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

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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

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This communication summarizes some of the work undertaken at the shellfish disease laboratory of La Tremblade concerning a herpes-like virus in *Crassostrea gigas*.

A herpes-like virus associated with high mortality levels among hatchery-reared larvae of the Pacific oyster, *C gigas,* was observed for the first time during the summer of 1991 by Nicolas *et al* (1992) in France and by Hine *et al* (1992) in New Zealand. Since these first reports, there have been similar outbreaks of the disease, during the summers of 1992 to 1994, associated with the detection of a herpes-like virus on the Pacific oyster larvae reared in several French hatcheries. Significant mortality levels were observed by day 6 of the infection, with 100% mortality attained by day 8–10 for most batches. Sporadic and abnormally high mortality rates (80–90%) also occurred in the summers of 1993 and 1994 among several batches of cultured spat of *C gigas* from different French locations (Renault *et al,* 1994a). Histological and electron microscopic examinations of the animals revealed that they were also infected by a herpes-like virus.

The major histological changes in the diseased larvae and spat consisted essentially of enlarged and abnormally shaped nuclei and abnormal chromatin patterns throughout the connective tissue (fig 1a and b). The inflammatory reaction around the infected cells was also reduced.

Morphological observation revealed that the herpes-like virus that was associated with the high mortality levels among the hatchery-reared larval Pacific oysters (fig 1c) seemed to be the same as the one reported in Pacific oyster spat (fig 1d) (Renault *et al,* 1994b). They had the same structural characteristics, the same cellular localizations and were of comparable size. Moreover, the morphological characteristics of the viruses described here are closely related to those of the viruses reported among the Pacific oyster by Nicolas *et al* (1992) and Hine *et al* (1992).

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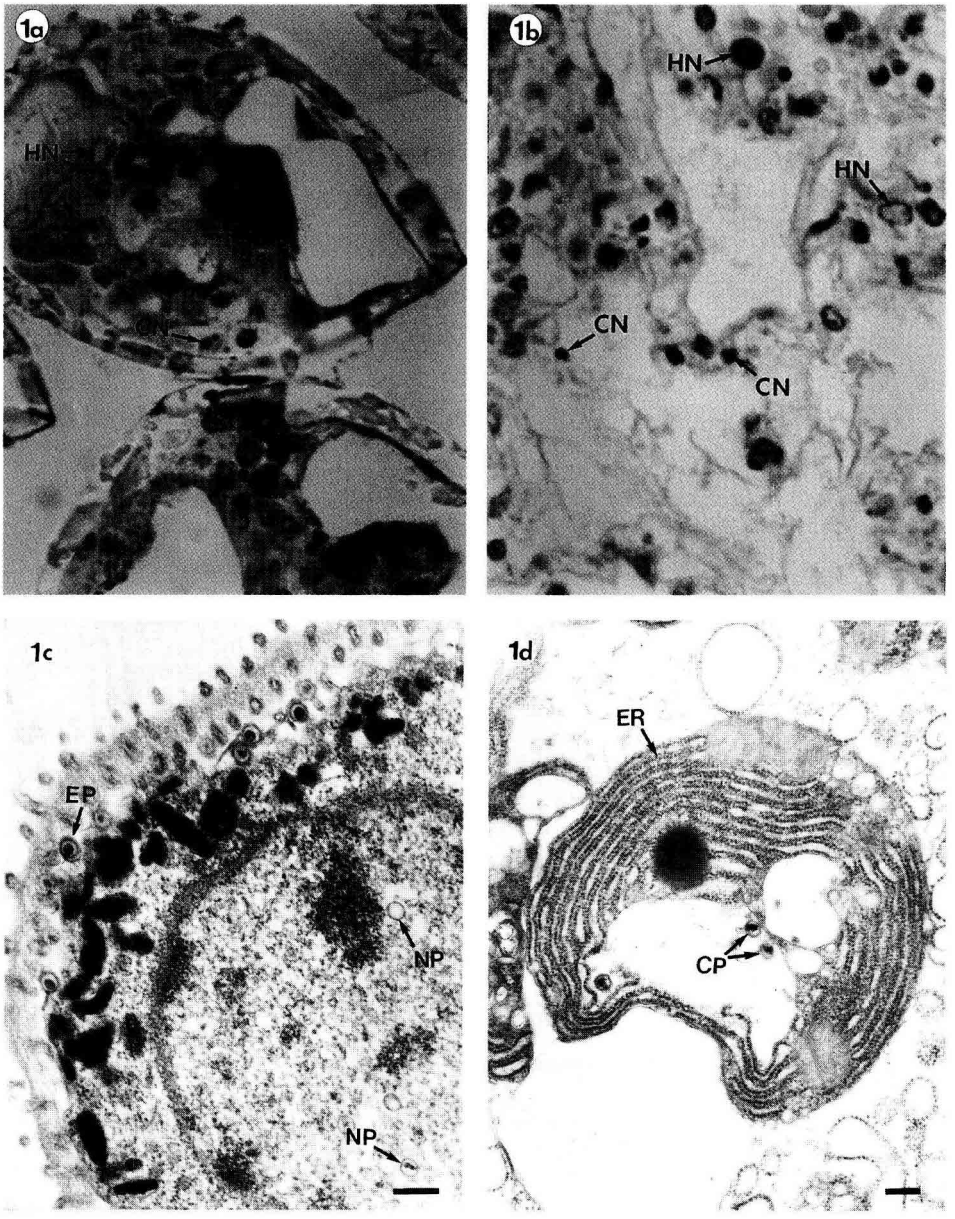

