Spread of Pathogens from Marine Cage Aquaculture – A Potential Threat for Wild Fish Assemblages Under Protection Regimes?

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1. Introduction

The spread and the control of pathogens represent a serious problem in aquaculture, implying important environmental and economic issues (Huntington, 2006; Sapkota et al., 2008).

With the rapid development of aquaculture in recent decades (Food and Agriculture Organization [FAO], 2008), an increasing number of pathogens involved in fish disease, and the range of susceptible fish species have been identified and described. The increasing use of molecular tools has helped improve our knowledge in this area.

Few studies have been performed to evaluate the interactions host/pathogen in the natural environment (Hedrick, 1998), or to investigate the mechanisms of infection and interaction between farmed and wild specimens, nor the ecological role assumed by infected wild hosts has been explored yet.

In view of the current decline of fish stocks, which has recently been attributed not only to habitat loss and overfishing, but also even to the spread of pathogens (Daszak, 2000; Gozlan et al., 2005), understanding these mechanisms is essential for a proper conservation and management of marine and fisheries resources.

The effect of parasites and other pathogens on fish populations under protection regimes has received virtually no attention (McCallum et al., 2005). Marine Protected Areas (MPAs) can provide unique protection for critical areas and spatial escape for overexploited species and are regarded as essential tools for the conservation of marine environments (Lubchenco et al., 2003; Pauly, 2005; Roberts et al., 2005). However, the effectiveness of MPAs is greatly limited by several anthropogenic and natural impacts acting at scales much larger than that encompassing by the reserve boundaries. Examples of such impacts toward which MPAs cannot offer any direct protection are represented by climate change (Graham et al., 2008; McClanahan, 2000), with all the problems related to it (Keller et al., 2009), the spread of pollutants (Jarman et al., 1992; Loganathan & Kannan, 1994; Terlizzi et al., 2004), invasive

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species (Carlton et al., 1990; Katsanevakis et al. 2010; Trowbridge, 1995), and pathogens (Lessios, 1988; Littler & Littler, 1995; Rasmussen, 1977; Steinbeck et al., 1992).

In this chapter we briefly review the potential threats to wild population represented by the spread of pathogens from marine cage fish aquaculture. As an emblematic example we focus particularly on the effects of a serious disease, namely the **Viral Nervous Necrosis** (VNN), affecting several reared fish species worldwide.

In order to warn about how the spread of pathogens could represent a risk for natural populations, even under protection regimes, we report on the results of a preliminary survey for the detection of viral particles responsible for VNN in the gonads and brain homogenates in some wild specimens collected in two no take-no access Marine Protected Areas in the Southern Italy (South Adriatic and North Ionian Sea).

### 2. The spread of pathogens from marine cage aquaculture

There are several ways by which new pathogens can attain natural populations.

For example, a benign organism can undergo a genetic change that may increase its degree of pathogenicity (Bull, 1994; Cunningham, 2002). A pathogen may also be introduced through the expansion of the range of wild species in new regions as a result of global warming, the removal of barriers, or the transport of animals for commercial and recreational purposes (Gozlan et al., 2006).

It is now known that the activities of aquaculture farms, particularly those of offshore floating cages, are a source of various types of environmental (Pusceddu et al., 2007) and biological (Terlizzi et al., 2010) impact, including the facilitation of the spread of potential pathogens (Krkošek et al., 2006). The high density to which organisms are reared and the unnatural and stressful conditions to which they are subjected may in fact facilitate the emergence and spread of diseases, which can potentially extend to wild organisms (Naylor et al., 2005).

The use of net pen or cages systems involves a higher risk for disease transmission because there is no impermeable barrier between the farm and the aquatic environment (Huntington et al., 2006). There is therefore a strong potential for contamination between reared organisms and the wildlife of the surrounding environment.

Many species of wild fish are attracted by the presence of the cages, which provide them food and shelter (Diamant et al., 2000), often succeeding to get into cages, passing through the mesh. Farmed species may be able to escape in the environment too. This phenomenon may have important biological implications (e.g. in terms of genetic contamination) on wild populations. In addition, escaped individuals can carry infections they acquired at the culture systems (Conseil International pour L’Exploitation Scientifique de la Mer Méditerranée [CIESM], 2007).

