

Reprint from:  
**Fish**  
Ecotoxicology and Ecophysiology  
T. Braunbeck, W. Hanke, H. Segner (Eds.)

## **Acquired immunity to infectious diseases in fish: implications for the interpretation of fish disease surveys**

*Kai Lorenzen* \*

Institute for Marine Sciences, University of Kiel, Dusternbrooker Weg 20, D-W-2300 Kiel 1, Federal Republic of Germany

### **Abstract**

Fish disease surveys form a part of many biological monitoring programmes in European marine waters. Prevalence patterns of infectious diseases within wild fish populations are strongly influenced by the acquisition of immunity in individual fish. Hence, the interpretation of observed prevalence patterns requires understanding of the role of acquired immunity and immunomodulation in disease population dynamics. This review attempts to amalgamate information on the immune response in individual fish with theoretical considerations of its population consequences. Different approaches to the detection of acquired immunity in wild fish are discussed and recommendations for further research are made.

Keywords: epidemiology, epizootiology, population dynamics, serological surveys

### **Introduction**

Biological monitoring is concerned with the measurement of anthropogenic changes to organisms and populations. In the aquatic environment, fish and their disease conditions have long been at the focus of interest of both monitoring scientists and the public. This is especially true for the North Sea area, where fish disease surveys form a major part of national and international monitoring programmes (Anders and Moller 1991, Bucke and Watermann 1988, Dethlefsen 1980, 1984, Dethlefsen et al. 1987, Moller 1985, 1988, 1990, Vethaak 1985).

\* Present address: Renewable Resources Assessment Group, Imperial College of Science, Technology and Medicine, 8 Princess Gardens, London SW7 1NA, United Kingdom

VCH Verlagsgesellschaft mbH, D-6940 Weinheim, 1993

Many disease conditions found in demersal marine fish in Europe and used for biological monitoring are infectious. Lymphocystis, an iridovirus infection (Anders 1989, Wolf 1988), and skin ulcers often associated with *Vibrio anguillarum* and other bacteria (Ullrich 1991) are common and easily recognized. Other conditions are due to fungi, protozoa, helminths and crustaceans, but are less conspicuous, and, hence, less well suited for monitoring. I will therefore confine my discussion to viral and bacterial infections, with special emphasis on lymphocystis and ulcers.

Fish disease surveys are concerned with populations (in the sense of organisms of the same species sharing a certain habitat at a certain time) rather than individual fish. The statistic usually measured is the prevalence of disease, i.e., the proportion of fish showing gross pathology. The prevalence of an infectious disease is the visible outcome of the interaction between a population of pathogens and a population of fish hosts. One of the central processes in this interaction is the acquisition of immunity: immunity is acquired by contracting and surviving an infection, and it prevents the host from showing any further visible interaction with the pathogen. On the population level, immune fish are in effect removed from the host population. Hence, it is important to assess which proportion of a population sampled during a fish disease survey is in the immune state.

Only a few publications have addressed the problem of acquired immunity in wild fish populations explicitly, but there is a collection of circumstantial evidence. My task is that of assembling a jigsaw puzzle with some pails missing while others cannot yet be assigned a definitive place. Following a brief introduction to acquired immunity against viruses and bacteria in teleost fish, I will review some methods which can be used to detect acquired immunity in wild fish and some results obtained from them. I will then discuss the consequences of acquired immunity on the population level. Finally, I will try to identify some areas where progress is most needed if our understanding of the role of acquired immunity in the emergence of prevalence patterns is to be improved.

## The immune response to infectious diseases in fish

The immunology of fish is less well understood than that of mammals or even birds, although considerable progress has been achieved in recent years in areas relevant to the vaccination of farmed fish. The immune response of fish under natural conditions is even more of a grey area.

Resistance to and recovery from first infection are the results of complex interactions between non-specific and specific defense mechanisms. Acquired immunity to reinfection is mediated by lymphocytes and effected mainly through antibodies which can neutralize viruses, facilitate phagocytosis of pathogens through opsonization, and activate complement via the classical pathway (Sakai 1984). Only one class of antibody has been demonstrated in teleost fish, corresponding to the IgM class in mammals on the basis of heavy chain molecular weight (Dorson 1981, Ellis 1989). Fish IgM are usually tetrameric as opposed to the pentameric structure of mammalian IgM. The role of fish antibodies in the recovery from disease has not been studied in detail, but high activities of neutralizing or agglutinating antibodies can be induced by vaccination and provide complete protection against certain infections (Ellis 1989).

