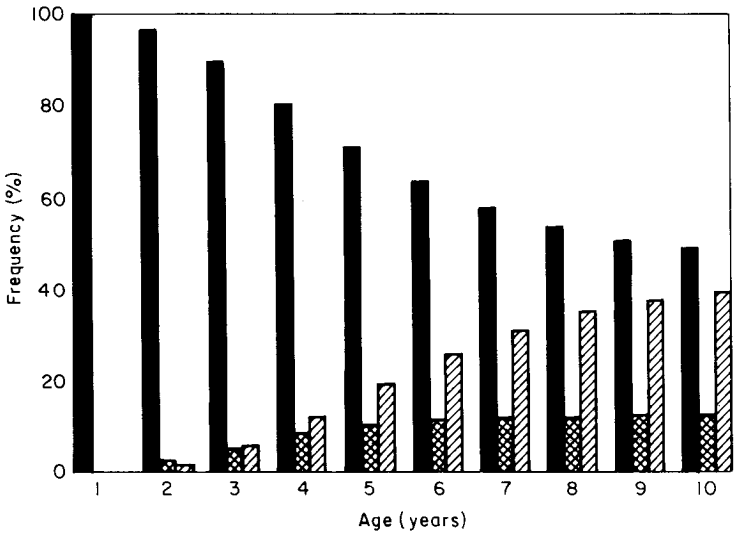

FIG. 2. Single cohort: change in disease status with age. Frequency of ■, susceptible; ⊠, diseased (i.e. infected and infectious fish) and ▨, immune fish.

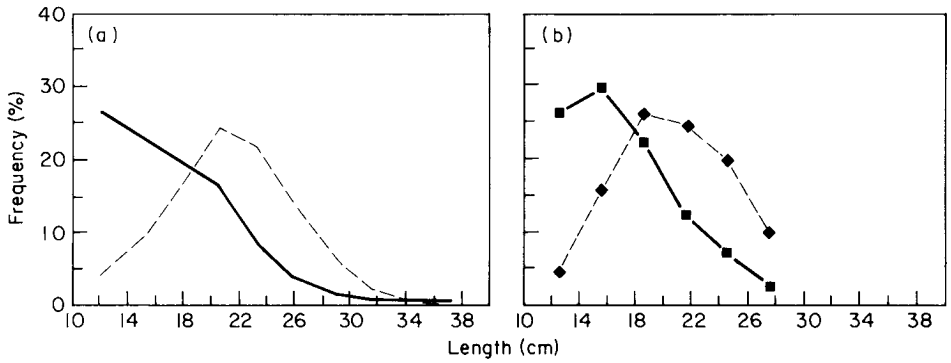

FIG. 3. Single cohort: length frequency distributions, (——) all fish, (— —) diseased fish. (a) predicted by the model, (b) average of observations on flounder in the Elbe estuary 1981–1986. In any one length group the standard error is around 0·4 for all fish and 4 for diseased fish.

Length structure of the population

Age-based model predictions are transformed to length using the VBGF. The length-frequency distribution given by the model is very similar to the observed average length-frequency distribution in Elbe estuary flounder (Fig. 3). The estuarine population is dominated by young and immature fish, smaller than 20 cm. Although prevalence is highest in old and large fish (Fig. 2), their low density means that they contribute little to disease transmission.

Population density and duration of acquired immunity

Predicted length-prevalence profiles [Fig. 4(a) and (b)] compare well with observed averages in Elbe estuary flounder given in Fig. 4(c). Lymphocystis is directly communicated from infectious to susceptible individuals thus making

