Vol. 78: 181–188, 2008
doi: 10.3354/dao01874

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published January 24

Herpesvirus infection in European flat oysters *Ostrea edulis* obtained from brood stocks of various geographic origins and grown in Galicia (NW Spain)

P. Mirella da Silva[1], Tristan Renault[2], José Fuentes[1], Antonio Villalba[1,*]

[1]Centro de Investigacións Mariñas, Consellería de Pesca e Asuntos Marítimos, Xunta de Galicia, Aptdo. 13, 36620 Vilanova de Arousa, Spain

[2]Laboratoire Génétique et Pathologie, IFREMER, 17390 La Tremblade, France

ABSTRACT: We evaluated differences in productive traits and disease susceptibility among *Ostrea edulis* stocks. We produced 4 to 5 families from each of 4 oyster populations (Irish, Greek and 2 Galician) in a hatchery. Spat corresponding to 19 different families were transferred to a raft in the Ría de Arousa (Galicia, Spain) for grow-out. Samples of each family were histologically processed every month for 2 yr. One of the pathological conditions disclosed by histological examination was characterised by the occurrence of numerous abnormal cells throughout the connective tissue of various organs, showing hypertrophied nuclei with marginated chromatin and a characteristic large intranuclear acidophilic inclusion. Ultrastructural examination showed that the abnormal cells contained herpesvirus-like particles. *In situ* hybridisation assay using a DNA probe specific for Ostreid herpesvirus 1 (OsHV-1) confirmed that the abnormal cells were infected by OsHV-1 or a closely related herpesvirus. All cases of this pathological condition, except one, were detected during the first year of grow-out; thus it was mostly restricted to juvenile stages. The disease was detected in oysters of each origin but it was not found in all families of each origin, thus suggesting significant parental influence in the susceptibility to this disease or significant influence of the infective status of the parents on the infection of the progeny (vertical transmission). This pathological condition was likely responsible for oyster mortality to some extent during the first year of grow-out.

KEY WORDS: *Ostrea edulis* · Ostreid herspesvirus 1 · Bonamiosis · Histology · Transmission electron microscopy · *In situ* hybridisation

INTRODUCTION

Overfishing of flat oyster *Ostrea edulis* beds (Andreu 1968) and spread of bonamiosis (Polanco et al. 1984), after uncontrolled mass introduction of oysters from other countries, have contributed to the exhaustion of natural beds in Galicia (NW Spain). High oyster mortality due mostly to bonamiosis constrains *O. edulis* farming in the region. A programme to produce an *O. edulis* strain resistant to bonamiosis has been planned (da Silva et al. 2005) to rehabilitate natural oyster populations and the oyster farming industry. As a first step, comparison of disease susceptibility and productive traits among European flat oyster stocks, which had been shown to be genetically different, was performed to identify favourable oyster populations for selective breeding (da Silva et al. 2005). This first stage involved hatchery-nursery production of oyster spat families from each of 4 oyster brood stocks of different geographic origin, and the comparison of their performance through grow-out to market size in the Ría de Arousa (Galicia, Spain). This resulted in the first detection of a herpesvirus infection in oysters cultured in Galicia.

Herpes-like viruses and herpesviruses have been associated with mortality outbreaks in bivalve mollusc

*Corresponding author. Email: villalba@cimacoron.org

larvae, spat and adults in different countries. Since the first report of a herpes-like virus infecting the eastern oyster *Crassostrea virginica* (Farley et al. 1972) additional cases have been reported from different bivalve species, including the oysters *Crassostrea gigas* (Hine et al. 1992, Nicolas et al. 1992, Renault et al. 1994a,b, 1995, 2001a, Burge et al. 2004), *Ostrea edulis* (Comps & Cochennec 1993, Renault et al. 2000a), *Ostrea angasi* (Hine & Thorne 1997) and *Tiostrea chilensis* (Hine et al. 1998); the Manila clam *Ruditapes philippinarum* (Renault et al. 2001a,b); and the great scallop *Pecten maximus* (Arzul et al. 2001a). Most cases of herpes-like virus infection were found in larvae and spat rather than in adults; however, Arzul et al. (2002) noticed that herpesviruses can be present without inducing disease or mortality in a great proportion of adult individuals of Pacific oyster populations. Therefore, adults are a potential reservoir of the virus either in the natural environment or in hatchery and nursery-rearing conditions, supporting the possibility of vertical viral transmission. Inter- and intraspecific transmission of Ostreid herpesvirus 1 (OsHV-1) may occur efficiently (Renault et al. 2000a, 2001a, Arzul et al. 2001b,c). The pathogenicity of OsHV-1 was demonstrated by experimental transmission of the disease to axenic *Crassostrea gigas* larvae (Le Deuff et al. 1994).

Important advances in the field of molecular genetics have contributed sensitive techniques to viral diagnosis such as PCR and *in situ* hybridization (ISH) (Renault et al. 2000b, Renault & Arzul 2001, Arzul et al. 2002, Lipart & Renault 2002, Burge et al. 2004), which facilitate virus detection and confirmation. Nevertheless, transmission electron microscopy (TEM) is still very important for identification of the virus type, since capsid symmetry and presence or absence of an envelope are 2 criteria of great value for virus classification (Roizman & Baines 1991).

This article reports the first occurrence of herpesvirus infection in oysters *Ostrea edulis* cultured in the Ría de Arousa (NW Spain). Differences in susceptibility to this infection between stocks and families are analysed and the association of this viral infection with oyster mortality is evaluated.

