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FEATURE ARTICLE

Recruitment of the Pacific oyster *Crassostrea gigas* in a shellfish-exploited Mediterranean lagoon: discovery, driving factors and a favorable environmental window

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ABSTRACT: In the context of increasing demand for environmental recovery, aquatic systems may face the challenge of evolving under oligotrophication. This is the case in Mediterranean lagoons, in particular the shellfish-farmed Thau lagoon in France, where we studied recruitment of the Pacific oyster *Crassostrea gigas*. Oyster spat and environmental parameters were monitored at several sampling sites for 3 yr (2012 to 2014) using an original method with a temporal overlap deployment of collectors to study pre- and post-settlement processes and to identify the best conditions for recruitment. Contrary to the 'no Pacific oyster reproduction' paradigm in Mediterranean lagoons, our study showed that recruitment of this introduced species is possible in the Thau lagoon at levels comparable to those in other traditional French breeding basins. We identified a favorable environmental window for recruitment characterized by high water temperature (>26.5°C) and high nanophytoplankton and *Chaetoceros* spp. abundances (>4.3×10^6 and 345 $\times 10^3$ cells l^{-1}, respectively). In these favorable conditions, we hypothesize that the ecosystem functions as an autotrophic system, in contrast to the heterotrophic system that characterizes unfavorable conditions. Under heterotrophic conditions, high abundances of mixotrophic and heterotrophic organisms (ciliates and dinoflagellates) limited the metamorphosis of *C. gigas* larvae, leading to poor recruitment. This study provides new knowledge on the reproduction of the Pacific oyster in a Mediterranean lagoon under warming and oligotrophication. The shellfish industry will profit from the discovery of spatfields to develop new nursery practices that are eco-friendly and limit risks of transfers with other spatfall areas.

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Discovery of oyster spat recruitment in the French Mediterranean Thau lagoon.

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KEY WORDS: *Crassostrea gigas* · Oyster spat · Pediveliger · Metamorphosis · Recruitment · Oligotrophication · Larval ecology

INTRODUCTION

Ecosystems change over time under the constraints of combined global warming and anthropogenic impacts. More visible than global warming, marked local effects due to coastal urban development and/or pollution lead to environmental changes that seriously affect ecological processes. Hence, the increase

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in nutrient intakes in recent decades has led to the eutrophication of aquatic ecosystems (Nixon 1995, De Jonge & Elliott 2001, Smith & Schindler 2009). In the context of increasing demand for the recovery of ecosystem services (Bullock et al. 2011) and good environmental status (Vethaak et al. 2017), these ecosystems now face the challenge of evolving under oligotrophication (Jeppesen et al. 1998, 2005, Duarte et al. 2009, Yanagi 2015).

Oligotrophication (Cloern 2001, Boesch 2002) caused by ecosystem and wastewater management (Leruste et al. 2016) and increasing temperature (Collos et al. 2009) are 2 major trends that have been observed in Mediterranean lagoons. Oligotrophication causes a shift in the structure of the phytoplankton community from diatoms, cryptophytes and green algae to mixotrophic dinophytes (Leruste et al. 2016, Gowen et al. 2015). The combination of global warming and reduced nutrient loads may lower phytoplankton biomass (Collos et al. 2009, Lie et al. 2011, Saeck et al. 2013). This reduction may affect the breeding stocks of bivalve suspension feeders in shellfish basins (Dame 2011), possibly jeopardizing their sustainability.

The Pacific oyster *Crassostrea gigas* is one of the most economically important invertebrate species. According to FAO Fishery Statistics, the global production of this species was estimated to have reached 4.4 million t by 2003 and its global aquaculture production to be 625 925 t in 2014 (http://www.fao.org/fishery/culturedspecies/Crassostrea_gigas/en). This oyster species was introduced into France in 1970 for social and economic reasons (Grizel & Héral 1991) and was highly productive from the 1980s onwards in both the Atlantic and Mediterranean (Héral & Deslous-Paoli 1991, Deslous-Paoli et al. 1993). About 10% of French oyster production comes from the Mediterranean Thau lagoon (Robert et al. 2013), located in southern France.

C. gigas has been widely studied all over the world, and its life history (reproduction, larval cycle and recruitment phase) has been relatively well described in both controlled environments (Fabioux et al. 2005, Enriquez-Diaz et al. 2009, Rico-Villa et al. 2010) and coastal tidal ecosystems (Dutertre et al. 2010, Thomas et al. 2016, Bernard et al. 2016). Fewer studies have been conducted in nanotidal environments such as Mediterranean lagoons (Tagliapietra & Ghirardini 2006). The gametogenesis and spawning behavior of *C. gigas* were only recently explored in the Thau lagoon by Ubertini et al. (2017). According to these authors, the spawning behavior of *C. gigas* within Thau lagoon appeared to be slightly different from that on the Atlantic coast, with several spawning events occurring from June to October. The minimal temperature observed for spawning was 23°C, which is much higher than the temperature mentioned in the literature for this species. Additionally, a strong relationship was found between phytoplankton communities and gametogenesis, the latter being improved by a higher diatom/dinoflagellate ratio.

The Mediterranean oyster industry is based on spat supplied from Atlantic nursery basins (mainly Arcachon and Marennes-Oléron) and hatcheries (Buestel et al. 2009). The massive spat mortality observed in France since 2008 (Pernet et al. 2010, 2012, 2014) emphasized the dependence of the Mediterranean industry on outside sources, highlighting the need for a native supply. However, reproduction of *C. gigas* is believed to be impossible (Debos et al. 1972, Drullion 2002) or irregular (Goulletquer 1995) in the Thau lagoon, possibly due to unfavorable hydrological conditions (e.g. temperature, salinity, insufficient food and high concentrations of antifouling paint) (Deslous-Paoli et al. 1982, His & Robert 1985, His et al. 1986).

A limited number of studies on marine invertebrates have suggested that recruitment in the Thau lagoon is highly spatially heterogeneous, potentially related to plankton depletion due to intensive shellfish culture (Lam-Hoai et al. 1997, Souchu et al. 2001) and hydrodynamic circulation (Borsa & Millet 1992). However, recruitment information on *C. gigas* in the Thau lagoon remains limited, and the aim of the present work was to fill this gap.

To this end, several larval and oyster spat monitoring sites were created at different locations in the Thau lagoon to precisely monitor both pelagic larval phases and benthic settlement of *C. gigas* over a period of 3 yr. The general hypothesis of this study is that the 'no-recruitment' paradigm in Mediterranean lagoons is false, and that lagoon ecology offers recruitment windows, in the general concept of several conceptual frameworks such as supply-side ecology (Grosberg & Levitan 1992), match–mismatch (Cushing 1990), transport and retention (Bishop et al. 2006). Our objectives were to (1) identify patterns of oyster recruitment in space and over time, (2) characterize variations within different pelagic and benthic larval stages and (3) explore the effects of environmental factors on larval development and recruitment success. Using a correlative approach, we paid particular attention to the food sources known to play a major role in the development of bivalve mollusk larvae (His et al. 1989, His & Seaman 1992).

MATERIALS AND METHODS

Study site

The Thau lagoon is the largest nanotidal lagoon in the Occitanian region in southern France (Fig. 1). It covers an area of 7500 ha (19 × 4.5 km) on a northeast–southwest axis and has a mean depth of 3.5 m. Seawater inputs from the Mediterranean Sea enter by artificial channels. Four spatfall sites (Fig. 1) were monitored to assess pre-settled oyster larvae and post-settled spat abundances in pelagic and benthic habitats: 3 inside shellfish-farming zones (Bouzigues, Meze and Marseillan) and one outside (Listel).

Larval and spat abundances

Crassostrea gigas pelagic and benthic larval abundances were assessed from June to September in 2012, 2013 and 2014. Pelagic larvae assessments were carried out twice a week using a standard protocol provided by the National Larval Network (Pouvreau et al. 2013, 2016) where the size of 'D-larvae' varies between 60 and 100 µm and 'large umbo larvae' between 180 and 300 µm. A sampling volume of 1.5 m^3 was pumped and filtered through a 40 µm plankton net.

