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Spatial variability in growth and reproduction of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) along the west European coast

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Abstract

The Pacific oyster *Crassostrea gigas* was introduced in Europe for commercial purposes in the mid 1960s. It was initially thought that low winter temperatures would restrain this species' reproduction and settlement; however, its present distribution in areas where no introduction has taken place suggests that natural invasion and expansion has occurred. Along the European coast, wild populations of Pacific oysters are already found from northern Germany to southern Portugal. Whether *C. gigas* will continue to further expand through northern waters will depend on its physiological performance. In this study, the performance of wild oyster populations has been studied in terms of growth and reproduction at three stations: La Rochelle (France; 46°N), Yerseke (Oosterschelde estuary, The Netherlands, 51°N), and Texel (Wadden Sea estuary, The Netherlands, 53°N). The French population had the lowest somatic-shell mass ratio and an increase in maximum shell length, somatic and gonadal mass was observed from France to the Netherlands. In addition, mean oocyte diameter decreased significantly from south to north. The combination of increasing gonadal mass and decreasing oocyte volume suggests an increasing reproductive output in terms of egg numbers from France to The Netherlands. Differences in temperature between locations will at least be partly responsible for the observed patterns; however, other environmental factors (such as food availability, predation pressure, sediment type and/or seston concentration) cannot be excluded. Since smaller eggs (oocytes) are thought to have a longer development time, the environmental conditions along the Dutch coast may result in increased larval dispersal and possibly in further population expansion.

Keywords: *Crassostrea gigas*; Pacific oyster; Growth; Reproduction; Oocyte size; Age; Cathodoluminescence

1. Introduction

Coastal environments have been extensively invaded by exotic (non-indigenous) species, many of them associated with mariculture (for the Wadden Sea see Reise et al., 1998; for the Netherlands see Wolff, 2005). Many introductions have failed, but some have a strong impact on the ecosystem. In the Wadden Sea, some species such as the North American spionid polychaete *Marenzellaria* cf. *wireni* (Essink et al., 1998), the American razor clam *Ensis directus* (Beukema and Dekker, 1995; Armonies, 2001), and the Pacific oyster

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Crassostrea gigas (Reise et al., 1998; Dankers et al., 2004, 2006) have even become abundant. The Pacific oyster was introduced for aquaculture purposes in several European coastal waters during the 1960s and 1970s (Meixner, 1973; Walne and Helm, 1979; Grizel and Héral, 1991; Drinkwaard, 1999; Wolff and Reise, 2002; Dankers et al., 2004; Smaal et al., 2005). It was thought that this species would not be able to reproduce in many of these areas because of its natural distribution in relatively warm waters (Drinkwaard, 1999), but its present distribution in northern Europe does not seem to be the result of active introductions only. Natural invasion and expansion are suggested by the recorded new presence, and subsequent increase, in areas where no deliberate introduction has taken place. As a consequence, wild populations are now found along a long stretch of the Atlantic European coast, from northern Germany to southern Portugal (Wehrmann et al., 2000; Dankers et al., 2006; Pouvreau, pers. comm., 2005; Peralta, pers. comm., 2005; Iglesias, pers. comm., 2005).

Larval settlement of the Pacific oyster requires the presence of hard substrates. Thus, in Dutch waters, spat were initially found on dikes (Bruins, 1983). Subsequently, invasion and colonisation of Pacific oyster spat in intertidal areas occurred mainly on mussel and cockle beds (Dankers et al., 2004; Diederich et al., 2004). In contrast to other areas (Ren et al., 2003; Diederich et al., 2004), a time lag of about 15-20 years was observed between initial invasion of Pacific oysters at the intertidal Dutch Wadden Sea and population expansion (Dankers, pers. comm., 2005). Larval supply may have been the limiting factor for population growth. On the one hand, larval and juvenile survival could have been affected by low winter temperatures because the minimum temperature tolerance of juvenile Pacific oysters is about three weeks at 3 °C (Child and Laing, 1998). On the other hand, summer temperatures could have been too low to reach the spawning threshold since spawning seems to be induced at around 22 °C (Kobayashi et al., 1997). In corroboration of temperature being a limiting factor in the Wadden Sea, it has recently been noted that invasion and expansion of the Pacific oyster in the German Wadden Sea were accelerated by high late summer water temperatures (Diederich et al., 2004). In summary, water temperature appears to be an important factor for reproduction, survival and further expansion of northern oyster populations. A general latitudinal trend in temperature is observed along the European coast, with average water temperatures decreasing with increasing latitude (http://www.ifremer.fr/; http://www.surf-forecast.com/breaks/; http://www.hmcz.nl; http://www.bsh.de; http://www.nioz.nl; http://www.dmu.dk; http://www.cefas.co.uk).

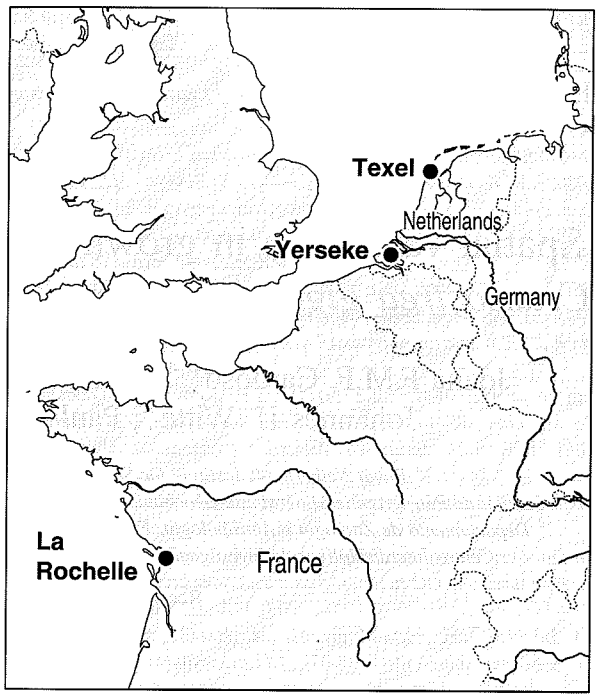

Fig. 1. Sampling locations of *Crassostrea gigas*: La Rochelle (France), Yerseke (Oosterschelde, The Netherlands) and Texel (Wadden Sea, The Netherlands).

