Spatial and temporal variability in growth and reproduction of the Japanese cupped oyster *Crassostrea gigas* (Thunberg, 1793): are they adapting to northern European waters?

Joana F.M.F. Cardoso[1], Denis Langlet[2], José F. Loff[1], Ana R. Martins[1], Johannes. IJ. Witte[1] and Henk W. van der Veer[1]

[1]*Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg Texel, The Netherlands*
[2]*Section de Pétrographie-Minéralogie-Géochimie, Musée Royal de l'Afrique Centrale, MRAC-Tervuren, Leuvensesteenweg 13, B3080 Tervuren, Belgium*

Abstract

The Japanese cupped oyster, *Crassostrea gigas*, is native to Japan and was introduced in Europe for commercial purposes in the early 1960's. In France and in the south of the Netherlands, this species is now a very important cultured species. Initially, it was thought that the cold northern waters of the Netherlands would restrain the species' reproduction and settlement but surprisingly *C. gigas* is reproducing and expanding in several estuaries, and has already been seen in northern Germany. This suggests that over time eco-physiological changes have occurred, indicating either phenotypic and/or genotypic plasticity. In order to understand how this species was able to invade northern habitats, the energy flow over growth and reproduction in *C. gigas* was studied along a latitudinal gradient from La Rochelle, France to Eastern Scheldt and Wadden Sea in the Netherlands. Overall, the French population had a lower body condition than the two Dutch populations had. Animals from the north of the Netherlands (Wadden Sea) showed the highest gonad mass ratio, although the smallest egg size. Since egg volume directly affects development time and hence reproductive success, we will present evidence that there is a trade off between egg size and larval development time. In fact, a clear latitudinal trend in egg size was found from France to the German Wadden Sea. We will discuss the results in the light of the different life-history strategies of each population in terms of energy allocation over growth and reproduction and we will postulate a hypothesis explaining how the Japanese oyster has passed from "an immigrant to a permanent member".

Corresponding author. E-mail address: cardoso@nioz.nl

1. Introduction

Coastal environments have been extensively invaded by exotic (non-indigenous) species, many of them being associated with mariculture. Although many introductions have failed or these species had little effect on native ones, in some cases introduced species have strong impact in the ecosystem. An example is the introduction of the Japanese cupped oyster *Crassostrea gigas* in the Netherlands. The Japanese oyster was first introduced for aquaculture purposes in the southern part of the Netherlands (Eastern Scheldt estuary) in the 1960's. Although this warm-water species is characterized by a very high fecundity (50 to 100 million eggs / female / year) and a relatively long pelagic larval phase (18 to 30 days), which allows a large dispersal range (Quayle 1969, Bruins 1983), it was thought that the low water temperatures would limit and prevent natural reproduction (Drinkwaard 1998). Nevertheless, since 1980, natural reproduction occurred, and *C. gigas* started to spread out, being also observed in the north of the Netherlands (Wadden Sea estuary) and is still rapidly expanding (Wehrmann et al. 2000, Wolff 1999). At present the introduction of the species has resulted in changes in community structure and ecosystem functioning, mainly by the colonization of tidal flats and possibly by causing food limitation for other bivalves.

So despite the apparent suboptimal environmental conditions, within a few decades after its introduction, *C. gigas* managed to reproduce successfully. This suggests that phenotypic and/or genotypic plasticity has resulted in eco-physiological changes over time. Since we lack information from the 1980 and 1990, temporal changes in the various natural populations cannot be followed. Instead an indirect approach was embraced by analysing the spatial variability in population structure. We analysed the energy flow over growth and reproduction in *C. gigas* along a latitudinal gradient from France (La Rochelle) to the northern part of the Netherlands (western Dutch Wadden Sea), based on the fact that for successful reproduction and dispersal, at least three aspects are of importance: first of all a significant egg production, followed by survival of the eggs and a significant survival of the resulting larvae. Particular attention was paid to egg size, since bivalve larvae rely mainly on stored egg (oocyte) nutrients, and their survival is strongly dependent on the size of these reserves i.e. the size or volume of the oocytes (Honkoop et al. 1999, Massapina et al. 1999). Additionally, development time seems to be related to egg size (Kooiman 2000; Cardoso et al. 2004).

2. Materials and Methods

2.1. Sampling areas and processing methods

Samples were collected at three stations (Fig. 1): La Rochelle (France), and Yerseke (Oosterschelde estuary, The Netherlands) and Texel (Wadden Sea estuary, The Netherlands). In principle around 100 individuals of intertidal *Crassostrea gigas* were collected monthly by hand from June 2003 to May 2004 at La Rochelle and from October 2002 to November 2003 at Yerseke and

Texel at stations with a more or less similar emerging time. At each sampling, water temperature was measured and compared with existing long term temperature series. Texel data were obtained from the long term series from the Marsdiep (van Aken, per. comm.), data from Yerseke were obtained from the "Hydro Méteo Centrum Zeeland" (via http://www.hmcz.nl) and data from La Rochelle were provided by "IFREMER, REPHY monitoring network" (via http://www.ifremer.fr/envlit/region/index.htm).

