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Can the protozoan parasite *Bonamia ostreae* infect larvae of flat oysters *Ostrea edulis*?

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ABSTRACT

Bonamia ostreae is an intracellular protistan parasite affecting flat oysters *Ostrea edulis*. It can be detected in juveniles but mortalities mainly affect oysters which are more than 2 years old. The parasite is usually observed inside haemocytes and sometimes free, notably in gill epithelia suggesting a parasite release through this organ. However, the infective form and ways of entry and release remain undetermined. Flat oysters incubate their larvae in their pallial cavity for 8–10 days before releasing them into the water column. Flat oysters in Bay of Quiberon in South Brittany (France) are known to be infected with *B. ostreae* since 1979 and is the most important area in France for *O. edulis* spat collection. Flat oysters incubating larvae were sampled in this area during summertime between 2007 and 2009. Both adults and larvae were preserved and assayed by PCR and *in situ* hybridisation (ISH). PCR tests revealed the presence of parasite DNA in some adults and larvae. Specific labelling could be detected by ISH in gills, digestive system, gonad and mantle in adults and in the epithelium surrounding the visceral cavity of some larvae. Our results demonstrate that larvae can be infected with *B. ostreae*. Larvae might thus contribute to the spread of the parasite during their planktonic life. In addition, their transfer for aquaculture purpose should be controlled especially when they are exported from infected zones.

1. Introduction

The flat oyster *Ostrea edulis* is a native species from Europe. It occurs along the western European coast from Norway to Morocco, through the Mediterranean Sea, and into the Black Sea. Naturalized populations also occur along the eastern coast of North America from Maine to Rhode Island following intentional introductions in the 1940s and 1950s (Hidu and Lavoie, 1991; MacKenzie et al., 1997). This species has been endangered by overfishing, cold winters, predation pressure and diseases and is today in the OSPAR (Oslo and Paris Conventions for the protection of the marine environment of the North-East Atlantic) list of threatened and/or declining species and habitats (OSPAR agreement 2008-6). Specifically, French flat oyster production estimated at 28 000 tonnes in 1960 dramatically decreased because of two protozoan diseases namely marteiliosis due to *Marteilia refringens* and bonamiosis due to *Bonamia ostreae* (Meuriot and Grizel, 1984; Goulletquer and Héral, 1997). The production of this endemic species has remained low, less than 2000 tonnes per year since the

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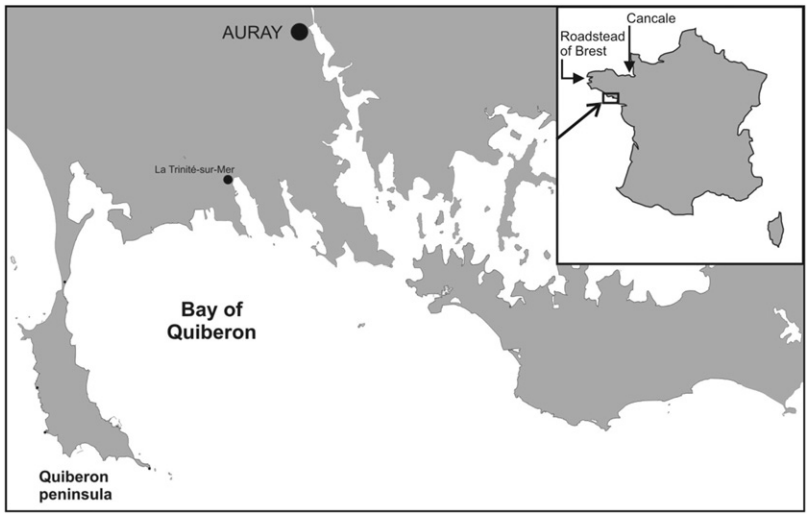

Fig. 1. Main French sites concerned by the production of flat oyster, *Ostrea edulis*: spat is mainly collected in Bay of Quiberon and the Roadstead of Brest; some of the oysters are then moved to Cancale for grow-out. Detail of Bay of Quiberon, Southern Brittany, France.

emergence of these two diseases and is now located in a few specific areas (Fig. 1). Data from the last census on shellfish culture in France carried out in 2001 showed that most of the spat is collected in the Bay of Quiberon and to a lesser extent in the Bay of Brest (Girard et al., 2005). Spat collected in the Bay of Brest and some of the spat collected in the Bay of Quiberon are moved to Cancale, North Brittany for grow-out when the spat is 10 months old. In 2001, the production of flat oysters was estimated at 1960 tonnes (FAO, 2008).

Few data are available on pathogens affecting young flat oysters. However, herpes-like viruses were reported in 5-month-old spats collected in northern Brittany (Comps and Cochennec, 1993). Similar viruses were associated with the mortality of flat oyster larvae in a hatchery (Renault et al., 2000; Arzul et al., 2001). *Vibrio* strains were shown to be pathogenic to larvae of flat oysters by inducing mortalities in hatcheries (Jeffries, 1982; DiSalvo et al., 1978; Tubiash et al., 1965). Young prespawning flat oysters (1–3-month old to 18-month old) are susceptible to infection by *B. ostreae* and can develop a high prevalence and intensity of infection over a 6-month period (Lynch et al., 2005). Mortality associated with infection with *B. ostreae* has even been described in 6-month-old juveniles (Lallias et al., 2008). However, individuals older than 2 years appear more susceptible to the disease (Balouet et al., 1983; Culloty and Mulcahy, 1996; Grizel, 1985; Robert et al., 1991) and death usually occurs concurrently with the highest level of infection intensity (Bréhelin et al., 1982; Caceres-Martinez et al., 1995; Montes et al., 2003). While adults and juveniles are known to be susceptible stages to bonamiosis there is no data on the possible role of larvae in the cycle of the parasite.

Fig. 2. Flat oyster incubating larvae.

B. ostreae life cycle is unknown, but the disease can be transmitted directly between oysters in a population or experimentally by cohabitation or inoculation (Elston et al., 1986; Hervio et al., 1995), suggesting that an intermediate host is not required for the parasite to complete its life cycle. Observation of free parasites in gill epithelia potentially associated with gill lesions supports the hypothesis of a parasite release through this organ (Montes et al., 1994). However, the infective form and routes of entry and release remain undetermined. A controversial description proposed that *B. ostreae* was an ovarian tissue parasite for part of its life cycle (Van Banning, 1990) but this hypothesis was not confirmed. In spite of several management practices, diseases have drastically affected wild and cultured flat oyster populations. The main solutions for the industry rely on transfer restrictions and on the development of resistant strains which require a better understanding of host pathogen interactions.

The Bay of Quiberon, South Brittany, France (Fig. 1) is an interesting site to study bonamiosis in flat oyster populations because flat oysters there have been infected since 1979 with prevalence of infection ranging from 2 to 37% and a mean around 13% (Arzul et al., 2006). In addition, it is the most important bay for flat oyster spat collection in France and surveys on the reproduction of this species have been carried out there since 1996. Flat oyster female gametes are liberated into the pallial cavity where they are