This decrease in temperature with latitude might potentially determine the ultimate northern limit of the geographical distribution of *C. gigas*.

Further expansion of *C. gigas* will depend on its physiological performance. So far no information exists on growth and reproduction of wild oyster populations. Therefore, in the present paper, we studied growth and reproduction of three wild oyster populations along a latitudinal gradient, from France (La Rochelle, 46°N) to the northern part of the Netherlands (western Dutch Wadden Sea, 53°N). Spatial and temporal variation in growth and reproductive output was assessed by analysing:

(1) age composition and maximum growth rates;
(2) mass allocation to growth and reproduction, by determining amounts of soma and gonads throughout the year;
(3) oocyte size of the various populations to determine the trade-off between egg numbers and egg size.

So far, any analysis of growth in the Pacific oyster has been hampered by the fact that no reliable method was available for age determination. Only recently has a validated method become available based on the seasonal incorporation of manganese in the shell

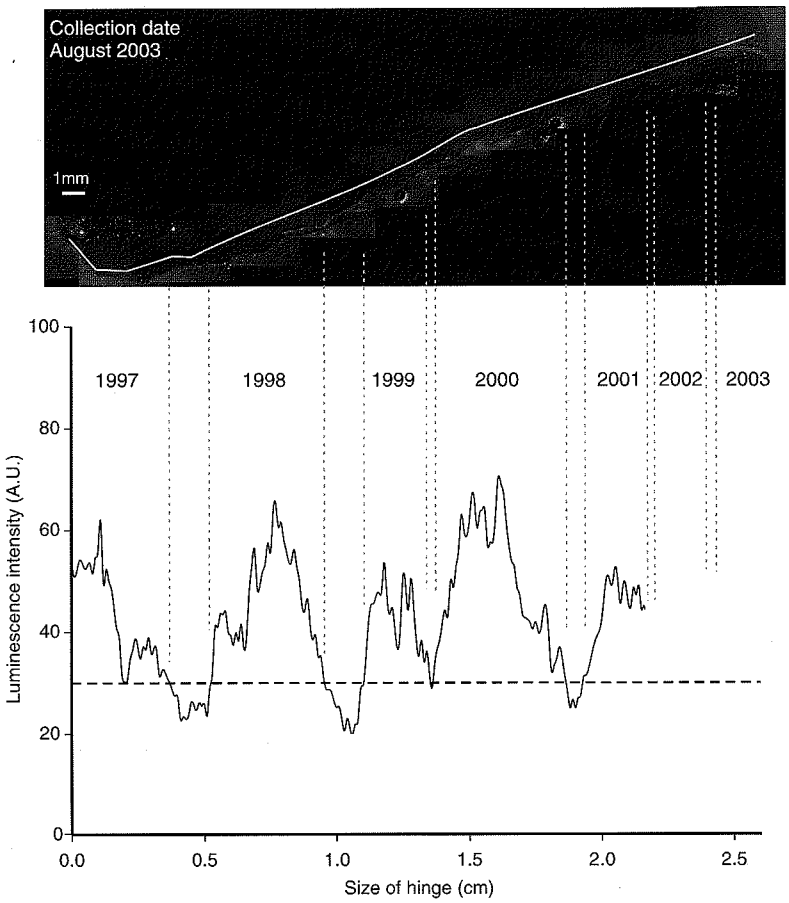

Fig. 2. Cathodoluminescence microphotograph (upper panel) of the longitudinal section of the hinge of a 6-year-old *Crassostrea gigas* (17.8 cm shell length) from Yerseke, showing successive areas with contrasting natural luminescence; and profile of luminescence intensity (lower panel), measured along the marked line on the photograph, showing 7 peaks corresponding to 7 summer seasons. Horizontal dashed line indicates the threshold under which the luminosity was considered to represent the winter period (see text).

(Langlet, 2002; Langlet et al., 2006), and this method will be applied in the present study.

2. Materials and methods

2.1. Identification

Due to the deliberate introduction of Pacific oysters and other *Crassostrea* species from different origins into European waters, it cannot be excluded that along European coasts a mixture of (sub)species was introduced over time and still occurs. Therefore, before starting the sampling program, the presence of *C. gigas* in the sample populations was confirmed by genetic analysis. To that end, the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using primer pair LCO1490-HCO2198 according to Folmer et al. (1994) and resulting sequences were submitted to GenBank (accession codes DQ417690-DQ417696).

2.2. Sampling

Samples were collected at three intertidal stations (Fig. 1) at a similar intertidal level (they were exposed for about 5 h per tidal cycle): La Rochelle (France, 46°N), Yerseke (Oosterschelde estuary, The Netherlands, 51°N) and Texel (Wadden Sea estuary, The Netherlands, 53°N). Salinity conditions were around 33 ppm at La Rochelle and 25-28 ppm at Texel and Yerseke. Around 100 individuals over the whole size range observed were collected randomly in an area of a few km^2, if possible once a month. Sampling took place from June 2003 to May 2004 at La Rochelle and from October 2002 to November 2003 at Yerseke and Texel. After collection, all samples were stored dry at 5 °C, transported to the laboratory and processed within the next 48 h.