The occurrence of fish IgM is not limited to the serum. Antibodies are also found in the mucus covering fish epithelia, and these IgM may be of local origin rather than derived from the serum (Fletcher and White 1973, Lobb and Clem 1981, Paleteiro and Richards 1985). Ellis (1988b) suggested that the immune system of fish could be viewed as consisting of a systemic and a mucosal part. Following oral vaccination against *Vibrio* spp. bacteria, specific serum antibody activity indicates protection. Often, however, some degree of protection is acquired in fish not showing serum antibody activity (Croy and Amend 1977). This may be caused by mucosal antibodies against *Vibrio* spp., which have been found in fish with no or little demonstrable serum antibody (Fletcher and White 1973, Kawai et al. 1981).

Lymphocyte proliferation and antibody titres in fish take longer to reach a peak after challenge with a pathogen, and antibody titres in the secondary response are less increased with respect to the primary response than in mammals (Ellis 1989). Acquired immunity in fish seems to be of transient nature, but may persist for well over a year after vaccination (Johnson et al. 1982, Paterson et al. 1981) and possibly longer following acute disease.

Is acquired immunity in wild fish always preceded by gross pathology? This question has not yet been addressed explicitly, but is of considerable importance, when it comes to assessing the population consequences of immunity. Conventional wisdom holds that serum antibody production occurs only when infections have become systemic (Dorson 1981). This is probably true for viral infections, where a high level of antigen is available only after virus replication in the host. Bacterial pathogens may be present in high density in the environment and the normal microflora of fish, but can they elicit an immune response without generalized pathology? Stolen et al. (1983) induced serum antibody activity to sewage sludge-associated bacteria, many of which are not pathogenic to fish, by bath and in *situ* exposure. Whether antibodies acquired in this way provide reliable protection remains to be shown, but is not unlikely. Moreover, as mentioned above, mucosal antibodies may provide sufficient protection and their production does not require invasion of the host (Lobb 1987).

The immune reaction in fish is influenced by endogenous rhythms (Zeeman 1986) and environmental parameters, of which temperature is by far the most important. Low temperatures generally impair the ability of fish to mount a specific immune response, though the details are subject to controversy (Avtalion 1981, Ellis 1981, 1989) and differences exist between species. Another important factor is nutrition (Henken et al. 1987, Landolt 1989), which may be subject to enormous variation within and between wild populations.

A wide range of chemicals are known to modulate the immune response of fish. These include agricultural pesticides (Zeeman and Brindley 1981) as well as antibiotics used in fish farming (Grondel et al. 1987, Rijkers et al. 1980). Immunomodulation (more specifically, immunosuppression) is thought to be one possible mechanism relating the prevalence of infectious diseases to pollution. In the case of viruses, which, unlike bacteria, cannot benefit from increased organic loads in the environment, immunomodulation is the only such mechanism. The important question in relation to the prevalence of infectious disease, however, is: How, if at all, does immunomodulation influence the pathogenesis

of disease? Does it result in increased disease-induced mortality, delay recovery, impair the development of immunological memory or increase susceptibility to first infection? I will attempt a theoretical and somewhat speculative discussion of the population consequences of immunomodulation-related changes in disease pathogenesis in the last section. First, however, I will review some methods that can be used to assess the immune status of wild fish.

### **Detecting immunity in wild fish**

Classical fish disease surveys quantify the relative frequency of fish showing gross pathology and those that do not. In order to understand why an observed pattern of prevalence has developed, it is necessary to separate the "apparently healthy" group into the epidemiologically relevant susceptible and immune (latent in some cases) classes. Immunity here refers to complete protection, hence, the aim of such an investigation is to reach a "yes or no" decision on the immune status of individual fish.

Surveys of acquired immunity in wild fish populations may also provide a means of estimating disease-induced mortality, provided that all immune individuals are survivors of gross pathology. However, the estimation of mortality rates requires indices of the absolute abundance at age (as opposed to proportions) of susceptible, diseased and immune fish.

Several methods can be employed to detect acquired immunity in wild fish, all of which have some advantages and problems. I will discuss three of them: challenge experiments, the detection of antibodies and the measurement of lymphocyte response to challenge with a pathogen. A number of challenge experiments and serological surveys have been conducted in the past, usually with an aim other than to estimate the proportion of immune fish in a population. I will mention some results, but should point out that none of these studies have actually demonstrated acquired, protective immunity to a pathogen in individual fish.

