CHRONIC MORTALITY IN A BIVALVE MOLLUSC HATCHERY: AN INVESTIGATION IN PROGRESS

*by L. F. Richens *[1], R-M. Le Deuff[1], A. E. Bayley[1], P. D. Martin[1], K. S. Bateman[1] and K. V. Thomas[2]*

CEFAS
The Centre for Environment, Fisheries & Aquaculture Science

Introduction

Persistent, chronic high mortalities of bivalve larvae have been a continuing problem in a British bivalve hatchery. Species affected are Pacific oyster (*Crassostrea gigas*), Manila clam (*Tapes philippinarum*) and carpet shell (*Tapes decussatus*). The peak mortality of Pacific oyster larvae is between five and six days post hatch, whilst the larvae of the Manila and carpet shell clams die pre-settlement, typically at ten to thirteen days post hatching. Chronic high mortality has been experienced in 90% of spawned batches of clams, each of around 100 million larvae, over several production seasons. Samples of larvae and water were examined for the presence of viral or bacterial pathogens that might be associated with significant mortalities in larval molluscs. Seawater from the affected site was also screened for the presence of toxins using the TIE method.

Materials and Methods

Bacteriology

Larvae

Batches of approx. 1000 larvae suspended in 0.1ml of sterile saline and were homogenised using micro-pestle attached to a hand-held, battery-operated impeller. Suspensions made up to 1ml using sterile saline and diluted to 10^{-6}. 100µl of each concentration was then spread onto TSA, Sea Water Agar and TCBS agar using a sterile spreader and incubated for 36 hours. Plate counts were made at 24, 48 and 36 hours.

Sea water

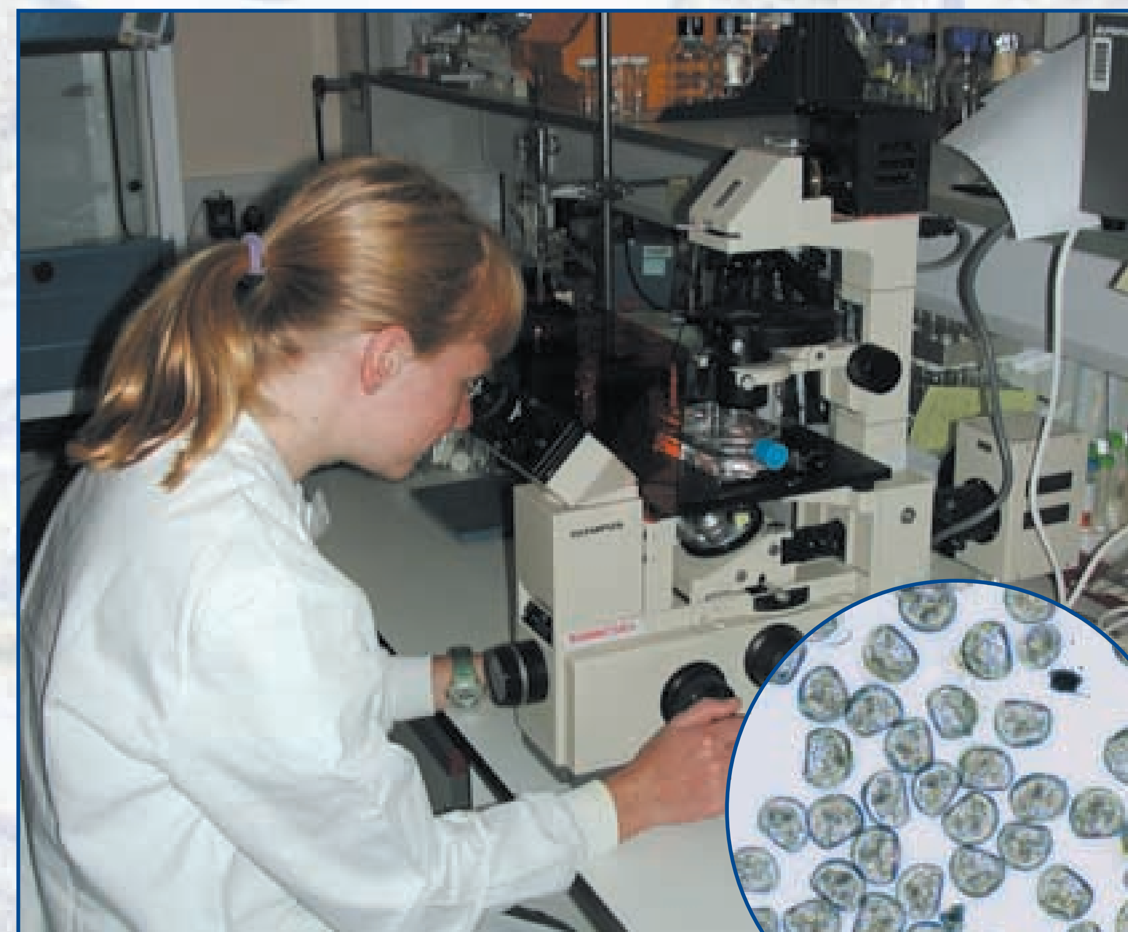
1) 500 ml seawater filtered using 0.2 micron filter.
2) Filters put into universal with 10ml sterile seawater and vortexed for 2 minutes.
3) Diluted to 10^{-7} and 100µl of each dilution spread onto SWA as above and incubated for 36 hours. Plate counts after 24, 36 and 48 hours as above.