Farmed fish stocks may prove receptive to pathogens from the surrounding environment too. The exchange of pathogens between farmed and wild fish has been repeatedly observed in different geographical regions (Diamant et al., 2000; Kent, 2000; Mc Vicar, 1997; Nowak et al., 2004; Paperna, 1998; Sepulveda et al., 2004). Bacterial infections by *Mycobacterium marinum* and *Streptococcus iniae*, which are common among farmed fish stocks, have been described to cause mortality even in wild fish (Colorni et al., 2002).
Pathological events in populations of wild abalone (Alstatt et al., 1996) suggest a potential susceptibility that makes them able to influence or be influenced by, reared individuals. Such pathogen exchange relationship seems to occur frequently between farmed and wild fish populations, as in the case of a disease known as Infectious Hematopoietic Necrosis, which affects mainly salmons, and which appears to be transmitted between wild and farmed specimens in both directions (Naylor et al., 2003).

Transmission of pathogens from aquaculture to wild fish can occur at different stages of the life cycle: infection can start from the earliest stages of reproduction and larval rearing (hatchery) and spread in the later stages (growing) contaminating wild stocks and causing dangerous epidemics (Naylor et al., 2005).

Krkošek et al. (2011) studied the effects of sea lice (Lepeophtheirus salmonis) from salmon farming facilities on populations of wild salmon (Onchorhynchus gorbusha and O. keta), noting that juveniles of these species, when parasitized, show a reduced ability to escape predators that consume selectively parasitized salmon. Therefore, the mortality of juvenile wild salmon may be higher than previously believed and this has important implications for the development of appropriate conservation policies.

The importation of feed for aquaculture can also be considered vehicle of sources of pollutants and of pathogens between fish stocks very distant from each other (Dalton, 2004). Parasites and pathogens can therefore play an important role for fish stocks, reducing both the number and yield, increasing mortality, altering the reproductive potential, affecting the structure of the population size and/or reducing the commercial value of harvested stocks (Dobson & May, 1987; Kuris & Lafferty, 1992).

Being able to influence the survival of the host and his reproductive success, pathogens should be taken into great consideration in the design and management of MPAs (McCallum et al., 2005). Also, even if the survival or reproduction are not affected, as in the case of infections by Anisakis simplex and Pseudoterranova decipiens, pathogens may pose a problem for fisheries management-oriented reserves, because the infected hosts have a lower market value and can represent a health risk when consumed as food by humans.

3. The Viral Nervous Necrosis (VNN)

The genus Betanodavirus, family Nodaviridae is a widespread and well-known viral pathogen in the field of fish farming. It is the etiologic agent responsible for a severe disease known as Viral Encephalopathy and Retinopathy (VER), or Viral Nervous Necrosis (VNN). It affects more than 40 species of marine fish worldwide (Panzarin et al., 2010). VNN disease is regarded as one of the most devastating viral infections among marine fish.

Affected fish, particularly juvenile forms, show spiralling swimming, darkened colour and hyper inflated swim bladders. Internal disease signs include pale livers, empty digestive tracts and intestines filled with brownish fluid. In affected fish the virus propagates in the eye, brain and distal spinal cord causing marked vacuolations leading to encephalopathy and retinopathy (Fig. 1). Such lesions in the Central Nervous System and in the retina (nervous tissue of the eye) have always been typical of larval and juvenile stages in the sea bass (Bovo et al., 1996; Breuil et al., 1991) and in other species sensitive to nodaviriosis (Glazebrook et al., 1990; Grotmol et al., 1997; Mori et al., 1991; Nguyen et al., 1996). For this
Fig. 1. Histological preparation of a sea bass larva (age 45 days) with the typical vacuolar necrosis of the brain tissue and in the retina (indicated by white and black arrows, respectively). After the collection, the sample has been readily fixed in 10% neutral buffered formalin for not more than 7 days. Thereafter it has been included in paraffin to be cut in to sections of 5 µm and then colored for histological analysis. The dye used in this histological section is hematoxylin-eosin.

The virus also multiplies in the gonad, livers, kidney, stomach and intestine (Grotmol et al., 2000; Munday et al., 2002).