#### *Challenge experiments*

Challenge experiments are commonly used to assess the efficacy of vaccines in aquaculture (Ellis 1988a): fish are exposed to a pathogen and their resistance assessed from the resulting prevalence of disease or mortality. Resistance, however, comprises both specific (acquired) and non-specific immunological mechanisms, as well as possible genetic factors. Challenge experiments are to be supplemented by other studies, e.g. of specific antibody activity, if the investigation focuses on acquired immunity. A practical problem with challenge experiments is that they require the maintenance of a large number of fish in captivity, and that handling stress may seriously affect their immune response.

Robohm and Sparrow (1981) conducted an interesting challenge experiment on summer flounder (*Paralichthys dentatus*). They injected a total of 22 fish from polluted and unpolluted waters with four strains of formalin-killed bacteria, two of which (*Aeromonas salmonicida* and *Vibrio anguillarum*) were fish pathogens, and analyzed the develop-

ment of agglutinating antibody titres. Summer flounder from the most polluted area developed significantly higher antibody titres to all but one (*Aeromonas salmonicida*) strains of bacteria than those from less polluted areas. A booster injection was necessary in all cases to prompt an increase in antibody titres. This led the authors to conclude they were observing a primary response, and to attribute the observed differences to genetic selection of high antibody responders in polluted areas. The authors selected experimental fish for not showing antibody activity prior to challenge. The fact that none of the selected fish seemed to show a secondary response points to the value of antibodies as indicators of immunity. Nevertheless, if experiments of this type were conducted on all fish sampled and using live rather than inactivated pathogens, they could establish not only the proportion of immune fish, but also the precise relationship between antibody levels and protective immunity.

### *Detection of antibodies*

Antibodies are the principal indicators of acquired immunity to pathogens. Their detection using enzyme or radio-immunoassays (ELISA or RIA) is relatively simple and very sensitive. Unfortunately, the relationship between antibody levels and protective immunity is less than straightforward and needs additional experiments to be established. The protective role of antibodies must be demonstrated for the pathogen in question. On the other hand, immunological memory may persist for a longer period than do elevated antibody titres, and, hence, the prevalence of immunity is likely to be underestimated by serological surveys.

Although modern immunoassays are very sensitive, their results may not be easy to analyse. This is partly because the blood chemistry of fish is highly dependent on environmental conditions, nutrition and other factors. Nonspecific reactions in immunoassays may vary by an order of magnitude between fish caught at the same time and place, and may eventually obscure specific antibody activity. Moreover, positive and negative controls are often unavailable for surveys of wild fish populations. The assessment of a sample as antibody positive is then based on its "obvious" or statistically significant difference from the mean level of antibody activity in the population.

Few serological surveys of marine fish populations have been published, and no information is available on mucosal antibodies. Robohm et al. (1979) studied agglutinating antibodies against 36 bacteria in three fish species caught in polluted and unpolluted marine waters. They found antibodies against a greater diversity of bacteria and generally higher antibody titres in summer flounder (*P. dentatus*) and weakfish (*Cynoscion regalis*) from polluted waters than in those from unpolluted waters. This effect was less marked in winter flounder (*Pseudopleuronectes americanus*) sampled in the winter months, which may reflect seasonality in antibody production. The authors present population means of antibody titres rather than the proportion of antibody positive-fish, hence, their data is of limited relevance to the population dynamics of bacterial disease.

Stolen et al. (1985) measured agglutinating antibody activity to sludge-associated bacteria including the fish pathogen *Aeromonas hydrophila* in several species of marine fish caught off the mid-Atlantic coast of the United States. Antibody activity was found

against all bacteria tested, but the proportion of positive fish was highly dependent on the time of year when the samples were taken. The highest antibody activities were recorded against *Aeromonas hydrophila*, indicating that such fish pathogens are better immunogens than other bacteria. Robohm and Sparrow (1981) have reported a similar observation from their challenge experiments.

Lorenzen and Dixon (1991) employed an ELISA to detect antibodies to lymphocystis virus in European flounder (*Platichthys flesus*). This survey was specifically designed to provide information relevant to the population dynamics of lymphocystis disease. Hence, individual flounders were aged and scored as antibody-positive or -negative on the basis of a statistically defined antibody activity threshold. The proportion of antibody-positive flounders was found to increase rapidly with age. This fits in well with predictions obtained from a mathematical model of lymphocystis population dynamics (Lorenzen et al. 1991) and suggests that acquired immunity is indeed an important factor influencing lymphocystis prevalence patterns. However, caution is necessary, because the relationship between antibody levels and protective immunity has not been established for lymphocystis. The statistically established threshold may lack biological relevance.