Benthic oyster abundances were estimated every 2 wk at 3 different settler stages: pre-settled larvae, young postlarvae and newly-settled spat (Arakawa 1990). An original method with a temporal overlap of collector deployment was used (Hughes et al. 2000, Arnold & Steneck 2011). Pediveligers were observed on the collectors' plates, with prodissochonch 2 shell size ranging from 180 to 300 µm. This pediveliger stage precedes metamorphosis, a vulnerable phase of their life cycle (Coon et al. 1990, Pechenik 2006). The main physiological transformations of metamorphosis are complex (Bishop et al. 2006), basically converting the larval body plan into an adult body plan (Wray 1995). In bivalves, the evolution of important organs such as gills during metamorphosis remarkably affects the feeding mechanism (Veniot et al. 2003, Cannuel & Beninger 2007, Cannuel et al. 2009). Metamorphosis is defined as the transformation of pediveliger into postlarvae. Postlarvae are strictly benthic and cemented with a dissoconch shell. Their size ranges from 300 to 1000 µm. Oyster spat follow postlarvae at 4 wk of age (maximum), with a size range from 1 to 8 mm. To collect these benthic stages, the sites were equipped with 3 replicated sets of 2 collectors (Fig. 2). Each collector was composed of 44 white plates (15 cm diameter; 250 cm^2), measuring 110 cm (Fig. 2). Collectors were vertically submerged 2 m below the surface, suspended under shellfish farming structures, or outside farming structures on specially designed mooring systems (Fig. 2a). The mooring systems were positioned so that the top of the uppermost collector was 2.5 m below the surface. The systems were designed to support 2 sets of collectors immersed for 4 wk at 2 wk intervals. Pediveliger and postlarvae abundance was assessed on the 2-wk collector and oyster spat abundance was assessed on the 4-wk collector. The 4-wk collector was replaced; therefore in the system 1 collector was replaced every 2 wk throughout the summer.

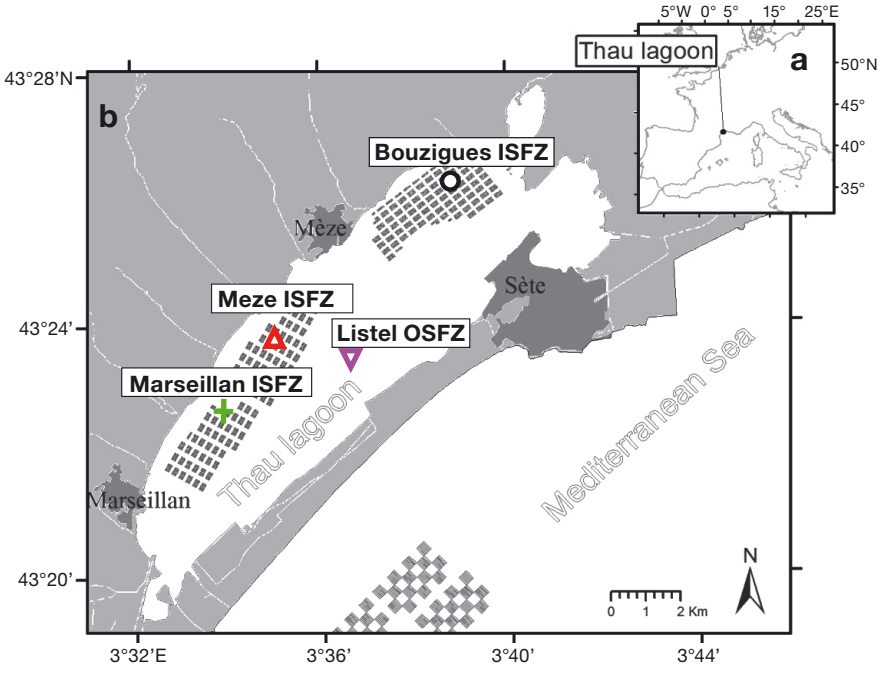

Fig. 1. (a) The Mediterranean Thau lagoon in France and (b) sampling sites within the Thau lagoon. Labelled are the 4 sampling sites (Marseillan, green cross; Meze, red triangle; Bouzigues, black open circle; and Listel, purple triangle) where pelagic and benthic Pacific oyster larvae, spat abundances, hydrological and plankton data were monitored. ISFZ: Inside the shellfish farmed zone (monitoring took place under farmed structures); OSFZ: outside the shellfish farmed zone (monitoring took place at specially designed mooring systems, see Fig. 2a). Grey boxes: shellfish farms; dark grey areas: towns

Each collector was sampled at 3 vertical levels: close to the top (39th plate), middle (22th plate) and bottom (5th plate). Both sides of the plates

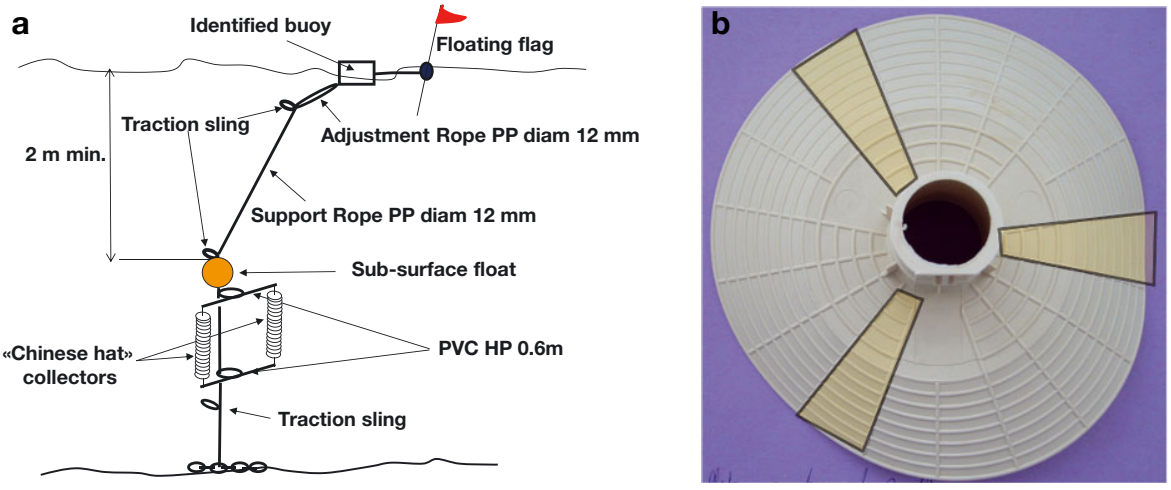

Fig. 2. (a) Mooring system used OSFZ in the Thau lagoon. The gear supported a set of 2 'Chinese hat' collectors with plates. The first collector was immersed for 2 wk and the second for 4 wk before being replaced by a new collector. The sampling period lasted from June to October. (b) Top view of a collector plate with counting subunits in yellow, in this case: 14 cm^2

(above and below) were examined under a binocular microscope to assess the mean abundance of pediveligers, postlarvae and newly settled spat, or macroscopically when possible. On each occasion, counting was carried out on subunits, i.e. from 1 to 4 basic subunits (14 to 56 cm^2), replicated 3 times per side (Fig. 2b), for a total of 54 counts by date and site (3 collectors × 3 levels × 2 sides × 3 subunits). The abundance per plate was averaged from subunit counts and converted into total individual abundances per plate.

Pediveliger and spat abundances were classified in 5 categories according to a recruitment scale proposed by Pouvreau et al. (2013) for the French coast: zero (0 ind. $plate^{-1}$), low (1 to 20 ind. $plate^{-1}$), medium (21 to 200 ind. $plate^{-1}$), high (201 to 2000 ind. $plate^{-1}$) and overabundant (>2000 ind. $plate^{-1}$). An additional qualitative recruitment factor was created to compare pediveliger (Pedi) and spat (Spat) abundances (Fig. 3). The recruitment factor terms 'Pedi–' and 'Spat–' summed the abundances from 0 to 20 ind. $plate^{-1}$, and Pedi+ and Spat+ summed the abundances >20 ind. $plate^{-1}$. The recruitment factor combined terms to define 3 conditions: Pedi– indicated low pediveliger supplies, Pedi+Spat– indicated metamorphosis failure and Pedi+Spat+ indicated successful metamorphosis.