Experiments with *Pseudocaranx dentex* (Arimoto et al., 1992; Mushiake et al., 1994; Nishizawa et al., 1996) have shown that the virus transmission can occur either vertically, from broodstock to larvae through the eggs or sperm, either horizontally, through the contact between healthy fish and infected larvae (Arimoto et al., 1993). Experimental contamination with infected tissue homogenates (Arimoto et al., 1993; Glazebrook et al., 1990; Grotmol et al., 1999; Tanaka et al., 1998) or addition of purified virus from infected individuals (Nguyen et al., 1996) or produced in SSN-1 cells (Péducasse et al., 1999) is also able to transmit the virus. The ability of the betanodavirus to resist to a wide pH range, varying from 2 to 9 (Frerichs et al., 1996), and its resistance in sea water at 15°C for more than one year (Frerichs et al., 2000) increases the probability of horizontal transmission (Munday et al., 2002). The transmission of the betanodavirus from infected, asymptomatic specimens is also possible. Some Authors (e.g. Castric et al., 2001) have shown how specimens of *Sparus aurata*
experimentally infected but apparently healthy, are able to transmit the virus to individuals of *Dicentrarchus labrax* placed in the same cage.

The finding, in Norway (Aspehaug et al., 1999), of adults of Atlantic halibut (*Hippoglossus hippoglossus*) positive for betanodavirus suggests a possible danger of using wild animals as breeding stock.

There is obviously also the possible risk of transmission occurring in the opposite direction, namely that the virus originating from aquaculture facilities can infect wild healthy individuals. Supports to this hypothesis, in addition to the above mentioned evidences, which show that betanodavirus can spread both horizontally and vertically within a system, are provided by the detection of the virus in several wild marine species, although the infection in the examined subjects was asymptomatic (Barker et al., 2002; Gagné et al., 2004; Gomez et al., 2004; Gomez et al., 2008; Thiéry et al., 2004).

Recent studies carried out along the Italian coast have identified the etiologic agent responsible for the disease in wild organisms belonging to a wide panel of species. From a virological examination conducted by Maltese et al. (2005) on samples from the northern Adriatic and the Strait of Sicily, the presence of viruses has been detected in the sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), white grouper (*Epinephelus aeneus*), dusky grouper (*Epinephelus marginatus*), red mullet (*Mullus barbatus*), axillary seabream (*Pagellus acarne*), poor cod (*Trisopterus minutus*) and in the black goby (*Gobius niger*). The study highlighted a possible widespread of the virus in nature, the role of *S. aurata* as an asymptomatic carrier and the high sensitivity of the genus *Epinephelus*, for which episodes of mortality in other geographical areas have been reported. The study also confirmed the considerable sensitivity of *D. labrax* to the betanodavirus, which had already been proven in the context of intensive farming.

### 4. VNN and protected populations: A limited effectiveness of MPAs?

The two areas considered for sampling are the MPA of Porto Cesareo, located along the southwestern coast of Apulia (Ionian Sea, 40°15' N - 17°52' E) and the MPA of Torre Guaceto, located along the east coast of Apulia (Adriatic Sea, 40°43' N - 17°48' E). Both areas are characterized by the past presence of extensive aquaculture activities within or close to the reserve boundaries. The survey focused mainly on specimens of *D. labrax* but, whenever possible, considered, as a preliminary screening, some other species of commercial interest for which positive serology for the betanodavirus has been already reported in literature. For all specimens, samples of brain and gonadal tissue, the main target organs of the virus were taken in double aliquot for DNA extraction and biomolecular analysis. Virological analyses were performed by the laboratories of the Istituto Zooprofilattico Sperimentale delle Venezie (Padua, Italy). Samples were analysed through real-time TaqMan PCR. This technique proved to be highly sensitive, is suitable for a wide range of organic matrices and is able to detect the four betanodavirus genotypes currently recognized. The experimental protocol followed the procedure described in Panzarin et al. (2010). More particularly, samples were processed for RNA extraction using the NucleoSpin® RNA II (Macherey-Nagel GmbH & C., Düren, Germany). Real-time PCR was performed using the LightCycler 2.0 system and carried out in 20 µl with “LightCycler® TaqMan® Master” (Roche Diagnostics GmbH, Mannheim, Germany), 0.9 µM of each primer, 0.75 µM of probe and 5 µl
of cDNA template. The thermal profile consisted of a 10-min incubation at 95°C followed by 45 cycles of 10 s denaturation at 95°C, 35 s annealing at 58°C and 1 s elongation at 72°C, followed by an additional 30-s cooling step at 40°C.

The analysis involved the collection of 28 specimens, 9 of which coming from the MPA of Torre Guaceto and 19 from the MPA of Porto Cesareo. Results are summarised in Table 1.