MATERIALS AND METHODS

Production of oyster families. Four oyster populations from different geographic locations were selected as sources of broodstock oysters; northern Ireland (IR), Greece (GR), and 2 populations from Galicia (Ría de Ortigueira [OR] and Coroso [CO, Ría de Arousa]). In December 2000, adult oysters from those populations were transferred to the hatchery of the Centro de Investigacións Mariñas (CIMA) where they were distributed in tanks (5 tanks per stock, 15–20 oysters per tank) and conditioned for spawning as previously described (da Silva et al. 2005). The larvae from each batch originated from a single mother, thus being either one-half or full sibling families. Nineteen larval families were reared following the procedure described by da Silva et al. (2005), adapted after Román (1992), comprising 5 families from each origin, except for IR from which only 4 families were obtained. Families were named with the initial letters of their respective origins (IR, GR, OR and CO) and numbered from 1 to 5. Once spat exceeded 1 cm in shell height, they were transferred to a raft for grow-out.

Experimental design for oyster raft grow-out. The experiment for oyster grow-out was designed to determine various parameters (growth, mortality and disease susceptibility) in a research programme previously described in da Silva et al. (2005). In September 2001, approximately 4000 oysters from each family were transferred to a raft located near Cambados (Ría de Arousa, Galicia, NW Spain). Ten oysters per family were randomly collected monthly for disease diagnosis until June 2002. Beginning in July 2002, 6 oysters were taken monthly from each family. Mortality corresponding to each family was estimated monthly. Sampling finished in September 2003 after 2 yr of raft culture.

Histology and TEM examination. The occurrence of pathological conditions was evaluated by examining histological sections. Oysters were shucked and from each a sagittal section approximately 5 mm thick containing gill, visceral mass and mantle lobes was fixed in Davidson's solution and embedded in paraffin. Histological sections (5 µm thick) were stained with hematoxylin and eosin (H&E) and some with Feulgen picromethyl blue (Howard & Smith 1983) for DNA. In addition, the paraffin-embedded tissues from 2 oysters showing intranuclear inclusion bodies in histological sections were processed for TEM examination. These were de-waxed in xylene (60°C, 3 h), hydrated through serial ethanol baths, fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.2 for 2 h and post-fixed for 2 h in 1% osmium tetroxide in the same buffer. Ultrathin sections were examined in a JEOL JEM 1010 transmission electron microscope at 80 kV.

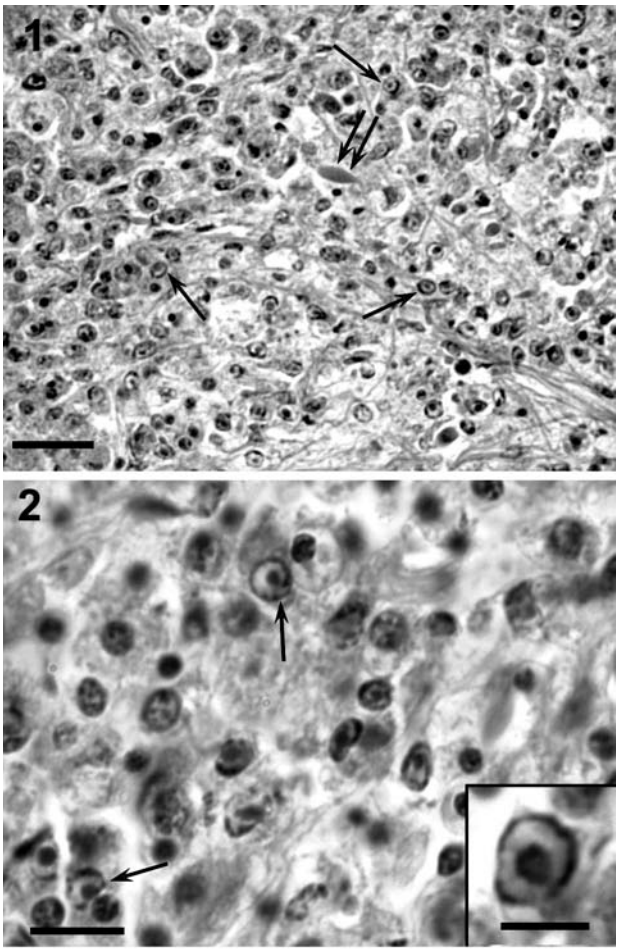
***In situ* hybridisation.** A herpesvirus-specific polynucleotide probe was synthesised by incorporation of digoxigenin-11-dUTP during PCR using OsHV-1 specific primers C5 (5′-ccgtgacttctatgggtatgtcag-3′) and C13 (5′-cctgaggtagctttagatacccacc-3′) as previously described (Barbosa-Solomieu et al. 2004, Renault et al. 2004). Extracted viral DNA (100 pg μl^{-1}) was added in a volume of 1 µl as template. The tissues of 4 oysters that had been included in paraffin blocks and whose histological sections had shown tissue lesions were used for ISH assay. The oysters corresponded to 3 dif-