Algae

Algae samples mixed and diluted to 10^{-7} and spread onto TSA. Plate counts made after 24, 36 and 48 hours.

Table 1:

Species	Average number of organisms per larvae		
	Vibrio spp. (10 strains)	*Pseudoalteromonas*	*Vibrio splendidus* Type 1
Tapes decussatus	109	905	-
Tapes phillippinarum	30	-	601
Crassostrea gigas	-	395	764



Microscopic evaluation of larval challenge assay

Virology

The known oyster larval virus infections cannot be isolated into tissue culture since no oyster cell lines exist that might be used. A PCR method developed for the diagnosis of OsHV-1 (Renault *et al.*, 2000) was performed. Additionally see histopathology.

Histopathology

Apart from OsHV-1 identification, which is relatively well documented, there is no other specific method available for the direct diagnosis of other bivalve mollusc viruses. The histological examination, implemented with TEM, remains the ultimate method of diagnosis. As these methods allow direct observation of tissues, they are also an effective way to screen for new viruses or parasites.

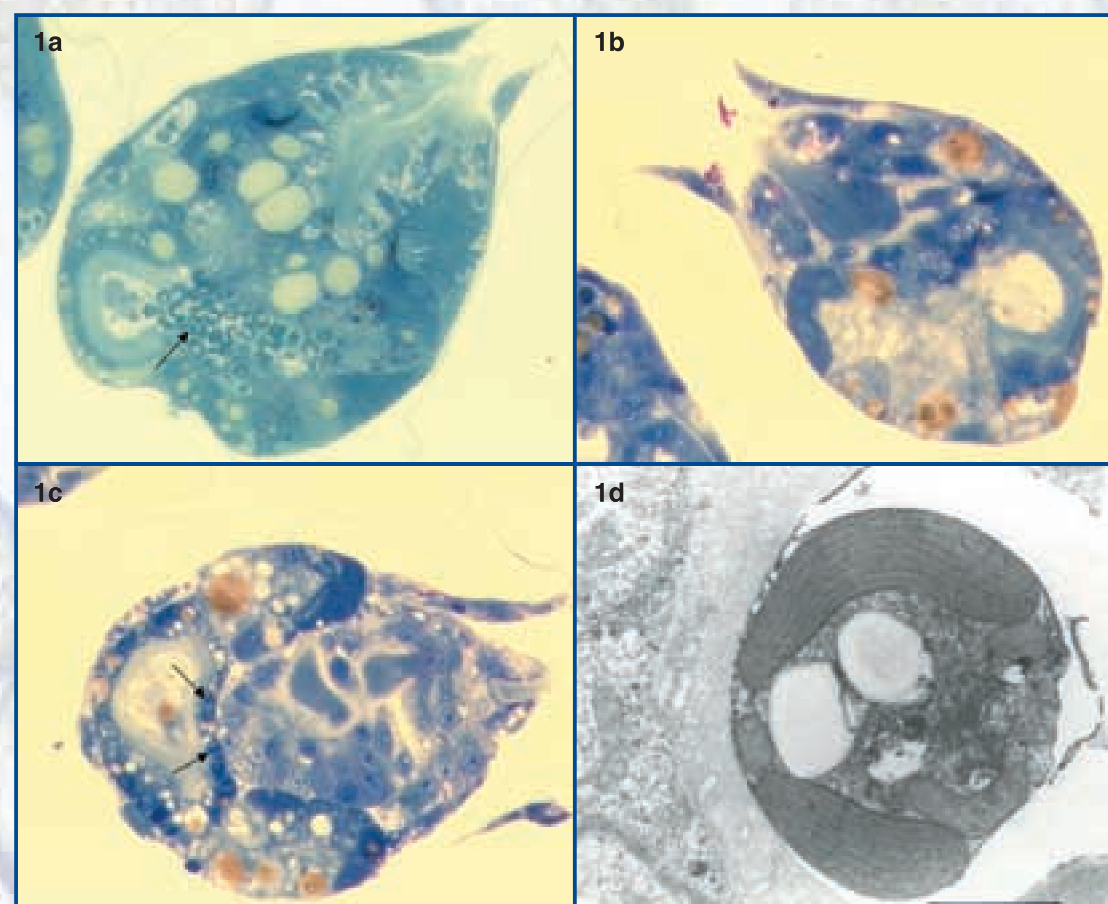


Figure 1: (1a) Spherical bodies of a *Chlorella*-like (?) organism (CLO) in the lumen of the stomach of a *C. gigas* larvae (arrow). (1b) Deposit of dense unidentified material is present mainly in the velum cells (arrow) in association with CLO in this sample of *T. decussatus* larvae. (1c) Possible dividing forms of CLO were observed in the tissues surrounding the stomach epithelium (arrows). (1d) TEM of CLO in lumen *T. decussatus* larvae. Bar: 1µm.

Mollusc hatchery larval incubation columns

Toxicity Identification Evaluation (TIE)

Samples of water from the hatchery are evaluated using an oyster (*Crassostrea gigas*) larvae bioassay. The growth of larvae in the sample water is compared to growth in reference seawater. This growth is measured by image analysis and results compiled by a sophisticated computer program.

Results and discussion

- Although *Vibrio splendidus* was found frequently in *Tapes phillippinarum* and *Crassostrea gigas*, larval challenge assays with this strain do not indicate particular pathogenicity. *Pseudoalteromonas* isolates, frequent from *Tapes decussatus* and *Crassostrea gigas* had no effect even at 100,000 CFU/ml. Larval challenge assay work is ongoing.
- No toxicants have been identified in hatchery water using TIE.
- No trace of oyster velar virus (OVV, *Iridoviridae*) could be detected in the samples of larvae examined by histopathology or TEM. PCR and histological showed no evidence OsHV-1 infection in 2000-2001 samples, however, OsHV-1 was found in a 1997 sample of larval *C. gigas*. No other evidence suggesting viral aetiology was found.
