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Correlation between perkinsosis and growth in clams *Ruditapes* spp.

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ABSTRACT: Perkinsosis is one of the most widespread diseases affecting commercially important species of molluscs globally. We examined the impact of *Perkinsus* spp. on shell growth at the individual scale in 2 clam species: *Ruditapes decussatus* from Mundaka Estuary (Spain) and *R. philippinarum* from Arcachon Bay (France). At Arcachon, 2 contrasting sites in terms of environment and *Perkinsus olseni* presence were chosen: Arguin (disease-free) and Ile aux Oiseaux (infected site). We monitored the dynamics of perkinsosis over the course of the experiment at Mundaka and Ile aux Oiseaux. Prevalences were high (>70%), and intensities were around 10^5 cells g^{-1} wet gills at Ile aux Oiseaux, and 10^6 cells g^{-1} at Mundaka. No significant differences in prevalence or intensity were observed over time. A 2 yr field growth experiment of tagged-recaptured clams was performed to determine individual clam growth rate, condition index (CI), and *Perkinsus* spp. infection intensity. Clams were collected at Ile aux Oiseaux and transplanted to Arguin. The growth rate was always significantly and negatively correlated with *Perkinsus* spp. infection, and positively correlated with CI. CI and *Perkinsus* spp. infection explained 19% and 7% of the variability of the growth rate at Mundaka and Ile aux Oiseaux, respectively. In experimental clams at Arguin, *P. olseni* infection explained 26% of the variability of the growth rate at the lower tidal level. Our results suggest that at a concentration of between 10^5 and 10^6 cells g^{-1}, perkinsosis affects the physiological functions of the clams, highlighted by its impact on the growth rate.

KEY WORDS: Clams · *Perkinsus* spp. · *Perkinsus olseni* · *Ruditapes philippinarum* · *Ruditapes decussatus* · Growth

INTRODUCTION

Among molluscan diseases, perkinsosis has led to the most severe economic losses, prompting considerable study of *Perkinsus* spp. parasites. Protozoans of the genus *Perkinsus* have been associated with mortalities and/or population declines in various commercially important species of molluscs, including *Perkinsus marinus* in the eastern oyster *Crassostrea virginica* in the USA (Andrews & Hewatt 1957), and *Perkinsus olseni* in the tridacnid clam *Tridacna gigas* in Australia (Goggin & Lester 1987), in the carpet-shell clam *Ruditapes decussatus* in Spain and Portugal (Ruano & Cachola 1986, Azevedo 1989, Villalba et al. 2005), and in the Manila clam *Ruditapes philippinarum* in Korea and Japan (Choi & Park 1997, Park & Choi 2001, Yoshinaga et al. 2010). In Europe, *Perkinsus* spp. has been observed in *R. philippinarum* and *R. decussatus* from Spain (Figueras et al. 1992, Navas et al. 1992, Elandaloussi

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et al. 2009), Italy (Da Ros & Canzonier 1985, Da Ros et al. 1998), and France (Goggin 1992, Lassalle et al. 2007, Flye-Sainte-Marie 2007).

Perkinsus spp. infection progresses through clam host tissues, causing lesions that may eventually result in host death. The inflammatory reaction induced by *Perkinsus olseni* in venerid clams primarily involves encapsulation of the parasite cells by hemocytes (Navas et al. 1992, Sagristá et al. 1995, Montes et al. 1996). Heavy infections produce lesions that are sometimes visible to the naked eye. The normal structure of host tissues is lost, and white milky nodules or pustules appear (Lee et al. 2001, Choi et al. 2002).

To develop and proliferate, a parasite absorbs nutrients at the expense of its host. Defense responses against *Perkinsus marinus* and *P. olseni* have been found to be energetically costly for their hosts (Park et al. 2006). Diseases reduce bivalve fitness and alter their filtration rate. Therefore, a high concentration of *Perkinsus* spp. in bivalve gills may decrease filtration efficiency. Da Ros et al. (1998) observed a higher clearance rate in *Ruditapes philippinarum* from sites with lower *Perkinsus* spp. infection, while clearance rate tended to decrease in *R. decussatus* heavily infected by *P. olseni* (Casas 2002, Villalba et al. 2004). Yoshinaga et al. (2010) did not obtain clear evidence of *Perkinsus* spp. on clam physiology, including clearance rate, although infection intensity ranged among high values (10^5 to 10^6 cells gill^{-1} wet weight). Clearance rate tended to decrease in *R. decussatus* heavily infected by *P. olseni* but the effects were not statistically significant (Casas 2002). *Perkinsus* spp. infection, if it alters filtration, could lead to a decrease of oxygen and food absorption for clams and have direct repercussions on clam metabolism. A defense reaction, in addition to the energy absorbed by *Perkinsus* spp. directly from the clam, could consume much energy at the expense of physiological activities such as growth and reproduction. Thus, *Perkinsus* spp. may alter reproduction, growth, and condition index of infected hosts. Nevertheless, there is a lack of information at the individual level regarding these detrimental effects in clams at a sub-lethal level of infection.