#### *Lymphocyte activation and proliferation assays*

Lymphocyte activation or proliferation assays have not yet been applied to the detection of acquired immunity in wild fish. Lymphocyte activation and proliferation are the processes at the heart of acquired immunity, and their measurement might be the best way to establish the immunological status of a fish. Moreover, the experiments are conducted *in vitro*, so that conditions can easily be standardized and it is not necessary to maintain large numbers of live fish. Hopefully, this method will receive more attention in the future.

### **Population consequences of acquired immunity**

The use of fish disease surveys in biological monitoring is based on prevalence, a population parameter. Hence, I will now explore the role of acquired immunity in the population dynamics of fish diseases and its influence on prevalence patterns. The term „acquired immunity" refers to a principle rather than the complex mechanisms it entails: it is defined here as protection against gross pathology caused by an infectious agent, acquired by previous infection with the same agent. Usually the previous infection will have been linked to gross pathology with subsequent recovery. The infectious agent may have been eliminated from the host completely in the course of recovery, or it may persist without causing pathology.

Although acquired immunity is invariably of great importance in determining the prevalence of infectious disease, its precise implications depend on the life-history characteristics of both the pathogen and its host. Hence, a number of questions need to be answered, before the role of acquired immunity on the population level can be assessed for any disease: Is the agent an obligatory pathogen, and is it specific to the host species

under study? Does the agent persist in immune fish and, if so, is it continuously being shed? How long does acquired immunity last in the host? Finally, structure and dynamics of the host population have to be investigated. This is a task on its own, but a necessity if the emergence of disease prevalence patterns is to be understood.

A useful and convenient framework for studying the population consequences of acquired immunity is provided by compartmental models of the host population (see, e.g., Anderson and May 1982). The population of fish hosts is divided into compartments containing susceptible, infected, diseased and immune (latent?) individuals. The processes governing the transition of fish between compartments are then studied in detail and, in the model, are expressed as fixed or variable rates. Within this framework, I will discuss two diseases, which are quite different in terms of the questions posed above: lymphocystis and ulcer disease.

### *Viral and bacterial diseases in closed model populations*

Closed populations form reproductive units and are not subject to immigration or emigration. For the moment, I will also assume that individuals within closed populations mix homogeneously, i.e., there is no segregation of sexes or age classes.

I will start by looking at lymphocystis disease because the essentials of its dynamics are readily understood on the basis of virus transmission, acquisition of immunity and the interaction of the two. Lymphocystis virus is a generally host-specific, obligatory fish pathogen transmitted via the water from clinically diseased to susceptible fish only. Acquisition of immunity to the virus probably requires a period of clinical disease. It is unknown whether the virus persists in immune fish. If it does persist, it is unlikely to reproduce and be shed without causing clinical pathology.

The population dynamics of lymphocystis has been studied in some detail (Lorenzen et al. 1991) using a mathematical model. A simplified flowchart of the model is shown in Fig. 1, where arrows indicate the possible transition of fish and transmission of the pathogen between compartments of the fish population. All fish are born susceptible and some acquire the infection as they grow older, while others die in the susceptible state. The infected and diseased compartments are merged, reflecting the assumption that infection is usually (after a short incubation period) followed by clinical disease. This implies that exposure to the pathogen is the principal factor determining the rate at which new infections are acquired by susceptible fish. Experiments have shown that the disease can be readily transmitted to some hosts by addition of virus to the water in aquaria (Anders 1984). Mortality associated with lymphocystis is usually low (Anders 1989, Wolf 1988), the majority of diseased fish recover and acquire temporary immunity. When immunity declines, fish either become susceptible to reinfection or, if the virus lies dormant in recovered individuals, may pass straight into the diseased state again.