ferent families: CO1, CO2 and OR2. ISH was carried out adapting the protocols described previously (Renault & Lipart 1998, Arzul et al. 2002, Lipart & Renault 2002). Tissue sections (7 µm thick) on silane-prep™ slides were de-waxed in xylene (2 × 5 min), treated in absolute ethanol (2 × 5 min) and air-dried at room temperature (15 min). Sections were then permeabilised with proteinase K (100 µg ml^{-1} in distilled water) for 30 min at 37°C in a humid chamber. Proteolysis was stopped by one 3 min wash in 0.1 M Tris, 0.1 M NaCl buffer (pH 7.5) at room temperature. Sections were dehydrated in 95% ethanol for 1 min, absolute ethanol for 1 min and air-dried (15 min). A prehybridisation step was carried out with pre-hybridisation buffer (50% formamide, 10% dextran sulfate, 4× SSC [0.06 M Na_3 citrate, 0.6 M NaCl, pH 7], 250 µg ml^{-1} yeast tRNA and 10% Denhart) for 30 min at 42°C in a humid chamber. The prehybridisation buffer solution was replaced with 100 µl of hybridisation buffer solution containing 50 µl of digoxigenin-labelled probe (5 ng $µl^{-1}$) and 50 µl of hybridisation buffer (50% formamide, 10% dextran sulfate, 4× SSC, 250 µg ml^{-1} yeast tRNA and 10% Denhart). Slides were covered with plastic coverslips (Polylabo). Target DNA and digoxigenin-labelled probe were denatured at 95°C for 5 min and the hybridisation was carried out overnight at 42°C in a humid chamber. After hybridisation, coverslips were removed carefully and slides were washed for 10 min in 1× SSC (0.2% BSA) at 42°C. Specifically bound probe was detected using a peroxidase-conjugated mouse IgG antibody, again digoxigenin (Boehringer Mannheim) diluted 1:250 in 1× PBS (1 h at room temperature). Unbound peroxidase-conjugated antibody was removed by 6 washes in 1× PBS (5 min). Diaminobenzidine (DAB) tetrahydrochloride was diluted in 1× PBS (0.7 mg ml^{-1}). The colour solution was added to tissue sections (500 µl) and incubated at room temperature in the dark for 20 min. The reaction was stopped with two 1× PBS washes. Slides were stained for 20 s in Unna Blue (RAL) followed by ethanol dehydration and mounted in Eukitt via xylene. Negative controls included samples without digoxigenin-labelled probes in the hybridisation mixture or omitting the peroxidase-conjugated antibody. A positive control consisted of OsHV-1 infected *Crassostrea gigas* spat previously characterised by TEM and PCR.

RESULTS

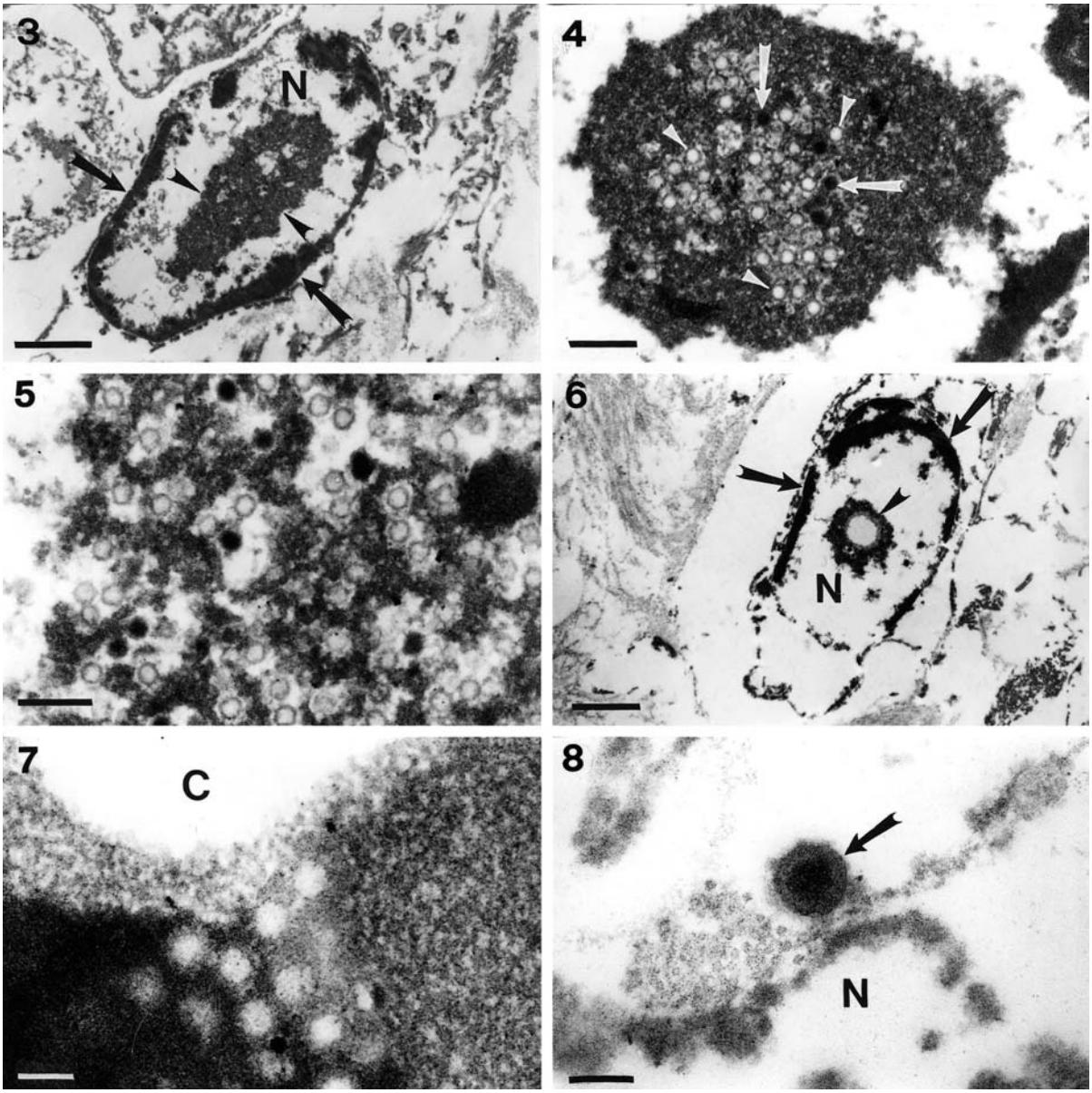
One of the pathological conditions disclosed by histological examination was characterised by the occurrence of numerous abnormal cells throughout the connective tissue of visceral mass, gills, labial palps and mantle (Fig. 1). These abnormal cells had hypertrophied nuclei with marginated chromatin and a characteristic large intranuclear acidophilic inclusion (INAI, Figs. 1 & 2), spherical to ovoid in shape and positive for Feulgen picromethyl blue stain. The nuclei of the abnormal cells were 5.14 ± 0.127 µm in length (range 4.0–7.0 µm, N = 35) and the INAIs were 1.96 ± 0.118 µm long (range 1.0–3.5 µm, N = 35) in the longest axis. The nuclei of normal cells in the connective tissue of the visceral mass were 3.54 ± 0.094 µm in length (range 2.5 to 5.0 µm, N = 40). Alteration of connective tissue also involved disappearance of vesicular cells and occurrence of numerous bundles of fibres (Fig. 1).