The purpose of this study was to investigate the impact of *Perkinsus* spp. on growth rate in 2 clam species (*Ruditapes philippinarum* and *R. decussatus*) in environmentally-contrasted sites, through an *in situ* caging experiment with tagged bivalves. The 2 study sites were Arcachon Bay (France) and Mundaka estuary (Spain). In Arcachon Bay, it is probable that any *Perkinsus* infection will be *P. olseni*, as this is the only *Perkinsus* species that has been detected in the Bay (Arzul et al. 2012). However, there is as yet no precise identification of *Perkinsus* species for Mundaka clams. Consequently, we will not reject the possibility that we worked with a *Perkinsus* spp. complex in this site, as *P. chesapeaki* was also detected in *R. decussatus* from Leucate (a lagoon on the Mediterranean coast) as well as in *R. philippinarum* from the Atlantic coast of Charente-Maritime (Arzul et al. 2012).

MATERIALS AND METHODS

Study areas

Carpet-shell clams *Ruditapes decussatus* were collected by hand from Mundaka Estuary, Spain (43° 22′ N, 2° 43′ W) and Manila clams (*R. philippinarum*) from Arcachon Bay, France (44° 40′ N, 1° 10′ W) (Fig. 1).

Mundaka Estuary is a shallow, 13 km^2 meso-tidal estuary in the southeast of the Bay of Biscay (Fig. 1). This system is dominated by euhaline waters at high tide and by polyhaline waters at low tide (Villate

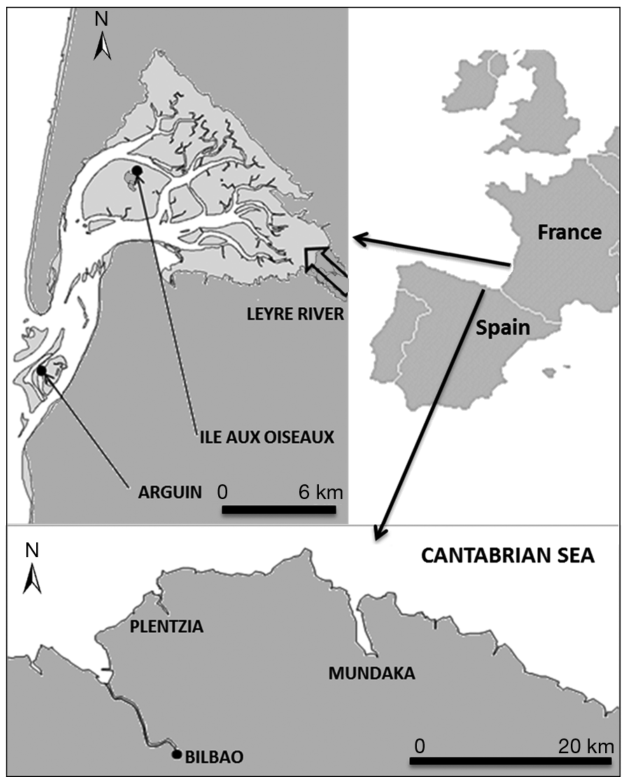

Fig. 1. Sampling sites of (a) Arguin and Ile aux Oiseaux in Arcachon Bay, and (b) Mundaka Estuary

1997). Carpet-shell clams were collected from the outer part of the estuary on a stony bank.

Arcachon Bay is a 180 km^2 semi-sheltered lagoon in the southwest coast of France (Fig. 1). Fresh waters are released into the bay mainly by the Leyre River but also by many streams around the lagoon. The lagoon receives marine waters from the Atlantic Ocean. The mixture of marine and continental water inputs, as well as the slow renewal by tide, result in temperature and salinity gradients throughout the bay (Plus et al. 2006). Muddy intertidal flats colonized by a vast *Zostera noltii* seagrass bed dominate the inner part of the lagoon (110 km^2), whereas intertidal sand flats (Arguin) are located at the mouth of the lagoon where it meets the Atlantic Ocean. Manila clams inhabit both of these 2 contrasting environments. The 2 studied sites were at Arguin (oceanic site) and Ile aux Oiseaux (inner bay; Fig. 1). A preliminary survey showed that perkinsosis was absent at Arguin but that clams from Ile aux Oiseaux were heavily infected by *Perkinsus olseni* (Arzul et al. 2009, Dang et al. 2010a). Sediments at Ile aux Oiseaux and Arguin differ in median grain size (97 and 360 µm), organic matter content (13 % and 1 %, respectively) and silt and clay proportion (42.4 % and 3.5 %, respectively). Sediment temperature fluctuations are high at both sites (Arguin: min. = –0.2°C, max. = 30°C, mean = 15.1°C; Ile aux Oiseaux: min. = 0.2°C, max. = 37.9°C, mean = 16.1°C). The salinity is higher and more stable at Arguin (min. = 1.2, max. = 35.4, mean = 34.2) than at Ile aux Oiseaux (min. = 12.1, max. = 34.8, mean = 29.6) (Dang et al. 2010b).