Lymphocystis virus is shed by diseased fish only so that the exposure of susceptibles is directly related to the density of diseased (infectious) fish. Immune fish do not participate in the transmission process, hence, acquired immunity is beneficial not only for the individual, but for the population. Because transmission is density dependent, the propor-

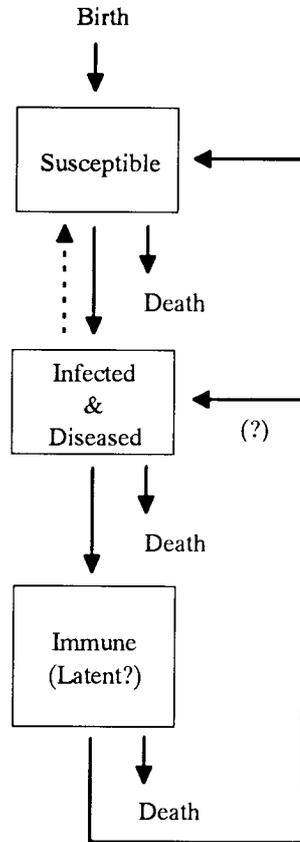


Fig. 1. Compartmental model of the lymphocystis disease in a wild fish population. Heavy solid lines indicate the possible transition of fish between the compartments, dashed lines indicate the transmission of the pathogen.

tion of immune fish and the importance of acquired immunity in the transmission dynamics of disease increases with population density.

In a closed population, the age-distribution of immunity to lymphocystis can be inferred from the prevalence pattern because immunity is acquired by passing through the diseased state. If all fish are born susceptible, the prevalence in young fish depends on their exposure to the virus. Many older individuals will have acquired immunity, and this "history parameter" may be the principal determinant of prevalence in these age-groups. This insight pinpoints the need for an age-structured approach to both theory and field studies of fish diseases.

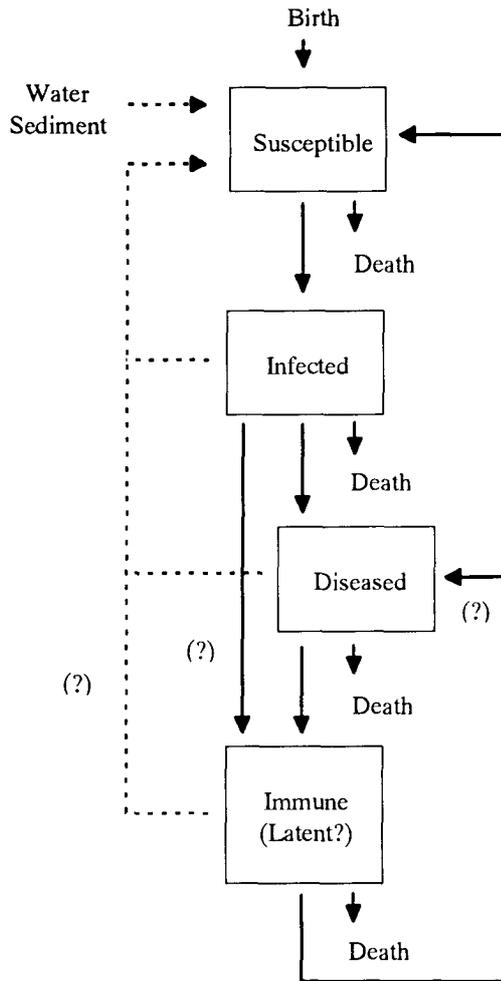


Fig. 2. Outline of a compartmental model of bacterial ulcer disease in a wild fish population. Heavy solid lines indicate the possible transition of fish between compartments, the dashed lines the transmission of pathogenic bacteria.

How does the suppression of specific immune mechanisms affect the prevalence of lymphocystis? The consequences of immunosuppression for the pathogenesis of lymphocystis and ulcers are not known, but I will consider possible effects theoretically. To understand the conclusions, it is important to remember that prevalence is proportional to both the incidence (the rate at which new infections are acquired) and the duration of disease. Increased susceptibility to first infection is beyond the scope of this review, but recovery from disease is aided by the specific primary response and initiates

the acquisition of protective immunity. If the relevant mechanisms are suppressed, the result is most likely delayed recovery, rather than increased mortality, because lymphocystis is generally a benign infection. Delayed recovery would increase prevalence both directly and via increased transmission. If immunosuppression would increase disease-induced mortality, the duration of disease would be shortened, reducing prevalence both directly and via reduced exposure of susceptibles. Toxicants could also impair the development of immunological memory, and, hence, shorten the duration of acquired immunity. This would, of course, increase the density of susceptible individuals and result in increased prevalence especially among older fish.