Ultrastructural examination of the abnormal cells showed nuclei with margined heterochromatin and a large electron-dense deposit occupying most of the



Figs. 1 & 2. *Ostrea edulis*. Histological sections. Fig. 1. Connective tissue of the visceral mass showing numerous abnormal cells (arrows) with hypertrophied nucleus enclosing intranuclear acidophilic inclusions and bundles of fibers (double arrows). Scale bar = 20 µm. Fig. 2. Detail of the abnormal cells (arrows) showing the hypertrophied nucleus with an intranuclear acidophilic inclusion and margined chromatin. Scale bar = 10 µm. Inset: Enlargement of an abnormal cell. Scale bar = 5 µm

nucleus, which corresponded to the INAI (Fig. 3). The INAI consisted of a coarsely granular electron-dense matrix enclosing virus-like structures. Nucleocapsid-like structures containing an electron-dense core and empty capsids were observed scattered within the INAI. They were spherical to hexagonal in shape and 73 and 71 nm in diameter, respectively (Figs. 4 & 5). Empty capsids were more abundant than the nucleocapsid-like structures. Circular to ovoid structures, consisting of a central electron-lucent area surrounded by a finely granular electron-dense halo, were observed within some INAIs (Fig. 6). Capsids and nucleocapsids were rarely seen in para-crystalline array (Fig. 7). Few virions (106 nm in diameter) consisting of



Figs. 3 to 8. *Ostrea edulis*. Transmission electron micrographs. Fig. 3. An abnormal cell with a hypertrophied nucleus (N), marginated heterochromatin (arrows) and a large central inclusion (arrowheads). Scale bar = 1 µm. Fig. 4. An intranuclear inclusion showing an electron-dense granular matrix with empty capsids (arrowheads) and nucleocapsids (arrows) enclosing an electron-dense material. Scale bar = 300 nm. Fig. 5. Intranuclear inclusion showing empty capsids and nucleocapsids with hexagonal outlines located in spaces of the electron-dense matrix. Scale bar = 200 nm. Fig. 6. Abnormal cell with hypertrophied nucleus (N) with marginated heterochromatin (arrows) and a large central inclusion showing an electron-lucent circular structure (arrowhead). Scale bar = 1 µm. Fig. 7. Enlargement of an intranuclear inclusion showing empty capsids in paracrystalline array close to an electron-lucent circular structure (C). Scale bar = 100 nm. Fig. 8. Virion (arrow) with nucleocapsid surrounded by an envelope, located in the cytoplasm of the host cell in close proximity to the nucleus (N). Scale bar = 100 nm

a nucleocapsid surrounded by an envelope were seen in the cytoplasm near the nuclear membrane (Fig. 8).

The ISH assay gave rise to positive results in 3 of the tested oysters and negative results in the fourth. The cytoplasm and the nucleus of positive cells were stained brown (Fig. 9) and were restricted to connective tissues. The nucleus of the cells that did not react with the probe showed a 'healthy' aspect with chromatin evenly distributed within the nucleus. A similar positive signal was detected in OsHV-1 infected spat (positive control), whereas no signal was detected when digoxigenin-labelled probe or peroxidase-conjugated antibody were omitted. Hybridisation consistency and signal intensity varied between the 3 positive oysters.

Most cells with INAI were detected during the first year of grow-out, with peak prevalence occurring during April–May 2002; only 1 case was detected during 2003 (April), corresponding to the family CO2 (Fig. 10). Remarkably, abnormal cells with INAI were detected as early as the first sampling in IR1 (Fig. 11), just when the spat batches were transferred to grow-out from the hatchery-nursery facilities. Abnormal cells with INAI were observed in 3 families from each origin (Fig. 11). The highest mean prevalences during the entire grow-out period corresponded to the families IR1 (6.2%) and CO2 (5.4%), both of which showed the highest monthly prevalence value (30%). With regard to oyster origin, the mean prevalence was higher in Irish (2.4%) and Coroso (1.3%) oysters and lower in Ortigueira (0.9%) and Greek oysters (0.4%).