Perkinsus spp. infection in total body burden vs. gills

A preliminary objective was to ensure that the *Perkinsus* spp. intensity in analysed gills was representative of the intensity in the total clam individual. In order to correlate the total number of *Perkinsus* spp. per gram of wet tissue and the number of *Perkinsus* spp. per gram of wet gills, 50 adult *Ruditapes philippinarum* were sampled at Ile aux Oiseaux, Arcachon Bay, and their gills and the rest of the body processed to quantify *Perkinsus olseni* infection by the fluid thioglycolate medium (FTM) method of Ray (1952), modified by Choi et al. (1989). The gills and the rest of the body were separately incubated in FTM supplemented by antibiotics (streptomycin and penicillin G) and antifungals (Nystatin) for 5 d in the dark at room temperature. They were subsequently harvested by centrifugation and digested twice with 2 N NaOH at 60°C for 1 h. The resulting solution was centrifuged and the supernatant discarded. The pellet was rinsed twice in a Phosphate Buffered Saline (PBS 1×) solution and finally resuspended in 1 ml of PBS. *P. olseni* hypnospores were enumerated using a Malassez chamber under light microscopy. Finally, *P. olseni* was recorded as number of parasite cells per gram of wet gill organ or per gram of wet remaining tissues. The intensity of infection was then calculated as the mean number of parasite cells per diseased clam. The threshold of detection was <20 cells in 1000 µl.

Prevalence and intensity of perkinsosis

In order to assess the dynamics of *Perkinsus* spp. burden in clams in the experiment, prevalence and intensity of infection were monitored every 2 mo — from November 2005 to October 2007 at Mundaka, and from December 2005 to February 2007 at Ile aux Oiseaux. No survey was conducted at Arguin, since the parasite is absent in the natural population of clams there (Dang et al. 2010a). At each sampling, 30 adult clams (30 to 40 mm) were randomly collected by hand at Mundaka and at Ile aux Oiseaux, and their gills were excised and processed for quantification of the parasite, according to the previously described method.

Enclosure experiments

Mundaka Estuary. In order to assess the relationships between *Perkinsus* spp. infection, clam growth rate (GR) and condition index (CI), carpet shell clams between 5 and 48 mm shell length were collected in December 2005 at Mundaka Estuary. Each clam was labelled with a numbered tag and measured to the nearest 0.1 mm shell length with a calliper. Clams were subsequently planted out in 3 enclosures at a density of 320 ind. m^{-2}. Enclosures were emptied of native bivalves before the experiment. Each enclosure occupied a 0.25 m^2 surface, and sides were covered with a 2 mm mesh plastic net to avoid clam migration. We assumed that enclosures represented a barrier for clams but had little effect on local environmental conditions. The 3 enclosures were at the same elevation above low tide level (i.e. 1.7 m), as the distribution area of this species in the estuary was small and the difference in elevation was negligible. Clams were recaptured at completion of the trial in October 2007.

Arcachon Bay. The same procedure was performed in Arcachon Bay at Arguin and at Ile aux Oiseaux. However, the 3 enclosures were located at different tidal levels. Manila clams with shell lengths between 6 and 44 mm were collected at Ile aux Oiseaux (infected site) in December 2005. Each clam was tagged, measured and released into enclosures at either Ile aux Oiseaux or Arguin at a density of 320 ind. m^{-2}. At Ile aux Oiseaux, enclosure LTL (low tidal level) was situated at 1.8 m, enclosure MTL (medium tidal level) at 2.03 m and enclosure HTL (high tidal level) at 2.6 m. At Arguin, enclosures LTL, MTL and HTL were positioned at 1.8, 2.1 and 2.9 m respectively. In October 2007, clams were recaptured and analysed.

Clam analyses

At the end of the experiment, clam shells were measured individually to the nearest 0.1 mm. Growth rate was calculated by the following equation:

$$G = [(L_f - L_i) \times 1000]/(t_f - t_i) \quad (1)$$

where G = growth rate in µm d^{-1}, L_f = final shell length (mm), L_i = initial shell length (mm), t_f = final time (i.e. the number of days accumulated at completion of the experiment), and t_i = initial time. Remaining tissues (total flesh without gills that were removed for *Perkinsus* spp. analysis) were collected in order to calculate CI (Walne & Mann 1975) following the relation:

$$\text{CI (‰)} = \text{dry flesh weight (mg)/dry shell weight (g)} \quad (2)$$

The total dry flesh weight was calculated by adding dry flesh weight and dry gill weight. Dry gill weight was obtained by multiplying the wet gill weight by 0.153 (Flye-Sainte-Marie 2007).

Statistical data treatments

A linear regression was performed between the number of *Perkinsus olseni* in the gills and in the total body. A 1-way ANOVA was used to compare the intensity of infection over time. Maximum Type I error rates were set at $\alpha = 0.05$. Prior to ANOVA, homogeneity of variance was confirmed using Cochran's test. Data were log-transformed to follow homogeneity requirements. Where ANOVA was significant, differences between treatments were separated by a Tukey test of mean comparison. When variances were not homogeneous, the non-parametric Mann-Whitney *U*-test was used. The impact of the tidal level on the GR was assessed by a 1-way ANOVA.

