handling of fish may also contribute towards the formation of reversible opacities.

The mechanism by which reduced temperature caused the observed temporary opacities is unknown. However, this effect may perhaps be attributed to a general phenomenon in fish whereby low molecular weight lens proteins have been shown to precipitate *in vitro* at reduced temperatures (Smith, 1972; Loewenstein and Bettelheim, 1979).

This experiment indicates the importance of considering any recent temperature fluctuations together with effects of fish handling procedures when fish are examined for the presence of eye opacities. The importance of differentiating between reversible opacities and other lesions of the eye could be important when determining the general health of fish.

Summary

Salmon post-smolts developed a reversible eye opacity following a 20 min exposure to water temperatures below ambient. The number of opacities was greater in fish exposed to 5°C compared with fish exposed to 9°C. However, 60 mins after the return of fish to ambient temperatures, the number of opacities in each of the two groups was similar. Reductions in the incidence of eye opacity were observed 220 mins after returning to ambient temperatures. The number of opacities in the control groups were low. It was concluded that a knowledge of water temperature fluctuations together with effects of fish handling procedures are important for studies which report on eye opacities.

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MYTILICOLA INTESTINALIS AND *PROCTOECES MACULATUS* IN MUSSEL *(MYTILUS GALLOPROVINCIALIS* LMK.) BEDS IN SPAIN

BY J.A F. ROBLEDO, J. CACERES-MARTINEZ & A. FIGUERAS(*).

Galicia (NW Spain) is the largest producer of cultured mussels in the world (Figueras, 1989). During decades of culture no mass mortalities have been recorded in mussels from this area (Figueras *et al.,* 199l); however, in the last few years there has been a tendency towards a decrease in production (Figueras, 1989). Despite its economic importance, there is a lack of information about the health status of the mussel stocks which may be a consequence of the absence of mortalities. Most work in this area has been carried out on cultured mussels (Figueras and Figueras, 1981; Figueras *et al.,* 199l; Villalba *et al.,* 1993). There are no studies on pathology of mussels from natural beds.

In this paper, we present the results of a base-line study of *Mytilicola intestinalis* and *Proctoeces maculatus* in *Mytilus galloprovincialis* from natural mussel beds (the main source of mussel spat for culture) and from culture rafts in the inner and outer areas of the Ria de Vigo.

Materials and Methods

Between November 1991 and February 1993, adult mussels *(M. galloprovincialis)* were collected from mussel beds in San Adrian, Cabo Home and from the floats of rafts in areas close to the mussels beds in Ria de Vigo. The animals were processed for histology following the usual protocols.

Results and Discussion

Sporocysts containing cercariae of *P. maculatus* were found with low prevalence (Table l) mainly in the mantle but also in the digestive gland and gills. The presence of the parasite caused suppression of gametogenesis as the reproductive organs were atrophied (Fig. l). An intense host reaction to the sporocyst was found, in some cases sufficient to destroy the parasite. The prevalence found was higher than the prevalence recorded by Ferrer (1981) for cultured mussels from the same area (1%). *P. maculatus* has been already identified in *M. galloprovincialis* from the Mediterranean Sea and from the Atlantic Ocean

Table 1. R. Rafts, MB. Mussel Beds, M i. *Mytilicola intestinalis,* P. m. *Proctoeces maculatus*. The infestation is indicated as prevalence.