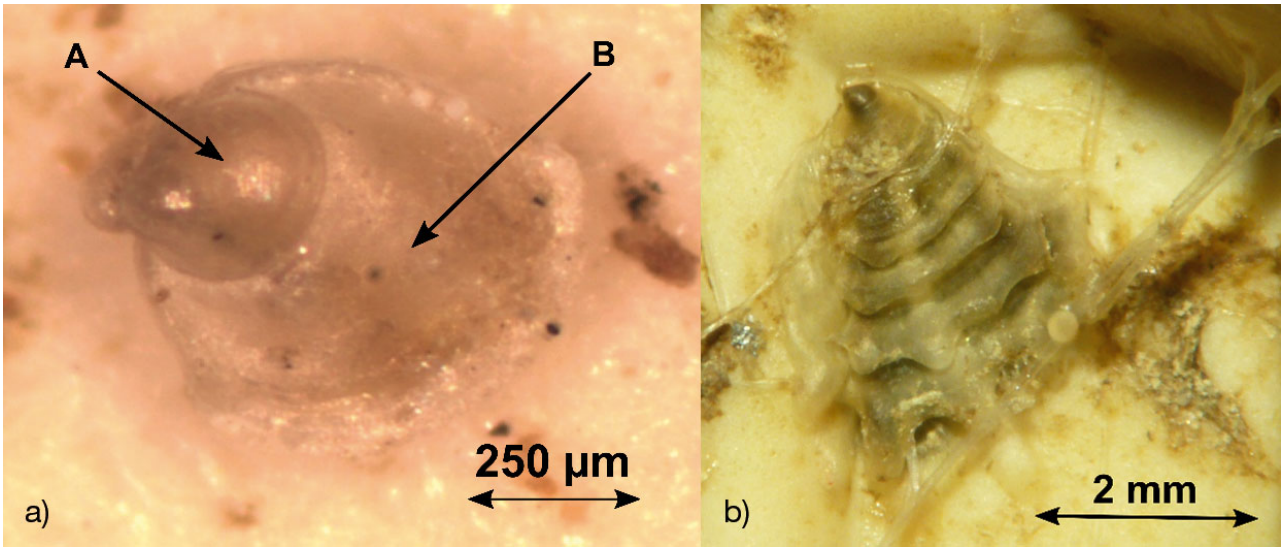

Fig. 3. Settled Pacific oyster larvae on a collector plate. (a) Shell of pre-settled larvae (pediveliger; A) and shell of young postlarvae after metamorphosis (B) on collectors immersed for 2 wk; and (b) newly-settled spat on a collector immersed for 4 wk

Environmental measurements

Environmental parameters (hydrological and plankton samples) were recorded every Monday morning from first of June to the end of September in 2012, 2013 and 2014. Samples were taken on spatfall sites at Listel and Mèze. Bouzigues and Marseillan spatfall sites were located less than 1 km from where environmental measures were monitored (data from sampling sites for the REPHY dataset — French Observation and Monitoring program for Phytoplankton and Hydrology in coastal waters (2017) — were used in this study). For Bouzigues and Marseillan, hydrological and plankton samples associated with spatfall sampling sites were in biocoenosis, characterized by the same phytoplankton, zooplankton and benthic populations (Jarry et al. 1990, Jouffre et al. 1991, Guelorget et al. 1994).

Hydrological monitoring

Temperature and salinity were measured twice a week and weekly averaged with WTW® probes positioned between 1 and 1.5 m below the surface. Oxygen concentrations were measured once a week at the Bouzigues site at the bottom of the water column. Data were collected from June to September in 2012, 2013 and 2014.

Plankton monitoring

Our sampling strategy provided 135 observations: 3 sites × 15 wk × 3 yr. Each phytoplankton and protozooplankton sample was collected by sampling site and date using subsamples from a 4 l sample. Samples were collected weekly using a Standard Water Sampler acc. to Ruttner (Hydro-Bios) at 3 sampling sites yr^{-1}. No analytical replicates or sampling replicates were used. The Bouzigues, Meze and Marseillan sites were sampled in 2012, while the Bouzigues, Listel and Marseillan sites were sampled in 2013 and 2014.

For total chlorophyll *a* (total_chloa) measurements, seawater samples (200 ml) were filtered (Bec et al. 2005, 2011) under vacuum (<10 cm Hg) on Whatman GF/F membranes (0.7 µm porosity) and stored in glass tubes at –20°C. Filters were ground in acetone (90%) and extracted for 24 h at 4°C in the dark. Chl *a* biomass was also determined after size fractioning (200 ml for picophytoplankton and 200 ml for nanophytoplankton from the 4 l samples) through Nuclepore membranes (3 and 20 µm) to determine the contribution of picophytoplankton (<3 µm), nanophytoplankton (3 to 20 µm) and microphytoplankton (>20 µm) to total phytoplankton biomass. The pigment content (µg chl *a* l^{-1}) was measured using a spectrofluorometer (Perkin-Elmer LS50b) (Neveux & Lantoine 1993).

Abundances of picocyanobacteria (<1 µm), autotrophic picoeukaryotes (<3 µm) and nanophytoplankton (3 to 20 µm) were determined on the basis of a sampled volume of 1 ml with a Becton Dickinson FACSCalibur flow cytometer (Bec et al. 2011). Total picophytoplankton abundances were estimated as the sum of the picocyanobacteria and picoeukaryote abundances. Among nanophytoplankton, cryptophytes were distinguishable from other photosynthetic organisms by their strong orange fluorescence and their size. Abundances were expressed in 10^6 cells l^{-1}.

To measure bacterial abundance, samples (1 ml from the 4 l samples) were fixed with prefiltered (0.2 µm) buffered formaldehyde (2% final concentration) and stored in liquid nitrogen. Abundances were determined by a FACSCalibur flow cytometer Becton Dickinson method (Marie et al. 1997). The procedure was slightly modified as higher concentrations of fluorochrome (SYBR Green I) were used (Bouvy et al. 2016). A total of 1 ml of fixed sample was incubated with SYBR Green I (Molecular Probes) at a final concentration of 1/375 for 15 min at 4°C in the dark. Stained bacterial cells excited at 488 nm were determined according to their side-scattered light and green fluorescence collected using a 530/30 nm filter. Fluorescent beads (0.94 µm; Polysciences) were added to each sample.

Protozooplankton (heterotrophic flagellate, 30 ml sample and ciliates, 100 ml sample) and total phytoplankton flora (10 ml sample) were estimated using the standard Utermöhl method NF-EN-152014, 2006. For the total phytoplankton flora, 2 main classes ('diatoms', aggregating 52 taxa and 'dinoflagellates', aggregating 38 taxa) were first used to explore the dataset. In addition, we paid particular attention to the diatom genus *Chaetocoeros* spp. because of its high abundance and its known role in the biological cycle of oyster recruitment (Rico-Villa et al. 2006, Blanchard et al. 2008, Ben Kheder et al. 2010). Protozooplankton and total flora were expressed as the number of individuals per liter. Total phytoplankton flora were not monitored in 2012 at the Meze sampling site.

For analysis of the heterotrophic flagellate, the 30 ml samples were preserved in an 8% formaldehyde solution and stored in a cold room at 4°C in the dark until analysis. A 10 ml subsample was stained using

4′,6-diamidino-2-phenylindole (DAPI) at a final concentration of 2.5 µg ml^{-1}. The heterotrophic flagellate counts were performed using an epifluorescence microscope (Olympus AX70) with UV illumination (Sherr et al. 1993). For the naked ciliate and tintinnid counts and analyses, 100 ml samples were preserved in 2% Lugol's iodine solution and kept in a cold room at 4°C in the dark until analysis. Naked ciliates and tintinnids were identified, measured and counted with an inverted microscope (Olympus IX70) after a 100 ml sample was left to settle in an Utermöhl chamber for 24 h (Utermöhl 1931).

Mesozooplankton were sampled using subsurface horizontal net tows. AWP2 net type with a mesh size of 80 µm and an opening diameter of 50 cm was used. In general, this plankton net was towed at an average speed of 3 km h^{-1} for 2 to 3 min. The volumes of water filtered by the file averaged 20 to 30 m^3 and were calculated precisely for each trawl, taking into account the speed of the boat and the time of immersion of the net. Once the net had been brought back on board, the contents of the collector were sieved over 80 µm to concentrate the sample and then transferred to a 250 ml sample bottle fixed with 4% stabilized formaldehyde. The samples were then stored at room temperature until analysis (diversity and abundance). Abundances of mesozooplankton considered as potential predators and trophic competitors of *Crassostrea gigas* larvae were estimated using a binocular microscope with taxonomic identification (Rose 1933). The 'trophic competitors' group was determined as the sum of copepod nauplii, annelids and barnacle larvae, ascidia and gastropod larvae. 'Potential predators' were assessed as the sum of cladocerans (*Penilia avirostris*, *Podon* spp., and *Evadne* spp.), decapod larvae, mysids and hydrozoa (*Obelia* spp.). Mesozooplankton are expressed as individuals per cubic meter.