All animals were stored dry in the refrigerator at 5ºC and processed within the next 48 hours. After cleaning the surface of the shells, shell length of each individual was measured to the nearest 0.01 mm with electronic callipers. Bivalves were then opened and all flesh was removed. Gonads were separated from body mass under a microscope (6,4x). Dry mass and ash mass of each part were determined to the nearest 0.01 mg, by drying for 4 days at 60ºC and incinerating for 4 hours at 560ºC, respectively. The ash-free dry mass of each part was then determined by taking the difference between dry mass and ash mass. Shells were left to dry at room temperature during 24 hours and weighed to the nearest 0.01 g. Age was determined by analysing the manganese luminescence of thin shell sections under a microscope, according to Langlet (2002).

Egg size was determined in the laboratory after induced spawning of individual animals from each location. For that, animals were collected at each station and forced to spawn several times between June and August 2003. After collection, animals were stored for one night in the refrigerator at 10ºC. The day after, animals were placed individually in glass pots (200 to 1000 ml) and a thermal shock was given by adding seawater between 25 and 30ºC. Every hour the water was renewed. In the first hour, 0.5ml of "Fluoxetine 20mg" was added to the water as described in Honkoop (1999). A random sample of freshly spawned eggs were collected separately from each female, placed on a microscope slide and digital photographs were made with a Pixera View Finder digital camera fitted to a Zeiss stereo microscope with a final resolution of 1510 pixels per millimetre. Afterwards, sharply focussed eggs were measured using the ImageJ™ software package (http://rsb.info.nih.gov/ij/). Egg size of at least 5 round eggs (defined as 4p*area/perimeter^2 > 85%) was represented by the mean of the longest and shortest axis, according to Thorsen and Kjesbu (2001). In addition, in the summer of 2004, egg development time from fertilization to the appearance of D-larvae was determined at 24ºC in relation to egg volume of eggs from individual oysters from different locations. For that, ripe animals from Yerseke, Texel and Sylt (Germany) were chosen and stripped for collection of sperm and eggs. Concentrations of eggs and spermatozoids were determined for each animal and eggs were fertilized in beaker glass pots of 300ml with a concentration of 2.000 eggs/ml. Fertilized eggs were examined every hour and developing eggs were classified and counted. Hatch was defined as the time necessary for 50% of the developing eggs to become D-larvae.

2.2. Data analysis

The investment in gonad and somatic mass was analysed by means of the Somatic-Shell mass ratio (SSM) and Gonad-Shell mass ratio (GSM) which are defined as the somatic ash-free dry mass (mg) divided by shell mass (g) and the gonad ash-free dry mass divided by shell mass (g), respectively. The relative investment in reproduction was provided by calculation the Gonadosomatic ratio (GSR), described as the gonad ash-free dry mass divided by the somatic ash-free dry mass. Due to deviations from normal distribution, data were treated with the square root. A general linear model was used to compare differences in somatic mass between locations along the year. As it was not possible to sample all stations in the same month, a sinus effect was introduced in order to treat statistically the data. The effect of time was then calculated in two parts, as an overall monthly effect and as a seasonality effect given by the expression Sinus Month = sin[(month-x/12)*2*3.14] (different sinus functions were made for each station). A model was run using Station as a categorical factor and Month and Sinus Month as independent variables. Differences in peaks of gonad mass between locations were analyzed on a similar way, using Station as a categorical factor and Month and Peak as independent variables. Shell mass (mg), somatic ash-free dry mass (mg) and gonad ash-free dry mass (mg) were plotted against age (days) and the Von Bertalanffy growth parameters for maximum growth were iteratively estimated.

Differences in egg sizes along the summer, from induced spawning in the lab, were compared using a mixed model with station, month and female as categorical factors. As no significant differences were found between months of the same station (p>0.05), differences in egg size between stations were compared using a two-level nested ANOVA with station and female as categorical factors.

All statistical analysis was done using the software package SYSTAT (Wilkinson 1999).

3. Results

3.1. Temperature

Water temperatures measured during sampling fitted the long-term data series available for each area (not shown). The annual pattern was similar for the three areas with lowest values between December and February and highest temperatures around June to August (Fig. 2). The seasonal fluctuations in temperature were lowest in La Rochelle, mainly because of relatively higher temperatures in winter. In some years small differences between Yerseke and Texel could be observed. On Texel and Yerseke, temperatures were similar during winter but higher in Yerseke during the summer. On average, water temperatures during the winter period (defined as the period 1 November – 31 March) were around 9ºC in La Rochelle and 7ºC in Yerseke and Texel during the winter, while in the summer (defined as the period 1 April – 31 October) water temperatures were around 19ºC, 17ºc and 15ºC for La Rochelle, Yerseke and Texel, respectively. In 2003, temperatures were higher than in 2002, with higher temperature values in the summer and winter for the three locations. While in all areas the maximum temperature was almost similar, especially

the minimum temperature in winter differed between about 5ºC in La Rochelle and 1-3ºC at Yerseke and Texel.