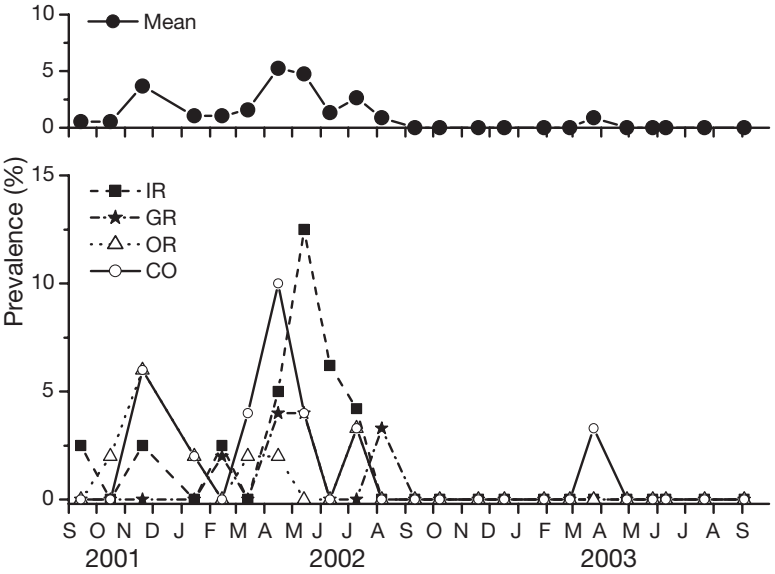


Fig. 10. *Ostrea edulis*. Monthly variation in the prevalence of the pathological condition characterised by abnormal cells with intranuclear acidophilic inclusions in oysters through the grow-out period. Top: Mean prevalence considering all the oysters of each monthly sample. Bottom: Prevalence corresponding to each stock origin (IR: Ireland; GR: Greece; OR: Ortigueira; CO: Coroso)

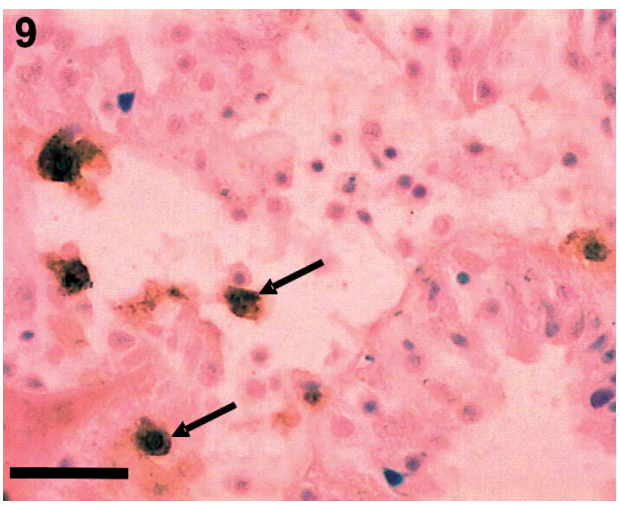


Fig. 9. *Ostrea edulis*. Micrograph of a histological section after *in situ* hybridisation with an OsHV-1 specific DNA probe showing connective tissue cells with strong positive reaction (brown deposits, arrows) in cytoplasm and nucleus. Scale bar = 20 µm

Mortality was not systematically monitored during hatchery-nursery rearing, thus accurate mortality estimations during that period are lacking. Nevertheless, higher mortality was observed in the Irish spat than in those of other origins. The families in which INAI were not detected showed the lowest cumulative mortality of their respective origins (Fig. 11). Conversely, the 2 families (IR1 and CO2) with the highest prevalence showed the highest (CO2) or close to the highest cumulative mortality of their respective origins (Fig. 11).

DISCUSSION

The results obtained by examination of histological sections, TEM and ISH led to the conclusion that the occurrence of the abnormal cells described in this study corresponded to a herpesvirus infection. The histological features of hypertrophied nuclei, marginated chromatin and Feulgen-positive INAI resembled those observed in herpes-like virus infected cells from *Ostrea edulis* (Comps & Cochennec 1993) and *O. angasi* (Hine & Thorne 1997). The intranuclear inclusions are frequent signs observed in cells infected by herpesvirus in oysters (Farley et al. 1972, Comps & Cochennec 1993, Hine & Thorne 2000, Cáceres-