Data analysis

All data analyses were performed with R statistical software (R Core Team 2015). Decimal logarithm and square-root transformations were used to tend towards linearity of response variables and to linearize the relationship with explanatory variables (Table 1).

An ANOVA was performed to test the effect of year and sampling site on the recruitment of observed spat abundances with Power Box-Cox transformation (lambda = −0.63). Normality and heteroscedasticity of residuals were checked by visual inspection. Oyster spat recruitment was graphically described using comparison of means with 95% confidence intervals.

The relationships between abundances of larvae at different stages (i.e. small, large, pediveliger, postlarvae and newly settled spat) were studied using a series of non-parametric Spearman correlation tests and linear models. In addition, we used the ratio of pediveliger to spat abundance to quantify successful metamorphosis. The Listel sampling site was excluded from the D-larvae analysis because it is located outside the geographic location of shellfish farming, thus inducing very few small larvae supplies.

To study the influence of environmental (hydrological and planktonic) variables on the different life stages, we integrated the data over periods that matched the time scale of the process under study. Hence, to characterize the environment of the pelagic larval and settling phases, we averaged, respectively, the environmental variables over 2 periods: a 22 d period before retrieval of the collectors (suffix variable LARV for larval phase) and over a 14 d period preceding retrieval of the collectors to characterize the environment during metamorphosis (suffix variable MET for metamorphosis). Small and large pelagic larvae abundances are represented with their maxima in both the LARV and MET periods.

A decision tree method was used to explore the links between target variables (abundance of pediveliger, abundance of spat and survival after metamorphosis) and environmental variables, with the package 'party' provided by the CRAN-R Project, with conditional inference trees ('ctree') (Hothorn et al. 2006a,b). As only the first splitting path of the decision trees was significant, we split the dataset in 2 and performed simple graphical and statistical tests such as boxplots to observe the quantitative effects of significant variables on the dataset.

A principal component analysis (PCA), based on plankton data with recruitment factor representation, is presented to explore and illustrate interactions between the pediveliger and spat classes. Kruskal-Wallis tests were used to assess significant environmental factors on the classes Pedi−, Pedi+, Spat−, Spat+ and significant favorable or unfavorable parameters of the recruitment windows to identify the optimum recruitment windows.

RESULTS

Remarkable abundance of Pacific oyster spat were observed each year at each of the 4 sites sampled in the Thau lagoon (Fig. 4). Over the 3 yr, we assessed 12 events of medium spat recruitment (21 to 200 ind. $plate^{-1}$), 19 events of low recruitment (1 to 20 ind.

Table 1. Variables characterizing the interactions between the environment and Pacific oyster larvae. Each environmental variable was averaged over a 22 d period preceding retrieval of the collectors and over a 2 wk period preceding the retrieval of the collectors to characterize the environment for metamorphosis

Variables	Description	Units	Transformation	Abbreviation
Target variables				
Oyster spat	Abundance	ind. plate^{-1}	$\log_{10}(x + 1)$ or power Box-Cox (lambda = −0.63)	log_spat or pbc_spat
Postlarvae	Abundance	ind. plate^{-1}	$\log_{10}(x+1)$	log_post
Pediveligers	Abundance	ind. plate^{-1}	$\log_{10}(x+1)$	log_pedi
Environmental variables				
Max. small larvae	Maximum small pelagic larvae abundance	ind. m^{-3}	$\log_{10}(x+1)$	log_max_SL
Max. large larvae	Maximum large pelagic larvae abundance	ind. m^{-3}	$\log_{10}(x+1)$	log_max_LL
Pediveliger/spat survival	Ratio of pediveliger abundance on oyster spat	–	–	–
Oxygen concentration	Daily average	mg l^{-1}	–	–
Temperature	Daily average	°C	–	–
Salinity	Daily average	No units	–	–
Bacteria	Abundance	10^6 cells l^{-1}	–	log_bact
Total picoeukaryotes	Abundance	10^6 cells l^{-1}	square root(x)	sqrt_peuk_tot
Total cyanophycae	Abundance	10^6 cells l^{-1}	square root(x)	sqrt_cyan
Picoeukaryotes + cyanophycae	Abundance	10^6 cells l^{-1}	$\log_{10}(x + 1)$	log_pico_tot
Nanophytoplankton	Abundance	10^6 cells l^{-1}	$\log_{10}(x + 1)$	log_nano
Cryptophycae	Abundance	10^6 cells l^{-1}	$\log_{10}(x + 1)$	log_crypto
Nanophytoplankton + cryptophycae	Abundance	10^6 cells l^{-1}	$\log_{10}(x + 1)$	log_nano_tot
Heterotrophic flagellates	Abundance	cells l^{-1}	$\log_{10}(x + 1)$	log_HF
Ciliates	Abundance	cells l^{-1}	$\log_{10}(x + 1)$	log_ciliates
Tintinnidae	Abundance	cells l^{-1}	$\log_{10}(x + 1)$	log_tinti
Diatom	Abundance	cells l^{-1}	$\log_{10}(x + 1)$	log_diatom
Dinoflagellates	Abundance	cells l^{-1}	$\log_{10}(x + 1)$	log_dinoflagellates
Chaetoceros spp.	Abundance	cells l^{-1}	$\log_{10}(x + 1)$	log_chaetoceros
Total chlorophyll *a*	Biomass	µg chl *a* l^{-1}	–	total_chloa
Picophytoplankton	Biomass	µg chl *a* l^{-1}	–	pico
Nanophytoplankton	Biomass	µg chl *a* l^{-1}	–	nano_3_20
Picophytoplankton + nanophytoplankton	Biomass	µg chl *a* l^{-1}	–	nano_low20
Microphytoplankton >20 µm	Biomass	µg l^{-1}	–	micro
Competitors	Abundance	ind. m^{-3}	$\log_{10}(x + 1)$	log_comp
Predators	Abundance	ind. m^{-3}	$\log_{10}(x + 1)$	log_pred

plate^{-1}) and 65 events of null recruitment. The analysis was performed using the whole dataset over the 3 yr period but spat abundances did not differ significantly among years (Table 2). In contrast, the site factor had a significant effect on oyster spat abundances (pbc_spat, lambda = −0.63, $p < 0.05$) (Table 2).

In 2012, 2 medium intensity spatfall events occurred, the first in mid-August, with 126 and 47 ind. plate^{-1} at the Listel and Bouzigues sites, respectively, and the second at the end of September with 45 and 21 ind. plate^{-1} at the Bouzigues and Listel sites, respectively (Fig. 4). In 2013, there was a single medium spatfall event lasting from the middle to the end of August at all 4 sites, with maximum values of 188, 187, 91 and 49 ind. plate^{-1} at Bouzigues, Marseillan, Listel and Meze, respectively. In 2014, a medium spatfall event occurred at Listel only, but lasted longer, from the end of August to the end of September. To summarize, medium spatfall events were observed each year at Listel, in 2 yr at Bouzigues and only in 1 yr at Marseillan and Meze. It is interesting to note that medium spat recruitment occurred any time from August to September, but never in July or October.