Ulcer disease in flatfish is linked to bacteria, mainly *Vibrio anguillarum* and several species of *Aeromonas* (Anderson and Conroy 1968, Haastein and Holt 1972, Ullrich 1991). It is possible, however, that these bacteria are secondary invaders rather than the primary pathogen. Such has been demonstrated for the cod ulcer syndrome, where the primary pathogen appears to be a virus (Jensen et al. 1979, Jensen and Larsen 1982, Larsen 1983). *Vibrio anguillarum* and *Aeromonas* spp. form part of the normal microflora of both the water and the intestine of fish (Austin and Austin 1987). Ullrich (1991) was unable to establish a correlation between the level of potentially pathogenic bacteria in the environment and the prevalence of ulcers. This suggests that transmission of the bacteria is of minor importance in the population dynamics of ulcer disease. A direct consequence is that the acquisition of immunity to bacteria in some individuals does not diminish the exposure of others. However, if the bacteria are secondary invaders, we may be looking at the wrong pathogen populations altogether. Also, the virulence of *Vibrio* is linked to the presence of a plasmid (Crosa 1980, Crosa et al. 1977), the population dynamics of which might eventually turn out to be of key importance in the epidemiology of ulcers.

A compartmental model of ulcer disease is outlined in Fig. 2, assuming bacteria to be the primary pathogens. Although fish are born susceptible, some of them may soon become infected without developing gross pathology. The circumstances crucial in the development of pathology are yet unknown, but, besides the plasmid already mentioned, these are likely to involve a weakening of nonspecific immune mechanisms. Possibly, some individuals may by-pass the diseased state and move straight from the infected to the immune compartment. When the pathogens are ubiquitous in the environment, the question of their persistence in the recovered host is of little interest.

The influence of acquired immunity on ulcer prevalence patterns is apparently limited to the protection of individual fish against gross pathology. If immunity can be acquired without passing through the diseased state, it is impossible to infer the age-distribution of immunity from the age-prevalence pattern.

The conclusions drawn about possible influences of immunosuppression on the prevalence of lymphocystis are valid for ulcer disease as well. This is because the influence of immunosuppression on pathogen transmission only adds to the effects of changes in the duration of disease or immunity.

*The role of repeated exposure*

In the above discussions, acquired immunity was assumed to persist for a fixed (though unknown) period. In reality, the length of this period may depend on the degree to which immunity is "boosted" by repeated exposure of immune fish to the pathogen. This is a potential, prevalence-stabilizing mechanism for directly transmitted obligatory pathogens, where prevalence would otherwise be highly dependent on host population density. Moreover, continuous challenge by bacteria in polluted waters may itself provide another explanation for the lack of correlation between bacterial numbers in the environment and the prevalence of ulcer disease.

*Acquired immunity and the interpretation of prevalence patterns in real populations*

Real fish populations are subject to extensive migrations, and individuals do not mix homogeneously. Often, young and mature fish are spatially segregated. A set of fish sampled in a certain area is an assemblage of individuals of different origins and history. Whether or not these fish are showing gross pathology, will depend on their disease history at least as much as on current exposure to a pathogen or environmental conditions. Did the fish acquire immunity against a certain pathogen earlier in their lives, or are they persistently infected? Hence, the interpretation of prevalence data requires the investigation of these history parameters, especially the measurement of acquired immunity.

**Concluding remarks**

There is no doubt that acquired immunity is one of the principal factors determining the prevalence patterns of infectious diseases in wild fish populations. In order to understand its contribution more comprehensively, progress is needed in the following areas.

The role of specific immune mechanisms in the pathogenesis of fish diseases has to be studied in detail. Also, the duration of protective immunity to different pathogens should be established in laboratory experiments.

Techniques for the detection of acquired immunity in wild fish need to be improved. This should help to bridge the gap between the theoretical concept called "acquired immunity" and parameters which can actually be measured in fish. The improvement of techniques would also involve the study of seasonal differences in the detectability of immunity.

Finally, immunological studies should form an integral part of fish disease surveys. All data should be recorded in relation to the age of fish to facilitate understanding of the disease's population dynamics.

*Acknowledgement*

I am grateful to D. Bucke, Dr P. F. Dixon, Dr H. Møller and S. Ullrich for their comments on an earlier draft of the manuscript.