3.2. Growth

Significant relationships were found between shell length (mm) and total body wet mass (g) (Fig. 3a). Although relationships were observed for each of the three stations, the scatter in the data was considerable. The scatter decreased in case shell mass was taken instead of shell length (Fig. 3b). Although in the first plot there was a trend with increasing body mass from La Rochelle to Texel, in the second case, a trend with increasing body mass with shell mass was found from Texel to La Rochelle.

Annual growth rings were determined for in total 129 specimens (La Rochelle: 24; Yerseke: 42; Texel: 63). Individuals up to 6 years-old were found in all three areas. Shell length showed considerable variation with age for each location (Fig. 4a), and the same was true for somatic mass (Fig. 4b) and gonad mass (Fig. 4c). When Von Bertalanffy curves were fitted through the maximum values observed at each age group, significant different relationships were found (Table 1, Fig. 4). Due to the scatter of the data, some high values from Texel were excluded from the gonad mass-at-age curve. Nevertheless, it appeared to be a clear latitudinal trend of increasing shell length and somatic and gonad mass with age from La Rochelle to Texel.

Seasonal growth in somatic and gonad mass was analysed by standardizing for shell mass instead of shell length due to the lower variability in mass compared with length (see above). Similar cycles of somatic-shell mass ratio were observed at the three locations (Fig. 5). An increase in ratio was observed during spring with maximum values around June for Texel, July in Yerseke and August at La Rochelle. However, in Texel and Yerseke there was a slight suggestion for another peak of somatic-shell mass ratio in April/May. Significant differences were found between locations (Table 2), being all the effects and interactions significant. On Texel, there was a trend of increase of ratio along the year, as indicated by the sinus function. The decrease in somatic-shell mass ratio at the end of 2003 is not as strong as the decrease in this ratio the winter before. The increase during the year was lowest in La Rochelle, about $1 - 1.5$ mg g^{-1}, while in Yerseke and Texel, the increase amounted 2 mg g^{-1}.

Gonad-shell mass ratios showed very low values in winter and spring and strong peaks during summer and autumn (Fig. 6). Highest values were reached in June/July on Texel, August to September on Yerseke and July/August at La Rochelle. Significant differences were found between locations (Table 3) mainly due to differences in values between peaks of the different locations. Highest values of gonad-shell mass ratio were found on Texel. In Yerseke and La Rochelle maximum values were about the same although in the latter, values were not so low during winter. In Yerseke a longer period of high ratios was seen. Gonadosomatic ratio showed two peaks in Yerseke and one peak on Texel and La Rochelle (Fig.7). Highest values of gonadosomatic ratio were reached on Texel in July, with about 65% of gonad mass in relation to somatic mass. In

Yerseke, the two peaks had similar values of approximately 50% in June and October. In La Rochelle, maximum values of gonadosomatic ratio were reached in July with ratio of about 48%.

3.3. Reproduction

Spawning was induced in about 20 females for each location. In total 56 individuals were forced to spawn. The mean egg diameter of ripe female oysters followed a decreasing trend with latitude, from about 53 µm in La Rochelle, about 50.5 µm at Yerseke to about 48.5 µm in Texel (Fig. 8). The differences between stations were highly significant (Table 4).

Fertilization was successful in 4 batches from individuals from Yerseke, 7 batches from female oysters from Texel and 4 from Sylt. Mean egg volume was smaller on Texel than in Yerseke and in Sylt and there was an inverse relationship between development time and egg volume (Fig. 8)

4. Discussion

4.1. From invading species to permanent resident

The processes determining whether a species will be able to persist in an area after its introduction by either transoceanic shipping or by aquaculture are always the same: first of all becoming mature and being able to reproduce, and subsequently eggs and larvae survival must be substantial to guarantee a next generation. This means that the species must be able the close its life cycle in the area [the so-called member-vagrant hypothesis of Sinclair (1988)]. Over the past decades various introduced species have successfully invaded the Wadden Sea (Reise et al. 1998). Some of them have even become spatially abundant such as for instance the North American spionid polychaete *Marenzellaria* cf. *wireni* (Essink et al.. 1998), the American razor clam *Ensis directus* (Beukema & Dekker 1995, Armonies 2001) and the Japanese oyster. After their first introduction, in most species there is a time lack before they are able to expand and to become abundant as if it takes some time before they are able to close their life cycle in the area. This might point to the fact that adaptations to the local circumstances take their time.

In case of the Japanese oyster, it was originally thought that the species would not be able to reproduce in the area because of its natural distribution in relatively warm waters (Drinkwaard 1999) and the fact that temperature should rise above 23ºC before gonad tissue development occurred (Koyayashi et al. 1997). However, despite the apparent suboptimal environmental conditions (temperature), within a few decades after its introduction, successful recruitment of *C. gigas* was observed in the Eastern Scheldt and more recently in the Dutch and German Wadden Sea. The main question is why did it take so long and what was the bottleneck: adaptations in adult physiology to become able to reproduce at lower temperatures or adaptations in the egg and larval stages to increase survival and produce successful settlement. As stated before, direct

measurements are lacking and therefore an indirect approach is applied by comparing the life history of Japanese oyster in populations over its latitudinal range of distribution. Instead of focussing on spatial patterns only by analysing covariation in life history parameters along a latitudinal gradient (cf Appeldoorn 1995), also temporal variability is considered by year-round sampling of various locations.