Surface water temperatures were available from long-term data series in subtidal areas in the close vicinity of the sampling stations. Data for La Rochelle were provided by

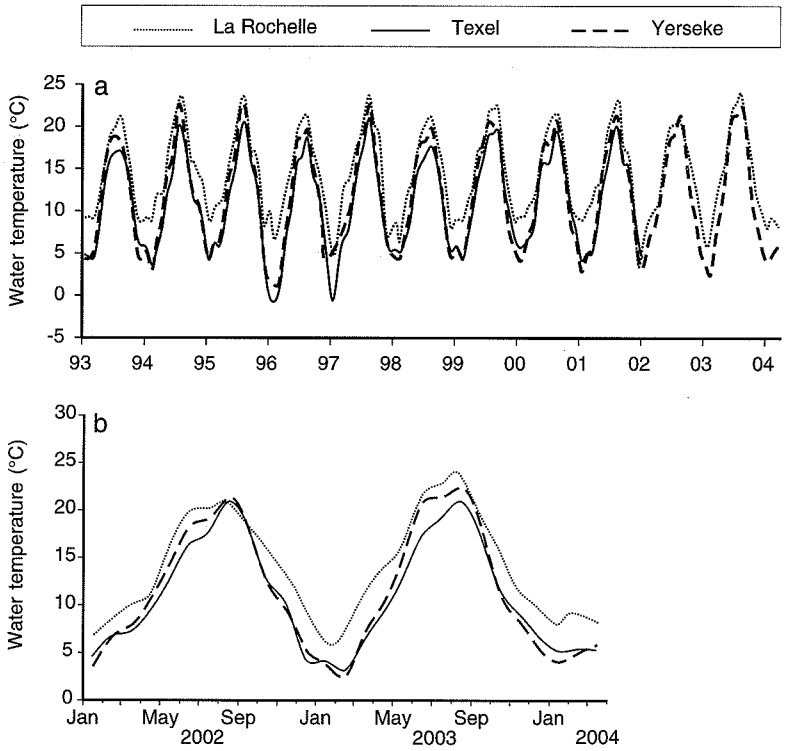

Fig. 3. Water temperatures (°C) at the three locations. (a) Monthly mean temperature patterns from the last 10 years. (b) Temperature measurements during the sampling period.

the 'IFREMER, REPHY monitoring network' (via http://www.ifremer.fr/envlit/region/), data for Yerseke were obtained from the 'Hydro Méteo Centrum Zeeland' (via http://www.hmcz.nl) and data for Texel taken from the 'Royal NIOZ' long-term series (via http://www.nioz.nl, go to Research, Scientific Departments, Physical Oceanography, Ferry and Jerry Observations).

2.3. Age determination

Age determination was done by analysing the seasonal incorporation of manganese in the shell according to Langlet (2002) and Langlet et al. (2006). A total of 218 specimens (La Rochelle: 29; Yerseke: 98; Texel: 93) were analysed. Annual growth rings (Fig. 2) were determined by analysing the fluctuations in cathodoluminescence of manganese ions on a section of the hinge.

For that, shells were cleaned and shell length (defined here as the longest distance from the hinge to the shell edge) of each individual was measured to the nearest 0.01 cm with electronic callipers. Subsequently, bivalves were opened and all soft parts removed. Shells were left to dry at room temperature for 24 h and weighed to the nearest 0.01 g. After shells were cleaned and dried, the left valves (with hinge) were placed face down in a plastic mould and embedded in epoxy resin (Poly Service, THV-500 epoxyhars and Harder 355), following Ropes (1985). Once hardened, the blocks were sectioned longitudinally through the hinge (Witbaard, 1997; Witbaard et al., 1999). The sectioned half valves were then ground flat, wet polished and stuck on a glass slide before being sliced. The facing was ground and wet polished with decreasing grain size polishing suspension (down to 1 μm), leaving a section with a thickness of approximately 500 μm. Digital pictures were then taken with an exposure time of 8 s. On the pictures, the variations in luminescence intensity were measured with the ImageJ™ software package (http://rsb.info.nih.gov/ij/) according to the method described by Langlet (2002) and Langlet et al. (2006), whereby the number of peaks of luminosity along a transect through the shell section corresponds with the age of the individual. The intensity of luminescence at the different locations was analysed and a threshold of 30 arbitrary units (A.U.) of luminescence intensity was selected, under which the luminescence was so low that it was considered to correspond to the winter period (for more details see Langlet et al., 2006).