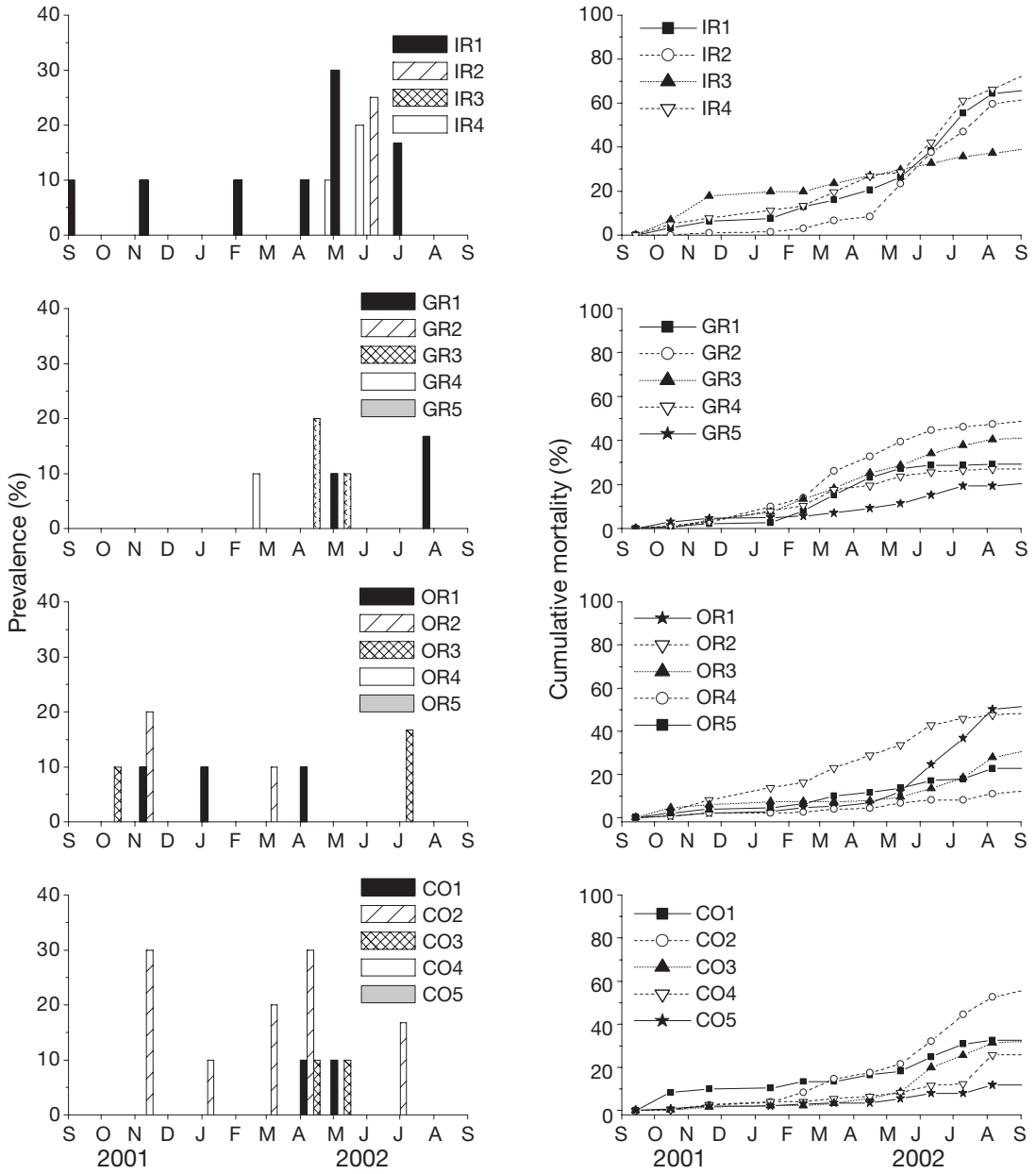


Fig. 11. *Ostrea edulis*. Left: Monthly variation in the prevalence of the pathological condition characterised by abnormal cells with intranuclear acidophilic inclusions in oysters of each family from every origin through the first year of grow-out. Right: Progression of the cumulative mortality of each family from every origin through the first year of grow-out. Families of each origin are identified by numbers 1–5. IR: Ireland; GR: Greece; OR: Ortigueira; CO: Coroso

Martínez & Vásquez-Yeomans 2003), and the enlargement of the host cell nucleus is characteristic of vertebrate herpesvirus infections (Roizman & Baines 1991). Although INAI were not reported in association with herpes viral infection of *Crassostrea gigas* spat, hypertrophied nuclei with marginated chromatin were patent in the affected cells (Renault et al. 2000a), and the reported occurrence of the infected cells in the connective tissue of visceral mass, mantle and labial palps is coincident with our observations in *O. edulis*.

Use of paraffin-embedded tissues for TEM was not the most appropriate technique, but new attempts to process fresh material were not successful. Juvenile oysters were no longer available and the abnormal condition was not detected in the newly selected older individuals. Nevertheless, TEM images allowed us to determine that the size and morphology of capsid and nucleocapsids, the occurrence of nucleocapsid assembly inside the host cell nucleus, and the occurrence of enveloped virions in the cytoplasm are in agreement

with characteristics of the family Herpesviridae (Roizman & Baines 1991). The ultrastructural characteristics of the viral particles observed in the present study are similar to those previously described from *Crassostrea gigas* and *Ostrea edulis* larvae and spat in France (Comps & Cochennec 1993, Renault et al. 1994a, 2000a, Le Deuff et al. 1996), from adult *C. virginica* in the USA (Farley et al. 1972) and from larvae of the Manila clam *Ruditapes philippinarum* and the great scallop *Pecten maximus* reared in France (Arzul et al. 2001a, Renault et al. 2001a,b). Larger sizes were reported for herpesviral particles from *C. gigas* larvae (Hine et al. 1992), adult *O. angasi* (Hine & Thorne 1997), and *Tiostrea chilensis* larvae (Hine et al. 1998) from Australia and New Zealand. Virion dimensions can vary according to the technique used to process the specimen. In this study, the de-waxing process could have affected the envelope and, thus, the virion size. The herpesvirus envelope consists mainly of lipids and glycoproteins (Roizman & Baines 1991) that can be easily altered. Complete virions were not observed inside the host cell nuclei, but only in the cytoplasm near the nuclear membrane. This observation is in agreement with the virogenesis described for the herpesviridae family, which begins in the nucleus where nucleocapsids are assembled and then continues through the nuclear membrane to acquire the envelope (Roizman & Baines 1991). Likely, the tissue processing procedure used for ultrastructural examination did not allow observation of a tegument between the capsid and the envelope, which is a typical herpesvirus structure (Roizman & Baines 1991). The circular to ovoid structures that were observed with a central electron-lucent area surrounded by a finely granular electron-dense halo occurring in some INAIs have also been reported in *O. angasi* (Hine & Thorne 1997). Comps & Cochennec (1993) did not mention this structure in their report of herpes-like virus from *O. edulis*, although a similar structure can be seen in their Fig. 2. Paracrystalline arrangement of capsids and nucleocapsids were also reported in larvae of *C. gigas* (Renault et al. 1994a,b), *T. chilensis* (Hine et al. 1998) and *R. philippinarum* (Renault et al. 2001a,b).

