Diseases in Mollusc Hatcheries and their Paradox in Health Management

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ABSTRACT

Global molluscan aquaculture production is continuously increasing, dominated by five species, among which the Pacific cupped oyster, *Crassostrea gigas*, predominates. Hatchery production is accompanying this increase to assist consistent availability of juveniles for restocking, fishery enhancement, genetic improvements as well as for species diversification. Hatchery development contributes significantly to the demand for international transfers of live molluscs; a consequence of which pathogen transfer via transfer of live molluscs is currently recognised as a major cause of epizootic disease outbreaks. Diseases are a primary constraint to mollusc aquaculture growth and sustainability, severely impacting socio-economic development in many countries. Several diseases which occur in hatcheries could be disseminated with live transfers to grow-out areas. On the other hand, hatchery production may also be a way to provide disease-free juveniles and therefore be a pivotal tool to prevent the transfer of infected stocks to susceptible areas. After reviewing the importance of hatcheries for molluscs and mollusc diseases in hatcheries, this paradox in health risk and management is discussed.

INTRODUCTION

Hatchery production involves use of broodstock to produce fertilised eggs, larvae and juveniles for growth to market size or for enhancement. Although mastery of such a process may appear pivotal in the development of aquaculture, traditional mollusc culture in many countries is based on the use of wild stocks and populations. In Europe, for example, culture of the edible flat oyster, *Ostrea edulis*, has existed since Roman times, long before any technical hatchery development (Coste, 1861).

However, marine molluscs are an important commodity, many species providing a cheap protein source and some being luxury items. Mollusc aquaculture has increased over the past decades, averaging a growth of 11.5% p.a. in the nineties, and continues to increase (FAO, 2002). In 2000, world production of molluscs reached about 10.7 million metric tonnes. These data underline the increased demand for juveniles of molluscs.

A rapid overview reveals a rich variety of species and physical environments where mollusc culture is practised. However, natural populations often do not fulfil the market demand. Many reasons may lead to this situation such as poor value of the product, over-fishing of the resource, lack of availability of juveniles or impact of diseases on stocks. A solution to

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this has frequently been the introduction of new stocks or new species (Goulletquer and Héral, 1997).

Few basic criteria have dictated which mollusc species are good candidates for aquaculture. Existing mollusc aquaculture industries are based on a limited number of species suitable for mass cultivation. Forty-two mollusc species contribute to global production, however only five species dominate: the Pacific cupped oyster, *Crassostrea gigas*, the Manila clam, *Ruditapes philippinarum*, the Yesso scallop, *Patinopecten yessoensis*, the blue mussel, *Mytilus edulis*, and the blood cockle, *Anadara granosa* (FAO, 2002).

The Pacific oyster, *C. gigas*, illustrates this situation by its geographic distribution and economic weight. It is the second most widely cultivated farmed aquatic species by weight at 3,944,042 metric tonnes and currently represents over 36% of global mollusc aquaculture production. As a consequence of its success, there have been many transfers and introductions of *C. gigas* throughout the many countries culturing this species.

These movements have had very positive impacts and provided strong support to the development of the oyster-culture sector, however, they have also had some unforeseen detrimental consequences.

A well documented example is given by the parasite *Haplosporidium nelsoni* (Farley, 1968; Andrews, 1984), the causative agent of MSX disease of the American oyster, *Crassostrea virginica*. A parasite morphologically similar to *H. nelsoni* but causing no disease was reported from Pacific oysters, *C. gigas*, on the west and east coasts of the United States (Friedman *et al*., 1991). The parasite was later identified as *H. nelsoni* by the use of a specific DNA probe (Stokes and Burreson, 1995). It is thought to have been introduced in *C. gigas* imported from Japan onto the west coast of the United States, and from there to the east coast, where it jumped host from *C. gigas* into *C. virginica* (Friedman, 1996; Burreson *et al*., 2000). It was also probably introduced into France in *C. gigas* (Renault *et al*., 2000). This highlights, if needed, the inherent risk associated with the international trade of live molluscs (Hine, 1996; Grizel, 1997).