4.2. Latitudinal gradients in life history parameters: spatial and temporal variability

The similarity in seasonal pattern in water temperature in the three areas does not suggest a strong gradient in environmental conditions. However, on the other hand the various length-at-age and body mass-at-age data indicate large differences not only between areas but also within each area. A potential bias might be introduced by the age determination, because simple counting of annual rings is impossible in the Japanese oyster. However, the alternative method in which the manganese luminescence of thin shell sections is analysed under a microscope has been extensively validated by Langlet (2002). Therefore, the observed variability in both length- and body mass-at-age is considered to be a refection of variability in growth conditions. The maximum observed growth shows a clear gradient with increasing length and mass from La Rochelle towards Texel. In La Rochelle, maximum length and total body mass remain relatively low, about 10 cm and 10 g wet mass, compared with about 20 cm and 40 g in Yerseke and even more (25 cm; 60 g) at Texel. The increase in somatic-shell mass ratio during the year is also clearly lower at this location than on Texel and Yerseke. The absence of a similar gradient in abiotic environmental conditions (temperature), suggests that this might reflect differences in food conditions for the Japanese oysters between the areas. In all areas most individuals do not show such a maximum growth, which means that in addition to the overall differences between areas also within an area large differences in growth conditions occur between individuals. For the dense intertidal colonies competition for food seems likely to occur. An independent test of food competition and limitation in the Japanese oyster would be an analysis of the growth of individual oysters present on hard substrate such as boulders in harbours and sheetpile walls. It is expected that these solitaire individuals will suffer no or at least less competition for food. The latitudinal trend in growth is also reflected in the development of the gonad mass with age. Oysters from La Rochelle built up relatively small gonads of maximal 250 mg ADFM, while about ten times higher values were found in Yerseke and even higher values in Texel. This is also confirmed by the low peak of gonad-shell mass ration in La Rochelle when compared with the other two locations. Gonad mass clearly increases with latitude. Since egg volume decreases from La Rochelle to Texel, the overall effect is an enormous increase in egg production with latitude.

The observed pattern of variation in life history parameters of the Japanese oyster with latitude does not fit with information on other species. In *Macoma balthica* growth decreased with increasing latitude along the American coast and did show maximum growth at intermediate latitude along the European coast (reference). Also maximum size decreased with latitude (Beukema &

Meehan 1985). In *Mya arenaria* a similar pattern was found with decreasing growth and egg size with latitude (Appeldoorn 1995). However, in these studies a much larger latitudinal gradient is covered and these gradients also represented a marked environmental gradient in terms of temperature, in contrast to the present study. This means that the present study needs to be extended over a larger latitudinal gradient. Nevertheless, the present study has indicated that plasticity in life history parameters of the Japanese oyster occurs even over relatively small distances.

4.3. How has the Japanese oyster succeeded in becoming a permanent resident?

In contrast to the expectations, the introduction of the warm-water Japanese oyster in the Eastern Scheldt and the Wadden Sea did not result in a reduced growth but in much faster potential growth most likely due to more optimal food conditions. As a consequence, development of gonads and reproduction occurs in all areas and is even more successful in terms of energy flux than near La Rochelle. It is therefore very unlikely that becoming mature and being able to reproduce has been a bottleneck for the Japanese oyster either in the Eastern Scheldt or in the Dutch Wadden Sea. This implies that the substantial egg, larvae and juvenile survival necessary to guarantee the next generation has been the bottleneck in the past. At low abundances fertilization might be a problem and only under favourable conditions will fertilization be successful. Also egg, larval and juvenile survival might be critical in the beginning, shortly after introduction. For instance, temperature tolerance of juvenile Japanese oyster is in the range of minimum temperature values of about 3°C observed in winter (Child & Laing 1998). Therefore, it is required a period of mild winters to guarantee sufficient juvenile survival to build up the population and to ensure closing of the life cycle: to be able to build up members according to the member-vagrant hypothesis of Sinclair (1988). In the East Frisian Wadden Sea, the first occurrence of *C. gigas* was in 1998 represented by one living specimen, most probably originated from the spatfall of 1996 during which strong spatfall of several bivalve species was recorded (Wehrmann 1999). In the following years, no marked recruitment in bivalve larvae was observed and only in spring of 2000 juveniles were found (Wehrmann 1999). The incidental occurrence of successful fertilization and survival might be part of the explanation of the observed time lack between introduction and rapid increase in population abundance some years later.