In situ hybridisation assay using an OsHV-1 specific DNA probe suggested that the virus infecting Galician flat oysters could be OsHV-1 or a closely related virus. Although the resolution of ISH enables viruses to be typed, ISH can only confirm identification in relation to known species or strains. The lack of labelling in one of the 4 analysed oysters might be due to a lower infection level. Hybridisation consistency and signal intensity may vary between individuals (Lipart & Renault 2002). Human error or, less likely, a different viral strain could be also hypothesised. An extensive study including both more individuals without lesions and highly infected oysters is needed in order to confirm these first results. This is the first detection of herpesvirus infection of the flat oyster *Ostrea edulis* on the Spanish coast. The first report of herpes-like virus detection in the European oyster *O. edulis* was made by D. J. Alderman (in Farley 1978) followed by Comps & Cochennec (1993) and Renault et al. (2000a). Arzul et al. (2001b) demonstrated that OsHV-1 can be transmitted from infected *Crassostrea gigas* to healthy *O. edulis*. The occurrence of the virus in the broodstock with vertical transmission to progeny is plausible despite the absence of INIA in the broodstock samples, because herpes-like virus may be present asymptomatically in adult oysters (Arzul et al. 2002, Barbosa-Solomieu et al. 2005).

In this study abnormal cells with INAI cellular lesions were almost restricted to juvenile stages. That coincided with previous observations of herpes-like virus infections in *Ostrea edulis* spat (Comps & Cochennec 1993) and larvae (Renault et al. 2000a) and with the report of herpesviral infection causing disease in *Crassostrea gigas* larvae and spat with asymptomatic adult oysters (Arzul et al. 2002, Barbosa-Solomieu et al. 2005). The viral infection was observed in oysters from each origin but not in all families, suggesting significant parental influence in susceptibility, which could be due to gene inheritance conferring increased susceptibility or vertical transmission of the viral infection. The stock of IR origin was the most heavily affected; oysters from this origin showed the worst performance (slowest growth, highest mortality and highest disease susceptibility) through the grow-out period (da Silva et al. 2005). The families in which the viral infection was not detected also showed significantly lower prevalence of bonamiosis and lower index of the overall incidence of pathological conditions through the grow-out period (da Silva et al. 2005). The families in which the viral infection was not detected showed lower cumulative mortality at the end of the first year than other families of the same origin. Therefore, the herpesviral infection might be responsible for oyster mortality to some extent during the first year of grow-out, but its contribution was difficult to evaluate because disseminated neoplasia was also prevalent through the entire grow-out period (da Silva et al. 2005). The fact that the virus was detected early in the Irish stock when transferring to grow-out from the hatchery further suggests that viral infection may have contributed to mortality during the nursery stage, which was higher in Irish oysters. Since herpes-like viruses have been blamed for mortalities in larvae and spat of *O. edulis* and other oyster species (Hine et al. 1992, Comps & Cochennec 1993, Renault et al. 1994b, 1995, Hine et al. 1998, Arzul et al. 2001a,b), this infection should be considered in genetic improvement programmes looking for *O. edulis* strains resistant to disease.

Acknowledgements. M. I. Seoane and V. Rodríguez supervised all the work to produce oyster spat in the CIMA hatchery facilities. Jose Maria Daporta Leiro e Hijos, S.L., shellfish farmers L. Nogueira, J. L. Nogueira and M. Nogueira, and the Confraría de Pescadores Nosa Señora do Carmen de Cariño helped with oyster grow-out and provided broodstock oysters. J. Méndez and I. Pazos (CACTI, Vigo) helped with TEM techniques. E. Penas, I. Meléndez, M. Andrade, P. Comesaña, I. Fernández, A. I. González, M. V. Gregorio, A. C. Iglesias and C. Rodríguez provided field and laboratory technical assistance. This work was partially supported by funds from the Secretaría Xeral de Investigación e Desenvolvemento Tecnolóxico da Xunta de Galicia, through the project PGIDT-CIMA 01/1. P.M.S. was supported by successive scholarships from the Agencia Española de Cooperación Internacional (AECI) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of the Brazilian Government.

LITERATURE CITED

Andreu B (1968) Pesquería y cultivo de mejillones y ostras en España. Publ Téc Junta Est Pesca 7:303–320

Arzul I, Nicolas JL, Davison A, Renault T (2001a) French scallops: a new host for ostreid herpesvirus-1. Virology 290:342–349

Arzul I, Renault T, Lipart C (2001b) Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission. Dis Aquat Org 46:1–6

Arzul I, Renault T, Lipart C, Davison AJ (2001c) Evidence for interspecies transmission of oyster herpesvirus in marine bivalves. J Gen Virol 82:865–870

Arzul I, Renault T, Thébault A, Gérard A (2002) Detection of oyster herpesvirus DNA and proteins in asymptomatic *Crassostrea gigas* adults. Virus Res 84:151–160

Barbosa-Solomieu V, Miossec L, Vázquez-Juárez R, Ascencio-Valle F, Renault T (2004) Diagnosis of Ostreid herpesvirus 1 in fixed paraffin-embedded archival samples using PCR and *in situ* hybridization. J Virol Methods 119:65–72

Barbosa-Solomieu V, Dégremont R, Vázquez-Juárez R, Ascencio-Valle F, Boudry P, Renault T (2005) Ostreid herpesvirus 1 (OsHV-1) detection among 3 successive generation of Pacific oysters (*Crassostrea gigas*). Virus Res 107:47–56