Given that fisheries harvests have markedly declined in recent years for many species, aquaculture will have an increasing role to provide future supply of many molluscs. Hatcheries will be needed for reseeding and stock enhancement, genetic improvement of cultured species and technological improvement of mollusc aquaculture. Thus, economically sound and viable hatchery technologies are being developed or improved for many species.

In this context, I review and discuss the apparent paradox of mollusc hatcheries, seen as a potential for spreading pathogens or preventing their spread.

DISEASES OF MOLLUSCS IN HATCHERIES

Several diseases are known to occur in hatcheries. Crowding of animals and stress are inherent conditions in hatcheries, recognised to promote disease outbreaks. Disease may affect broodstock survival or health, quality of spawn, survival of larvae or juveniles when seeded in grow-out areas. This paper is not intended as an exhaustive review of the literature on diseases in mollusc hatcheries, as there are too many papers to make such a review manageable. Valuable information may be retrieved from a previously published synopsis of infectious diseases and parasites of commercially exploited shellfish (Bower *et al*., 1994), and only a few examples will be cited here.

On the Atlantic coast of Canada and the United States, tissue lesions and mortality occurs in Atlantic bay scallop broodstock, *Argopecten irradians*, being conditioned for spawning when water temperatures exceed 20°C (McGladdery *et al*., 1991). Parasites spread throughout the body and proliferation results in haemocytic infiltration and epithelial disruption. Timing of this proliferation appears to coincide with gonadal maturation; however, effect of the parasite is difficult to distinguish from normal post-spawning mortality, characteristic of bay scallops. Transmission from adult to larvae of the pathogen, formerly described as *P. karlssoni* though now known not to belong to the genus Perkinsus (Goggin *et al*., 1996), can occur during spawning (McGladdery *et al*., 1993), and therefore, scallops originating from areas with records of the disease should not be used as broodstock in hatcheries.

A viral gametocytic hypertrophy has been associated with papova-like viruses (Elston, 1997). This infection causes massive hypertrophy of individual gametes and gametogenic epithelium associated with viral replication in the host nucleus. It was reported in *C. virginica* from the Atlantic coast of North America. Other species, such as the Pacific oyster, *C. gigas*, mangrove cupped oyster, *C. rhizophorae*, Sydney rock oyster, *Saccostrea glomerata*, Olympia flat oyster, *Ostrea lurida*, silverlip pearl oyster, *Pinctada maxima* and soft-shell clam, *Mya arenaria*, were reported to be also infected by papova-like viruses. The host response to infection is apparently negligible and level of infection generally low with no indication of associated mortality. However, given the apparent broad spectrum of host species, this infection should be scrutinised by investigators and diagnosticians in hatcheries.

Another example of disease affecting broodstock is given by *Marteilioides chungmuensis* (Comps *et al*., 1987; Itoh *et al*., 2002). This paramyxean parasite occurs in *C. gigas* from Korea and Japan and has been known since 1970 (Chun, 1970). Parasites infect the cytoplasm of oocytes and can affect large areas of reproductive follicles. During the spawning season, infected oysters develop lumps or nodule-like gonads on their body and are unacceptable in the market, resulting in a serious economic loss to oyster farmers. Though, infected eggs may be liberated, infected oysters often carry large numbers of ripe but infected eggs. This suggests that the infection leads to spawning failure by delaying spawning, as well as the destruction of ripe oocytes.