**References**

- Anders K (1984) Die Lymphocystis-Krankheit der Fische. Verlag H. Møller, &el, 102 pp.
- Anders, K (1989) Lymphocystis disease in fishes. In: Ahne W, Kurstak D (eds) Viruses of lower vertebrates. Springer, Heidelberg, pp 141-160.
- Anders K, Møller H (1991) Epidemiologische Untersuchungen von Fischkrankheiten im Watterunser. Ber Inst f Meereskunde 207, &el, 166 pp.
- Anderson JIW, Conroy DA (1968) The significance of disease in preliminary attempts to raise flatfish and salmonids in seawater. Bull Off Int Epic 69: 1129-1137.
- Anderson RM, May RM (1982) Directly transmitted infectious diseases: control by vaccination. Science 215: 1053-1060.
- Austin B, Austin DA (1987) Bacterial fish pathogens: disease in farmed and wild fish. Ellis Horwood, Chichester, 364 pp.
- Avtalion RR (1981) Environmental control of the immune response in fish. Crit Rev Environ Control 11: 163-188.
- Bucke D, Watermann B (1988) Effects of pollutants on fish. In: Salomons W, Bayne BIDuursma EK, Förstrier U (eds) Pollution of the North Sea. An assessment. Springer, Berlin, pp 612-623.
- Crosa JH (1980) A plasmid associated with virulence in the marine fish pathogen *Vibrio anguillarum* specifies an iron-sequestering system. Nature 283: 566-568.
- Crosa JH, Schiewe MH, Falkow S (1977) Evidence for plasmid contribution to the virulence of the fish pathogen *Vibrio anguillarum*. Infection and Immunity 18: 509-513.
- Croy TR, Amend DF (1977) Immunization of sockeye salmon (*Oncorhynchus nerka*) against vibriosis using the hyperosmotic infiltration technique. Aquaculture 12: 317-325.
- Dethlefsen V (1980) Observations on fish diseases in the German bight and their possible relation to pollution. Rapp P V Reun Cons Int Explor Mer 179: 110-117.
- Dethlefsen V (1984) Diseases in North Sea fishes. Helgol Meeresunters 37: 353-374.
- Dethlefsen V, Watermann B, Hoppenheit M (1987) Diseases of North Sea dab (*Limanda limanda* L.) in relation to biological and chemical parameters. Arch FischWiss 37: 107-237.
- Dorson M (1981) Role and characterization of fish antibody. Develop Biol Standard 49: 307-319. Ellis AE (1981) Stress and the modulation of defence mechanisms in fish. In: Pickering AD (ed) Stress and fish. Academic Press, London, pp 147-169.
- Ellis AE (1988a) General principles of fish vaccination. In: Ellis A-E (ed) Fish vaccination. Academic Press, London, pp 1-19.
- Ellis A-F- (1988b) Optimizing factors in fish vaccination. In: Ellis A-E (ed) Fish vaccination. Academic Press, London, pp 32-46.
- Ellis AE (1989) The immunology of teleosts. In: Roberts W (ed) Fish pathology, 2nd edn. Bailliere Tindall, London, pp 135-152.

