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A NEGATIVE TEST FOR INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) IN CRUSTACEAN DIETS FED TO LARVAL ATLANTIC HALIBUT, *HIPPOGLOSSUS HIPPOGLOSSUS*

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Abstract

An IPNV virus test was carried out on the typical crustacean feeds for Atlantic halibut at a Scottish west coast halibut farm. The result was negative and so does not implicate the feeds in a recent IPNV outbreak at the farm.

Introduction

Atlantic halibut is a mariculture species of increasing interest and development in Scotland. It has been reported by Mortensen *et al.* (1990) and Biering *et al.* (1994) that small fry are extremely susceptible to IPNV and a similar experience has also been found in Scotland in 1996 at a halibut rearing site which showed marked mortality of fry and juveniles (Wood, Bruno and Ross, 1996). At the time of the mortality, water at this site was being filtered to 5 mm and treated with UV at a level of 30 mJ/cm^2.

It was thought of practical and thereotical interest to find out if the living crustacean feeds *viz Artemia salina* (the brine shrimp) and the cultured copepods *Euretemora velox* used as the halibut feeds for larval stages could be the source of the virus, therefore a virus test was carried out using standard culture techniques. A negative result could therefore help in deducing the possible source of the virus and put into practice preventative measures on the farm site.

An IPNV test on the feed was especially relevant since birnaviruses have been previously detected in rotifer feeds. A mortality in sea bass fry was associated with a birnavirus, seabass birnavirus (SBV) and previous feeding of rotifers was suspected as the origin (Bonami *et al.*, 1983). Also a mass mortality of rotifers was attributed to rotifer birnavirus (RBV), causing basophilic inclusion bodies and high density paracrystalline arrays (Comps *et al.*, 1991).

Materials and Methods

65 ml of Artemia and copepod suspensions were received after chilled shipment for 24 h. The organisms were sedimented at 2,000 g for 20 mins at 4°C. The supernatants were discarded and the pellets washed in 10 ml Hanks Balanced Salt Solution (1x). The supernatants were again discarded and each pellet approximated to 2 g. 2 ml HBSS was added to each pellet to give 4 ml of suspension.

Each was sonicated in a bath sonicator at 550 watts for 60 secs. (Heat Systems, Farmingdale, NY, USA - model XL 2020). The sonicate was diluted 5x further in HBSS to give 10x w/v dilution and final dilutions made to 50x, 200x, 800x using HBSS 1x. Each dilution was clarified by sedimentation at 2,000 g for 20 mins. Sub-confluent cultures of chinook salmon embryo cells (CHSE-214) in 25 cm^2 flasks were inoculated with 1 ml of the above supernatant passed through a Millipore disposable HV filter (0.45 mm porosity). An IPNV positive control was set up and uninoculated cell controls were included. Cultures were incu-

Table 1: IPNV testing of halibut feeds

		Culture	
Feed type	Dilution	Primary	Pass
(w/v) x			
Artemia	50	-ve	-ve
	200	-ve	-ve
	800	-ve	-ve
Copepods	50	-ve	-ve
	200	-ve	-ve
	800	-ve	-ve
IPNV Sp control		++	++

bated at 15°C, with microscopic observation at 2 and 27 days post infection. Blind passage was then performed and cultures read 12 days later after further incubation at 15°C.

Results and Discussion

All cultures were negative and no marked cytotoxic effects on the cultures were observed at the lowest dilution of the crustacean sonicates (Table 1).

This result points to a source of the virus other than the feeds cultured for the young stages. This statement can be qualified by saying that the IPN test is a valid negative for the two copepod stocks only at the time of year tested, ie late August and that it pertains only to the sample numbers actually obtained.

Obvious possibilities for alternative sources of the IPN virus isolation are the water source itself, infected farmed stocks of salmonids within the boundaries of the farm or the halibut broodstock. Preventive practices could be put in place to isolate the water supply for this unit from other water supplies. Intense UV treatment of the sea water supply at a level of 120 mJ/cm^2 (Yoshimizu *et al.*, 1986) could be a suitable preventative measure for a rearing site for halibut, in the vicinity of IPNV infected salmonid stocks which may be harbouring IPN virus. An alternative measure for recirculation systems would be ozone disinfection, with a suitable biofilter placed after the ozonator to adsorb toxic hypobromite salts. Milt and ovarian fluid samples from the broodstock could be tested for IPN virus and fish testing positive removed from the system. This would eliminate any possible vertical transmission of the virus.

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