- A significant number of spherical bodies were noted in the stomach of larvae (Figure 1a) in five of the six batches examined histologically. Dilation of the stomach was observed but no significant tissue necrosis. An abnormal deposit of dense material was observed, mainly localised in the velum, in *T. decussatus* (Figure 1b). These observations seem to correlate to a condition described by Elston (1980) as *digestive tract impaction of oysters*, associated with the presence of a "*Dermocystidium*-like organism". In this case the organism is not *Dermocystidium*, nor is it *Hyalochlorella* Poyton (1970), also associated with digestive tract impaction in oyster larvae. In this case chloroplasts were observed using TEM. The organism does not appear to be a larval feed species. Temporarily the term "Chlorella-like-organism" (CLO) is being used. Possible dividing forms of the organism were observed. (Figure 1c). Ultrastructural investigations are on-going (Figure 1d), but further identification is not yet complete.

Conclusion

Bacterial and viral aetiologies have been investigated, as well as the possibility of water toxicity. There was no conclusive evidence to suggest that any one of these agents was the primary cause of the mortality events. Currently investigations are concentrating on the potential digestive tract impacting organism described above.

References

Elston, R., (1980). Ultrastructural aspects of a serious disease of hatchery reared larval oysters, *Crassostrea gigas* Thünberg. *J. Fish Dis.*, **3**(1): 1-10.

Renault, T., LeDeuff, R.M., Lipart, C. and Delsert, C. (2000). Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. J.Virol. Methods, **88**(1), 41-50.

Poyton, R.O. (1970). The characterization of *Hyalochlorella marina* gen. et. sp. nov. a new colourless counterpart of Chlorella. *J. Gen. Microbiol.* **62**, 171-188.

Acknowledgements

This work was conducted under MAFF contracts C1033 (LINK Aquaculture), F1136 and EU contract FAIR-CT98-4334.

We should also acknowledge colleagues from IFREMER labs in Brest (J. -L. Nicolas) and La Tremblade (Dr T. Renault and Dr F. Leroux) for their help and expert advice. This study would not have been possible without the valuable contribution of private shellfish hatcheries'.

[1] The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory, The Nothe, Barrack Road, Weymouth, Dorset DT4 8UB, UK. http://www.cefas.co.uk
[2] The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Burnham Laboratory, Remembrance Avenue, Burnham on Crouch, Essex CM0 8HA, UK. http://www.cefas.co.uk

© Crown Copyright 2001