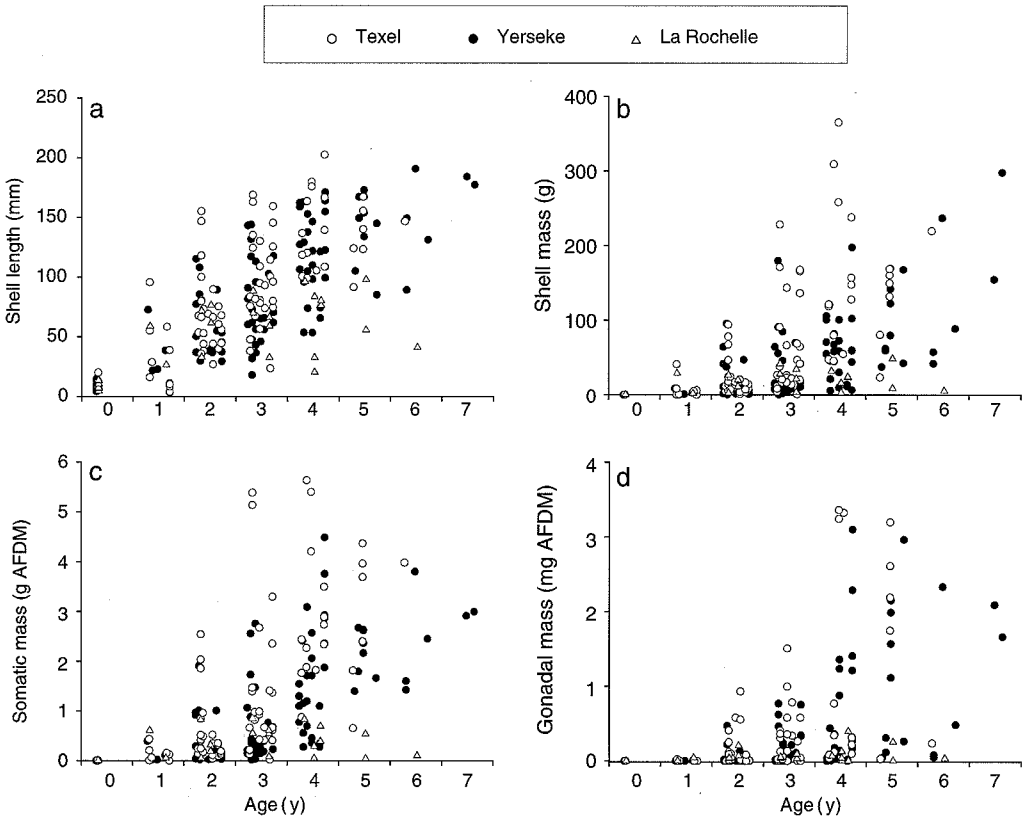

Fig. 4. Relationship between age (y) of *Crassostrea gigas* and (a) shell length (mm); (b) shell mass (g); (c) somatic mass (mg AFDM) and (d) gonadal mass (mg AFDM) (open circles: Texel; full circles: Yerseke; triangles: La Rochelle).

2.4. Oocyte size determination

Between June and August 2003, about 50 animals were collected at each station and forced to spawn in the laboratory. After collection, animals were stored one night at 10 °C. Subsequently, they were placed individually in glass jars (200 to 1000 ml) and a thermal shock was given by adding seawater between 25 and 30 °C, at a constant salinity of 33 ppm. From each spawned female, a random sample of freshly spawned oocytes was collected, placed on a microscope slide and digital photographs were taken with a Pixera View Finder digital camera fitted to a Zeiss stereo microscope with a final resolution of 1510 pixels per mm. Then sharply focused oocytes were measured using the ImageJ™ software package (http://rsb.info.nih.gov/ij/). Oocyte size of at least five round eggs per female was determined according to Thorsen and Kjesbu (2001).

2.5. Data analysis

Gonads were separated from somatic tissue under a microscope (6.4x). Dry and ash mass of each part were determined to the nearest 0.01 mg, by drying for 4 d at 60 °C and incinerating for 4 h at 560 °C. The difference between dry and ash mass represented the ash-free dry mass (AFDM). Investment in somatic and gonadal mass was analysed by means of the somatic-shell mass ratio (SSM) and gonad-shell mass ratio (GSM) which are defined as the somatic AFDM (mg) divided by shell mass (g) and the gonadal AFDM (mg) divided by shell mass (g), respectively. By dividing AFDM by shell mass, animals of different size could be compared in terms of condition. The relative investment in reproduction was provided by calculation of the gonadosomatic ratio (GSR), described as the gonadal AFDM divided by the total AFDM (soma plus gonads). The extent to which variability in SSM ratio could be accounted for by seasonal variability was examined by ANOVA. Due to an imbalance in the sampling scheme over the year, the effect of time could not be described in terms of differences among all sampling months (that is, by using sampling month as a categorical variable). Instead, we used a linear trend over time in combination with

Table 1
Mean shell length (cm) per age group of *Crassostrea gigas* at Texel, Yerseke and La Rochelle

	Texel		Yerseke		La Rochelle	
Age	Mean±SE	n	Mean±SE	n	Mean±SE	n
0	10.0±1.7	8	9.8±1.3	8	9.1±1.0	9
1	26.9±7.7	13	27.8±11.8	4	42.6±16.2	2
2	70.2±6.4	25	58.2±7.0	16	60.6±9.7	4
3	97.2±7.3	27	76.6±6.3	28	63.3±9.0	5
4	140.0±9.6	13	118.5±6.9	26	65.0±12.6	6

a sinusoidal seasonal effect. The overall time effect was, therefore:

$$\beta_1 Time + \beta_2 \sin(2\pi((Month - \beta_3/12)),$$

in which β_1, β_2, and β_3 are parameters, *Time* is a continuous variable that runs from the first day of observation to the last day, and *Month* is a continuous variable that runs from the first month of observation to the last month. Note that this model is only a linear model when β_3 is known beforehand. For that, we ran this linear model, which further included the factor site, for all 12 possible values of β_3 (i.e. the values 1 to 12) and selected the model with the lowest residual mean squares. In order to obtain normality, SSM data were transformed using a squared root transformation.

A similar analysis was used for the analysis of gonadal-shell mass ratio (GSM). However, instead of a sinusoidal function, a block function was used, which divided the year into two periods: a period with a low GSM and one with a high GSM. These periods could differ between sites. Individuals with no gonadal mass were excluded from this analysis. Again, a squared root transformation was used.