Fig 1. Changes in herpes-like virus infected tissues of Pacific oyster larvae (**a**) and spat (**b**) with condensed nuclei (CN) and hypertrophied nuclei presenting marginalization of chromatin (HN). Transmission electron micrographs of herpes-like virus infecting Pacific oyster larvae (**c**) and spat (**d**). NP: nuclear virus particles. CP: cytoplasmic virus particles. EP: extracellular virus particles. ER: proliferation of endoplasmic reticulum in infected spat cell. Bars: 200 nm.

The virus was experimentally transmitted to Pacific oyster larvae (Le Deuff *et al*, 1994) using virus suspensions obtained from infected larvae. These were milled in sea water and filtered through a 0.2 µm filter. The virus suspensions were inoculated into 2-day-old axenic larvae. Four to 6 d post inoculation, 100% mortality was reached. Analysis by electron microscopy confirmed the presence of the virus in the inoculated axenic larvae.

A common feature to all the herpes-associated deaths among ostreids is their occurrence during the hotter summer period. It

Table I. Transmission electron microscope (TEM) and light microscope (LM) examination of fixed samples of Pacific oyster larvae held at different temperature: 22–23°C (22) or 25–26°C (25).

Days	*LM*				*TEM*					
	%NA		*N*		*%NA*		*%VP*		*N*	
	22°C	*25°C*	*22°C*	*25°C*	*22°C*	*25°C*	*22°C*	*25°C*	*22°C*	*25°C*
Broodstocks from Brest										
6	0		90		0		0		5	
8		0		70		0		0		3
10	0		35		0		0		5	
13										
15	0	0	22	31	0	0	0	0	5	4
Broodstocks from Arcachon										
6	0	100	100	50	0	100	0	100	6	4
8	0	*	70	*	0	*	0	*	10	*
10	10	*	40	*	8	*	0	*	12	*
13	25	*	32	*	0	*	0	*	6	*
15		*		*		*		*		*
Broodstocks from Marennes										
6	6	29	117	80	60	100	0	100	5	5
8		*		*		*		*		*
10	ND	*	ND	*	40	*	0	*	5	*
13		*		*		*		*		*
15	0	*	14	*	40	*	0	*	5	*
Broodstocks from La Tremblade										
6	0	0	90	60	0	0	0	0	6	4
8	0	49	95	45	0	100	0	100	9	4
10	ND		ND		0		0		6	
13	3	100[a]	35	45[a]	0	[b]	0	[b]	2	4
15	10.5	*	19	*	0	*	0	*	2	*

Observation of nuclear alterations (NA) and/or the presence of herpesvirus-like particles (VP) are indicated as a percentage calculated from the indicated number of animals examined in each case (N). ND: value not determined. [a] Among 45 larvae observed, 41 were empty shells, but 100% nuclear alterations were found in the 4 shells of larvae containing tissues. [b] Only empty shells of larvae were found, observation of virus particles was therefore not possible. * 100% mortality occurred, no analysis could be performed later.