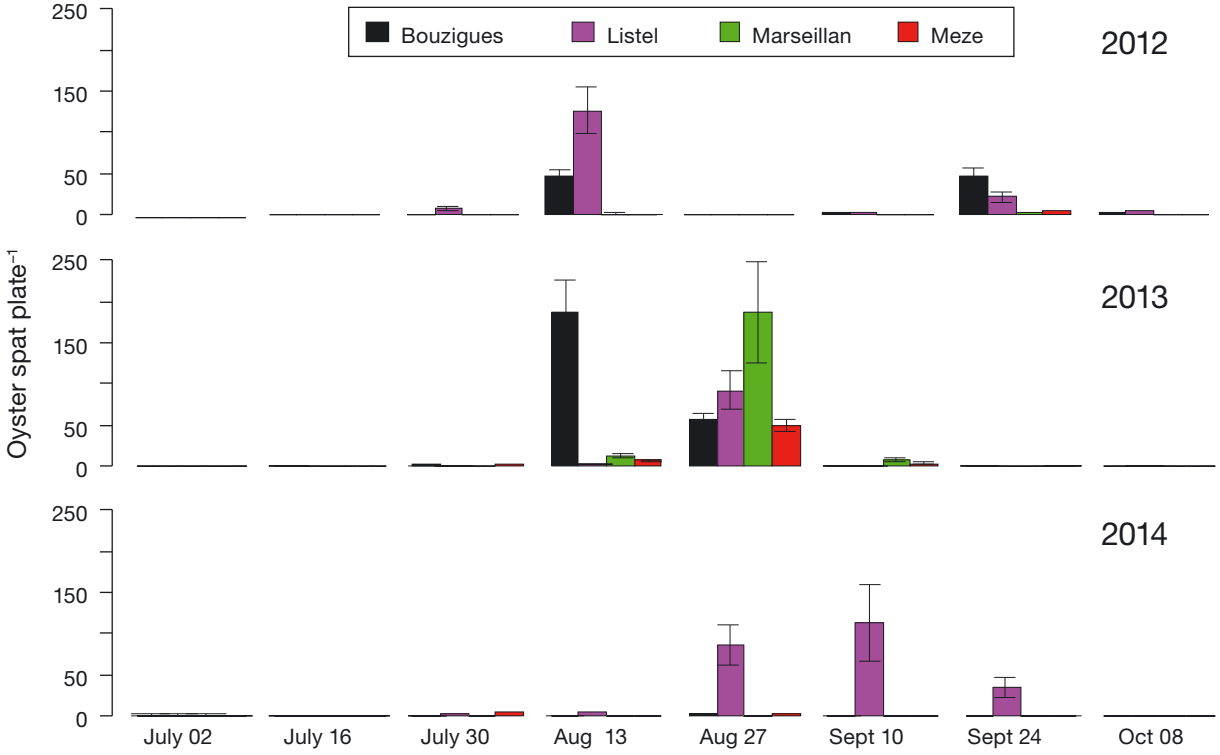

Fig. 4. Mean (±95% CI) Pacific oyster spat abundance per plate observed at the 4 spatfall sampling sites (Bouzigues, Listel, Marseillan and Meze) at 2 wk intervals throughout the summer (2012, 2013, 2014). Spat abundances were estimated after 4 wk of immersion (n = 54 per date and sampling site)

Table 2. ANOVA examining the effect year and site factors on Pacific oyster spat abundance per plate (power Box-Cox transformation; lambda = −0.63, n = 96). Significant values in bold ($p < 0.05$)

	df	SS	MS	*F*	Pr(>F)
Site	3	2.528	0.8425	3.353	**0.0227**
Year	2	0.080	0.0400	0.159	0.8529
Site × year	6	3.210	0.5351	2.130	0.0582
Residuals	84	21.105	0.2512		

Significant correlations were found between the abundance of small D-larvae and the abundance of pediveligers, specifically in the shellfish-farmed area (Fig. 5a; n = 75, ρ = 0.44, p < 0. 001). At the lagoon scale, a significant correlation was found between large larvae and pediveligers (Fig. 5b; n = 100, ρ = 0.45, p < 0.001). The relationship between large larvae and pediveliger abundance, assessed using a linear model, was strong (log_pedi = 0.864 log_max_LL).

Pediveliger and postlarvae abundances were on average significantly correlated (Fig. 5c; n = 100, ρ = 0.47, R^2 = 31%, p < 0.001) and the relationship between pediveligers and postlarvae (log_postlarvae = 0.41 log_pedi) was successful or ranged from low to zero (failure of metamorphosis). Among 69 harvest events with less than 2 spats (Spat–), 26 (38%) had a relatively high number of pediveligers 2 wk before (>20 ind. plate^{-1}, i.e. Pedi+) whereas 43 (62%) did not (<20 ind. plate^{-1}, i.e. Pedi–).

Oyster spat abundances were highly correlated with postlarvae abundances (Fig. 5d; n = 96, ρ = 0.69, R^2 = 65%, p < 0.001). The linear model between postlarvae and oyster spat was, on average, defined as log_spat = 0.59 log_post_larvae.

Concerning environmental drivers and based on preliminary analyses of the decision trees, we found that (1) spat abundance was significantly higher (p = 0.034) when the temperature (throughout the larval pelagic period; LARV) was above 26.5°C (Fig. 6a); (2) the abundance of pediveligers was significantly higher (p = 0.012) above a threshold value of *Chaetoceros* abundance (~345 × 10^3 cells l^{-1}; Fig. 6b) during the larval cycle; and (3) the success of metamorphosis survival was significantly higher (p = 0.014) with high nanophytoplankton biomass (throughout the MET period, >4.3 × 10^6 cells l^{-1}; Fig. 6c).

A PCA of the environmental dataset with the recruitment factor (categorized as Pedi–, Pedi–Spat– and Pedi+Spat+) showed that the 2 first axes ex-

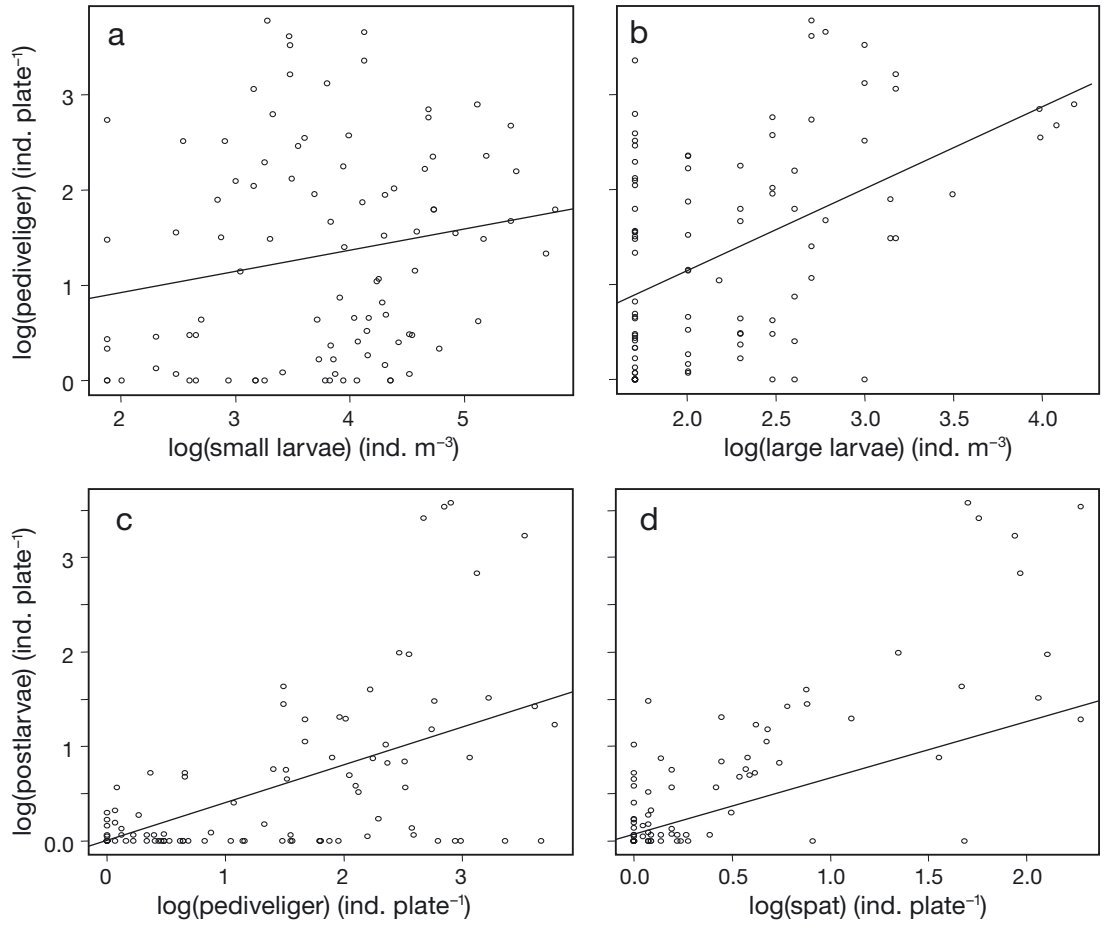

Fig. 5. Relationships between Pacific oyster pediveliger and (a) small larvae, (b) large larvae and postlarvae and (c) pediveliger or (d) spat abundances for the years 2012, 2013 and 2014