For the analysis of growth, shell length (mm), shell mass (g), somatic AFDM (mg) and gonadal AFDM (mg) were plotted against age (years) for each location. Differences in oocyte size (µm) between locations were compared using a two-level nested ANOVA with station and female as categorical factors. All statistical analyses were done using the software package SYSTAT (Wilkinson, 1996).

3. Results

3.1. Temperature conditions

Temperature patterns over the last 10 years show that differences between stations occurred mainly in winter (Fig. 3a), with milder winters prevailing at La Rochelle. The minimum winter water temperature during this period was 3.5 °C at La Rochelle, while in Yerseke and on Texel it was around 1.7 °C and −0.6 °C, respectively. Summer temperatures were similar for the three stations, although in most years higher maximal temperatures were observed at La Rochelle. During the sampling period, temperatures followed the same trend, with La Rochelle presenting milder winters and slightly warmer summers than the other two locations (Fig. 3b). Overall, lowest temperatures occurred between December and February and highest temperatures from June to August. Small differences in temperature between Yerseke and Texel occurred mainly

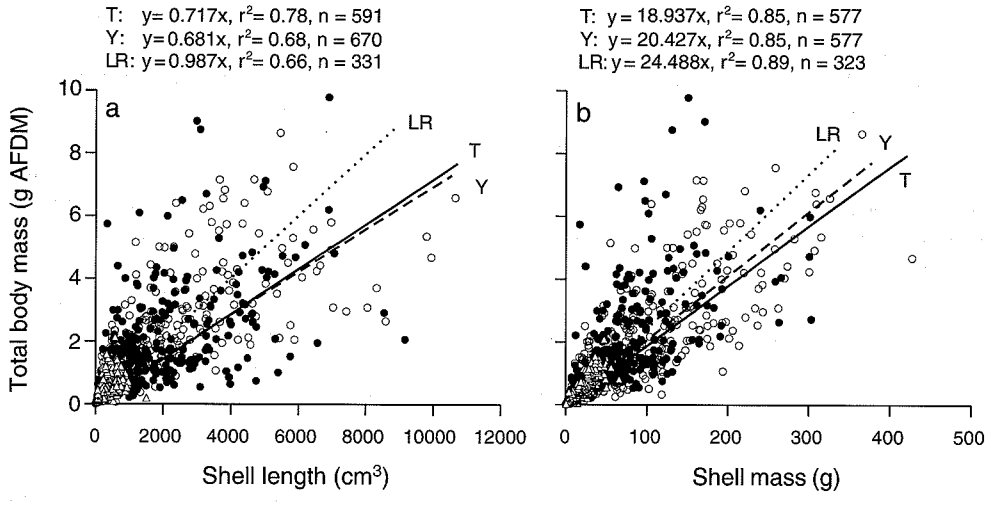

Fig. 5. Relationship between total body wet mass (g) of *Crassostrea gigas* and (a) cubic shell length (cm^3) and (b) shell mass (g), with weighted regression lines. T: Texel; Y: Yerseke; LR: La Rochelle.

