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Infection of the cockle *Cerastoderma edule* in the Baie des Veys (France) by the microsporidian parasite *Steinhausia* sp.

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ABSTRACT: We report the occurrence of the microsporidian parasite *Steinhausia* sp. in the oocytes of the common cockle *Cerastoderma edule* in a natural population in France, where high mortalities occurred. *Steinhausia* sp. appeared primarily as sporocysts containing many small spores, and putative earlier developmental stages were also observed. Both its prevalence and infection intensity were low, and no host defence reaction was recognized, suggesting that *Steinhausia* sp. had no detrimental effect on *C. edule*. Its prevalence was higher in cockles lying on the sediment surface, but the significance of this observation could not be explained given the poor knowledge of the *Steinhausia* life cycle. The present data did not allow specific identification of the parasite, and further studies are required to determine whether *Steinhausia* sp. in the cockle is a new species, or a microsporidian infecting multiple host species.

KEY WORDS: *Cerastoderma edule* · *Steinhausia* sp. · Microsporida · Oocyte · Baie des Veys

INTRODUCTION

The common cockle *Cerastoderma edule* (L.) is a suspension-feeding bivalve mainly distributed in the soft-bottom intertidal areas of numerous bays and estuaries in western Europe, where it lives buried in the sediment (a few cm depth). In bays where the species is abundant, natural populations are commercially exploited, but cockles are also occasionally cultured. In France, cockle production (1400 t yr^{-1}, FAO 2000) is mainly from natural populations distributed along both the Atlantic and English Channel coasts.

Numerous parasites have been reported in *Cerastoderma edule* (Bower et al. 1994, Azevedo 1997, Carballal et al. 2001), including microsporidians in the epithelial cells of the digestive gland (Comps et al. 1975). Microsporidians have been recorded in various tissues of numerous marine bivalve species: haemocytes forming aggregates in the connective tissue of the digestive gland of the scallop *Aequipecten opercularis* (Lohrmann et al. 2000); connective tissue surrounding the gut epithelium of the oyster *Ostrea lutaria* (Jones 1981); stomach epithelium of the clams *Ruditapes decussatus*, *Venerupis pullastra* and *V. rhomboides* (Villalba et al. 1993a,b); and digestive gland epithelium in the scallop *Chlamys varia* (Bodoy et al. 1991). In addition, microsporidians belonging to *Steinhausia* spp. have been described as infecting the oocytes of the European flat oyster *O. edulis* (Léger & Hollande 1917), the mytilid mussels *Mytilus galloprovincialis* and *M. edulis* (Field 1923, Sprague 1965, Figueras et al. 1991), the Sydney rock oyster *Saccostrea commercialis* (Anderson et al. 1995), and the clams *Macoma balthica* (Farley 1977) and *V. pullastra* (Villalba et al. 1993a,b).

More recently, a *Steinhausia*-like microsporidian was identified in the oocytes of *Cerastoderma edule* from 2 populations in Galicia (NW Spain), with both

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low prevalence (1 in 30 individuals were infected in each population) and low infection intensity (few oocytes were infected) (Carballal et al. 2001). We report herein the identification of a *Steinhausia* sp. microsporidian in *C. edule* from a natural population in the Baie des Veys, France.

MATERIALS AND METHODS

In the Baie des Veys (Fig. 1), natural populations of *Cerastoderma edule* are harvested, with an annual production ranging between 100 and 500 t (M. Letrouvé and P. Le Roland, Direction Départementale des Affaires Maritimes de la Manche and du Calvados, Directions Départementales, respectively, pers. comm.). In August 2001, high mortalities (up to 70%) were observed. As part of the sampling strategy of the French monitoring network REPAMO (IFREMER), which investigates the causes of mortalities observed in French production areas, 6 samples of ca. 30 living cockles each were collected on August 17, 2001 in order to evaluate their histopathological state. Three stations were sampled twice in the mortality area (Fig. 1), with 1 sample composed of cockles lying on the surface of the sediment ('surface' cockles) and 1 composed of cockles buried in the sediment ('buried' cockles).

Preliminary observations of whole-mount gonadal tissue of 23 additional specimens revealed the occurrence of a *Steinhausia*-like parasite in the oocytes. To study the extent of the parasite infection in the population, we carefully examined histological sections. Individuals were dissected and tissues fixed for 24 h in Davidson's fixative (Shaw & Battle 1957), and then dehydrated and embedded in paraffin. Sections (2 to 3 µm thick) were cut, stained with hematoxylin-eosin and examined with light microscopy for the presence of *Steinhausia* sp.

