

# Impact of *Perkinsus* sp. on Manila clam *Ruditapes philippinarum* beds

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**ABSTRACT:** Three million Manila clams (*Ruditapes philippinarum*, Adams & Reeve 1850) were sown in 3 sets: A, B and P (0.65, 0.66 and 0.79 g per clam respectively) in intertidal beds of the Eo estuary (Asturias, N Spain). In January 1994 *Perkinsus* sp. was detected in set P. Growth, survival and prevalence of *Perkinsus* sp. were periodically examined during a 2 yr period. Clam growth and survival were unaffected by the parasites in all sets, probably due to the low prevalence of *Perkinsus* sp. (which ranged from 2 to 9%). This low prevalence, in turn, may have been due to the fact that water temperature remained below 20°C. We suggest that, in most cases, clam mortality associated with *Perkinsus* sp. may be minimized with adequate management of clam beds. The disease could be controlled if stressful growing conditions such as high densities, harvesting, or overcrowding in depuration plants are avoided during the warmer months. Additionally, we recommend 2 prophylactic measures: the removal of sets with parasitized clams and the introduction of unparasitized seed in clam culture areas.

**KEY WORDS:** *Perkinsus* sp. · *Ruditapes philippinarum* · Manila clam · Management · Eo estuary

## INTRODUCTION

Parasitic protozoa of the genus *Perkinsus* (Apicomplexa, Perkinsea) are common in molluscs. Perkins (1993) reported *Perkinsus* spp. in 63 species of bivalves which ranged from temperate to tropical waters. *Perkinsus* spp. have historically been associated with high mortalities in cultured species, including *Crassostrea virginica* in the USA (Andrews & Hewatt 1957), *Tridacna gigas* in Australia (Goggin & Lester 1987), and *Ruditapes decussatus* in Portugal (Ruano & Cachola 1986) and Italy (Da Ros & Canzonier 1985). In Spain the disease was first detected in *R. decussatus* in 1985 (González et al. 1987), and later in both *R. philippinarum* and *R. decussatus* (Villalba & Navas 1988, Figueras et al. 1992).

Since the introduction of the Manila clam *Ruditapes philippinarum* in France (1972), England and Spain (1980) and in Italy (1982) (Breber 1985, Flassch & Leborgne 1992), its culture has been widely developed

in European waters. At present, *Perkinsus* sp. is not included in the legislation of the European Union as a 'pathogen of obligatory declaration' (93/54/EU), and therefore transplantations of Manila clams throughout European countries have resulted in the introduction of *Perkinsus* sp. to most clam culture areas.

The presence of *Perkinsus* sp. could endanger both cultured clams and clams harvested in natural beds. However, the effects of parasites on clam populations are not well known. In this study, the relationship of *Perkinsus* sp. to growth and survival of Manila clam beds was examined over a 2 yr period.

## MATERIALS AND METHODS

In June 1993, 3 sets, A, B and P, of 1 million clams each, of Manila clam spat (0.65, 0.66 and 0.79 g per clam respectively) from 3 different hatcheries were planted in intertidal beds of CULTIMAR S.A. in the Eo estuary (N Spain) (Fig. 1). The sets were planted in sand-gravel beds and covered with plastic netting (6 mm mesh size) at densities of approximately 260 clams m<sup>-2</sup>.