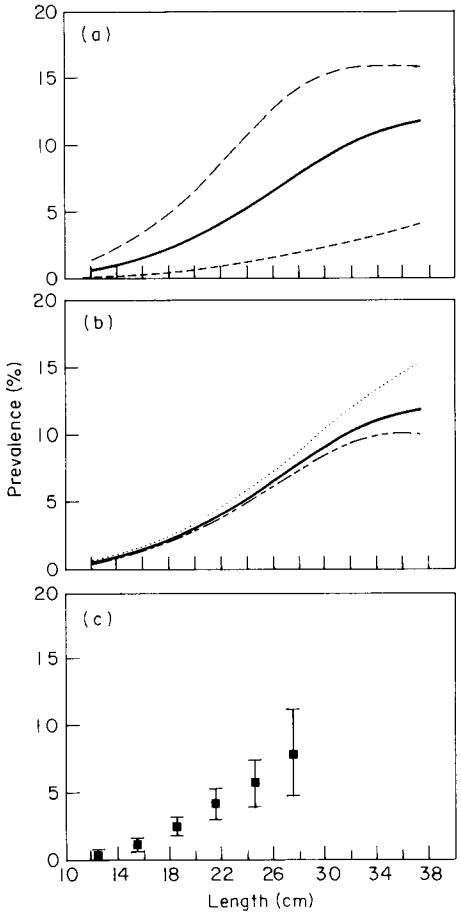

FIG. 4. Single cohort: predicted and observed length prevalence profiles. (a) Increasing the initial host density $N(0)$, with (– – –) for 900; (——) for 1000; and (— —) for 1100. (b) Increasing the duration of acquired immunity ($1/u$) from (· · ·) 1 year, to (——) 2 years, and (— – –) 3 years. (c) Observed average prevalence in flounder from the Elbe estuary 1981–1986, with 95% confidence limits.

transmission dependent on population density. A 10% variation in initial density (from 1000 fish, cf. Table 1) leads to dramatic changes in disease prevalence [Fig. 4(a)]. A higher density causes the disease to spread quicker, thus prevalence is higher and stabilizes earlier.

The duration of immunity has a different effect on the length prevalence profile, with longer lasting immunity leading to lower prevalence and early stabilization [Fig. 4(b)]. In young, fast growing fish, disease prevalence is chiefly determined by growing exposure to the pathogen, whereas in larger fish exposure changes little and duration of immunity becomes a prime determinant of prevalence.

111. MULTIPLE COHORTS

The population dynamics of a single cohort is equivalent to the dynamics of a multi-cohort population with recruitment and death continuous and equal in time. In temperate fish populations however, recruitment is seasonal, and can be expected to generate some periodicity in disease prevalence.

MODEL

Seasonal recruitment produces discrete generations recruited into the population as separate cohorts. Thus the basic model is extended by combining n cohorts. The densities of fish in the four compartments for the ith cohort are denoted X_i, H_i, Y_i and Z_i, for the susceptible, infected, infectious and immune fish, respectively, and N_i the density of each cohort, initially of all-susceptible fish. The multi-cohort population is represented by n sets of four ordinary differential equations:

$$\frac{dX_i}{dt} = -\{m(a_i) + \lambda(a_i, \bar{Y}(t))\} X_i(t) + u Z_i(t) \tag{7}$$

$$\frac{dH_i}{dt} = \lambda(a_i, \bar{Y}(t)) X_i(t) - (m(a_i) + d + s) H_i(t) \tag{8}$$

$$\frac{dY_i}{dt} = s H_i(t) - (m(a_i) + d + g) Y_i(t) \tag{9}$$

$$\frac{dZ_i}{dt} = g Y_i(t) - (m(a_i) + u) Z_i(t) \tag{10}$$

with $i = 1, \ldots, n$.

The overall density of infectious fish is now obtained by adding the densities of infectious individuals in all cohorts:

$$\bar{Y}(t) = \sum_{i=0}^{n} Y_i(t). \tag{11}$$

The force of infection remains as in equation (6), and all parameter values but the transmission coefficient are kept the same. A slightly different value for the transmission coefficient (Table I) is necessary to obtain seasonal prevalences that correspond to the average values. This is due to the change in population–age distribution from continuous to discrete.

MODEL PREDICTIONS

Annual variations in density of diseased fish and prevalence are shown in Fig. 5, for the youngest five cohorts. The density of diseased fish increases rapidly at first, to reach a peak at the onset of emigration 2.2 years after recruitment. In the multi-cohort extension, it becomes obvious that such a pattern of recruitment and emigration results in only two generations dominating the transmission process at any given time of the year [Fig. 5(a)].

During the year, prevalence [Fig. 5(b)] rises continuously in each cohort, although slowly for older fish, and distribution of diseased fish among the generations is determined both by increasing prevalence and decreasing cohort density.