Another important factor seems to be egg volume. With increasing growth larger gonads develop and largest eggs are being produced. Larger egg volume are thought to be associated with higher energy content and based on energetic principles this should result in a faster development rate (Kooijman, 2000). This is thought because more energy for a similar trajectory (hatching larvae) allows a faster speed (development time). This assumption was validated in this study for the Japanese oyster: development time of the eggs till hatching was inversely related to egg volume. This means that egg produced by the Japanese oysters in La Rochelle under relatively bad growth conditions will develop fastest. This will reduce pelagic stage duration and therefore also restrict

larval dispersal. In terms of the member-vagrant hypothesis of Sinclair (1988), this increases the chance of becoming a member instead of a vagrant. Populations suffering relatively low growth and reproductive rates require renewal by settlement of juveniles. Under more optimal conditions and increased reproduction, a reduced egg volume will increase larval dispersal and allow expansion of the population. The pattern has been observed in the East Frisian German Wadden Sea: introduction of the Japanese oyster occurred around 1980 near Texel and this population is thought to be the source of larval settlement in the 1990 in this area (Wehrmann et al., 2000).

In summary, we postulate the following mechanism:

[1] Introduction of the species either accidentally by transoceanic shipping or by aquaculture in relatively low numbers.

[2] Extinction of the adults or, in case of suitable environmental conditions, eco-physiological adaptations over time occur and the population is renewed by building up a local population after successful fertilisation and by substantial survival of locally produced eggs and larvae followed by settlement of juveniles.

[3] Expansion of the species by larval dispersal from the original population and rapidly building up other local populations.

The rapid expansion of the Japanese oyster into the East Frisian Wadden Sea suggests that it has not reached its eco-physiological limits yet and that in next future a further expansion can be expected.

5. Literature cited

Appeldoorn, R. S. (1995). Covariation in life-history parameters of soft-shell clams (*Mya arenaria*) along a latitudinal gradient. ICES Mar. Sci. Symp. 199: 19-25.

Beukema J. J. and B. W. Meehan (1985). Latitudinal variation in linear growth and other shell characteristics of *Macoma balthica*. Mar. Biol. 90: 27-33

Beukema, J. J. and R. Dekker (1995). Dynamics and growth of a recent invader into European coastal waters: The American razor clam, *Ensis directus*. Journal of the Marine Biological Association of the United Kingdom. Plymouth 75(2): 351-362.

Bruins, R. W. B. (1983). *Crassostrea gigas* (Thunberg, 1973) op Texel. Corresp. Bl. Nederl. Malac. Veren. 215: 1436-1438.

Cardoso, F. M. F. (2004). Intra- and interspecies comparison of the energy flow in various bivalve species in Dutch coastal waters by means of dynamic energy budgets. PhD thesis, Rijksuniversiteit Groningen, Groningen (in prep.).

Drinkwaard, A. C. (1998). Introductions and developments of oysters in the North Sea area: a review. Helgolander Meeresuntersuchungen 52(3-4): 301-308.

Drinkwaard, A. C. (1999). History of cupped oyster in European coastal waters. Aquaculture Europe: 7-11 + 41.

Essink, K., J. Eppinga and R. Dekker (1998). Long-term changes (1977-1994) in intertidal macrozoobenthos of the Dollard (Ems estuary) and effects of the introduction of the North American spionid polychaete *Marenzelleria* cf. wireni. Senckenb. Marit. 29: 25-35.

Honkoop, P. J. C., P. C. Luttikhuizen and T. Piersma (1999). Experimentally extending the spawning season of a marine bivalve using temperature change and fluoxetine as synergistic triggers. Mar. Ecol. Prog. Ser. 180: 297-300.

Kobayashi, M., E. E. Hofmann, E. N. Powell, J. M. Klinck and K. Kusaka (1997). A population dynamics model for the Japanese oyster, Crassostrea gigas. Aquaculture 149(3-4): 285-321.

Kooijman, S. A. L. M. (2000). Dynamic energy and mass budgets in biological systems. Cambridge University Press, Cambridge: 424pp.

Langlet, D. (2002). Enregistrement à haute fréquence des conditions environnementales par les tests de Bivalves - Application des techniques de marquage, cathodoluminescence, et chimie à l'huître creuse *Crassostrea gigas* de l'étang de Thau (Hérault, France). Thèse, Géologie Sédimentaire, Univ. P. & M. Curie, Paris: 231 pp.

Quayle, O. B. (1969). Pacific oyster culture in British Columbia. Fish. Res. Board Can. Bull. 168: 68pp.

Reise K., S. Gollasch, and W. J. Wolff (1998). Introduced marine species of the North Sea coasts. Helgolander Meeresunters., 52: 219-234.

Sinclair, M. 1988. Marine populations. An essay on population regulation and speciation. University of Washinghton Press, Seattle. 252 pp

Thorsen, A. and O. S. Kjesbu (2001). A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. Journal of Sea Research 46(3-4): 295-308.

Wehrmann, A., M. Herlyn, F. Bungenstock, G. Hertweck and G. Millat (2000). The distribution gap is closed -- first record of naturally settled Pacific oysters *Crassostrea gigas* in the East Frisian Wadden Sea, North Sea. Senckenbergiana Maritima 30: 3-6.