Prevalence is defined as the percentage of host individuals in a sample infected by a particular parasite species (Bush et al. 1997). Due to their location in the oocytes, *Steinhausia* microsporidians infect only females or hermaphrodites. Thus, although we also examined males, prevalence of the *Steinhausia* sp. infection was calculated only from female data (no hermaphrodite was encountered), as described by Villalba et al. (1997) for *S. mytilovum* in the mussel *Mytilus galloprovincialis*. In addition, only cases with sporocyst stages were considered, because identification of early developmental stages is not certain. The prevalence of the *Steinhausia* sp. infection between buried and surface cockles was compared using the non-parametric Wilcoxon-Mann-Whitney test (Scherrer 1984).

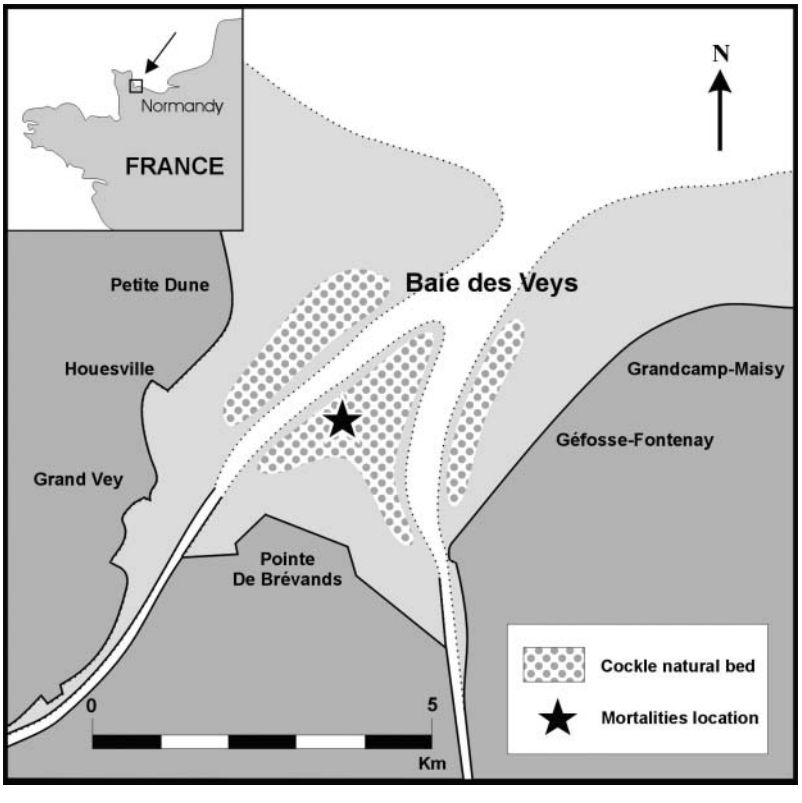


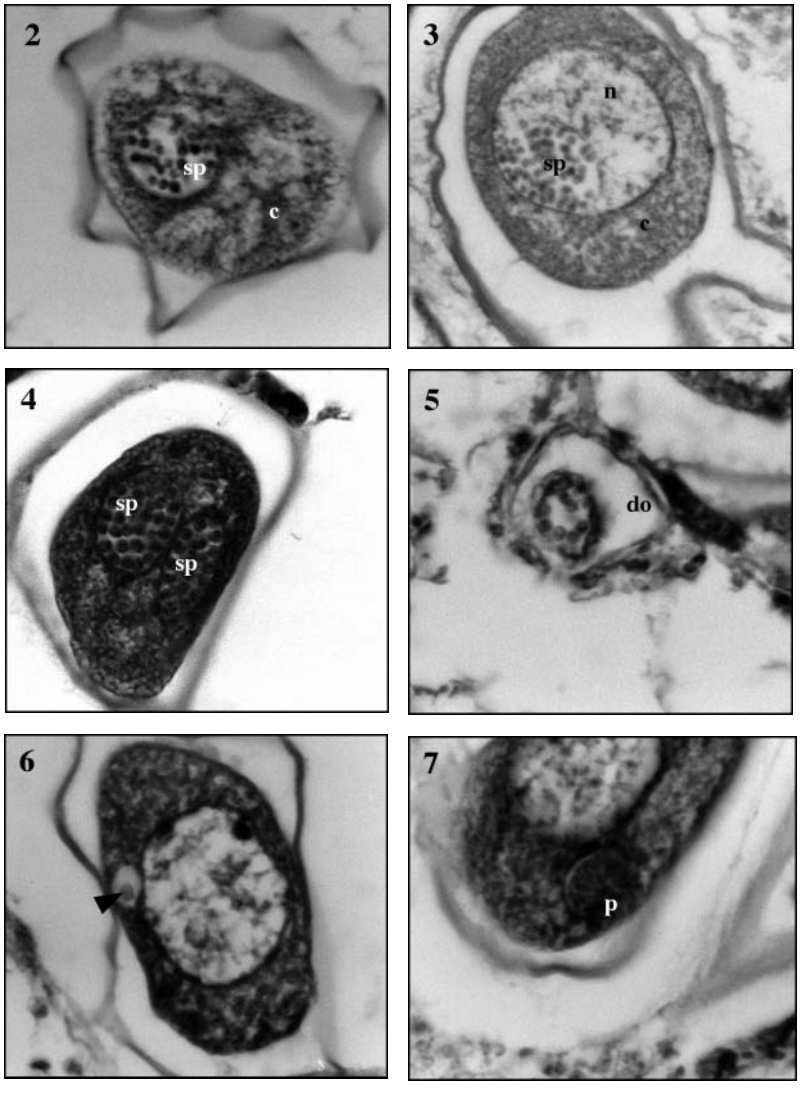
Fig. 1. Location of the sampled cockle bed in the Baie des Veys, France. 'Mortalities location' shows the area where mortalities occurred