	San Adrián (R)		San Adrián (M)		Liméns (R)		Cabo Home (MB)	
Date	*M.i.* (%)	*P.m.* (%)	*M.i.* (%)	*P.m.* (%)	*M.i.* (%)	*P.m.* (%)	*M.i.* (%)	*P.m.* (%)
Nov-1991	-	-	62.07	3.45	-	-	53.33	0.00
Dec-1991	64.29	0.00	51.72	0.00	10.34	3.45	20.83	0.00
Jan-1992	69.23	0.00	61.54	0.00	16.67	0.00	50.00	0.00
Feb-1992	44.44	0.00	92.31	4.17	3.85	0.00	72.73	0.00
Mar-1992	-	-	33.33	6.67	-	-	20.00	0.00
Apr-1992	-	-	43.33	3.33	-	-	13.04	0.00
Oct-1992	-	-	68.00	0.00	0.00	0.00	33.33	4.76
Nov-1992	65.22	0.00	69.23	3.85	-	-	71.43	7.14
Dec-1992	35.71	7.14	60.71	3.57	36.84	0.00	52.38	0.00
Jan-1993	70.83	4.17	84.62	0.00	42.86	4.35	-	-
Feb-1993	68.75	0.00	83.33	4.00	-	-		-

Limens (R) Cabo Home (MB)

(Martinez, 1973 and Ferrer, 1981). Although this parasite causes heavy lesions in the tissues where is localised, the low prevalence does not made it a true thread for the mussel.

M. intestinalis was found in the gut, but also in the stomach. The hooks of the antennae produced erosion of the intestinal epithelium, but as it as already indicated by Moore *et al.*, (1978) the damage can be rapidly repaired and no severe reaction in the host was induced. The copepod was present at all sites sampled, the highest prevalence being close to 100% in San Adrian (Table l). Results for the G-test for independence (NS P>0.05, *0.05>P>0.0l, **0.05>P>0.005, ***P<0.005, Sokal and Rohlf, 1981) showed that the prevalence of *M. intestinalis* was dependent of locality (df=l in each case, San Adrian (Raft) and Limens, n=292, G=19.061***; San-Adrian (Mussel Bed) and Cabo Home, n=643, G=4.848*) and location (natural mussel beds or rafts) in the outer part of the Ria (Limens and Cabo Home, df=l, n=258, G=13.898***) but not in the inner part of the Ria (San Adrian Raft and Mussel Bed, df=l, n=569, G=0.748 NS).

Infestation with *M. intestinalis* depends on a chance encounter between the copepodite and the host (Gee and Davey, 1986). With the highest density of cultured mussels in the inner part of the Ria a decrease of prevalence could be expected towards the outer part of the Ria. However, our results did not confirm this gradient, because the highest prevalence was found in mussels taken from mussel beds in San Adrian. In the outer part of the Ria we found differences between mussels taken from mussel beds where they are exposed to tidal cycles and mussels taken from rafts where they are submerged permanently. However opposite results were obtained from the inner part of the Ria.

Figueras and Figueras (1981) found that the highest infestation rate occurs in spring and autumn. Although the presence of the copepod fluctuates throughout the year, it was in the winter period that the prevalence was

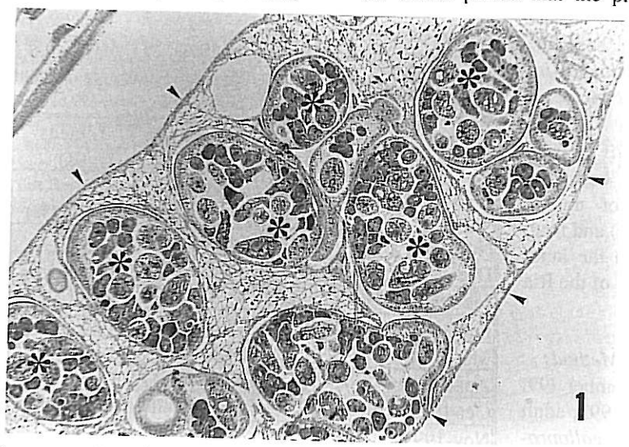

Figure 1 Sporocysts (asterisks) containing cercariae of *Proctoeces maculatus* in the mantle (limits of the mantle are indicated by arrows) of *Mytilus galloprovincialis*. Note the absence of follicles in the mantle caused by the presence of the parasite.

higher. The significance of the parasite does not seem to be great because, in a ten year survey of infested mussels in England, Davey (1989) pointed out that despite the intensity of infestation reaching levels higher than any reported for *Mytilus*, the host population was capable of sustaining the infestation indefinitely. Our results showed that the same is true for mussels cultured in NW Spain.