Herpesvirus infections are responsible for mass mortalities of molluscs, particularly larvae and juveniles (Arzul and Renault, 2002). They have been reported from various places in USA, Mexico, Tasmania, Australia, New Zealand and France, and are suspected to be widely distributed. Many host species have been recognised including *C. virginica, C. gigas, Ostrea edulis*, as well as the Australian mud oyster, *O. angasi*, the Chilean flat oyster, *Tiostrea chilensis*, the grooved carpet shell, *Ruditapes decussatus*, the Manila clam, *R. philipinarum*, and the great Atlantic scallop, *Pecten maximus* (Hine *et al*., 1992; Hine *et al*., 1998; Arzul *et al*., 2001). It is not known if the herpes-like viruses reported from various species of molluscs and various locations are the same; however, cases of inter-specific transmission are documented. Herpes-like virus quickly spread among *C. gigas* larvae, with cumulative mortality rates approaching 100% within a short period. Although no associated disease nor clinical signs, was reported in adult *C. virginica* (Meyers, 1981), or associated to concurrent infection with *Bonamia exitiosa* in adult *O. angasi* (Hine and Thorne, 1997). These viral infections may be complicated by bacterial infections which contribute to the severity of the case.

How important are these diseases in terms of impact is a difficult question to answer. Herpes-like viruses apparently do not affect the oyster industry in France. In Korea, oyster production has declined over the past decade. Several explanations have been proposed to account for this, such as slow growth in the highly intensive culture system (Kang *et al*., 2000), and more probably, shortage of healthy seed oysters (Park *et al*., 1999a; 1999b). Occurrence of herpes-like virus infection is also suspected (Comps, personal communication).

Above were discussed a few examples of hatchery related diseases. Many diseases are known to affect molluscs during the hatching process (Bower *et al*., 1994). Lack of scientific data with regards to diseases of molluscs has frequently been emphasised by authors (Hine, 1996; Berthe, 2001). However, one should question why little attention is paid to the available information. It can be anticipated that more pathogens will be detected with new developments of hatcheries and increased use of hatchery produced seeds and juveniles.

At this point, a rough categorisation of these diseases enables us to distinguish diseases affecting hatcheries, resulting in significant production losses, from diseases posing a threat to grow-out culture and wild stocks. Given the increasing contribution of hatcheries in mollusc aquaculture, the latter should be seriously considered. Moreover, improved management of diseases may lead to them being moved from the first category to the second.

Juvenile vibriosis caused by *Vibrio* spp., including *Vibrio* [*harveyi*] *carchariae*, are reported to affect abalone, *Haliotis* spp. (Elston and Lockwood, 1983; Nicolas *et al*., 2002). Systemic infection of the soft-tissues results in tissue necrosis and death. In abalone hatcheries, vibriosis is usually not considered to be a problem because of the relatively short larval period, and the stringent sanitary practices employed (Bower, 2000). Vibriosis appears to be directly related to poor and stressful rearing conditions that render young abalone more susceptible to infection. A consequence of husbandry improvements is the potential seeding of sub-clinically infected juveniles that may later experience disease during their economic life in open waters and thus contribute to pathogen dissemination.

Similarly, rearing conditions of *C. gigas* in a hatchery may prevent outbreaks of herpesvirus infection during larval stages. Cases are associated with warm water temperature; 80 to 90% mortality of larvae reared at 25 to 26°C were reported, while no mortality occurred in larvae reared at 22 to 23°C (Le Deuff *et al*., 1996). Moving oysters into cooler waters may apparently reduce the pathogenicity of the virus. However, the virus remains present and potentially infectious and has also been associated with high mortality among *C. gigas* spat.

This raises questions of diagnosis, prevention and treatment.

DIAGNOSIS, PREVENTION AND TREATMENT

Generally, disease control within hatcheries is a husbandry issue. Access to diagnostic tests that are rapid, reliable and highly sensitive is of the utmost importance for effective control of diseases in molluscs hatcheries. The larval period is usually brief; such is the time scale in hatcheries. Infection may quickly spread and cumulative mortality rates approach 100% within few days. Techniques for diagnosis should therefore meet the time constraints experienced under hatching conditions.