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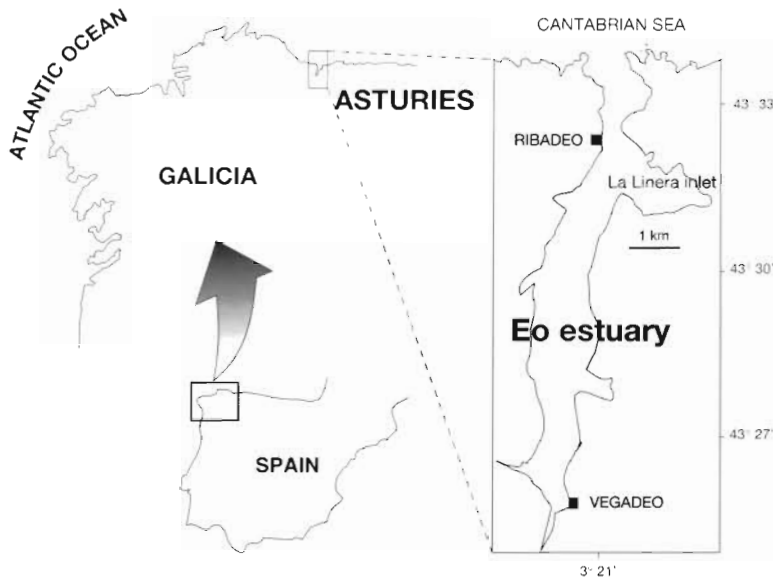


Fig. 1. Eo estuary. Culture took place in La Lina Inlet

We collected bi- or trimonthly samples from June 1993 to August 1995 in order to record growth and survival. The sampling method was as follows: a plastic square frame ( $0.25 \text{ m}^2$ ) was placed into the sandbed; a sand cut was taken out in a screen; it was sieved in seawater through a mesh of 4 mm and the number of live clams was counted. The procedure was repeated 15 times for each set to increase the precision of estimating mean survival ( $S$ ) (IFREMER 1988). A sample of 100 individuals was randomly selected from each set. Each clam was weighed ( $W$ ) to the nearest 0.01 g using a digital balance.

In January 1994, samples from 11 different Manila clam sets cultured in the Eo estuary were collected from each set and gill tissue (2 demibranchs per clam) was used following the thioglycollate procedure of Ray (1966) to detect the presence of the *Perkinsus* sp. It was detected in only 1 set (set P) from the 11 sets analyzed. To study the evolution of the parasitized set and to compare it with the unparasitized sets, 100 clams were collected trimonthly from set P and the closest (<60 m) sets A and B.

Temperature ( $^{\circ}\text{C}$ ) and salinity ( $\text{‰}$ ) values were recorded daily, at the high tide level and 1 m depth, near the clam beds (Fig. 2).

We used 1-way analysis of variance to study final survival data (in percentage). Previously we tested normality (Lilliefors' test,  $p > 0.1$ ) and homogeneity of variances (Bartlett's test,  $p > 0.05$ ).

## RESULTS

Initial and final weights, survival of Manila clams and prevalence of *Perkinsus* sp. in sets A, B and P are shown in Table 1. The growth rates of unparasitized clams in sets A and B were similar to the growth rates of clams in set P, which were infected with the parasite (Fig. 3). Similarly, no significant differences ( $p > 0.2$ ) were found in clam survival among sets (Fig. 4) at the end of the culture (summer 1995), indicating that there were no effects due to the parasite at the prevalences observed. As a prophylactic measure, a large number of parasitized clams (set P) were harvested in October–November 1994, and during this period a moderate mortality increase (14%) was observed (Fig. 4). All sets were harvested totally during summer 1995.

Water temperature in the Eo estuary (Fig. 2) varied from  $12^{\circ}\text{C}$  in December 1992 to  $20^{\circ}\text{C}$  in September 1994, always below the  $25^{\circ}\text{C}$  optimum for *Perkinsus* sp. (Vigario & Ruano 1992).

## DISCUSSION

During the 27 mo of our experiment, both growth and survival of Manila clams showed similar patterns in sets A, B and P. As usual, commercial clams (13 to 16 g per clam) were harvested after 2 yr of culture (Fig. 3), which indicates that clam growth was not affected by *Perkinsus* sp. at the prevalences observed during this study (never greater than 9%) (Fig. 4). Moreover, survival was similar in the 3 sets and only a moderate increase in mortality was observed following partial harvesting in set P (October–November 1994).

