ICES BIOLOGICAL EFFECTS OF CONTAMINANTS IN PELAGIC ECOSYSTEMS: MONITORING FOR VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

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Introduction

Until recently, viral haemorrhagic septicaemia (VHS) was considered to be a disease that primarily affected cultivated rainbow trout in Europe. Severe losses occur in both freshwater and marine cage culture. However, the causative virus (VHSV) has been isolated from an increasing number of marine or anadromous fish species, and the isolations from marine fish in Europe have all been from the North Sea, or waters linked to the North Sea such as the Baltic Sea (Smail 1995, Dixon *et al.* 1997, Mortensen *et al.* 1999). Rather than the virus being transferred from the freshwater environment to the marine environment, there is evidence that the reverse may have occurred (Meyers and Winton 1995, Dixon 1999), and that there is a reservoir for VHSV in marine fish; the herring is one species that has been suggested to be such a reservoir (Meyers *et al.* 1994, Meyers and Winton 1995, Dixon *et al.* 1997). The non-cultivated marine fish from which the virus has been isolated, particularly in European waters, have shown few external signs of disease, but the virus has been associated with external pathologies and mortality in marine fish from the Pacific Coast of North America (Meyers *et al.* 1994, Meyers *et al.* 1999, Traxler *et al.* 1999). It has also been suggested that exposure of fish to polynuclear aromatic hydrocarbons and other pollutants may reactivate a subclinical infection with VHSV, or make fish more susceptible to the virus (Carls *et al.* 1998, Marty *et al.* 1998, Dixon 1999, Marty *et al.* 1999). The herring has also been implicated as a possible reservoir of infectious salmon anaemia virus (ISAV) (Mullins *et al.* 1999, OIE 2000). That virus causes infectious salmon anaemia which is a serious disease of salmon, particularly those in seawater. Amongst other countries, the disease occurs in Norway and Scotland, both of which border the North Sea.

The objectives of this project were to take tissue samples from herring from contaminated and reference areas, try to detect VHSV or ISAV, and if successful, determine whether there is any correlation between virus incidence and sampling area.

Materials and Methods

Sample collection. Fish were collected by pelagic and/or bottom haul from three sites in the vicinity of an oil platform and one reference site in the Statfjord area, and three sites and a reference area on a contamination gradient in the German Bight (Figure 1) during a cruise of the Fisheries Research Services vessel *Scotia* between 17-28 March 2001. Individual fish (or pools of five small fish) were dissected and kidney, spleen, liver and heart were removed. Small portions, of approximately equal amounts, were dissected from each organ (total weight approximately 200 mg) and transferred to a tube containing 1 ml RNAlater™ (Ambion Inc, Austin, USA). Up to approximately 1 g (total) of the residual organ material was transferred to a tube containing 9 ml virus transport medium. A different set of instruments was used for each fish. The samples in transport medium and RNAlater™ were frozen at -20°C until they were subsequently processed at the laboratory.

Virus isolation. The samples in transport medium were thawed and the medium was removed. The tissues were weighed, ground using a mortar, pestle and sterile sand and maintenance medium was added to effect a 1:10 (w/v) dilution of the tissues. The tissues were further processed by the method stipulated by the Commission of the European Communities (CEC) for detecting VHSV (CEC 1996). *Epithelioma papulosum cyprini* (EPC) (Fijan *et al.* 1983) and bluegill fibroblast (BF-2) (ATCC CCL 91) cells were inoculated with 1:100 and 1:1000 (final) dilutions of extracts. The cells were incubated at 15°C for seven days then blind passaged at 1:10 and 1:100 dilutions and incubated for a further seven days. The tissues processed as above were also inoculated onto TO cells (Wergeland and Jakobsen. 2001) at a 1:1000 (final) dilution. Cells were incubated at 15°C, observed for 14 days and then blind passaged at a 1:10 dilution and incubated for a further 14 days. At the end of both incubation periods the ability of the TO cells to agglutinate salmon erythrocytes was determined (Smail *et al.* 2000).