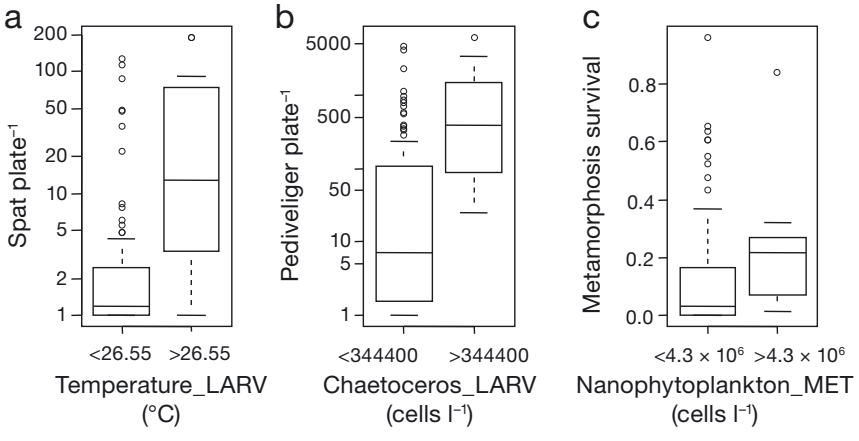

Fig. 6. Environmental and planktonic variables that have a significant effect on pre- and post-settled stages of Pacific oyster: (a) temperature averaged over the larval period (Temperature_LARV) on spat abundance; (b) *Chaetoceros* abundance averaged over the larval period (Chaetoceros_LARV) on the abundance of pediveligers; and (c) nanophytoplankton abundance averaged over the metamorphosis period (Nanophytoplankton_MET) on metamorphosis survival. Mid-line: median; box: 25th and 75th percentiles; whiskers: 1.5× the interquartile range; circles: outliers.

plained 49.4 % of the total variance of our dataset (Fig. 7). The first axis was built with the contributions of nanophytoplankton biomass (nano_low20^{B}, nano_3_20^{B}) and abundances (log_nano^{A}, log_nanotot^{A}) and total_chloa biomass (total_chloa^{B}). This first axis can be seen as a temporal gradient representative of fauna, flora and hydrological successions (Fig. 7). The second axis separated picophytoplankton abundance (sqrt_peuk_tot^{A}, log_pico^{A}), biomass (pico^{B}) and cryptophyte abundance (log_crypto^{A}) from microphytoplankton >20 µm biomass (micro^{B}) and *Chaetoceros* abundance (log_chaetoceros^{A}). On this axis, ciliate and dinoflagellate abundances are also opposed to picoeukaryote abundances, heterotrophic flagellate abundance

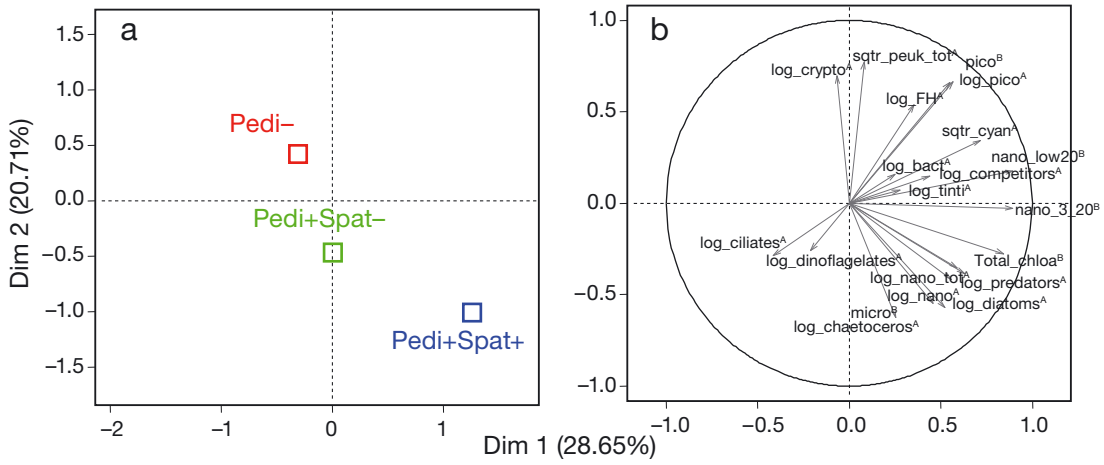

Fig. 7. Principal component analysis plots of (a) recruitment factors and (b) correlation circle of environmental transformed data integrated over a 22 d period before retrieval of the collector (superscript A: abundance; B: biomass). Recruitment factor categorizes Pacific oyster pediveliger and spat abundances 'Pedi–' and 'Spat–' by aggregating the abundances from 0 to 20 ind. plate^{-1}, and 'Pedi+' and 'Spat+' by aggregating abundances with >20 ind. plate^{-1}

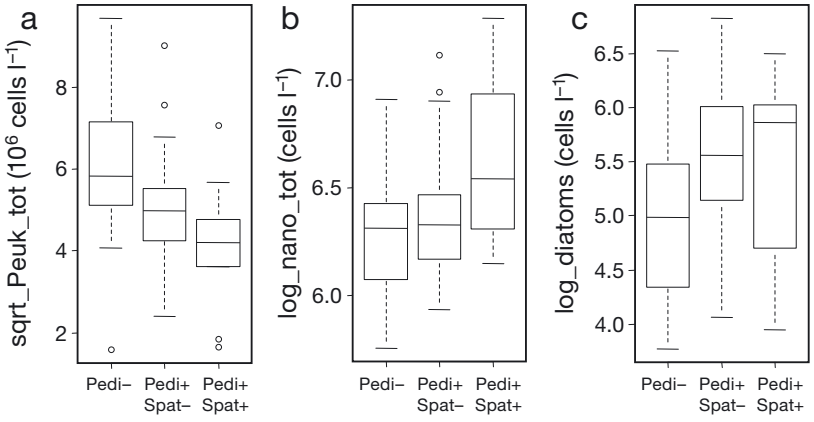

Fig. 8. Plankton variables that had a significant effect on Pacific oyster recruitment. The values on the *y*-axis are (a) the square root values of total picoeukaryote abundance and (b,c) $\log_{10}$ values of total nanophytoplankton and diatom abundances (n_{Pedi-} = 37, $n_{Pedi+Spat-}$ = 26 and $n_{Pedi+Spat+}$ = 9), respectively. Mid-line: median; box: 25th and 75th percentiles; whiskers: 1.5× the interquartile range; circles: outliers. See Fig. 7 and Table 1 for definitions

and picophytoplankton biomass. Their contribution to the building of the axis was, however, low. The second axis can be seen as a spatial gradient reflecting the effect of lagoon confinement and the effect of the shellfish biocoenosis on hydrobiology.

The barycenters of our recruitment factor (Pedi–, Pedi–Spat–, Pedi+Spat+) occurrences are well differentiated on the first PCA axis (Fig. 7a). Pediveliger abundance (Pedi–, Pedi+) was mostly correlated with the second axis, with positive effects of *Chaetoceros* abundance, microphytoplankton and nanophytoplankton, and negative effects of picophytoplankton (abundance and biomass) and cryptophyte abundance (Fig. 7b). The success of metamorphosis (i.e. the Spat– and Spat+ terms) was mostly differentiated on the first axis, with positive effects of nanophytoplankton biomass.

Non-parametric analyses (Kruskal-Wallis test) of the whole dataset distinguished 7 variables with significant effects on recruitment ($p < 0.05$). All 7 variables were integrated over the 3 wk pelagic larval phase (i.e. LARV).

High pediveliger abundance and/or successful metamorphosis were observed when picoeukaryote abundance was low (Fig. 8a), or when total nanophytoplankton levels (Fig. 8b) or diatom levels (Fig. 8c) were high. A total of 79% percent of the variability of diatom abundances ($p < 0.001$) was explained by *Chaetoceros* abundances. The effect of microphytoplankton abundance on recruitment (data not shown) was similar to the effect of diatoms.

Concerning hydrological variables, we found no effect on pediveliger supply but a significant effect of oxygen concentrations and temperature on recruitment, with more Pedi+Spat+ occurrence at lower oxygen concentrations (Fig. 9a) and higher temperatures (Fig. 9b).