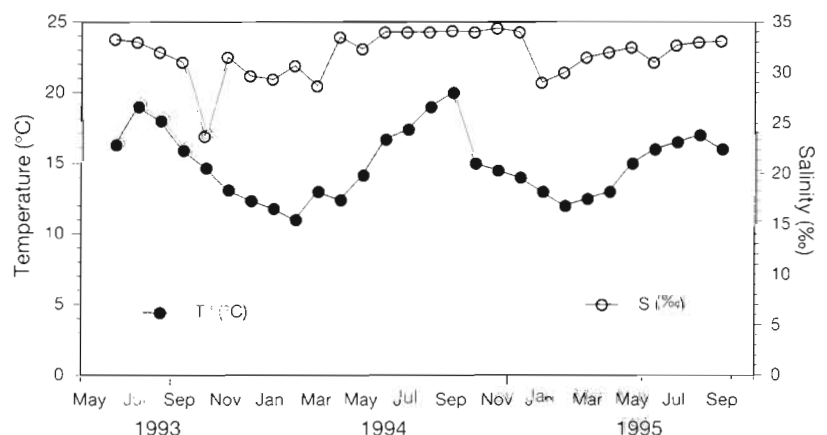


Fig. 2. Temperature ( $^{\circ}\text{C}$ ) and salinity ( $\text{‰}$ ) of Eo estuary

Table 1. Survival (*S*), weight (*W*) and prevalence of *Perkinsus* sp. (*P*) in Manila clam sets. Numbers represent the mean value ( $\pm 1$  SD) in percentage (survival, prevalence) and grams (weight) during the period June 1993–August 1995

Set	<i>S</i> <sub>initial</sub>	<i>S</i> <sub>final</sub>	<i>W</i> <sub>initial</sub>	<i>W</i> <sub>final</sub>	<i>P</i> <sub>initial</sub>	<i>P</i> <sub>final</sub>
A	100	53.8 (8.4)	0.65 (0.11)	14.1 (3.06)	0	0
B	100	56.3 (12.6)	0.66 (0.25)	13.3 (2.51)	0	0
P	100	53.4 (9.4)	0.79 (0.19)	15.5 (2.33)	?	7

Possibly, this mortality was due to harvesting, a phenomenon which has frequently been observed by the shellfish industry.

Since the parasite was not observed in clams from set A or B, prevailing conditions of temperature, salinity and density (always below  $2 \text{ kg m}^{-2}$ ) during this experiment may have prevented successful transmission of the parasite to the closest clams in set A or B in the Eo

estuary. Furthermore, *Perkinsus* sp. was not found in the natural populations of *Ruditapes decussatus* in the Eo estuary (C. Rodriguez unpubl. data). Apparently the source of *Perkinsus* sp. infections in the clams in set P was the hatchery stock from which they were derived, since other clams from the hatchery were found to be parasitized by the parasite as well (September 1994).

In contrast to other reports, *Perkinsus* sp. prevalence and clam mortality in our study showed no significant increases during high summer temperatures despite the fact that *Perkinsus* sp. is more active then. Vigario & Ruano (1992) demonstrated that infection and development of *Perkinsus* sp. in *Ruditapes decussatus* are favoured by water temperatures near  $25^\circ\text{C}$ , and temperatures below  $15^\circ\text{C}$  prevent parasite reproduction. Santmartí et al. (1995) reported variable mortalities (10 to 90%) of Manila clams during summer 1990 in the Ebro Delta (Spain) associated with high prevalences of *Perkinsus* sp. (83 to 100%).

Clam mortality attributed to *Perkinsus* sp. is usually associated with stress due to environmental factors (e.g. high temperature, since  $25^\circ\text{C}$  is the upper thermal threshold of *Ruditapes philippinarum*; Bernard 1983) and/or inadequate culture management (e.g. high clam densities). Although the lethality of *Perkinsus* sp. is clear (Villalba et al. 1993), disease develops only under optimal conditions. For example, F. Ruano (pers. comm.) in Portugal observed high prevalences of *Perkinsus* sp. in *R. decussatus* and high mortalities in dense beds ( $8 \text{ kg m}^{-2}$ ) when temperatures reached  $25^\circ\text{C}$ . Stress due to high temperatures and crowding facilitated the development of disease. Furthermore, high losses of clams were attributed to *Perkinsus* sp. in a Spanish clam depuration plant, while no mortalities were detected in natural beds with low parasite prevalences (Figueras et al. 1992). This difference in mortality may be explained by the lower prevalence of the parasite in natural beds and the lack of stressors such as the process of harvesting or/and holding in the plant. Similarly, McLaughlin & Farley (1995) suggested that *Mya arenaria* mortality due to *Perkinsus* sp. may occur when clams are heavily infected and/or stressed by other diseases and environmental factors.

