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Immunological *in situ* determination of *Pecten maximus* larvae and their temporal distribution

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An immunological technique has been developed, which discriminates the *Pecten maximus* larvae among all other species present in different plankton samples from Brest Bay. This tool was used on each plankton sample to target the temporal distribution of the larvae between summer 1997 and summer 1998. The least abundant period for larvae from April to July 1998, was confirmed and also identified *P. maximus* which would have been very difficult to recognize with only the help of the analysis of prodissoconch shape. Multi micro-cohort larvae principally from April to July 1998 corresponded probably with as many spawning events.

INTRODUCTION

Many infra-littoral species of pectinids are exploited commercially, constituting an important economic resource, as in the case of *Placopecten magellanicus* in Canada (Tremblay et al., 1987). Four species are commercialized in France: *Aequipecten opercularis*, *Chlamys varia*, *Pecten jacobaeus* and *Pecten maximus*. In Brittany, Brest Bay harbours a population of *P. maximus* that has been exploited since the beginning of the 20th Century. The studies of Priol (1930) and Faure (1966), as well as the more recent work of Boucher & Fifas (1995) based on fishing data, show inter-annual variations in abundance, which are irregular and independent of fishing demands. They are frequently for most pectinids exploited on a global scale, which is generally regarded as a disadvantage for recruitment (Conan & Shafee, 1978; Ansell et al., 1991; Dickie, 1995; Masso Rojas, 1995; Paulet et al., 1997). All events likely to interfere with the settling of new generations on gamete emission sites can disturb the recruitment of a species. Thus, in recruitment analysis, it is essential to monitor the development of organisms from the time of emission into water masses until settling in sites favourable to their benthic development. Most research on bivalve larvae is conducted in laboratories or hatcheries and not in the sea. These results, though not easily applicable to the natural environment, have improved our understanding of this stage of the biological cycle. Larval development is not easy to study in the sea, particularly because of the difficulty of recognizing individuals specifically and the impossibility of localizing them throughout the period of their planktonic existence. In France, the Programme National sur le Déterminisme du Recrutement (PNDR), initiated in 1985, is intended to unify research capabilities to achieve a better understanding of the mode of recruitment of several marine species, including *P. maximus*. The purpose of the present study, within the scope of PNDR objectives, was to use an immunological tool developed by Paugam et al. (2000) for rapid, specific identification of *P. maximus* larvae in plankton samples and with it to survey the annual distribution of scallop larvae within a natural environment (Brest Bay).

MATERIALS AND METHODS

Study site: Brest Bay

Between August 1997 and July 1998, plankton samples were obtained in the morning at four Brest Bay sites (Figure 1) using the IFREMER vessel 'Sainte Anne' and a rubber dinghy of the European University Institute of the Sea (IUEM). The sites: Sainte-Anne (48°21′N 4°33′W), Roscanvel (48°19′N 4°30′W), Ducs d'Albe (48°19′N 4°27′W) and Lanvéoc (48°17′N 4°26′W) were chosen because they contain adult *Pecten maximus* and their hydrological features are monitored. The four sites were always sampled on the same days and in the same tidal conditions and within a time interval generally of less than 3 h. Sampling frequency was increased during the periods considered most favourable to the reproduction of this species, i.e. late summer and early autumn (end of August to mid-October), spring (April and May), and early summer (June and July) (Paulet et al., 1997). A total of 150 samples were collected during 39 surveys. As tidal currents are rapid and widespread in Brest Bay (SHOM sea chart no. 7400), it was necessary before each excursion to plan a plankton collection route to ensure the hydrologic independence of the samples taken in each of the four sites. This route was determined according to predictions based on the hydrologic model (IFREMER, Brest) for the circulation of water masses in Brest Bay (Salomon et al., 1995), so that the four samples collected during a given excursion always differed significantly in hydrological terms.

Pumping

For each sample, 100 l of seawater were collected in less than 5 min using a Flyght model immersible pump