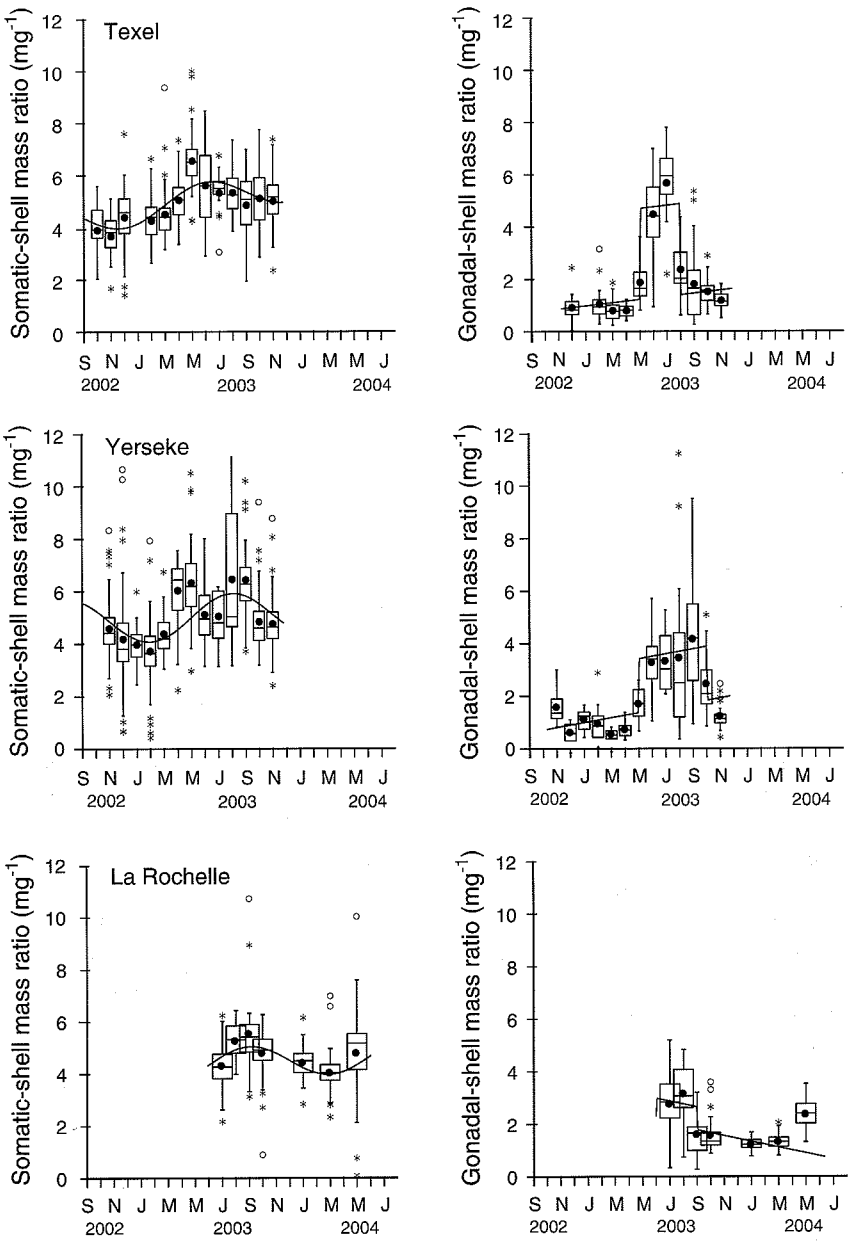

Fig. 6. Somatic-shell mass ratio (mg g^{-1}) and gonadal-shell mass ratio (mg g^{-1}) of *Crassostrea gigas* over the year. Original values were square-root transformed. Curves and lines are model predictions. Full dots and horizontal bars indicate mean and median value, respectively; boxes represent the range within which the central 50% of the values fall; bars represent the data range excluding outliers; outliers and extreme values are, respectively, observations more than 1.5 and 3 times the box range and are represented by asterisks and open circles.

in the summer of 2003, when Yerseke had slightly higher temperatures. On average, water temperatures during the winter period (defined as the period 1 November - 31 March) were around 9 °C at La Rochelle and 7 °C at Yerseke and Texel, while in summer (defined as the period 1 April - 31 October) water temperatures were around 19 °C, 17 °C and 15 °C for La Rochelle, Yerseke and Texel, respectively. In 2003, temperatures were higher than in 2002, with higher summer and winter temperatures at the three locations.

3.2. Annual growth

Individuals up to 6 years old were found at Texel and La Rochelle, and up to 7 years old at Yerseke. No missing age classes were observed at any of the stations,

indicating annual recruitment over the last 7-year period.

At the three locations, shell length, shell mass, somatic mass and gonadal mass showed a large scatter with age (Fig. 4). Nevertheless, differences between locations were found. Maximum shell length and mass observed were about 20 cm and 360 g at Texel, 19 cm and 300 g at Yerseke and 10 cm and 48 g at La Rochelle. Maximum somatic and gonadal masses observed followed the same pattern and were around 5.6 and 3.4 g at Texel, 4.5 and 3.1 g at Yerseke and 0.8 and 0.4 g at La Rochelle. The results suggested an increase in maximum shell length, shell mass and somatic mass from La Rochelle to Texel.

For comparison with previous studies, trials were done to fit Von Bertalanffy Growth (VBG) curves for growth of shell length. Due to a scarcity of individuals >5 y old in the samples, this was only successful for Texel and La Rochelle (Texel: $L_\infty = 23.8 \pm 6.8$ cm, $k = 0.001$ d^{-1}, n=93; La Rochelle: $L_\infty = 6.8 \pm 0.9$ cm, $k = 0.002$ d^{-1}, n=29). However, the large standard error of the parameter estimates indicates that results were not reliable. Mean values of shell length for each location per age group are presented in Table 1 (age groups >4 y old were excluded due to the low number of observations and very large variability).

Significant relationships were found between shell length and total body mass for the three stations (Fig. 5a); however, the scatter in the data was considerable. The scatter decreased when shell mass was taken instead of shell length (Fig. 5b). Therefore, seasonal growth in somatic and gonadal mass was analysed by standardising for shell mass instead of shell length.

3.3. Seasonal patterns

In all analyses, sexes were treated together because no significant differences in somatic- (SSM) and gonadal-shell mass ratios (GSM) were found between sexes (ANOVA, $p > 0.05$). The linear model run to determine the value of β_3 showed the lowest residuals when β_3 was 3 for La Rochelle, 8 for Yerseke and 6 for Texel. A seasonal pattern in SSM was found in all three locations (Fig. 6). An increase in ratio occurred during spring with maximum mean values at Texel in May and at La Rochelle in September. At Yerseke, two peaks in SSM were found, in April/May and in August/September. The decrease in SSM between the two peaks might be due to spawning. Significant differences were found between locations (Table 2), whereby the interactions Station*Month and Station*Season (sinusoidal function) were significant. The increase in SSM during the growing season was higher at Yerseke and Texel than at La Rochelle.

GSM ratios showed very low values in winter and spring, with peak values at La Rochelle in July/August, at Yerseke from June to September, and on Texel in June/July (Fig. 6). Significant differences between locations were found in the interaction term Station*Season (block function) (Table 3). These differences were significant between Texel and Yerseke, and Texel and La Rochelle, but not between Yerseke and La Rochelle (Fisher's LSD post-hoc test). At Yerseke and La Rochelle, maximum values were similar but during winter GSM was higher at La Rochelle. At Yerseke, a longer period of high ratios was seen and individuals without gonad were mostly found from November to January. At Texel, most individuals had no gonadal tissue between October and December while at La Rochelle many individuals were empty around March. At this last location, the proportion of animals that spawned completely was lower than at the other locations. Gonadosomatic ratio (used as a measure of reproductive effort) was highest on Texel: about 30% of the total body mass consisted of gonads in July (not shown). If only individuals with developed gonads were considered, this value would even increase to 50%.