Wilkinson, L. (1996). SYSTAT: the system for statistics. SYSTAT Inc. Evanston, Illinois.

Wolff, W.J. (1999). Exotic invaders of the meso-oligohaline zone of estuaries in the Netherlands: why are there so many? Helgolander Meeresuntersuchungen 52: 393-400.
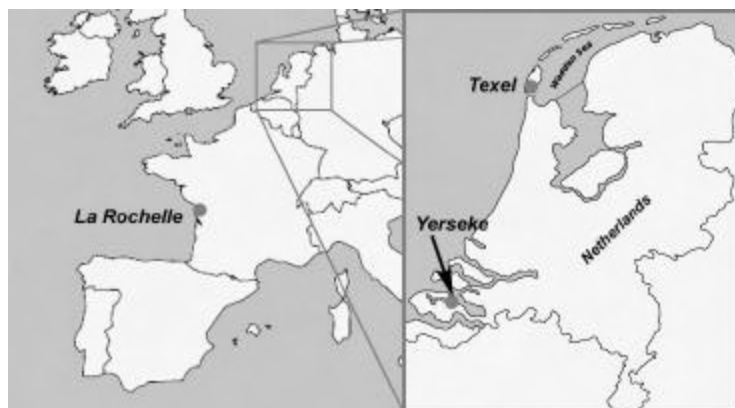
Figures and Tables

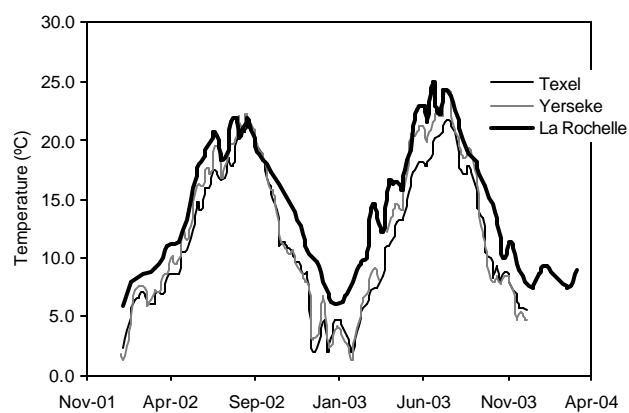**Fig. 1.** Map showing the sampling stations in the Netherlands and France.

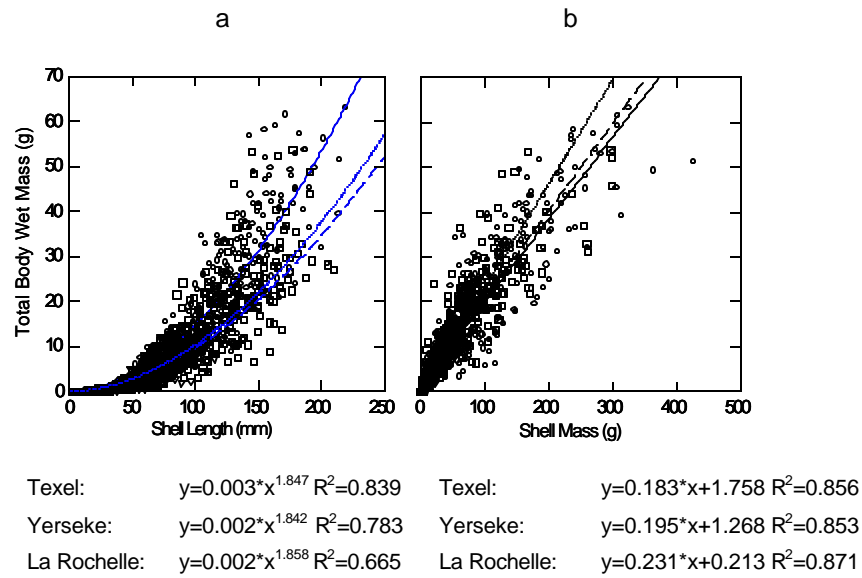**Fig. 2.** Water temperature (in ºC) at the three locations from January 2002 to March 2004.

Texel:	$y=0.003*x^{1.847}$ $R^2=0.839$	Texel:	$y=0.183*x+1.758$ $R^2=0.856$
Yerseke:	$y=0.002*x^{1.842}$ $R^2=0.783$	Yerseke:	$y=0.195*x+1.268$ $R^2=0.853$
La Rochelle:	$y=0.002*x^{1.858}$ $R^2=0.665$	La Rochelle:	$y=0.231*x+0.213$ $R^2=0.871$