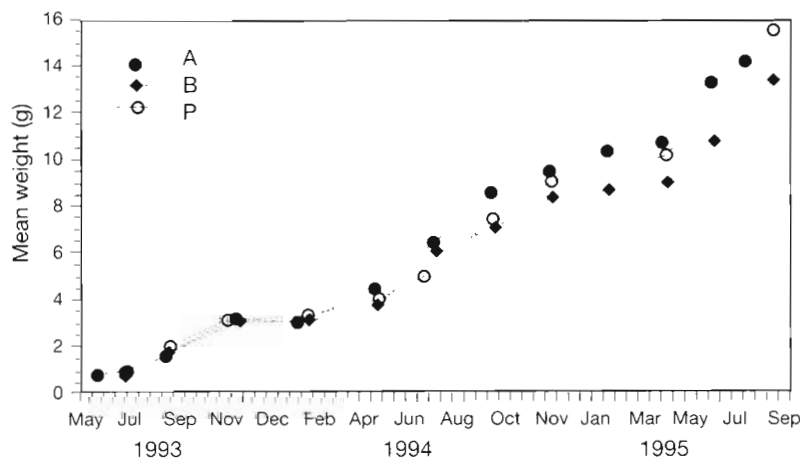


Fig. 3. *Ruditapes philippinarum*. Mean live weight of the 3 sets (A, B and P) of Manila clam

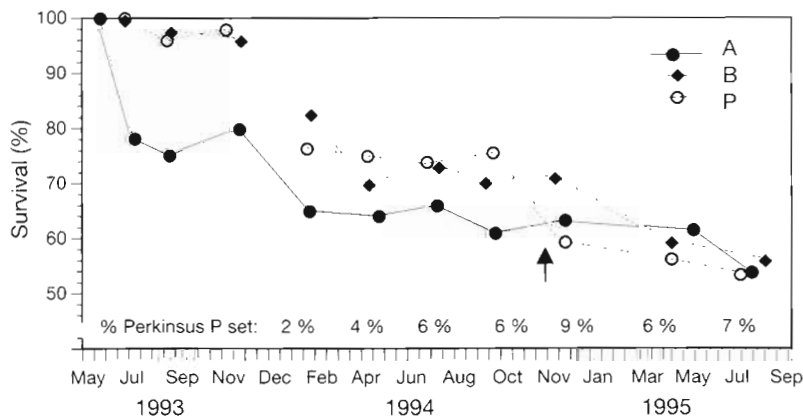


Fig. 4. *Ruditapes philippinarum*. Mean survival of the 3 sets (A, B and P) of Manila clam and percentage of parasitized clams in set P. Arrow indicates date of partial harvesting

Since temperature appears to be the main environmental factor in mortalities associated with *Perkinsus* sp., management techniques may be developed for 2 types of culture zones, according to temperature. Firstly, in areas where summer temperatures are below 20°C, the disease could be controlled if stressful growing conditions such as high densities, harvesting, and overcrowding in depuration plants are avoided. Secondly, in areas where maximum temperatures are near 25°C, the best technique, besides the above-mentioned precautions, would be to harvest all of the clam population before the temperature reaches 25°C and very heavy *Perkinsus* sp. pressure registered. In both zones we recommended 2 prophylactic measures: the total harvesting of sets with parasitized clams and the introduction of unparasitized seed in the culture areas.

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