To assess the association between CI, *Perkinsus* spp. infection and GR, multiple regressions were performed for each site. CI data represented the condition of the clam only at the experiment completion. CI values changed substantially over the course of the experiment, whereas GR values are more integrative and representative of the processes that had impacted the clam during the course of the experiment. Consequently, GR was considered in multiple regression as the dependent variable, and CI and perkinsosis were considered as independent variables. In the analysis, *Perkinsus* spp. values were considered in a logarithmic scale from 0 (uninfected clams) to 6 cells g^{-1} of wet gills. For each site, data from the enclosures were grouped or not, according to the results of the ANOVA comparing the GR at each tidal level.

RESULTS

Perkinsus spp. infection in total body vs. gills

Prevalence of *Perkinsus olseni* infection was found to be the same in the gills and in the total body of *Ruditapes philippinarum* (Fig. 2). A positive correlation was observed between the number of *P. olseni* cells g^{-1} in the gills and the total body burden (Fig. 2; $R^2 = 0.93$), and the origin of the curve is almost zero, suggesting that the *P. olseni* in the gill is representative of infection of the total clam individual.

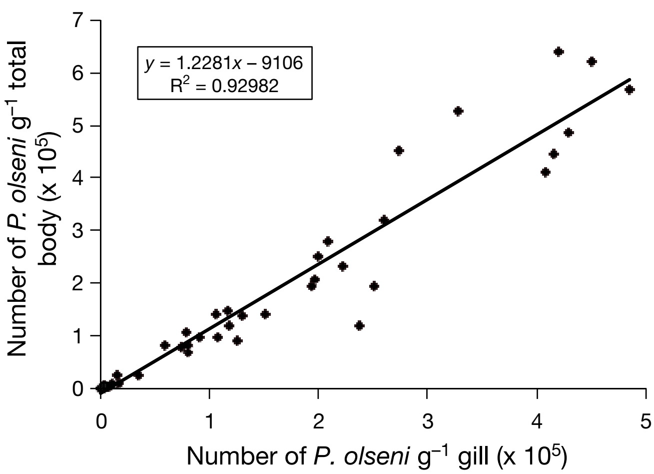

Fig. 2. *Ruditapes philippinarum.* Correlation between the total number of *Perkinsus olseni* per gram of wet tissue (total body) and the number of *P. olseni* per gram of wet gills

Prevalence and intensity of *Perkinsus* spp.

Prevalence of infection at both infected sites was high: between 68 and 100% at Mundaka and between 77 and 100% at Ile aux Oiseaux. Arguin was free of this parasite (Dang et al. 2010a). Mean intensities of infection were high: on a logarithmic scale between 5 and 6 at Mundaka and between 4 and 5 at Ile aux Oiseaux (Fig. 3). Average intensities (±SE) over the duration of the experiment were 1 115 428 ± 131 390 cells g^{-1} of wet gills at Mundaka, and 155 342 ± 18 028 cells g^{-1} of wet gills at Ile aux Oiseaux. No significant difference was observed between time either in prevalence (percentage comparison test, $p > 0.05$), or intensities for both sites ($p > 0.05$, 1-way ANOVA).

Enclosure experiment

The mortality rate inside the enclosures was relatively low for the 3 studied sites (on average, mortality rate, Z, is 0.67 yr^{-1}), with the exception of the lowest tidal level at Arguin and Ile aux Oiseaux, where clams were predated by the sting winkle *Ocenebra erinacea* (Dang et al. 2010b). Brown muscle disease (BMD) is also known to affect Manila clam populations in Arcachon Bay (Dang et al. 2008). However, observation of dead shells and live clams at the end of the experiment revealed a very low prevalence of BMD. Only 14 and 9 clams were infected at Arguin and Ile aux Oiseaux, respectively.

Relation between growth rate, CI, and *Perkinsus* infection

At the end of the experiment, the mean logarithmic infection intensity at Mundaka was 5.31 cells g^{-1} of wet gills, the mean GR was 24.4 µm d^{-1}, and the mean CI was 77.95 ‰ (Table 1). Multiple regression highlighted significant correlations between dependent and independent variables ($p < 0.001$, Table 2), with CI and *Perkinsus* spp. intensity explaining 19% of the variability of the GR (Table 2, Fig. 4a). Correlations were positive between GR and CI, whereas they were negative with the *Perkinsus* spp. intensity (Table 2, Fig. 4a).