Fig. 3. Total body wet mass (g) plotted against shell length (mm) (a) and plotted against shell mass (g) (b) for the three locations with fitted regression lines (Texel – circles and continuous line, Yerseke – squares and broken line, La Rochelle – triangles and dotted line).
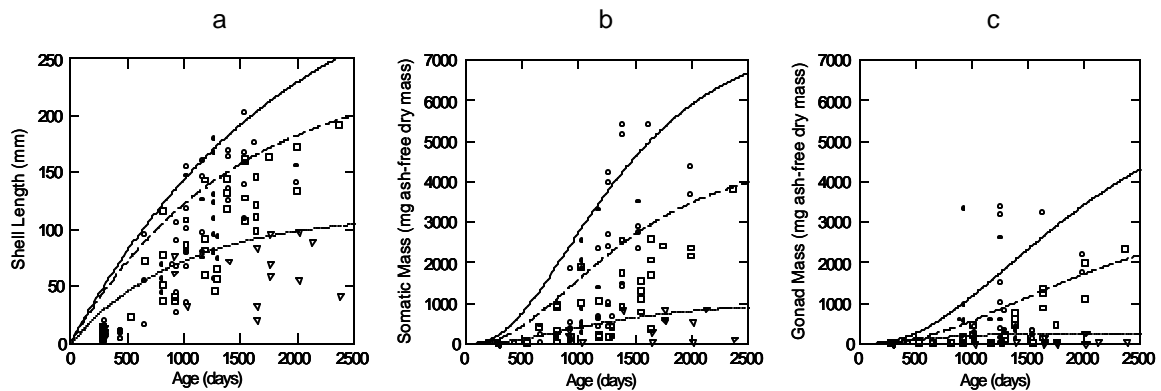

Fig. 4. Shell length (mm) (a), somatic ash-free dry mass (mg) (b) and gonad ash-free dry mass (mg) (c) of C. gigas plotted against age (days), with fitted Von Bertalanffy growth curves for maximum growth (Texel – circles and continuous line, Yerseke – squares and broken line, La Rochelle – triangles and dotted line).
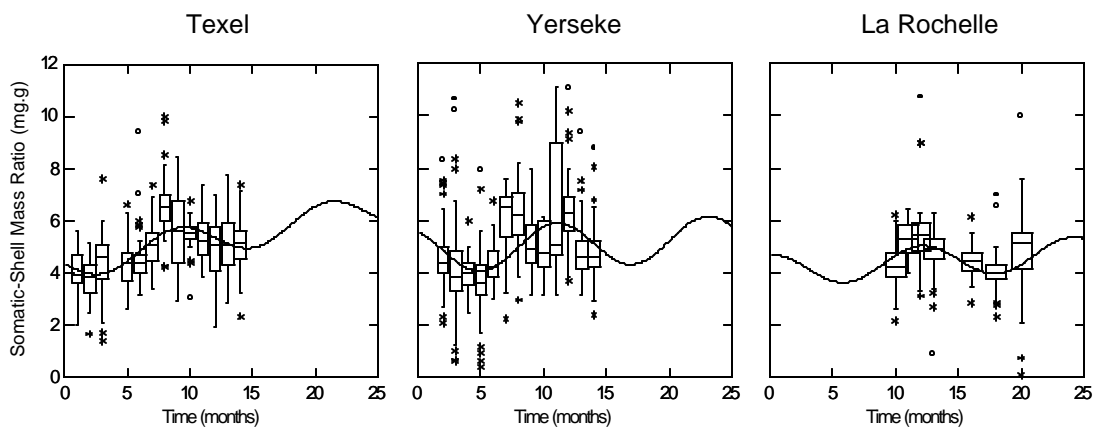
Fig. 5. Somatic-Shell mass ratio (mg.g^{-1}) plotted against time (in months) for the three stations. Original values were square root transformed. Month 1 is October 2002. Logistic curves are fitted to the data.
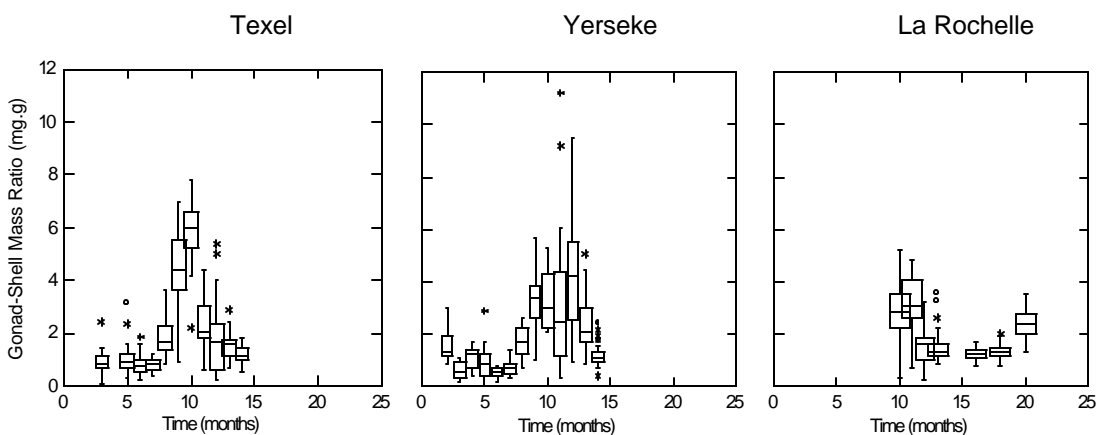