The changes in recruitment over time (aggregated over the 3 sampling years) in each of the 3 recruitment categories (Pedi–, Pedi–Spat+, Pedi+Spat+) defined above suggested a strong summer pattern independ-

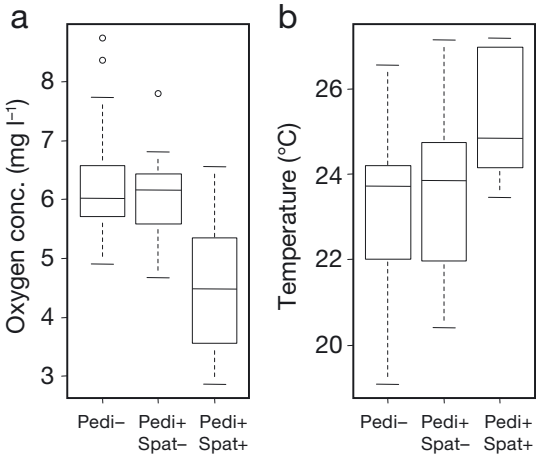

Fig. 9. Environmental variables that had a significant effect on Pacific oyster recruitment according to oxygen concentration ($n_{Pedi-Spat-}$ = 27, $n_{Pedi-Spat+}$ = 18, $n_{Pedi+Spat+}$ = 6) and temperature ($n_{Pedi-Spat-}$ = 46, $n_{Pedi-Spat+}$ = 38, $n_{Pedi+Spat+}$ = 12). See Figs. 7 & 8 for definitions

ent of the year (Table 3): no Pedi+Spat+ occurrences were observed in Weeks 24 to 29 (June and July) whereas Weeks 31 to 37 (August and September) saw the occurrence of Pedi+Spat+. Conditions were unfavorable at the end of the season (after Week 38), with no Pedi+Spat+ occurrence.

Kruskal-Wallis tests based on these new categories (favorable vs. unfavorable windows) revealed a significant difference ($p < 0.05$) for 13 of the 46 environmental variables: the 3 hydrological variables (temperature, oxygen and salinity) and the 10 transformed planktonic variables. Temporal windows that favor recruitment are associated with lower oxygen concentrations, higher temperatures and higher salinity, higher total_chloa, pico- and nanophytoplankton biomass, higher abundances of heterotrophic flagellates and trophic competitors, and lower abundances of ciliates and dinoflagellates (Fig. 10). The effects of unfavorable or favorable windows were similar for picocyanobacteria, picoeukaryote and picophytoplankton abundances. Only picophytoplankton results are presented here.

In unfavorable periods, ciliates were mainly represented by higher abundances of naked ciliates like Scuticociliates (1100 cells l^{-1} including Uronematidae, Philasteridae, Balanion), *Laboea* spp., *Favella erhenbergii* and tintinnid ciliates like *Eutintinus* spp. and *Tintinnopsis* spp. In the same period, dinoflagellates were represented by non-toxic species such as *Gyrodinium spirale* and *Prorocentrum micans* (both around 300 cells l^{-1}), and *Protoperidinium bipes* (around 40 cells l^{-1}).

DISCUSSION

Our general objective was to study recruitment of the Pacific oyster *Crassostrea gigas* by monitoring the different larval stages and exploring the effects of lagoon environmental conditions on successful larval development and recruitment. The present study showed that there was no statistical difference in spat abundance at an inter-annual scale, but there was strong temporal variability at an intra-annual scale, with clear favorable versus unfavorable windows. The unfavorable windows (June and July) showed good supplies of larvae (>20 ind. $plate^{-1}$) but metamorphosis failure, suggesting a possible biological obstacle at this step. Early summer months are usually characterized by rising temperatures, and increasing metabolism of poikilotherm filter feeders. Consequently, only small quantities of nano- and microplankton are available during that period since nano- and microplankton are mainly consumed by filter feeders (Dupuy et al. 2000). Indeed, the unfavorable recruitment windows in early summer were characterized by low pico-, nano- and microphytoplankton biomass. We therefore suggest that a top-down control of primary production by filter feeders explains the observed unfavorable recruitment period in Thau lagoon.

Picoplankton also support grazing by ciliate and flagellate protists (Lam-Hoai et al. 1997, Bec et al.

Table 3. Evolution of changes in recruitment categories over time. Recruitment factor categorizes Pacific oyster pediveliger and spat abundances Pedi– and Spat– by aggregating the abundances from 0 to 20 ind. $plate^{-1}$, and Pedi+ and Spat+ by aggregating the abundances with >20 ind. $plate^{-1}$. See Fig. 7 for definitions

Months		June		July				August					September				October
Weeks		24	26	27	28	29	30	31	32	33	34	35	36	37	38	39	41
Recruitment factor	Pedi–	4	4	0	4	0	3	2	2	3	5	3	7	0	5	1	3
	Pedi+Spat–	4	4	4	4	4	4	0	1	1	2	1	0	2	3	3	1
	Pedi+Spat+	0	0	0	0	0	1	2	5	0	1	0	1	2	0	0	0
Recruitment windows		Unfavorable						Favorable							Unfavorable		

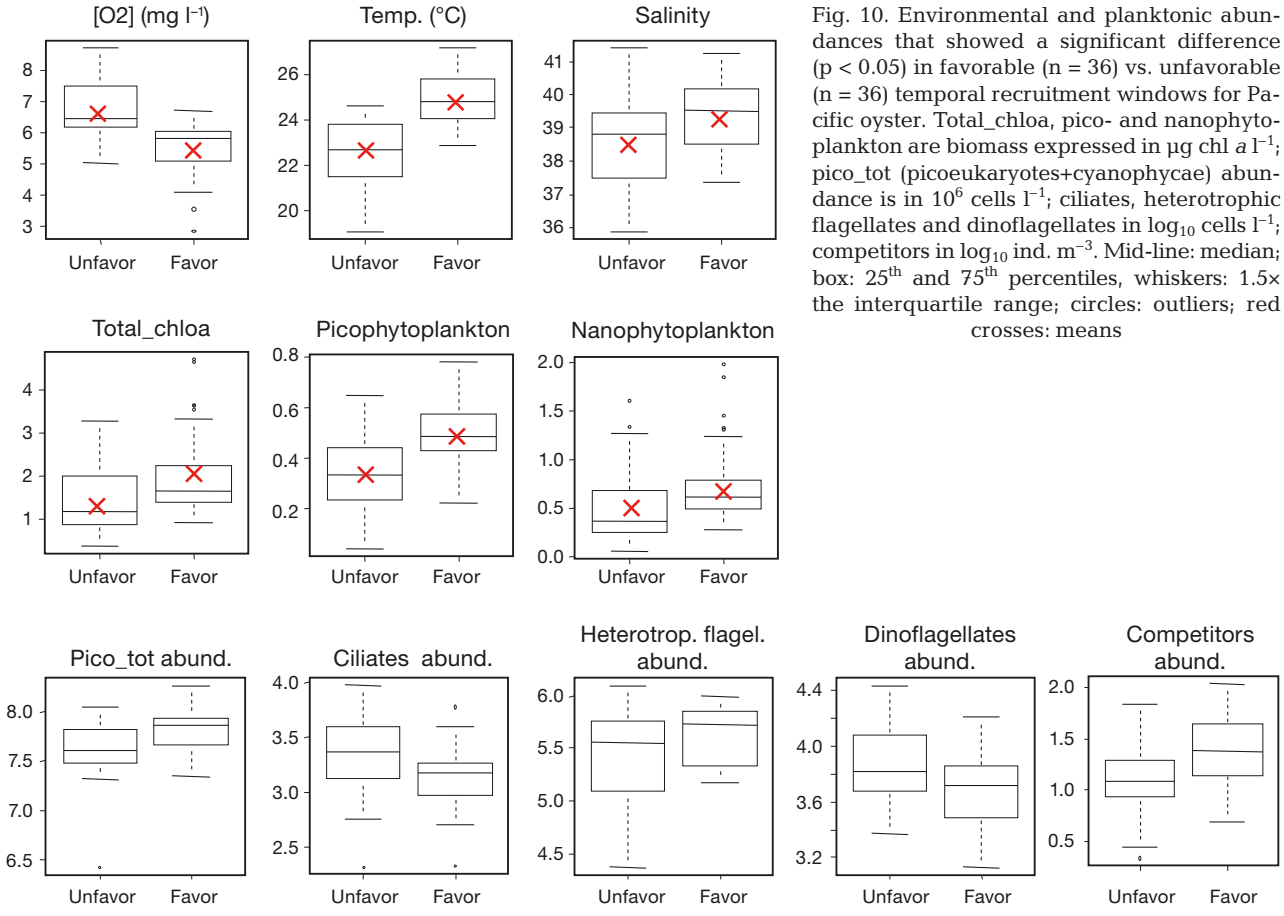

Fig. 10. Environmental and planktonic abundances that showed a significant difference ($p < 0.05$) in favorable (n = 36) vs. unfavorable (n = 36) temporal recruitment windows for Pacific oyster. Total_chloa, pico- and nanophytoplankton are biomass expressed in µg chl *a* l^{-1}; pico_tot (picoeukaryotes+cyanophycae) abundance is in 10^6 cells l^{-1}; ciliates, heterotrophic flagellates and dinoflagellates in $\log_{10}$ cells l^{-1}; competitors in $\log_{10}$ ind. m^{-3}. Mid-line: median; box: 25th and 75th percentiles, whiskers: 1.5× the interquartile range; circles: outliers; red crosses: means