At the completion of the experiment at Arguin, the mean logarithmic infection rates of *Perkinsus olseni* were 4.41, 4.50 and 4.46 cells g^{-1} of wet gills for LTL, MTL, and HTL, respectively (Table 1). Mean GR and mean CI are summarized in Table 1. The GR was significantly different for each tidal level (1-way

Table 1. Means of *Perkinsus* spp. infection intensity on a logarithmic scale, growth rate (GR) in µm d^{-1} and condition index (CI) in ‰ in clams from each enclosure. LTL: low tide level; MTL: medium tide level; HTL: high tide level

Site	*Perkinsus* intensity	GR	CI
Mundaka	5.31	24.4	77.95
Arguin LTL	4.41	15.8	49.86
Arguin MTL	4.5	20.9	62.73
Arguin HTL	4.46	13	78.62
Ile aux Oiseaux LTL	4.4	23.3	46.72
Ile aux Oiseaux MTL	4.13	23	38.6
Ile aux Oiseaux HTL	4.65	15.5	48.11

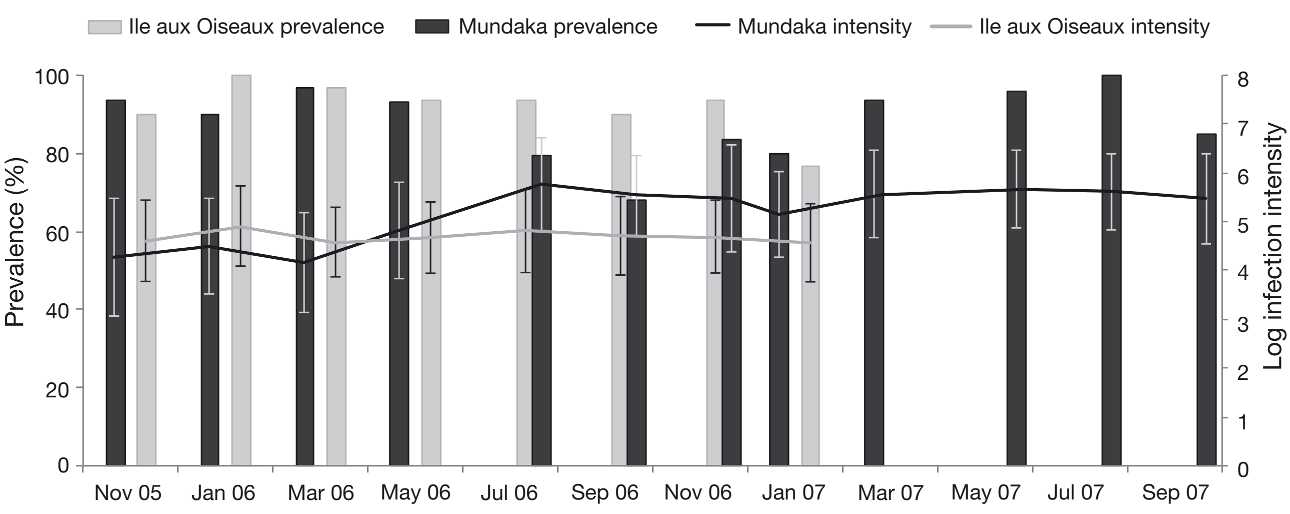

Fig. 3. Prevalences and intensities of *Perkinsus* spp. at Mundaka from November 2005 to October 2007, and at Ile aux Oiseaux from December 2005 to February 2007

Table 2. Multiple regression models predicting growth rate (GR) in functions of condition index (CI) and *Perkinsus* spp. intensity. β represents the coefficient of regression, i.e. the contribution of each independent variable to the prediction of GR. The direction of the relationship between variables is given by the sign of β

Site	Multiple regressions n	R^2	*F*	p	Predictor	β ±SE	p
Mundaka	227	0.19	25.98	<0.001	(Constant)	20.80 ±6.42	0.001
					CI	0.28 ±0.06	<0.001
					Perkinsus spp.	−3.48 ±0.71	<0.001
Arguin LTL	63	0.26	10.6	<0.001	(Constant)	27.30 ±6.57	<0.001
					CI	0.15 ±0.12	0.21
					Perkinsus	−4.47 ±0.98	<0.001
Arguin MTL	117	0.02	1.17	0.31	(Constant)	−1.35 ±19.2	0.94
					CI	0.48 ±0.34	0.16
					Perkinsus spp.	−2.20 ±1.98	0.27
Arguin HTL	129	0.17	13.03	<0.001	(Constant)	−15.44 ±7.18	0.03
					CI	0.42 ±0.09	<0.001
					Perkinsus spp.	−1.04 ±0.60	0.08
Ile aux Oiseaux	172	0.07	6.61	<0.001	(Constant)	12.47 ±10.04	0.22
					CI	0.42 ±0.18	0.02
					Perkinsus spp.	−2.53 ±1.01	0.01