RESULTS

A *Steinhausia*-like microsporidian parasite (referred to as *Steinhausia* sp. from this point) was recorded in the oocytes of 18 female *Cerastoderma edule*. The most abundant form of the parasite appeared as cysts containing many spores, the cysts being limited by a more or less thick membrane (Fig. 2) that was sometimes difficult to detect. Sporocysts were usually elliptical to circular in shape, with a size ranging from 16 to 31 µm (mean = 20.68 µm, SD = 4.06 µm, N = 13), containing up to 30 spores. Spores were almost spherical, with a diameter ranging from 2 to 3.2 µm (mean = 2.56 µm, SD = 0.35 µm, N = 73). The cysts were primarily located in the oocyte cytoplasm, with a single exam-

ple (i.e. 3%) being observed inside the nucleus (Fig. 3). Two oocytes (i.e. 6% of the infected oocytes) showed multiple infections, with 2 sporocysts per oocyte (Fig. 4). Distortion of the nuclear membrane due to the presence of a parasite in the cytoplasm was observed once (i.e. in a single oocyte). In a single case, a sporocyst was observed in a degraded oocyte (Fig. 5). In addition, putative other developmental stages were observed, including an early developmental stage (Fig. 6) and plasmodial stages containing numerous nuclei (Fig. 7). In no cases were major haemocyte infiltration associated with the infection. The intensity of infection was low, commonly with 1 to 5 infected oocytes per slide.

The prevalence of the *Steinhausia* sp. infection ranged from 11.8 to 20% in buried and surface cockles, respectively (Table 1). The non-parametric Wilcoxon-Mann-Whitney test indicated that prevalence was significantly higher in surface *Cerastoderma edule* (unilateral test, $p = 0.05$).



Figs. 2 to 7. *Steinhausia* sp. in the oocytes of the common cockle *Cerastoderma edule*. Histological sections in the gonadal tissue stained with hematoxylin-eosin (750×). Fig. 2. Sporocyst (sp) containing numerous spores in the cytoplasm (c) of an oocyte. Fig. 3. Sporocyst in the nucleus (n) of an oocyte. Fig. 4. Multiple infection with 2 sporocysts in the cytoplasm of a single oocyte. Fig. 5. Sporocyst within an apparently degraded oocyte (do). No cytoplasm is observed. Fig. 6. Putative early developmental stage (arrowhead) of *Steinhausia* sp. in the cytoplasm. Fig. 7. Putative plasmodial stage (p) of *Steinhausia* sp. in the cytoplasm

DISCUSSION

A *Steinhausia* sp. microsporidian parasite in the oocytes of *Cerastoderma edule* has been recorded for the first time in France. It was similar to that observed by Carballal et al. (2001) in *C. edule* from Spain, and also to the other different species of *Steinhausia* described to date. It occurred mainly as sporocysts containing many small spores. Spore sizes (2 to 3.2 µm) were in the range of those of *Steinhausia* spp. in *C. edule* from Spain (ca. 1.5 µm) (Carballal et al. 2001), *Saccostrea commercialis* from Australia (1.7 to 4.1 µm) (Anderson et al. 1995), *Ostrea edulis* from France (2.3 µm) (Léger & Hollande 1917), *Mytilus galloprovincialis* from Spain (1.5 to 2.5 µm) (Villalba et al. 1997, Sagristà et al. 1998), and *M. edulis* from the USA (3 µm, but spores as large as 6 to 8 µm were observed in fresh smears of gonadal tissue) (Sprague 1965). Infection by *Steinhausia* sp. in *C. edule* can be single or multiple (with 2 sporocysts in a single oocyte), as observed for other *Steinhausia* species (Léger & Hollande 1917, Sprague 1965, Anderson et al. 1995, Villalba et al. 1997). Sporocysts were mainly located in the oocyte cytoplasm, but were occasionally observed inside the nucleus, as already observed for *S. mytilovum* (de Vincentiis & Renzoni 1963, Sprague 1970, Sagristà et al. 1998). In the cytoplasm, we observed the parasite inducing an invagination of the nuclear membrane, but less frequently than in *S. mytilovum*, in which sporocysts are usually closely associated with the host-cell nucleus (Sprague 1965). Earlier developmental