Although behaviour of molluscs is usually extremely difficult to observe, the hatchery environment offers a conspicuous advantage of possible "real-time" observation and early warnings, avoiding after-the-event monitoring. Close observation can be made of broodstock and larvae in the rearing facilities. Feeding behaviour, food accumulation in tanks, pre-settlement of larvae onto the bottom, signs of weakening, etc. are some of the critical observations possible in hatcheries.

Histology and transmission electron microscopy are techniques frequently used in the investigation of a mollusc disease. They are time consuming, require experienced staff and high quality equipment and usually lack specificity (Miahle *et al*., 1995). Moreover, many pathogens are difficult to detect by these methods when clinical signs of disease are absent, a frequent situation in broodstock. Recent efforts to overcome these problems have led to the development of molecular methods (Berthe *et al*., 1999). The recognised need for taxonomic clarification and valid pathogen description has engaged during the last decade a number of research teams in sequencing genes of phylogenetic interest and developing DNA-based diagnostic techniques for mollusc pathogens. Their use is based on the fact that every species carries unique DNA sequences, or signatures, that can be targeted in an assay to differentiate it from other organisms. These techniques offer the advantages of high specificity, and possible rapid screening of hosts for the presence of a pathogen DNA. However, sensitivity of these methods is a frequently overemphasised quality, given that sensitivity is hampered by many factors in marine environments (Le Roux *et al*., 1999; Berthe *et al*., 1999).

To date, development of gene probes for diagnosis purpose has essentially been for detection of the most economically important pathogens of cultured molluscs (Walker and Subasinghe, 2000), which are of little concern in hatcheries. However, the approach and related techniques are quickly moving forward and likely to find an increased use in routine disease monitoring in mollusc hatcheries and efforts to prevent the spread of pathogens.

In the case of the herpesvirus discussed above, diagnosis was impeded by the lack of *in vitro* culture and limitations of histology and electron microscopy, although investigators were able to purify viral particles and characterise the genome of the virus (Le Deuff and Renault, 1999). This enabled the development of PCR assays and an *in situ* hybridisation test (Renault *et al*., 2000; Arzul *et al*., 2001; Lipart and Renault, 2002). These tools improve health management in hatcheries as well as supporting research programmes.

Molecular techniques should not be restricted to DNA based methods. Immunoassays and antibody based techniques still have promise and potential once taxonomy and phylogeny are clearly established.

Bearing in mind the development and interest in these diagnostic techniques, the potential for widespread application and the inherent problems currently associated with their use, it appears necessary to develop and validate molecular diagnostic techniques for mollusc pathogens in hatcheries. Development of new tools is needed to help in screening of brood-stock, monitoring of larvae and juveniles, and assist hatchery operations.

Another stake for development of diagnostic tools stands in the understanding of pathogen modes of action, namely virulence. An illustration of this is the frequent isolation of bacterial strains of the genus *Vibrio*, among which some are apparently virulent although con-specific

with strains showing no detrimental effect when tested in experimental challenges (Le Roux *et al*., 2002). In this recent study, *Vibrio* strains isolated from spat suffering abnormal mortality and related to *V. splendidus* biovar II were selected. Comparative analysis was performed by classical biochemical tests and PCR-RFLP of SSUrDNA, *rpo*D and *gyr*B genes and genomic similarities confirmed by quantitative DNA/DNA hybridisation. The only strain found to be closely related to a previously described virulent strain, was not virulent in experimental challenge. None of the phenotyping and genotyping methods used in the study enabled discrimination between virulent and a-virulent strains. This leads to questions about relationships with environmental strains and processes by which strains may acquire virulence factors. Furthermore, it shows the need for identification of new targets of our diagnostic tools (Berthe, 2002).