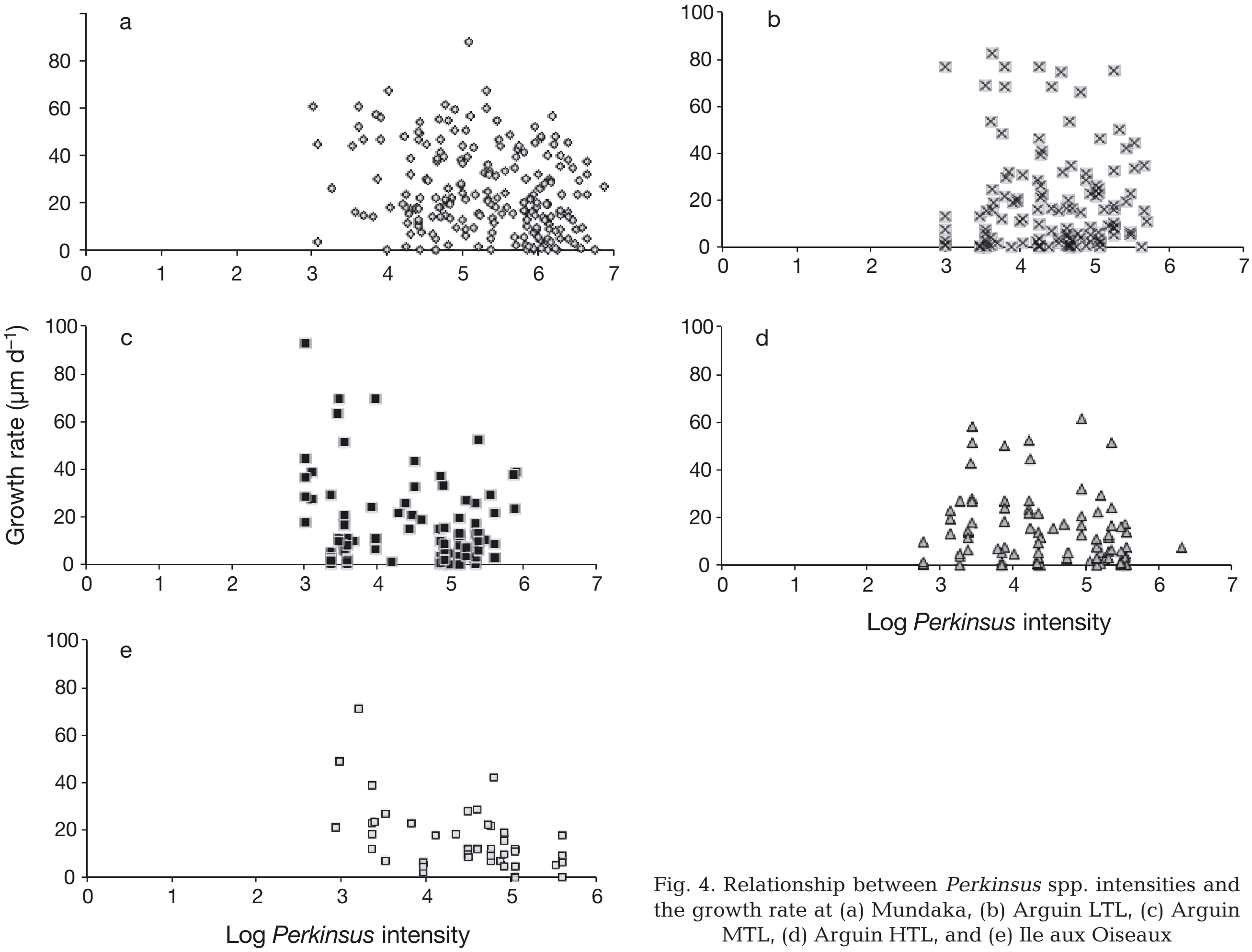

Fig. 4. Relationship between *Perkinsus* spp. intensities and the growth rate at (a) Mundaka, (b) Arguin LTL, (c) Arguin MTL, (d) Arguin HTL, and (e) Ile aux Oiseaux

ANOVA, $p = 0.01$) with GR increasing from the higher to the lower tidal level (Tukey test, $p < 0.05$). Consequently, multiple regressions were conducted for each enclosure. Significant correlations were found between the GR and independent variables (CI, *P. olseni* infection) for Arguin LTL and HTL ($p < 0.001$; Table 2, Fig. 4b–d), but not for MTL ($p = 0.31$; Table 2, Fig. 4b–d). *P. olseni* infection explained 26% of the variability in GR at LTL, whereas CI explained 17% of the variability at HTL (Table 2). Correlations were always positive between GR and CI, whereas they were always negative with the *P. olseni* intensity (Table 2).

At the end of the experiment at Ile aux Oiseaux, *Perkinsus olseni* infection intensities were on average on a log scale 4.40, 4.13 and 4.65 cells g^{-1} of wet gills for LTL, MTL and HTL respectively (Table 1). Mean GR and mean CI are summarized in Table 1. No significant effect of the tidal level was observed on the GR (Mann-Whitney *U*-test, $p = 0.12$), thus the 3 enclosures were included in the same multiple regressions. Significant correlations were observed ($p < 0.001$; Table 2, Fig. 4) and independent variables explained 7% of the variability of the GR (Table 2). As with the 2 other sites, positive correlations were observed between GR and CI, while GR and *P. olseni* infection were negatively correlated (Table 2, Fig. 4).

DISCUSSION

Our study showed that the *Perkinsus* spp. infection in the gills is representative of the infection in the total body, that prevalences and intensities of infection were stable over the course of the experiment, and that GR and *Perkinsus* spp. infection were negatively correlated for the 3 studied sites.