Table 1. *Steinhausia* sp. infection in the common cockle *Cerastoderma edule*. Prevalence of the parasite in females from 3 stations and 2 sediment positions (buried and surface) from the Baie des Veys. Only sporocysts were considered in the calculations, identification of earlier stages being not certain

Stn	Sediment position	Number of females	Number of infected females	Prevalence (%)
1	Surface	20	4	20.0
	Buried	18	3	16.7
2	Surface	10	2	20.0
	Buried	17	2	11.8
3	Surface	15	3	20.0
	Buried	22	4	18.2

stages of the parasite were also observed, similar to those reported for *S. mytilovum* in *M. galloprovincialis* (e.g. see Fig. 2 in Sagristà et al. 1998).

The prevalence of the *Steinhausia* sp. infection in *Cerastoderma edule* from the Baie des Veys (11.8 to 20% of the females were infected, which equals 6.3 to 13.3% of the total number of sampled individuals) was higher than that in *C. edule* from Galicia (3.3% of the collected cockles), but in this latter case, the proportion of males and females was not known (Carballal et al. 2001). Our values were in the range of those observed for *S. mytilovum* in *Mytilus galloprovincialis* from Galicia (7.5 to 28.3% of females) (Figueras et al. 1991, Villalba et al. 1997), but prevalence of up to 76% of females was reported in *M. galloprovincialis* from the Black Sea (Rybakov & Kholodkovskaya 1987). This low prevalence, associated with low intensity of infection, suggests that *Steinhausia* sp. probably had no lethal impact on cockles, and was not the main cause of the mortality observed in the analyzed populations. Moreover, no major haemocyte infiltration was observed, a reaction sometimes reported in *Steinhausia* spp. infections in *M. galloprovincialis* (Figueras et al. 1991, Villalba et al. 1997) and *Saccostrea commercialis* (Anderson et al. 1995). Our results did not allow us to evaluate the effect of *Steinhausia* sp. in *C. edule*. However, the parasite could affect the viability of the infected oocytes and cockle fecundity, as hypothesized for *S. mytilovum* in *M. galloprovincialis* (Field 1923, Figueras et al. 1991, Robledo et al. 1994, Villalba et al. 1997). The observation of a sporocyst inside a degraded oocyte supports this hypothesis.

Prevalence of infection was higher in surface females (non-parametric Wilcoxon-Mann-Whitney test, $p = 0.05$). Such a difference in infection levels between buried and surface cockles has already been shown for other parasites, including larval stages of digenean trematodes (Desclaux et al. 2002). In some digenean infections, this phenomenon could facilitate parasite transmission (Desclaux et al. 2002), with the parasite reducing the burrowing efficiency of its host (Jonsson & André 1992, Jensen et al. 1999). Unfortunately, in the present case the significance of differential infection for *Steinhausia* sp. in *Cerastoderma edule* could not be established due to the lack of knowledge of the parasite life-cycle and mode of transmission.

Our observation raises the question of species identification of *Steinhausia* sp. in *Cerastoderma edule*, and the possibility that *S. mytilovum* infects multiple host species, occuring with other microsporidian species (Pasharawipas et al. 1994, Fries et al. 2001). Indeed, *Mytilus edulis* and *M. galloprovincialis* from both natural and cultured populations located close to the Baie des Veys are infected by *S. mytilovum* (T. Comtet et al. unpubl.). The same situation is encountered at Illa de Arousa in Galicia, where *S. mytilovum* has been recorded in *M. galloprovincialis* (Figueras et al. 1991, Villalba et al. 1997), and where *C. edule* is infected with *Steinhausia* sp. (Carballal et al. 2001). To answer this question would require either a transmission electron microscopy examination of the spore ultrastructure, which we could not conduct in the present work because of the low intensity of infection, or the development of molecular tools for species identification (Berthe et al. 1999).

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