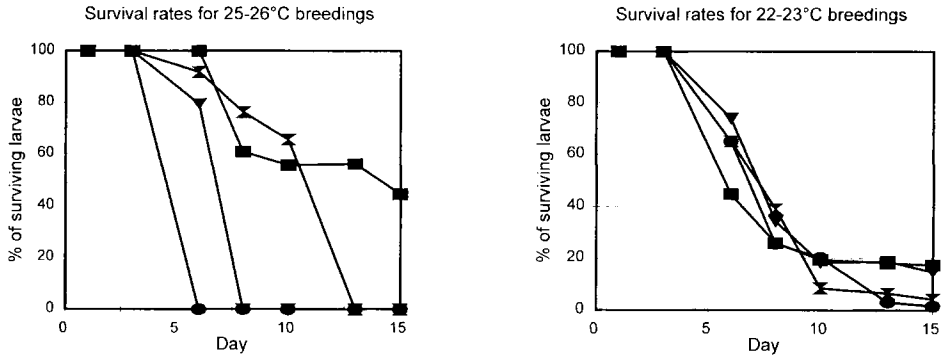

Fig 2. Survival rates of Pacific oyster larvae held at either 22–23°C or 25–26°C. Spawns were performed with broodstocks from 4 different French sites: Brest (■), Marennes (–⧗–), Arcachon (●) and La Tremblade (⧗). At 25–26°C, the survival rates of the larvae from Arcachon, Marennes and La Tremblade decreased suddenly and reached 0%on the 6th, 8th or 13th day post-fertilization respectively. Survival level for larvae from Brest was 44.4% on the 15th day. Decrease of survival levels in groups of larvae held at 22–23°C were more progressive. On the 15th day, they were lower than those of the 25–26°C groups for larvae from Arcachon (1.6%), Marennes (14.8%) and La Tremblade (4.2%). Survival level for larvae from Brest was 17.4% on the 15th day, which is a normal value when larvae are held at such low temperatures.

seemed possible, therefore, that temperature influences the rate of the viral productive cycle and may also be implicated in the activation of the infection from a latent to a productive phase. In order to investigate these hypotheses, we bred groups of *C gigas* larvae at different temperatures. These larvae issued from parents of different French origins: Brest; Arcachon; Marennes; and animals from Arcachon reared in La Tremblade for a period of several months. Fertilization was performed between males and females of the same origin. The spawn were further divided into 2 groups that were held at either 22–23°C or 25–26°C.

The analysis of the semi-thin sections of the oyster larvae from Arcachon, Marennes and La Tremblade cultivated at either 22–23°C or 25–26°C (table I) revealed condensed nuclei and nuclei presenting marginalization of their chromatin. No lesions were observed in the groups of oyster larvae from Brest. Analysis by transmission electron microscope (table I) only revealed the presence of herpes-like virus particles in the samples of oyster larvae from Arcachon, Marennes and La Tremblade held at the higher temperature (25–26°C). Herpes-like virus particles were never detected in the groups of larvae from the parents of all the various origins reared at 22–23°C or in the larvae from Brest. The herpes-like virus particles (fig 2) were found in the groups raised at 25–26°C in which sudden high mortality rates occurred. The nuclear alterations in the 22–23°C groups correlated with more progressive mortality rates.

In this study, viral particles could only be observed at 25–26°C. A similar feature was reported by Farley (1972) for a herpesviral infection among *C virginica*. The histology techniques used here, however, revealed nuclear alterations in the animals reared at 22–23°C, although these were not associated with the presence of viral particles at the ultrastructural level. These nuclear aberrations could result either from a slow viral productive cycle, in which the viral particles are very rare and are therefore not detected,

or from a non-productive state, a latent phase or an abortive cycle, in which progeny viral particles are not produced although some viral proteins may be synthesized (Girard and Hirth, 1989; Garcia-Blanco and Cullen, 1991). Consequently, the animals held at the low temperature (22–23°C), while being negative for viral-particle detection by transmission electron microscopy, could still be considered to be infected by the herpes-like virus. This means that larval rearing at 22–23°C is potentially hazardous because although acute infection and high levels of larval mortality are avoided, latent, asymptomatic, carrier animals may be produced.

In conclusion, it seems that both temperature and parental origin influence the expression of the herpes-like virus in *C gigas* larvae. Higher temperatures promote the early production of viral particles in association with high larval mortalities by activating the latent viral phase and/or by enhancing the rate of the viral productive cycle.

As the herpes-like virus is assumed to be carried by the parents, we propose to investigate the possible mechanism for the transmission of the virus to the larvae. The virus transmission and the development of infection in larvae may also be related to the larval breeding temperature and to stresses on broodstock oysters. We hope to be able to establish a breeding procedure that could avoid both the production and dissemination of larvae carrying the virus, which are susceptible to developing the disease in a later spat stage, or to transmitting the herpes-like virus to their progeny. Our future objectives also include the purification of the herpes-like virus. In our preliminary experiments, we obtained an enriched suspension of virus particles from infected larvae. A purified stock of the virus would permit the further development of diagnostic methods based on the use of nucleic acid probes or on the development of antibodies specific for this virus.

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