Table 2
Analysis of variance of the somatic-shell mass ratio (SSM; mg g^{-1}) of *Crassostrea gigas*, from October 2002 to April 2004, after square-root transformation

Source	Sum of Squares	df	Mean Square	F	p
Station	26.764	2	13.382	8.543	0.000***
Month	43.635	1	43.635	27.857	0.001***
Season	12.634	1	12.634	8.066	0.005**
Station*Month	21.327	2	10.663	6.808	0.001**
Station*Season	129.450	2	64.725	41.321	0.000***
Error	2437.300	1556	1.566		

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 3
Analysis of variance of the gonadal-shell mass ratio (GSM; mg g^{-1}) of *Crassostrea gigas*, from October 2002 to April 2004, after square-root transformation

Source	Sum of Squares	df	Mean Square	F	p
Station	1.263	2	0.632	0.528	0.590
Month	7.792	1	7.792	6.520	0.011*
Season	214.844	1	214.844	179.774	0.000***
Station*Month	3.912	2	1.956	1.637	0.195
Station*Season	75.488	2	37.744	31.583	0.001***
Error	893.922	748	1.195		

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

3.4. Oocyte size

Spawning was induced in 56 females and a total of 1619 oocytes were measured. Oocyte size did not differ significantly between months (ANOVA, $P>0.05$). The mean oocyte diameter of ripe female oysters decreased from south to north, from about 53 μm at La Rochelle, 50.5 μm at Yerseke to 48.5 μm at Texel, corresponding with a volume of 77912 μm^3 at La Rochelle, 67399 μm^3 at Yerseke and 59704 μm^3 at Texel. Variability in oocyte diameter within each location was low, but the mean diameter differed significantly between stations (ANOVA, $F_{(2,55)}=10.125$, $p<0.001$).

4. Discussion

4.1. Growth patterns

In this study of the Pacific oyster, large differences were found in the various length–at–age and mass–at–age data between the three areas. The maximum size observed showed a gradient with increasing shell and somatic mass from La Rochelle to Texel and the same pattern was observed for gonadal mass. Oysters from La Rochelle built up relatively small gonads while about 14 times higher values were found at Yerseke and even higher values at Texel. Somatic- and gonadal-shell mass ratios followed the same trend with latitude although differences were not so obvious. On average, somatic-shell mass ratio was lower at La Rochelle than at the other two stations and the peak in gonadosomatic ratio was highest at Texel. Besides, a large part of the individuals from La Rochelle did not spawn completely. Overall, a general trend of increase in terms of growth and reproduction was found from La Rochelle to Texel.

Trends in growth with latitude are generally related to temperature patterns. Along the European coast, mean temperatures vary between around 25 °C in summer and 14 °C in winter in southern Europe, to about 15 °C in summer and 2 °C in winter in northern Denmark and in England (http://www.ifremer.fr/; http://www.surf-forecast.com/breaks/; http://www.hmcz.nl; http://www.bsh.de; http://www.nioz.nl; http://www.dmu.dk; http://www.cefas.co.uk). Nevertheless, north-south trends in growth patterns in bivalve species are inconsistent, suggesting that local environmental conditions are important as well. In the Baltic tellin *Macoma balthica*, for example, growth along the American coast decreases towards the north (Gilbert, 1973; Beukema and Meehan, 1985), and along the European coast growth is maximal at intermediate latitude (Beukema and Meehan, 1985; Drent, 2004). In populations of *Mya arenaria* from the American coast, no clear pattern in growth was found (Brousseau, 1979; Brousseau and Baglivo, 1987), and in wild European populations of *C. gigas* maximum length was lower in the northern German Wadden Sea (180 mm; Diederich, 2006) than on Texel (220 mm; this study). In addition, maximum length of wild *C. gigas* from the south coast of Portugal was observed to be around 155 mm (Peralta, pers. comm., 2005), while in La Rochelle it was about 100 mm (this thesis). Apparently, there is not a clear latitudinal trend in growth in shell length of *C. gigas* along the European coasts.

The seasonal pattern in water temperature indicates a consistent gradient in temperature conditions between the sampled locations. Winter temperatures at La Rochelle are usually higher than at the other stations. Since minimum temperature tolerance of juvenile Pacific oysters is about three weeks at 3 °C in winter (Child and Laing, 1998), this may be an important factor determining oyster survival during early life. Differences in food conditions could also be responsible for the observed differences in growth. Suboptimal food conditions at Yerseke and La Rochelle could be induced by the high densities in culture plots; however, in both areas, sampling occurred far away from exploited cultures. Sediment type, seston concentration and predation pressure are other factors that could lead to growth differences between populations. These factors have been seen to affect growth of several bivalve species by hampering filtration and digestion processes (Kiørboe et al., 1981; Newell and Hidu, 1982; Navarro et al., 1992; Kamermans and Huitema, 1994; Gatenby et al., 1996; Iglesias et al., 1996; MacKenzie and McLaughlin, 2000; Carmichael et al., 2004). In oysters, such factors seem to play an important role as well. Predation by fish and gastropods has been observed to affect growth of spat and juveniles (Anderson and Connell, 1999; Villarroel et al., 2004). Also pollution is known to decrease filtration activity, and lead to inhibition of growth in larvae and adult oysters (Fichet et al., 1998; Encomio and Chu, 2000; Nice et al., 2000; Elfwing and Tedengren, 2002). Since these factors were not considered in this study, the causes of the observed differences in somatic and gonadal growth between locations cannot be accessed.

