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# Impact of *Perkinsus olseni* infection on a wild population of Manila clam *Ruditapes philippinarum* in Ariake Bay, Japan

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ABSTRACT

Many studies have addressed the production decline of Manila clam, *Ruditapes philippinarum*, in Japan, but infection of clams with *Perkinsus olseni* has received scarce attention. To evaluate the impact of *P. olseni*, infection levels and host density of a wild, unexploited clam population were monitored monthly or bimonthly on a tidal flat from June 2009 to January 2013. Real-time PCR analysis discriminating *P. olseni* and *Perkinsus honshuensis* detected only *P. olseni* in tested clams. The prevalence of *P. olseni* was 100% or nearly 100% in 7 cohorts throughout the study period, except in newly recruited clams. Infection intensity remained low and seldom reached $10^6$ cells/g wet tissue in newly recruited clams until the month of September. Infection intensity reached $10^6$ cells/g in autumn and remained high at $10^4$–$10^6$ cells/g until each cohort of clams disappeared. Clam density began to decrease in the autumn when the infection intensities reached ca. $10^6$ cells/g. Density was relatively stable in winter, increased in spring and decreased again in clams aged 1 year or older during summer and autumn in the following years. Survival of clams experimentally infected with *P. olseni* at ca. $10^6$ cells/g and placed in a cage in the tidal flat for 1 or 2 months was significantly lower than survival of uninfected control clams. Our results suggested that heavy infection with *P. olseni* was a major cause of the clam density decrease, although other environmental and biological factors also may have contributed to the decline in density. In addition, uninfected clams were deployed in cages for 1–2 months from June 2010 to January 2013 and prevalence and infection intensity were monitored. Parasite transmission and infection progression increased in summer and autumn.

## 1. Introduction

The Manila clam *Ruditapes philippinarum* is a common bivalve in tidal flats in Japan, and is important for recreational and licensed fisheries (Ito, 2002). In the early 1980s, Ariake Bay was a major site for Manila clam catch in Japan. According to the Food and Agriculture Organization of the United Nations (FAO) statistics, Manila clam production from the fishery and aquaculture in Japan was more than 100,000 tons from 1950 to 1986, the highest production worldwide. However, the annual catch dramatically declined to a minimum of 14,000 ton in 2015, probably because of the decline of the population in Ariake Bay since the late 1970s, and subsequent nationwide declines since the mid-1980s (Matsukawa et al., 2008). Environmental changes, overfishing, and predators, among other factors, are suspected causes of the decline in the Manila clam catch; however, the major causes of the decline are still unclear.

Protozoans in the genus *Perkinsus*, superphylum Alveolata (Siddall et al., 1997), infect mollusks (Villalba et al., 2004), and *Perkinsus olseni* is included in the list of notifiable mollusk diseases of the World Organization for Animal Health (OIE). In Japan, *Perkinsus* spp., primarily *P. olseni*, have been detected in most Manila clam populations since the mid-1990s (Choi et al., 2002; Hamaguchi et al., 1998, 2002; Momoyama and Taga, 2005; Sakai and Onodera, 2006; Takahashi et al., 2009; Umeda and Yoshinaga, 2012; Yoshinaga et al., 2010), except those on the north and east coasts of Hokkaido. *Perkinsus* spp. typically have two phases in their life cycle: the propagation phase in their hosts and the zoosporulation phase in seawater (Bordenave et al., 1995). Trophozoites propagate by repeated cell divisions in the host tissues. When trophozoites are exposed to anaerobic conditions generated by the death of the host, they transform into prezoosporangia. In seawater, the prezoosporangia transform into zoosporangia, which release infective zoospores (Bordenave et al., 1995).

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*Perkinsus honshuensis* and *P. olseni* have been reported from exploited and natural populations of Manila clam in Japan and have occasionally co-infected wild Manila clams (Dungan and Reece, 2006; Umeda and Yoshinaga, 2012). In studies conducted before the first detection of *P. honshuensis* (Dungan and Reece, 2006), *Perkinsus* spp. in Manila clams in Japan were reported as unidentified *Perkinsus* sp. or *P. olseni* because neither the Ray's fluid thioglycollate medium (RFTM) assay nor the PCR assay, both of which are genus specific, could distinguish the two species. Umeda and Yoshinaga (2012) developed a real-time PCR assay to distinguish *P. olseni* and *P. honshuensis*. They reported that *P. olseni* was predominant in all the tested Manila clam populations in Japan, and that the lower infection intensities of *P. honshuensis* were, therefore, negligible.

Recently, infection with *P. olseni* has been suspected to be a major cause of the Manila clam decline in Japan. High infection intensity and prevalence were reported in many areas, including Ariake Bay where clam catch has declined (Choi et al., 2002; Park et al., 2008; Umeda and Yoshinaga, 2012). Previous challenge experiments demonstrated that infection with *P. olseni* had serious negative effects on the survival and physiology of the clams (Shimokawa et al., 2010; Waki et al., 2012; Waki and Yoshinaga, 2013, 2015), however, the negative impacts of *P. olseni* infection on the survival of Manila clams in wild unexploited or exploited populations remained unclear.

The aim of this study was to evaluate the impact of *P. olseni* infection on wild Manila clam populations, and to quantify changes in population density of the wild clams after infection. We chose a survey site in a tidal flat of Ariake Bay where the annual Manila clam catch has dramatically decreased from 60,000 tons in the late 1970s to less than 10,000 tons in 2015. *P. olseni* infection, Manila clam population density and environmental factors were surveyed for 4.5 years in an area where clamming has been prohibited. We also examined the virulence of *P. olseni* under natural environmental conditions by placing experimentally challenged Manila clams, and unchallenged *Perkinsus*-free clams together in cages on the tidal flat. Additionally, seasonal fluctuations in parasite transmission were examined at the study site by placing uninfected clams at the site monthly or bimonthly and monitoring infection after 1–2 months of exposure.