2005). A few authors have reported a negative impact of ciliates on the development of mollusk bivalve spat (inhibition of settlement) (Plunket & Hidu 1978, Elston et al. 1999, Shimeta et al. 2012). Our results support this hypothesis since a negative correlation was observed between the abundance of ciliates and the metamorphosis success of *C. gigas*.

Dinoflagellate and diatom population dynamics are known to be asynchronous in lagoons with respect to nutrient inputs (Collos et al. 2008). In the present study, the unfavorable recruitment window was characterized by high abundances of mixotrophic dinoflagellates during the favorable period even though summer is not the best ecological window for dinoflagellates (Collos et al. 2008). We suggest that high concentrations of chemical contaminants, such as herbicides used in antifouling boat paints or in agriculture, favor these mixotrophic and heterotrophic organisms. Dinoflagellates are part of the diet of oysters (both adults and spat; Baldwin 1991, Baldwin & Newell 1995), but can inhibit the larval development and recruitment of bivalve mollusks (Mu & Li 2013, Mizuno et al. 2015).

The unfavorable recruitment window preceded a favorable window that systematically lasted from late July to early September. This favorable window was characterized by the availability of trophic variables for oyster larvae such as the abundance of nanophytoplankton and *Chaetoceros* spp. Larval food sources of *Crassostrea* spp. in the natural environment are multiple (types and particle sizes), and their consumption varies with larval stages: bacteria, phytoplankton, particulate and dissolved organic matter and protozoa (Douillet 1993, Raby et al. 1997, Sommer et al. 2000). At the beginning of August, warmer conditions (temperature >25°C) could cause a shift in the regeneration of organic matter and in remineralization favoring fast growing diatoms (Collos et al. 2003), most of which are *Chaetoceros* spp. (Collos 1986, Collos et al. 1997). As a result, the concentrations of nano- and microphytoplankton including diatoms are high and favor successful *C. gigas* recruitment with (this time) a bottom-up control of phytoplankton on larvae. The relationship between various pelagic cues and the improvement of benthic invertebrate recruitment is well known (Miron et al.

1995, Pineda et al. 2010). Particularly, the 'trophic settlement trigger' concept could indirectly affect recruitment by strongly improving the settlement rate of pediveliger larvae (Toupoint et al. 2012a,b). In addition to the match/mismatch theory (Cushing 1990), this concept shows the benefit of pelagic cues in favor of settlement success. Without augured benefit for *Chaetoceros* on pelagic larval development, our results appear to support the trophic settlement trigger hypothesis induced with the significant relationship between this diatom genus and *C. gigas* pediveliger abundance.

Our results showed extreme spatial heterogeneity of oyster spat collection among the sampling sites. The ecological conditions within the Thau lagoon are known to be highly contrasted (Troussellier & Deslous-Paoli 2001) and the spatial organization of oyster recruitment will consequently need to be studied more precisely. First, the exchange of water with the open sea and the circulation of water within the lagoon (Guelorget et al. 1994, Lam-Hoai et al. 1997) define the habitat and its ecological niches depending on the proportion of marine and lagoon water volumes (Fiandrino et al. 2017). In addition, the variability of the plankton communities in space and over time is significantly influenced by the hydrodynamic conditions, which in turn, depend on the wind (Millet & Cecchi 1992, Troussellier et al. 1993). Hydrodynamic circulation patterns are also one of the main factors that influence larval dispersal and spat distribution (North et al. 2008, Hubbard & Reidenbach 2015, Larsson et al. 2016). The use of hydrodynamic models, such as the Mars 3D model (Lazure & Dumas 2008, Fiandrino et al. 2017) would prove useful to quantify larval dispersal and connectivity once adapted for lagoon ecosystems in semi-closed nanotidal regimes. On the other hand, intensive shellfish-farmed communities structure the interactions between species and the supply of mollusk larvae (Borsa & Millet 1992). The Pacific oyster is an ecosystem engineer and keystone species, and has a significant impact on its own ecosystem, particularly at a high density in an enclosed system (Mazouni et al. 1998, Souchu et al. 2001, Bec et al. 2005). Significant chl *a* and particulate organic carbon depletion were measured in the shellfish farming zone (Souchu et al. 2001) along with a reduction in microzooplankton biomass compared with that in the middle of the lagoon (Lam-Hoai et al. 1997). Oyster larvae take place in the microzooplankton compartment and a full study of the effect of the breeding zone on the structure of oyster recruitment is now needed after this first ecological approach.

Mass mortalities in oyster juveniles have raised concerns about cultivation practices in Mediterranean lagoons, as the Thau basin depends on supplies of wild oyster juveniles from the Atlantic and individuals raised in French hatcheries. Thus it is important to increase knowledge of local reproduction and native oyster recruitment. Ubertini et al. (2017) showed that the reproduction window stretches from the beginning of June to the end of September. For these authors, phytoplankton concentrations and assemblages affected gametogenesis, with diatoms having a positive effect. No spawning events were observed below 22°C. The temperature of 23°C appears to be a temperature threshold for the occurrence of significant spawning events. Ubertini et al. (2017) also showed that the full and dark moon in combination with high temperature may enhance spawning events. In our 3 yr study (2012 to 2014), we observed natural recruitment of *C. gigas* for the first time in the Mediterranean Thau lagoon. When recruitment occurred, spat collection levels in the Thau lagoon were comparable with those in other French traditional breeding basins (Lagarde et al. 2015a), with recruitment classified from low (up to 20 ind. plate^{-1}) to medium (up to 200 ind. plate^{-1}) according to the classification proposed by the French oyster larvae monitoring network in the VELYGER project (Pouvreau et al. 2013, 2016). The discovery of spat-producing areas at a sampling site outside a shellfish-farming area is an important breakthrough and means that spat collection in Mediterranean lagoons may be possible in future (Lagarde et al. 2015b). Indeed, until now, attempts to collect spat have been restricted to shellfish-farming zones in the Thau lagoon, but amounts were too low to be profitable. Further technical experiments are necessary at an industrial scale to improve this novel shellfish-farming practice. There may also be other benefits in using native oysters; they may, for instance, have a better resistance to OsHv1-µvar (Petton et al. 2015). The use of native oysters may also be considered a more eco-friendly practice for spat supply.

Coastal lagoons are particularly sensitive to nutrient input of anthropogenic origin (Knoppers 1994, Cloern 2001, Kennish & Paerl 2010) due to restricted exchanges with the sea and intrinsic hydrodynamic patterns (Boesch 2002, Glibert et al. 2011). In French Mediterranean lagoons, anthropogenic nutrient inputs have been reduced, leading to oligotrophication (Leruste et al. 2016). Some authors suggest that the combination of global warming and reduced nutrient loads will reduce phytoplankton biomass, which would benefit picocyanobacteria (Collos et al. 2009).

In the Thau lagoon, the expected shift in the ecological community structure would presumably change the newly defined optimum recruitment window for the Pacific oyster. Results from the present study show that recruitment success or failure in this Mediterranean lagoon is related to the nursery function of summer biocoenosis trajectories of the lagoon. Further studies are necessary to define the *in situ* tolerance limits of *C. gigas*, in a strongly fluctuating ecosystem, at the frontiers of its ecological niche.

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