A strong positive correlation was observed in *Ruditapes philippinarum* between the number of *Perkinsus* spp. per gram of gills and the number of *Perkinsus* spp. in the whole animal, as previously observed in Japan by Choi et al. (2002), in Korea by Park et al. (1999), and in Spain by Rodriguez & Navas (1995). Thus, quantifying *Perkinsus* spp. infection in the gills is representative of the infection in the total body.

As previously observed by Dang et al. (2010b) in Arcachon Bay, we detected no seasonality in *Perkinsus olseni* prevalence or intensity in Arcachon Bay or in Mundaka Estuary. This stability could be a result of either the same quantity of parasites being acquired and purged from the host, or the stability of the *P. olseni* load in host tissues over the duration of the experiment. All clams came from the same infected site, and Arguin is free of the disease. Consequently, since the same parasite load was found in clams at Ile aux Oiseaux and Arguin at the end of the experiment, the second hypothesis is supported—that the *P. olseni* load did not change in clam tissues over the duration of the experiment.

Many studies have examined the impact of perkinsosis on growth and CI in bivalves (mostly clams and oysters at the population level) by studying the average CI and/or biometric parameters of a group of individuals (Andrews 1961, Crosby & Roberts 1990, Gauthier et al. 1990, Burreson & Andrews 1991, Goggin 1996, Villalba et al. 2000, Elston et al. 2004, Leite et al. 2004, Ford & Smolowitz 2007). To our knowledge, this is the first time the impact of perkinsosis on growth has been studied in clams at the individual level (i.e. tracked through time in individually identified animals). Menzel & Hopkins (1955), studying *Crassostrea virginica*/*Perkinsus marinus* interaction in Louisiana, USA, investigated the impact of the infection on growth by following individually marked oysters. They documented a negative correlation between parasite burden and shell growth. In the present study, we found a negative and significant correlation between *Perkinsus* spp. load and GR at all sites, with the exception of Arguin MTL and HTL. GR was integrative and representative of all processes that affected the clams during the experiment. Conversely, the GR was always positively correlated with the CI. We could not assess the impact of *Perkinsus* spp. on the CI over the duration of the experiment, as CI values were only representative of the end of the experiment. CI is known to vary greatly over time. However, the higher the GR, the lower the *Perkinsus* spp. load and higher the CI. So we may expect that *Perkinsus* spp. may also affect the CI. At Mundaka, CI and *Perkinsus* spp. load explained 19% of the variation of the GR of *Ruditapes decussatus*, whereas they explained 7% of the variability of the GR of *R. philippinarum* at Ile aux Oiseaux. However, even if the infection intensity was lower than at Mundaka Estuary (10^5 cells g^{-1} at Arcachon vs. 10^6 cells g^{-1} at Mundaka), the effect of *Perkinsus* spp. on GR was more significant at Arguin LTL, with perkinsosis explaining 26% of the GR variation. As a result, perkinsosis impacted the growth, although the difference in significance between sites at Arcachon with a similar range of infection highlights the importance of environmental conditions on host-pathogen dynamics. The presence of the parasite in the mantle of the clam may directly impact shell synthesis and consequently decrease the GR. Additionally, the animal could reallocate energy from growth and normal

Table 3. Effect of *Perkinsus* spp. on the survival, growth and condition index of bivalve hosts by location. Negative effect = −; positive effect = +; no effect = NE; not tested = …

Bivalve species/*Perkinsus* species Location	Survival	Growth	Condition index	Source
Crassostrea virginica/Perkinsus marinus				
Chesapeake Bay, USA	−	−	…	Burreson & Andrews (1991), Newell et al. (1994), Albright et al. (2007)
	−	…	…	Andrews & Hewatt (1957), Burreson & Ragone Calvo (1996), Ragone Calvo et al. (2003)
	…	−	…	Andrews (1961), Burreson & Andrews (1991)
	…	…	−	Hewatt (1951, cited in Andrew 1961), Ray (1952), Dittman (1993), Volety & Chu Fu-Lin (1994)
	…	−	−	Paynter & Burreson (1991), Paynter (1996)
	…	…	NE[a]	Chu & La Peyre (1993), Chu et al. (1993)
Louisiana, USA	…	−	…	Gauthier et al. (1990)
	…	…	−	Menzel & Hopkins (1955)
Delaware, USA	…	−	− [b]	Dittman et al. (2001), Ford & Smolowitz (2007)
South Carolina, USA	…	…	−	Crosby & Roberts (1990)
Mexico	−	…	…	Burreson et al. (1994)
***Ruditapes philippinarum/Perkinsus* sp.**				
Spain	NE	NE	…	Cigarría et al. (1997)
Korea	−	…	NE	Park & Choi (2001), Choi et al. (2002)
***Ruditapes decussatus/Perkinsus* sp.**				
Spain/Portugal	…	…	−	Villalba et al. (2000, 2005), Rodríguez Moscoso et al. (2002), Leite et al. (2004)
Patinopecten yessoensis/Perkinsus qugwadi				
British Columbia, Canada	−	…	…	Blackbourn et al. (1998), Bower et al. (1998)
Tridacna crocea/Perkinsus olseni				
Great Barrier Reef, Australia	…	…	NE	Goggin (1996)