## 2. Material and methods

### 2.1. Field survey

#### 2.1.1. Study area

Field sampling was conducted monthly or bimonthly from June 24, 2009 to January 30, 2013 at Station (St.) A (N32°52′46″, E130°30′13″) in the intertidal estuary zone of the tidal flat at Taimei, Kumamoto Prefecture, near the mouth of Kikuchi River, on the eastern side of Ariake Bay, Japan (Fig. 1). Aquaculture activities were absent in the area. Near St. A, clamming has been prohibited since 1989 to conserve Manila clams. At the station, we covered sediment with three polyethylene nets (37.5 mm mesh opening, 1 m × 2 m) from January 24, 2011 to January 30, 2013 to deter unexpected clamming. Sampling was conducted at an additional five stations: St. B (N32°52′59″, E130°30′24″), St. C (N32°52′34″, E130°30′7″), St. D (N32°53′2″, E130°30′13.31″), St. E (N32°52′54″, E130°30′6″), and St. F (N32°52′41″, E130°30′0″). These stations were set on a grid with spacing of ca. 500 m (Fig. 1). Sampling was conducted on June 5, 2012 (at St. B–F) and August 3, 2012 (at St. B and C) to determine the infection status of Manila clams in areas surrounding St. A.

Sediment temperature was measured at St. A at 10- or 15-min intervals from June 27 to December 22, 2010, January 23 to December 16, 2011, and February 2012 to January 30, 2013 with a temperature data logger (Bobo water temp pro V2, Onset Computer Cooperation; Bourne, MA, USA) buried to a depth of 5 cm in sediment. Water salinity was measured at 15-min intervals from January 2012 to January 30, 2013 with a salinity data logger (H24-002, Onset Computer Cooperation) set 5 cm above the sediment surface. The salinity data logger was replaced with a new logger monthly or bi-monthly. To avoid attachment of sessile organisms on the sensor, the salinity logger was covered with a 300 μm-mesh nylon bag from July 2012 to November 1, 2012 and the sensor was coated with a chemical barnacle repellent (Annex, Ecowel, Tokyo, Japan) mixed with water-based paint from November 1, 2012 to January 30, 2013.

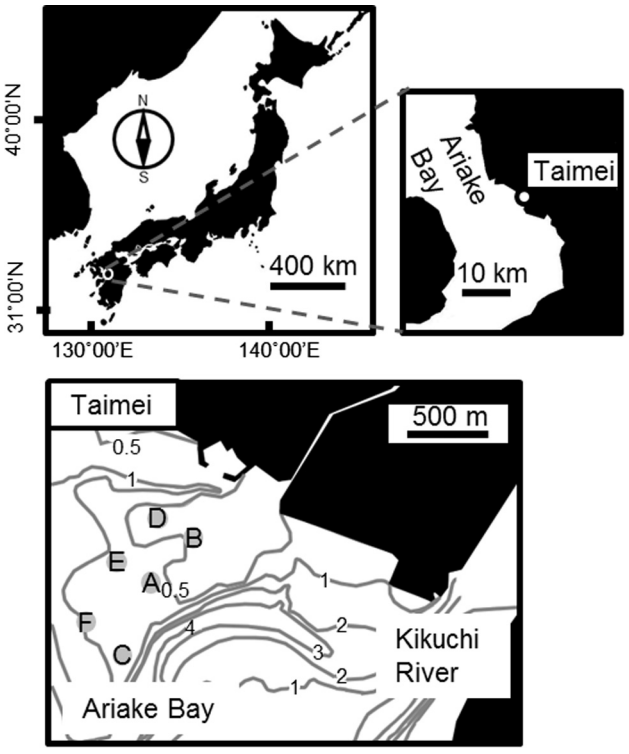


**Fig. 1.** Study stations at Taimei, Ariake Bay in Japan. A–F represent Stations. Gray lines represent depth contours.

We assumed that all clams determined to be positive in the RFTM assay were infected with *P. olseni* only (Umeda and Yoshinaga, 2012). Although some clams might have also been infected with *P. honshuensis*, the infection was considered to be negligible.

#### 2.1.2. Sampling procedure and analysis

Sediment samples were collected in triplicate within a quadrat (20 cm × 20 cm × 10 cm, L × W × D) at each station during the ebb tide. Each sediment sample was separately sieved through a 2-mm mesh, and trapped sediment was collected. Manila clams with two shells (apparently alive) were collected from the sediment and kept on ice or at 4 °C for transportation and storage for 1–2 days. The shell length of the clams was measured and their shells subsequently opened. Clams with soft tissue were classified as live clams and those without were classified as newly dead clams. To determine infection levels, 30 live clams were selected so that they would roughly represent the sizes of clams from the sediment sample containing the largest number of live clams. Each live clam was individually subjected to the whole-body RFTM assay (Ray, 1952), according to Waki and Yoshinaga (2013), Choi et al. (1989), and Almeida et al. (1999). When fewer than 30 clams were collected in a sediment sample, all of the clams were assayed.

To collect small Manila clams that passed through the 2-mm mesh (smaller than approximately 4 mm in shell length), sediment was sampled at depths of 0–1.5 cm and 1.5–5 cm in a small quadrat (10 cm × 10 cm × 5 cm, L × W × D) placed within the large quadrat (20 cm × 20 cm × 10 cm, L × W × D) at St. A from December 16, 2011 until the end of the survey. Sediment obtained from 0 to 1.5 cm depth with the 2-mm mesh was sieved and the samples were removed, while