In addition to the overall differences between areas, large differences in environmental conditions must also occur within an area, leading to the large variability in individual growth within each location. In fact, most individuals seem to show reduced growth. For bivalve species occurring in dense intertidal beds competition

for food seems likely. In the Pacific oyster, food limitation has been observed under field conditions (Fujisawa et al., 1987; Brown, 1988; Brown and Hartwick, 1988). During suitable temperature conditions, oysters from Canada showed higher growth rates in terms of shell length and body mass when food level was higher (Brown and Hartwick, 1988). Similar results have been recorded in other areas for other bivalve species such as mussels (Kamermans, 1993; Alunno-Bruscia et al., 2000), cockles (Kamermans, 1993; De Montaudouin, 1996) and *M. balthica* (Kamermans et al., 1992). The similarity in food source and timing of feeding in the various bivalve species might even suggest that inter– and intra-specific competition for food is a general phenomenon in bivalves. The observed relationship between the primary production of various estuarine systems and its macrofaunal biomass (Herman et al., 1999) supports this suggestion.

4.2. Reproductive patterns

In terms of oocyte volume, a significant decrease was observed from La Rochelle to Texel. Along the French Atlantic coast, Lango-Reynoso et al. (2000) found no significant differences between sites. However, these authors measured oocyte size in histological preparations of the gonad where oocytes in different stages of maturation can be found, causing a large variability in the data. During the spawning period, their largest measured oocyte was 61.4 μm and the smallest 19 μm, and the mean population oocyte size reported was of 34.9 μm. Measurements of oocyte size in mature individuals has also been done in other bivalve species and related to body growth. In populations of *Mya arenaria* along the north-eastern American coast, oocyte size was largest in southern populations and a positive relation was found between oocyte size and body growth (Appeldoorn, 1995). In the south, the more variable environmental conditions and high temperatures seemed to cause high juvenile mortality, leading to the necessity of investing more energy per egg and the production of larger (and more resistant) but fewer eggs (Appeldoorn, 1995). The same trend of growth and oocyte size was seen in the Wadden Sea population of *M. balthica* and *Cerastoderma edule* at different intertidal levels (Honkoop and Van der Meer, 1997). In these two species, growth and oocyte size were higher at the lowest tidal level, where submersion time and daily feeding periods are longer. However, in the present study, the opposite trend was observed, with northern populations presenting higher growth but smaller oocytes. Massapina et al. (1999) and Ren et al. (2003) observed a significant positive relationship between the condition of the spawning adults and the oocyte and gonad quality. This could mean that the inverse trend in growth and oocyte volume found between La Rochelle and Texel is a reflection of the fact that under good feeding conditions smaller eggs can be produced without negatively affecting their quality. A definitive answer can only be obtained by an analysis of oocyte quality and composition, in relation to oocyte volume and adult condition.

4.3. The potential for future population expansion of C. gigas

Conditions for growth and reproduction seem to be more optimal in the northern stations of Texel and Yerseke than at La Rochelle. Development of gonads and reproduction occurred in all areas but was much more successful in the northern stations. This suggests that the process of maturation and reproduction has not been the bottleneck for population expansion neither in the Oosterschelde nor in the Dutch Wadden Sea. Substantial egg and larvae survival necessary to guarantee the next generation may have been the key-factor for population growth in the past. At low abundances, fertilisation may be a problem and only under favourable conditions it will be successful. For instance, the first intensive spatfalls in the Oosterschelde and Wadden Sea were recorded in years with warm summers (Drinkwaard, 1999). Also juvenile survival may have been an important factor (Child and Laing, 1998) and mild winters may be required to guarantee sufficient juvenile survival to build up the population. The occurrence of successful fertilisation and survival of the planktonic stages may partly explain the observed time lag between introduction and rapid increase in population abundance some years later.

An important point in relation to the expansion of the species seems to be the observed trend in reproductive output. The decrease in oocyte size and increase in gonadal mass from south to north suggests that there is an enormous increase in numbers of eggs spawned, and, therefore, a capacity for further population expansion with latitude. An increase of energy investment per offspring (larger egg volume) results in either large offspring (larvae) or/and shorter egg development time (Kooijman, 2000). If within a species the size (volume) of the hatchling is constant, larger eggs result in shorter incubation (development) time (Kooijman, 2000), because more energy for a similar trajectory (till hatching) allows a faster development. In this way, the larger oocytes (and eggs) produced by oysters at La Rochelle,

under relatively poor growth conditions, will develop faster, reducing pelagic stage duration and therefore also restricting larval dispersal. The populations in the Oosterschelde and Wadden Sea area, which are under more optimal conditions, produce larger gonads containing smaller oocytes. The resulting higher egg numbers and longer development time will increase larval dispersal and allow expansion of the population. A consequence may be the pattern observed in the east Frisian German Wadden Sea: introduction of the Pacific oyster occurred around 1980 near Texel and this population is thought to be the source of larval settlement in the 1990s in the East Frisian area (Wehrmann et al., 2000).

The rapid expansion of the Pacific oyster in the Dutch and German Wadden Sea (Wehrmann et al., 2000; Diederich, 2005; Dankers et al., 2006) and the observed patterns in growth and reproduction suggest that this species has not reached its eco-physiological limits yet. In fact, the northern distributional limit of *C. gigas* already extends as far as Denmark (Bos et al., 2006; Dankers et al., 2006). Further expansion will depend on environmental conditions, such as food availability, suitable summer temperatures for spawning, presence of hard substrate for initial settling (Bruins, 1983) and mild winters, which determine the survival of juvenile oysters (Child and Laing, 1998). The Swedish west coast has a seasonal pattern in water temperature similar to that of the Wadden Sea, except that winter temperatures are slightly lower (Pihl and Rosenberg, 1982). Together with the extensive presence of hard substrate and the fact that mild winters have been more common during the last years, this suggests that the Pacific oyster may be able to extend its northern distribution.

To obtain more insight, a study of the performance of other wild European Pacific oyster populations over a more marked environmental gradient, not only in terms of temperature but also in terms of food conditions, is required.

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