Summary

The prevalence of the copepod *Mytilicola intestinalis* and the trematode *Proctoeces maculatus* in *Mytilus galloprovincialis* from natural beds in Ria de Vigo (NW Spain) was monitored for one year. The copepod was present at all the sites sampled with the highest prevalence in mussel beds from the inner part of the Ria. The trematode *P. maculatus* was of low prevalence, but caused severe damage to the tissues of the infected mussels.

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ISOLATION AND MORPHOLOGICAL INVESTIGATION OF A SHRIMP PATHOGENIC VIRUS

BY KEUN-KWANG LEE AND YOUNG-GILL KIM

Introduction

The Baculoviruses are subgrouped into the nuclear polyhedrosis viruses (NPV), the cytoplasmic viruses and the granuloviruses. These viruses have been found to infect cultured penaeid shrimps and to cause occasionally high mortality of the infected populations (Lightner, 1985; Lightner and Redman, 1981; Tsing and Bonami, 1984). Amongst the various shrimp pathogens so far investigated, viruses are regarded as very important from their wide-spread nature and high infection rates (Lightner, 1983). To date, three baculoviruses including baculovirus penaei (BP) (Couch, 1974), baculoviral midgut gland necrosis (BMNV) (Sano *et al.*, 1981), and *Penaeus monodon* -type baculovirus (MBV) (Lightner and Redman, 1981; Doubrovsky, 1988) have been reported from penaeid shrimp and cause unexpected mortalities in various species. These viruses may be the main causative agents for mass mortalities of the cultured penaeid shrimps that have occurred recently in Korea. The present study describes attempts to infect *Penaeus orientalis* and characterise the occlusion bodies of NPV in the infected animals in order to establish some general properties of the virus.

Materials and Methods

A nuclear polyhedrosis shrimp baculovirus was isolated from diseased *Penaeus orientalis*. A *Spodoftera frugiperda* cell line was used in this study. The cells were adapted and then propagated at 28ºC in TNM-FH medium, supplemented with 0.26% tryptose broth, 10% foetal bovine serum, 100 units penicillin and l00µg streptomycin per ml. The isolation of polyhedra was carried out as described by Lee and Lee (1989). The suspension was layered onto a 35-65% w/w) sucrose gradient and centrifuged for 1h at a speed of 100,000 x g. Virus multiplication was observed with a phase-contrast inverted microscope, and TEM and SEM observations were carried out as described by Oh and Lee (1990).

Results and Discussion

The baculoviruses subgroup, shrimp nuclear polyhedrosis virus successfully infected the continuous cell line. Polyhedral inclusion bodies (PIB) were formed after inoculation of monolayer cultures with the virus. Observation of inoculated cultures using phase-contrast microscopy indicated that the first sign of infection was seen within 20 hours post-infection (p.i). Infected cells lost their motility and then there was an enlargement of the cell due to nuclear hypertrophy. Within 20-24 hours a few cells may contain small, refractive polyhedra (Fig. 1B). At 28 C, 100% infection was achieved between 48 and 72 hours p.i (Fig. 1C,D). Polyhedron production and maturation were usually completed by 72-96 hours p.i (Fig. 1D). Polyhedra in the nuclei were released into the culture media at 84h p.i (Fig. 1E). These observed results are similar to other NPV infections in cells *in vitro* (Summers and Volkman, 1976; Oh and Lee, 1990). By the observation of NPV polyhedra and virions by phase-contrast and electron microscopy, polyhedral shapes were mostly tetragonal or hexagonal and polyhedra were about 3µm in size (Fig. 1, 2A,B). These virions were confined to the nucleus and appeared to be non-occluded, rod shaped with nucleocapsids ranging of 30-40 x 200-400nm in size (Fig. 2C,C'). The results were similar to those reported by Ackermann and Smirnoff (1983) and Oh and Lee(l990).