[a]Experimental challenge; [b]Depends on season

maintenance metabolism to resisting the parasitism, i.e. increasing phagocytic activity and repairing tissue damage (Crosby & Roberts 1990). Ford & Smolowitz (2007) observed negative and positive relations between growth of *C. virginica* and *P. marinus* infection intensity in 8% and 6% of the samples, respectively (Table 3). Paynter & Burreson (1991) reported that even very light infections in a population of oysters were sufficient to markedly slow the average GR. A reduction of *C. virginica* growth due to *P. marinus* has also been recorded at the population level (Andrews 1961, Burreson & Andrews 1991, Paynter & Burreson 1991, Newell et al. 1994, Paynter 1996, Dittman et al. 2001, Albright et al. 2007, Ford & Smolowitz 2007) (Table 3). Conversely, Cigarria & Fernandez (1998) did not note any effect of a *Perkinsus* sp. infection on the growth of *R. philippinarum,* although in that study, clams presented a very low prevalence (Table 3).

Other studies have documented the relationship between perkinsosis and CI. Conflicting results were reported according to the study and the studied species regarding the impact of *Perkinsus* spp. on CI of bivalves (Table 3). The oyster *Crassostrea virginica* infected by *Perkinsus marinus* mostly displayed a decline in CI according to the infection intensity (Ray 1952, Crosby & Roberts 1990, Gauthier et al. 1990, Paynter & Burreson 1991, Dittman 1993, Volety & Chu Fu-Lin 1994, Paynter 1996, Dittman et al. 2001, Ford & Smolowitz 2007) (Table 3). Villalba et al. (2000) and Rodríguez Moscoso et al. (2002) observed a decrease in clam CI with increasing infection intensity (Table 3). However, the effect could depend on the season (Dittman et al. 2001), and in other studies, no effect on CI has been noticed (Chu & La Peyre 1993, Chu et al. 1993) (Table 3). Leite et al. (2004), Choi et al. (2002), and Goggin (1996) did not find a significant relationship between *P. olseni* intensity and CI in *Ruditapes decussatus*, *R. philippinarum* and *Tridacna crocea* respectively, but Leite et al. (2004) noticed that the most heavily infected clams displayed the lowest CI (Table 3). Finally, CI is strongly related to the gonadal stage, but Casas & Villalba (2012) found no effect of *P. olseni* infection on gonadal index.

Our results suggested that at an average concentration of 10^5 and 10^6 parasites per gram of wet gills at Arcachon and Mundaka respectively, perkinsosis affected the physiological function of clams. Choi et al. (1989) calculated that 10^6 cells of *Perkinsus marinus* per gram of wet tissue in *Crassostrea virginica* may exceed the net production of the host oyster. Even if in some studies substantial mortalities were noticed when *P. marinus* burdens were less than 1×10^6 cells g^{-1} (Albright et al. 2007), it is accepted than 1×10^6 cells g^{-1} is the lethal body burden limit for the oyster *C. virginica* (Choi et al. 1989, La Peyre et al. 2003, Albright et al. 2007). To compare with our study, 10^6 cells g^{-1} in the total body of the Manila clam corresponds to 773 012 parasites g^{-1} in the gills. The difference in the lethal level between *C. virginica* and *Ruditapes philippinarum* could be due to the difference in the host or/and parasite species. The parasite species found in *R. decussatus* is more likely to be *P. olseni*, according to the presence of this parasite in carpet shell clams from the Atlantic coast of Spain (Villalba et al. 2005). However, Arzul et al. (2012) also observed *P. chesapeaki* in Manila clams of the Atlantic coast of Charente Maritime (France). La Peyre et al. (2008) demonstrated that *P. olseni* and *P. marinus* were different in terms of salinity and temperature tolerance as well as proliferation, but also in term of intrinsic metabolic activity. With a higher intrinsic metabolic activity, *P. olseni* would be expected to place a greater demand on the hosts' nutritional resource. The consequence of this greater nutritional demand, however, will likely depend on the extent of the demand and the capacity of the specific host species to tolerate or compensate for resource competition from the parasite (La Peyre et al. 2008). However, some studies on proteolytic enzymes produced by *Perkinsus* sp. revealed a lower virulence of *P. olseni* in *R. decussatus* than that of *P. marinus* in *C. virginica* (Casas et al. 2002 in Villalba et al. 2004). *R. philippinarum* may also be able to tolerate a higher level of infection than *C. virginica*.

In conclusion, *Perkinsus* spp. impacted the physiology of the clam during the course of the experiment, as highlighted by the significant and negative correlation between the GR and the parasite load. The effect of *Perkinsus* spp. on the host could vary according to the infection intensity level, to the bivalve and parasite species, as well as to the environmental conditions of the studied site.

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