Seasonality

Model predictions are now transformed into length using a normally distributed length at age with a constant standard deviation of 3 cm. The transformation is somewhat crude and the results are again intended to provide a basis for qualitative

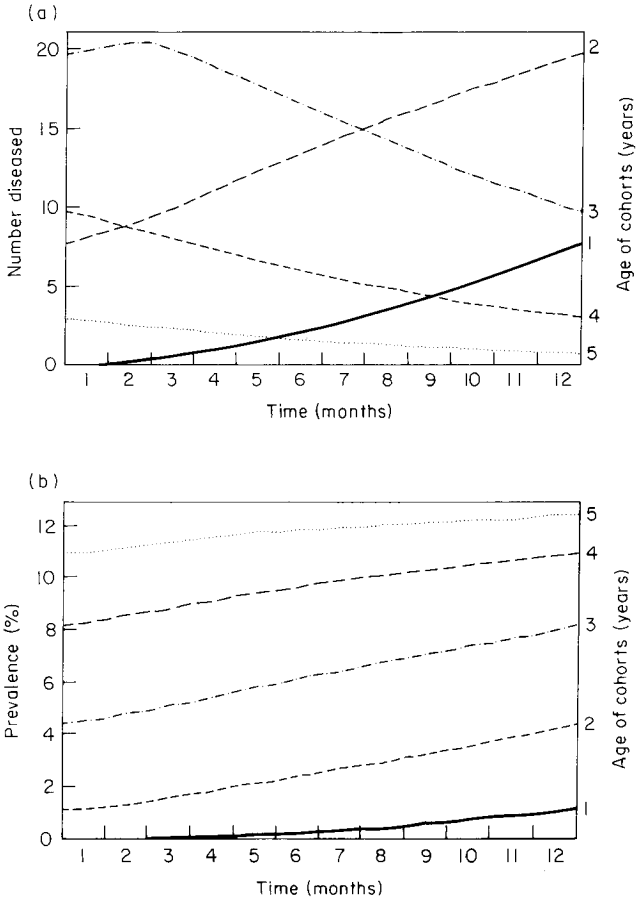

FIG. 5. Seasonal variations in a multi-cohort population: (a) Number of diseased fish for the five youngest cohorts, and (b) lymphocystis prevalence. Cohort age at the beginning of year (——) 0 years, (— —) 1 year, (— · —) 2 years, (– – –) 3 years and (· · ·) 4 years.

comparison only. Predicted prevalence and length-frequency distributions are averaged over 3 months, and compared to the averaged observations made between 1981 and 1986 in the Elbe estuary in Fig. 6.

As the youngest cohort grows and the predicted length increases from 15-5 cm in the autumn to 21.5 cm the following summer, the corresponding disease prevalence increases continuously. In each of the length groups however, prevalence shows a seasonal variation due to the changing age composition in the length group. Overall predicted and observed changes in length-prevalence patterns are remarkably similar, indicating that seasonal recruitment in the fish population is likely to drive seasonality in lymphocystis prevalence.

IV. DISCUSSION

Both fish population dynamics and duration of immunity are important factors controlling the prevalence of lymphocystis, crucial to the interpretation of observed prevalence patterns.