Very few ways to reduce the detrimental effect of pathogens on commercially exploited molluscs have been identified. Molluscs are usually reared in the open sea which strongly limits the help of chemotherapeutants, because of the quantity to be used and its impact on the environment. However, most of hatcheries use land based facilities and the use of antibiotics is frequent. This causes additional expense; and efficiency of antibiotic treatments for ubiquitous pathogens is seriously questioned. Alternatives exist, including use of probiotics, although these were not extensively applied until recently. Bacteriophages may be candidates as therapeutic agents in bacterial infections. Bacteriophages are viruses of bacteria which can lyse and kill the bacteria they infect. After their discovery in the early 20th century and enthusiastic beginning to phage therapy, the development of antibiotics has inhibited interest in phage therapy research. However, the alarming emergence of antibacterial resistance among bacteria has led to recent studies which confirmed that phages can be highly effective in treating many different types of bacterial infections (Duckworth and Gulig, 2002). Protective effects of phages against experimentally induced bacterial infections of cultured fish has been described (Nakai and Park, 2002). Use of phage therapy is a promising approach in aquaculture, particularly against well defined bacterial strains. Both approaches, phage therapy as well as probiotics, possess a common Damocles' sword, lying in the acquisition of virulence. The bacteriophage VHML is responsible for virulence conversion of toxin-producing *Vibrio harveyi*. The complete nucleotide sequence of VHML has recently identified a N6-adenine methyltransferase (Dam) gene potentially associated in this process (Oakey *et al*., 2002). New tracks could be opened in the near future and further research should lead to a better understanding of virulence of *V. harveyi* and, more broadly, vibriosis in molluscs.

Micro-algae are currently used as food in hatcheries, however, they also have a tremendous potential for various anti-microbial activities which has already been recognised (Kogure *et al*., 1979, see also mini review in Naviner *et al*., 1999). Although the effect of certain diatoms, *e.g. Skeletonema costatum*, on marine bacteria and more particularly vibrios was assessed, their possible use in the control of bacterial infections needs further understanding of the routes of action. Enhancement of antibiotic activities through genetic selection and co-culture will possibly permit micro-algae to be seen as a tool for prevention of infection besides their use as feed.

Further research is also needed to explore these fields and assess alternative methods of prevention and treatment.

CONCLUSION

Although hatcheries do not appear as a corner stone of mollusc aquaculture at the moment, they are likely to face an increased demand given their potential in aquaculture development. Mollusc hatcheries may therefore have an increased contribution to movements, transfers and introductions of live molluscs in the world. These movements are currently recognised as a major cause underlying outbreaks of mortality, epidemics and spread of diseases.

Paradoxically, building up locally based hatchery production would reduce the risk from exotic pathogens. This would also give the advantage of providing off-springs from broodstock naturally well adapted for the local growing conditions. These considerations as well as the growing stringency of international standards and guidelines should encourage local capacity building and countries to become less dependant on overseas broodstock and seed. Demonstration was recently made of cost effective technical solutions for small scale hatcheries. Building of good hatcheries and hatchery management should be regarded as a key point of mollusc aquaculture development.

A number of serious pathogens are already known to affect molluscs during the different stages of the hatchery process. Some of these pathogens can further affect mollusc aquaculture as well as natural resources. With this in mind, hatcheries should be underpinned as a risky segment of the mollusc aquaculture sector. Further research is needed towards a better identification of diseases in hatcheries, their distribution and impact.

On the other hand, and this is also where paradox exists, in mollusc aquaculture, hatcheries are unique in having closed facilities that enhance disease control capabilities. Hatcheries may produce checked mollusc progeny, namely specific pathogen free, and reduce circulation of infected stocks. Moreover, they are of a central importance in the development of health management strategies based on improved resistance to diseases.

Knowledge of diseases in mollusc hatcheries is currently under-developed when considering the increasing technical improvement of hatching and its potential of use in mollusc aquaculture. Tremendous needs are faced by research in the fields of diagnostic and control of diseases in mollusc hatcheries. Such a research is needed for hatcheries to play their pivotal role in mollusc aquaculture development and health management programmes.

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