Several specimens resulted positive to the VNN test. The individuals showing positivity to the molecular analyses were mostly represented by the sea bass (*Dicentrarchus labrax*) but the presence of the virus was also detected in the scorpion fish (*Scorpaena scrofa*), the gray mullet (*Mugil* sp.), the goby (*Gobius* sp.) and the comber (*Serranus cabrilla*). All positive individuals appeared asymptomatic.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>No. of examined fish</th>
<th>No. of positive samples</th>
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<tbody>
<tr>
<td>Torre Guaceto MPA</td>
<td></td>
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<tr>
<td><em>Dicentrarchus labrax</em></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><em>Diplodus sargus</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Porto Cesareo MPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dicentrarchus labrax</em></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>Scorpaena scrofa</em></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td><em>Mugil</em> sp.</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td><em>Gobius</em> sp.</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Serranus cabrilla</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Synodus saurus</em></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Serranus scriba</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Sarpa salpa</em></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Oblada melanura</em></td>
<td>1</td>
<td>0</td>
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Table 1. Summary of VNN tests performed in the two Marine Protected Areas (MPAs)

### 5. Discussion

The lack of clinical signs in infected fish specimens observed in this study is consistent with the results obtained in other recent studies of wild marine fauna (Baeck et al., 2007; Gomez et al., 2004), thus confirming how rare are the observations of clinical forms in wild fish populations (but see Gomez et al., 2009).

Some authors argue that most infections in wildlife are presumably latent and they do not evolve into a pathological condition until the fish becomes stressed (Barker et al., 2002).

Barker et al. (2002) firstly reported piscine nodavirus in wild winter flounder *Pleuronectes americanus* in Passamaquoddy Bay (New Brunswick, Canada) considering the possibility that the virus could be transferred between wild winter flounder and sea-caged halibut or cod.

Baeck et al. (2007) detected nodavirus in 21 different apparently healthy wild marine fish collected in coastal areas of Korea, close to aquaculture facilities and Gomez et al. (2004) reported
high prevalence for betanodavirus in apparently healthy cultured and wild marine fish near mariculture areas in Japan. In both studies, sampling areas were located near aquaculture facilities and the authors support the hypothesis of an horizontal transmission of the virus. The results here reported, although preliminary, make us suppose that the distribution of the betanodavirus is currently extending and not confined to marine cage aquaculture. The genetic characterization of isolated viral strains is currently under investigation and will likely help determining more precisely the range of dispersion and residence time of these possible sources of contamination, thus improving our understanding about the epidemiological mechanisms of the disease and consequently our ability to control it.

Our findings also confirm that MPAs are not able to cope with this particular form of impact, which clearly acts at a scale larger than that covered by the measures of protection (Allison et al., 1998).

The specimens considered, although asymptomatic, might still be reservoirs of infection, by acting as virus carriers, and further favoring the spread of the pathogen. The ecological role of these asymptomatic carriers in nature remains however unclear and the possible consequences on natural population still remain to be clarified. This is not an easy task, however, as disease disorders are difficult to observe in the wild because affected individuals are rapidly removed from the population by predators (Gozlan et al., 2006). These consequences are nevertheless conceivable since in some reared species such as the sea bass, the virus causes, in addition to the above-described damages to the nervous system, failure in reproduction (often sterilization), reduced hatching rate and high larval mortality.

In the view of the impact caused by the over-exploitation of marine resources and given the current trend of decline of commercially important fish stocks, increased mortalities and failure in reproduction due to diseases should be carefully considered and adequately addressed, especially in sound quantification of the effectiveness of MPAs.

Attempts to protect biodiversity through MPAs and/or restore ecosystem functioning (e.g., through the protection of over-exploited species that generate positive community-wide effect) might be frustrated and/or biased in their quantification by external pressures.

Given the wide spread of this virus and its possible consequences in asymptomatic forms it should be important to include the issue of pathogens in disease surveillance program and monitoring of biological health of each MPA as well as any area that has or has had a significant number of intensive aquaculture plants.

6. Acknowledgments

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7. References


Shellfish”, European Association of Fish Pathologists, Copenhagen (Denmark), 11-16 September 2005: Poster 8.19.


Aquaculture has been expanding in a fast rate, and further development should rely on the assimilation of scientific knowledge of diverse areas such as molecular and cellular biology, and ecology. Understanding the relation between farmed species and their pathogens and parasites, and this relation to environment is a great challenge. Scientific community is involved in building a model for aquaculture that does not harm ecosystems and provides a reliable source of healthy seafood. This book features contributions from renowned international authors, presenting high quality scientific chapters addressing key issues for effective health management of cultured aquatic animals. Available for open internet access, this book is an effort to reach the broadest diffusion of knowledge useful for both academic and productive sector.

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