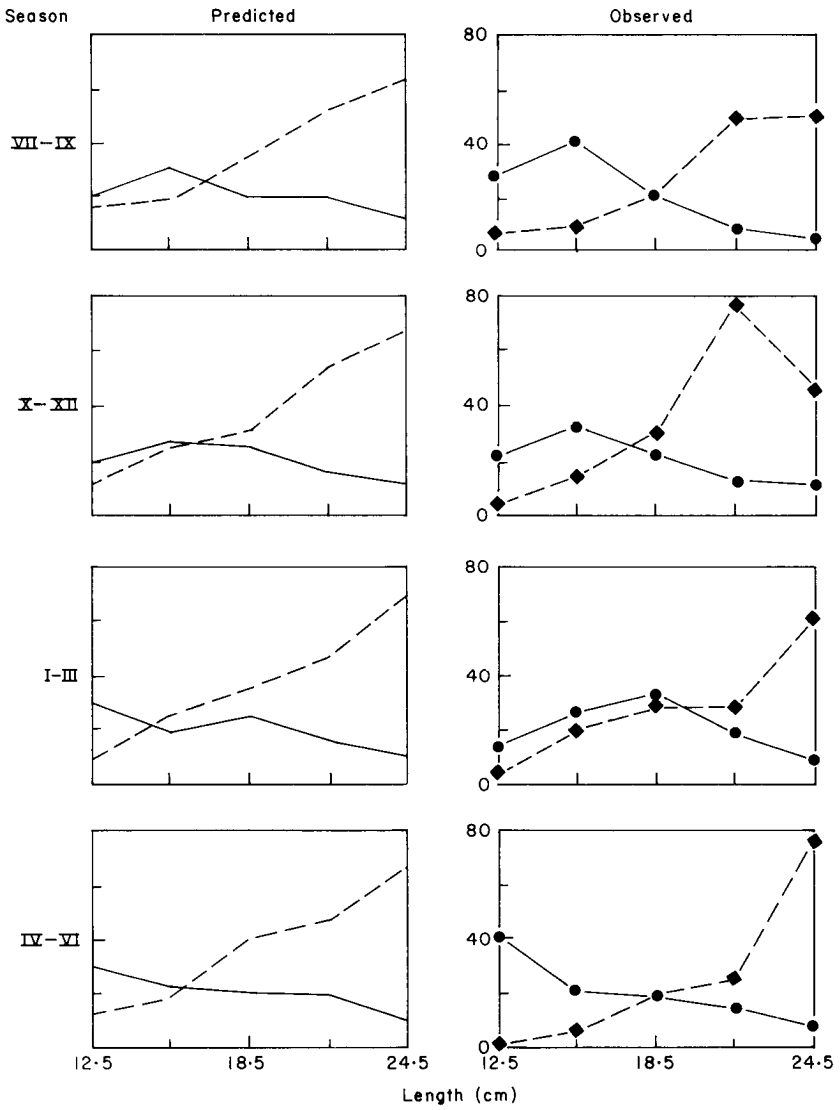

FIG. 6. Multi-cohort population: predicted and observed variations in the length–prevalence and length–frequency distributions over four seasons. ——, Length–frequency; – – –, length–prevalence × 10. Observations for flounders from the Elbe estuary, 1981–1986. In any one length group, standard error is below 1 for the length–frequency distribution, and ranges from 0·26 to 1·9 for the prevalence, with an approximately constant coefficient of variation of 0·3.

A link between exposure and gill surface area implies that the force of infection is more closely linked to the size and to the age of the host. Flounder are characterized by a high growth plasticity. Therefore a future more realistic model of lymphoeystis disease population dynamics may need to be both age- and size-structured. However, the hypothesis of size-dependent exposure needs to be tested explicitly. This may be achieved from surveys collecting information on both age and size of the fish.

This theoretical study indicates that acquired immunity is of major importance in the population dynamics of lymphocystis. Serological surveys and a more thorough understanding of the immune response to lymphocystis would facilitate the interpretation of observed prevalence patterns. Results of a first serological survey of Elbe flounder (Lorenzen & Dixon, 199 1) are in good accordance with the age-sero-prevalence profile predicted in Fig. 2.

Immunosuppression may link the dynamics of viral infections to environmental parameters (Vos et al., 1989). In theory immunosuppression can influence disease prevalence in different ways. If it increases the duration of visible disease, or decreases the duration of immunity, this will result in higher prevalence. If, on the other hand, immunosuppression increases disease-induced mortality, prevalence will be decreased. In practice it will be necessary to design laboratory experiments and serological surveys to assess the effect of specific environmental parameters on the immune response to and pathology of lymphocystis.

It is assumed here that the host population is evenly distributed in space and that any sub-populations mix homogeneously. Such assumptions may be valid in estuarine nursery areas, but do not hold for the spawning stock. Adult populations are highly aggregated during the spawning season and this is likely to drive most of the seasonality in disease prevalence. Segregation between the sexes during part of the year (Vethaak, 1985) is a further source of heterogeneity in adult fish.

The estuarine nursery is a unique environment for disease transmission in flounders. Susceptible fish form a high proportion of the population, and they are periodically supplied with new recruits, creating seasonal variation in disease prevalence. However, the combination of low exposure for small fish and emigration of mature fish means that only two year classes sustain the disease at any one time. Thus variable recruitment, through density-dependent transmission, can result in extremely variable disease prevalence.

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