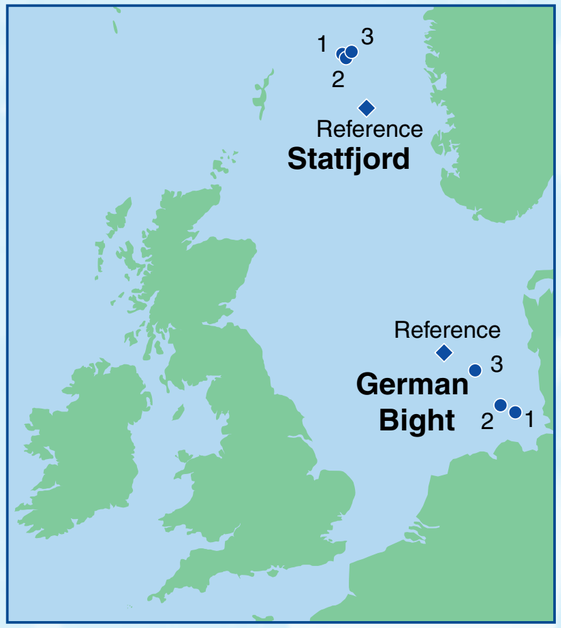

Figure 1: Locations of the three test and reference sites in the Statfjord and German Bight sampling areas.

Reverse transcription-polymerase chain reaction (RT-PCR). At the laboratory the RNAlater™ was removed from the tissues from herring only and total RNA was extracted using the Trizol™ reagent. The concentration of RNA was adjusted to 0.5 µg ml^{-1}. The RT-PCR for VHSV was carried out as previously described (Dixon *et al.* 1997, Strømmen and Stone 1998) using F3 (5' GAT-CAG-GTC-CCC-CAR-RTC-NGT 3') in place of F2 in the first round of RT-PCR.

Virus neutralisation. One sample produced cytopathic effect (CPE) in BF-2 cells which was not caused by VHSV. The CPE was suggestive of a birnavirus, and the cell culture supernatant, diluted to 1×10^{-4} and 1×10^{-5}, was incubated for 1 h with antisera against all nine birnavirus Serogroup A serotypes and the single Serogroup B serotype, each diluted to 1×10^{-3} and 1×10^{-4}, then inoculated onto fresh cell cultures. The findings of the initial assay were confirmed by a 50% plaque reduction assay.

Results and Discussion

It was intended that 50 juvenile herring would be sampled at each site, but at many sites that sample number was not achieved. The fish sampled are shown in Table 1; all except the saithe are known hosts of VHSV. However, neither VHSV nor ISAV were isolated in cell culture and the genome of VHSV was not detected by RT-PCR. The tissue extract from one individual whiting from the German Bight reference area produced CPE in BF-2 cells four days after the initial inoculation. A sample of the cell culture supernatant was not neutralised by VHSV antiserum, and did not produce a product in the VHSV RT-PCR. As the CPE was suggestive of a birnavirus, antisera against birnavirus serotypes were used in neutralisation tests with the cell culture supernatant. None of the antisera against the Serogroup A serotypes neutralised the virus, but it was neutralised by an antiserum against serotype B1. The preliminary neutralisation test was confirmed by a 50% plaque reduction assay. This is the first record of a birnavirus in the whiting.

The small numbers of herring captured in the Statfjord area meant that the original aims of the survey could not be achieved there, although whiting, being a host for VHSV was used as a substitute target species. The target number of herring was obtained at the German Bight sampling sites 1 and 2, but the small size of the fish meant that they had to be pooled, which reduced the chances of detecting the viruses. Again whiting were also taken as a substitute species. As only one sample yielded a virus of any type, the overall results are inconclusive. If this approach is to be repeated, a greater number of fish should be sampled if possible, and samples of caged herring should also be taken.

Table 1: Species and number of fish sampled at each site

Station	Species	Number
Statfjord area 1	Whiting (*Merlangius merlangus*)	30
Statfjord area 1	Atlantic herring (*Clupea harengus*)	9
Statfjord area 2	Atlantic cod (*Gadus morhua*)	2
Statfjord area 2	Whiting	28
Statfjord area 3	Saithe (*Pollachius virens*)	38
Statfjord area 3	Atlantic herring	2
Statfjord area 3	Whiting	19
Statfjord area reference	Atlantic herring	50
German Bight 1	Atlantic herring	10 x 5 fish pools
German Bight 2	Atlantic herring	10 x 5 fish pools
German Bight 3	Whiting	50
German Bight 3	Atlantic herring	3 x 5 fish pools
German Bight reference	Atlantic herring	25
German Bight reference	Whiting	14
German Bight reference	Atlantic cod	19

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