Fig. 6. Gonad-Shell mass ratio (mg.g^{-1}) plotted against time (in months) for the three stations. Original values were square root transformed. Month 1 is October 2002.
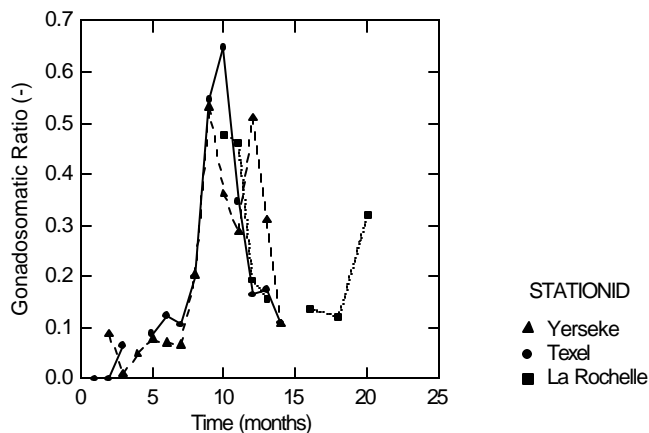

Fig. 7. Gonadosomatic ratio (-) plotted against time (months) for each location. Original values were square root transformed. Month 1 is October 2002.
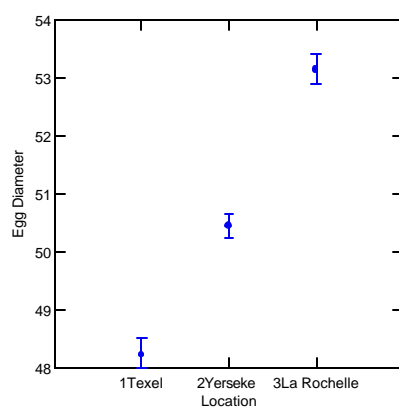
Fig 8. Mean egg diameter (μm) of *C. gigas* females from each location, after induced spawning in the lab. Error bars are ±SE.

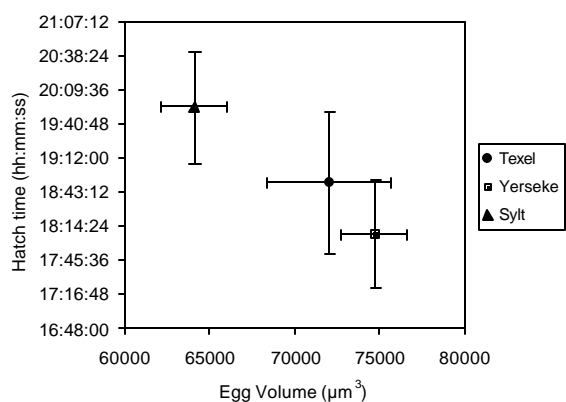**Fig. 9.** Hatch time (hh:mm:ss) plotted against egg volume (μm^3) for *C. gigas* from Yerseke, Texel and Sylt. Error bars are ±SD.

Table 1. Parameters of the Bertallanfy's growth curve for maximum growth in shell length, somatic mass and gonad mass for the three locations.

Location	Sum of Squares	df	Mean Square	R^2	Lmax	k
Length-at-age						
Texel	1281.452	5	256.290	0.978	356.824	0.515
Yerseke	1033.399	3	344.466	0.963	242.172	0.703
La Rochelle	368.656	4	92.164	0.947	113.165	1.021
Somatic mass-at-age						
Texel	3695817.235	8	461977.154	0.935	19.756	1.233
Yerseke	345564.442	3	115188.147	0.969	16.669	1.203
La Rochelle	15051.807	4	3762.952	0.977	10.033	1.307
Gonad mass-at-age						
Texel	1428700.904	6	238116.817	0.937	18.617	0.830
Yerseke	575234.500	10	57523.450	0.968	15.080	0.791
La Rochelle	1934.035	2	967.018	0.973	6.350	2.427

Table 2. Analysis of variance of the Somatic-Shell mass ratio (mg.g) of *C. gigas* from October 2002 to April 2004, after square root transformation.

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

Table 3. Analysis of variance of the Gonad-Shell mass ratio (mg.g) of *C. gigas* from October 2002 to April 2004, after square root transformation.

Source	Sum of Squares	df	Mean Square	F	*p*
Location	6.447	2	3.224	1.619	0.199
Month	0.539	1	0.539	0.271	0.603
Peak months	296.585	1	296.585	148.940	<0.001
Location*Month	13.117	2	6.559	3.294	0.038
Location*Peak	18.790	2	9.395	4.718	0.009
Error	1499.452	753	1.991		

Table 4. Nested analysis of variance of the egg size of *C. gigas* females, from the three locations, after induced spawning in the lab.

Source	Sum of Squares	df	Mean Square	F	*p*
Location	3209.202	2	1604.601	77.273	<0.001
Female(Location)	8716.073	55	158.474	7.632	<0.001
Error	32414.669	1561	20.765		