- Fletcher TC, White A (1973). Antibody production in the plaice after oral and parenteral immunization with *Vibrio anguillarum* antigens. *Aquaculture* 1: 417-428.
- Grondel JL, Nouws HM, van Muiswinkel YM (1987) The influence of antibiotics on the immune system: immunopharmacokinetic investigations on the primary anti-SRBC response in carp, *Cyprinus carpio* L, following oxytetracycline injection. *J Fish Dis* 10: 153-163.
- Haastein T, Holt G (1972) The occurrence of vibrio disease in wild Norwegian fish. *J Fish Biol* 4: 33-37.
- Henken AM, Tigchelaar AJ, Van Muiswinkel W13 (1987) Effects of feeding level on antibody production in African catfish, *Clarias gariepinus* Burchell, after injection of *Yersenia ruckeri* antigen. *J Fish Dis* 11: 85-88.
- Jensen NJ, Bloch B, Larsen JL (1979) The ulcus-syndrome in cod. III. A preliminary virological report. *Nord Vet Med* 31:280- 296.
- Jensen NJ, 1-arsen J1- (1982) The ulcus-syndrome in cod (*Gadus morhua*). IV. Transmission experiments with two viruses isolated from cod and *Vibrio anguillarum*. *Nord VetMed* 34: 136142.
- Johnson KA, Flyrm JIC, Amend DF (1982) Duration of immunity in salmonids vaccinated by direct immersion with *Yersenia ruckeri* and *Vibrio anguillarum* bacterins. *J Fish Dis* 5: 207213.
- Kawai K, Kusuda R, Itami T (1981). Mechanisms of protection in ayu orally vaccinated for vibriosis. *Fish Pathol* 15: 257-262.
- Landolt ML (1989) The relationship between diet and the immune response in fish. *Aquaculture* 79: 193-206.
- Larsen NJ (1983) the ulcus syndrome in cod (*Gadus moritua*): a review. *Rapp P V Reun Cons Int Explor Mer* 182: 58-64.
- Lobb CJ, Clem LW (1981) The metabolic relationships of immunoglobulins in fish serum, cutaneous mucus and bile. *J Immun* 127: 1525-1529.
- Lobb CJ (1987) Secretory immunity induced in catfish, *Ictalurus punctatus*, following bath immunization. *Dev Comp Immunol* 11: 727-738.
- Lorenzen K, Dixon PF (1991) Prevalence of antibodies to lymphocystis virus in estuarine flounder *Platichthys flesus*. *Dis aquat Org* 11: 99-103.
- Lorenzen K, des Clers SA, Anders K (1991) Population dynamics of lymphocystis disease in estuarine flounder, *Platichthys flesus* (L). *J Fish Biol* 39: 577-587.
- Moller H (1985) A critical review on the role of pollution as a cause of fish diseases. In: Ellis AE (ed) *Fish and shellfish pathology*. Academic Press, Undon, pp 169-182.
- Moller H (1988) *Fischbestand und Fischkrankheiten in der Unterelbe*. Verlag H. Maller, Kiel, 344 pp.
- Moller H (1990) Association between diseases of flounder (*Platichthys flesus*) and environmental conditions in the Elbe estuary, FRG. *J Cons Int Explor Mer* 46: 187-199.
- Paterson WD, Desautels D, Weber JM (1981) The immune response of Atlantic salmon, *Salmo salar* L, to the causative agent of bacterial kidney disease *Renibacterium salmoninarum*. *J Fish Dis* 4: 99-111.
- Peleteiro MC, Richards RH (1985) Identification of lymphocytes in the epidermis of the rainbow trout, *Salmo gairdneri* Richardson. *J Fish Dis* 8: 161-172.

- Rifters GT, van Osterom R, van Muiswinkel W13 (1980) The immune system of cyprinid fish. Oxytetracycline and the regulation of humoral immunity in carp (*Cyprinus carpio* L). *Vet Immunol Immunopathol* 2: 281-290.
- Robohm RA, Brown C, Murchelano RA (1979) Comparison of antibodies in marine fish from clean and polluted waters in the New York Bight: relative levels against 36 bacteria. *AppI Environ Microbiol* 38: 248-257.
- Robohm RA, Sparrow DS (1981) Evidence for genetic selection of high antibody responders in summer flounder (*Paralichthys dentatus*) from polluted areas. *Develop Biol Standard* 49: 273-278.
- Sakai DK (1984) Opsonization by fish antibody and complement in the immune phagocytosis by peritoneal exudate cells isolated from salmonid fishes. *J Fish Dis* 7: 29-38.
- Stolen JS, Kasper V, Gatin T, Lipeon V, Nagle JJ, Adams WN (1983) Monitoring environmental pollution in marine fishes by immunological techniques. The immune response of fish exposed by injection or bath to bacterial isolates from sludge or *in situ* exposure to sludge. *Biotech*. 1: 66-68.
- Stolen JS, Gahn T, Kasper V, Nagle JJ (1985) Natural and adaptive immunity in marine teleosts to bacterial isolates from sewage sludge. In: Manning MJ, Tatner MF (eds) *Fish immunology*. Academic Press, London, pp 207-220.
- Ullrich S (1991) Examination of bacterial fish diseases of wild fish in the Elbe and Eider estuaries. *Kieler Meeresforsch, Sonderh* 8: 252-256.
- Vethaak AD (1985) Prevalence of fish diseases with reference to pollution of Dutch coastal waters. RIVO-Report CA 85-01 E. Rijksinstituut voor Visserijonderzoek, IJmuiden, 70pp.
- Wolf K (1988) *Fish viruses and fish viral diseases*. Cornell University Press, Ithaca.
- Zeeman MG (1986) Modulation of the immune response in fish. *Vet Immunol Immunopathol* 12: 235-241.
- Zeeman MG, Brindley WA (1981) Effects of toxic agents upon fish immune systems. In: Sharma RB (ed) *Immunologic considerations in toxicology*, vol 11. CRC Press, Boca Raton, Florida, pp 160.