Burge CA, Estes RM, Stokes NA, Hargrove JS and others (2004) Identification of a herpes-like virus in Pacific oysters from Tomales Bay, California. J Shellfish Res 23:283

Cáceres-Martínez J, Vásquez-Yeomans R (2003) Presence of giant polymorphic cells in *Crassostrea gigas* cultured in Bahia Falsa, Baja California NW Mexico. J Shellfish Res 22:711–714

Comps M, Cochennec N (1993) A herpes-like virus from the European oyster *Ostrea edulis* L. J Invertebr Pathol 62: 201–203

da Silva PM, Fuentes J, Villalba A (2005) Growth, mortality and disease susceptibility of oyster *Ostrea edulis* families obtained from brood stocks of different geographical origins, through on growing in the Ría de Arousa (Galicia, NW Spain). Mar Biol 147:965–977

Farley CA (1978) Viruses and virus-like lesions in marine mollusks. Mar Fish Rev 40:18–20

Farley CA, Banfield WG, Foster WS (1972) Oyster herpes-type virus. Science 178:759–760

Hine PM, Thorne T (1997) Replication of herpes-like viruses in haemocytes of adult flat oysters *Ostrea angasi*: an ultrastructural study. Dis Aquat Org 29:189–196

Hine PM, Thorne T (2000) A survey of some parasites and diseases of several species of bivalve mollusc in northern Western Australia. Dis Aquat Org 40:67–78

Hine PM, Wesney B, Hay BE (1992) Herpesviruses associated with mortalities among hatchery-reared larval Pacific oysters *Crassostrea gigas*. Dis Aquat Org 12:135–142

Hine PM, Wesney B, Besant P (1998) Replication of a herpes-like virus in larvae of the flat oyster *Tiostrea chilensis* at ambient temperatures. Dis Aquat Org 32:161–171

Howard DW, Smith CS (1983) Histological techniques for bivalve molluscs. NOAA technical memorandum NMFS-F/NEC-25, Woods Hole, MA

Le Deuff RM, Nicolas JL, Renault T, Cochennec N (1994) Experimental transmission of a herpes-like virus to axenic larvae of Pacific oyster, *Crassostrea gigas.* Bull Eur Assoc Fish Pathol 14:69–72

Le Deuff RM, Renault T, Gérard A (1996) Effects of temperature on herpes-like virus detection among hatchery-reared larval Pacific oyster *Crassostrea gigas.* Dis Aquat Org 24:149–157

Lipart C, Renault T (2002) Herpes-like virus detection in infected *Crassostrea gigas* spat using DIG-labelled probes. J Virol Methods 101:1–10

Nicolas JL, Comps M, Cochennec N (1992) Herpes-like virus infecting Pacific-oyster larvae, *Crassostrea gigas.* Bull Eur Assoc Fish Pathol 12:11–13

Polanco E, Montes J, Outón MJ, Meléndez MI (1984) Situation pathologique du stock d'huitres plates en Galice (Espagne) en relation avec *Bonamia ostreae*. Haliotis Paris 14:91–95

Renault T, Arzul I (2001) Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR. J Fish Dis 24:161–167

Renault T, Lipart C (1998) Diagnosis of herpes-like virus infections in oysters using molecular techniques. Eur Aquac Soc Spec Publ 26, p 235–236

Renault T, Cochennec N, Le Deuff RM, Chollet B (1994a) Herpes-like virus infecting japanese oyster (*Crassostrea gigas*) spat. Bull Eur Assoc Fish Pathol 14:64–66

Renault T, Le Deuff RM, Cochennec N, Maffart P (1994b) Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France—comparative study. Rev Med Vet Toulouse 145:735–742

Renault T, Le Deuff RM, Cochennec N, Chollet B, Maffart P (1995) Herpes-like viruses associated with high mortality levels in larvae and spat of pacific oysters, *Crassostrea gigas*: a comparative study, the thermal effects on virus detection in hatchery-reared larvae, reproduction of the disease in axenic larvae. Vet Res 26:539–543

Renault T, Le Deuff RM, Chollet B, Cochennec N, Gérard A (2000a) Concomitant herpes-like virus infections in hatchery-reared larvae and nursery-cultured spat *Crassostrea gigas* and *Ostrea edulis.* Dis Aquat Org 42:173–183

Renault T, Le Deuff RM, Lipart C, Delsert C (2000b) Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. J Virol Methods 88:41–50

Renault T, Lipart C, Arzul I (2001a) A herpes-like virus infecting *Crassostrea gigas* and *Ruditapes philippinarum* larvae in France. J Fish Dis 24:369–376

Renault T, Lipart C, Arzul I (2001b) A herpes-like virus infects a non-ostreid bivalve species: virus replication in *Ruditapes philippinarum* larvae. Dis Aquat Org 45:1–7

Renault T, Arzul I, Lipart C (2004) Development and use of an internal standard for Oyster Herpesvirus 1 detection by PCR. J Virol Methods 121:17–23

Roizman B, Baines J (1991) The diversity and unity of Herpesviridae. Comp Immunol Microbiol Infect Dis 14:63–79

Román G (1992) Efecto del ciclo reproductivo y el acondicionamiento en el desarrollo larvario, la fijación y el posterior crecimiento de la semilla de *Ostrea edulis* Linné 1758. Publ Espec Inst Esp Oceanogr 9:1–172

Editorial responsibility: Richard Cawthorn, Charlottetown, Prince Edward Island, Canada

Submitted: June 2006; Accepted: October 2007
Proofs received from author(s): December 10, 2007