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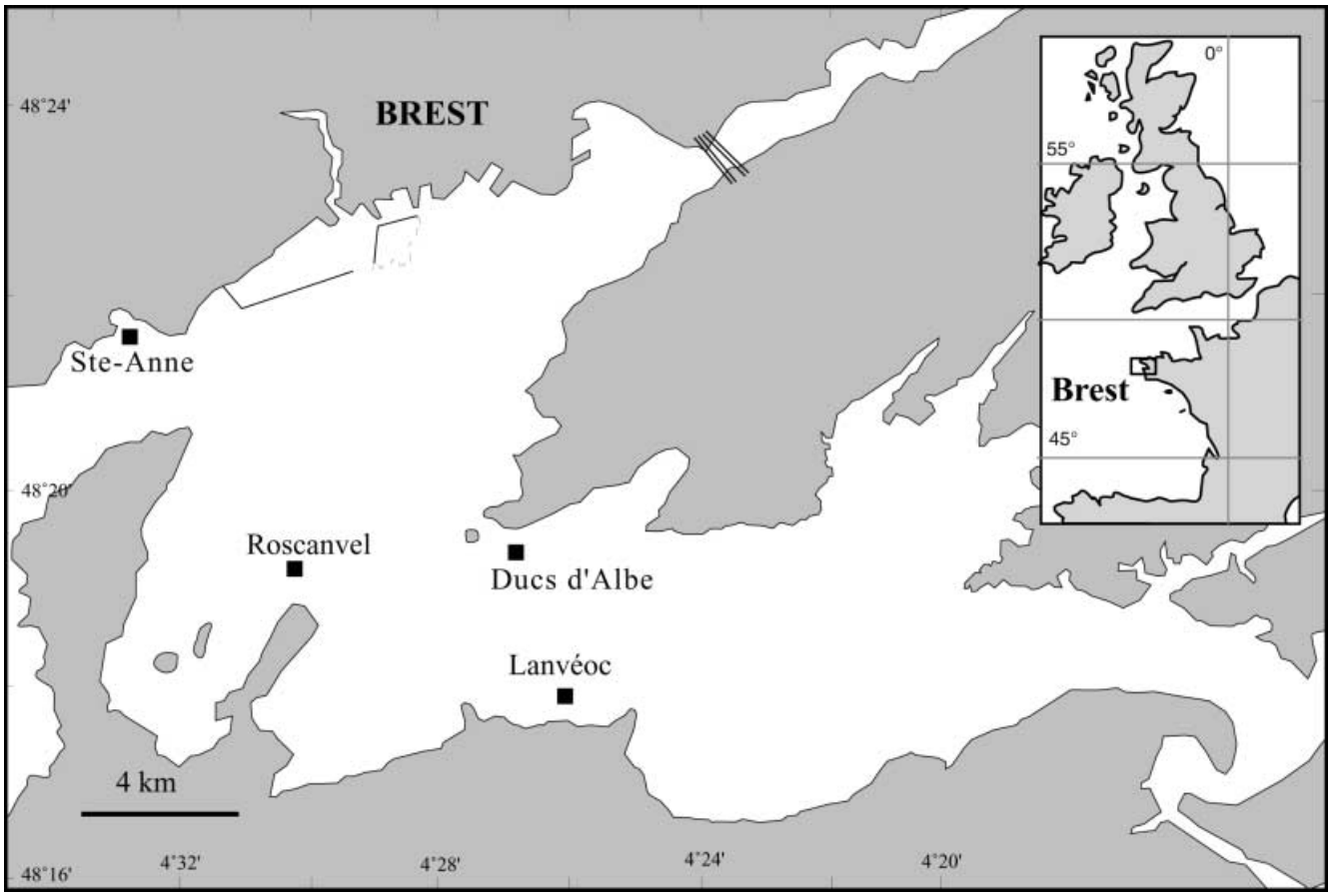

Figure 1. Distribution of the four plankton sampling sites: Ste-Anne, Roscanvel, Ducs d'Albe and Lanvéoc in the Bay of Brest.

(Tremblay et al., 1987). Pumping was performed for the entire water column by raising the pump regularly from the bottom to within a few centimetres of the surface. Plankton organisms were collected by filtering the water at the top of the column (25 cm in diameter). Only organisms between 60 and 300 μm were retained. Pump immersion depth was determined using a diving depth-meter (Scubapro NC 11). Water column height at the time of sampling was determined by the ship's sounding device.

Conservation of plankton organisms

Immunological detection requires suitable conservation of biological tissues, and the confirmation of species identification based on hinge observation in scanning electron microscopy (SEM) depends on the preservation of shell structures (Le Pennec, 1978). Accordingly, the planktonic organisms collected in the 60 μm filter were fixed with 70% alcohol and stored in a cold room (4°C) in darkness until use.

Sorting by density

This technique, based on that of Tremblay et al. (1987), was developed for rapid separation and elimination of elements with a density lower than that of the least dense *Pecten maximus* larvae. The lowest density of *P. maximus* D larvae, as estimated from monospecific batches supplied by the IFREMER Argenton hatchery (Brittany), was always greater than 1.30. Sorting involved careful sedimentation of all of the plankton organisms in a cushion solution with a density close to 1.30 to avoid damage to biological structures. After several attempts with different liquids, a commercial sugar cane syrup was finally chosen.

A first centrifugation of 300*g* of samples for 2 min allowed the plankton organisms to concentrate at the bottom of the tube (pellet 1). Supernatant 1 containing 70% alcohol was then separated from the rest of the sample. Pellet 1 containing the different plankton organisms was poured gently onto the surface of 20 ml of cane syrup in another

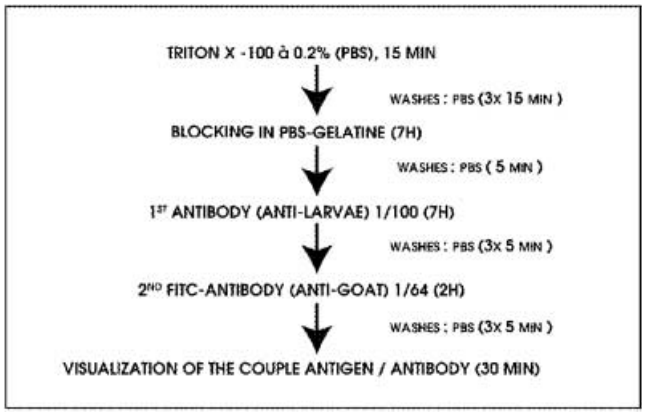

Figure 2. Summary of plankton sample preparation for fluorescence labelling of *Pecten maximus* larvae.

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centrifugation tube. Centrifugation for 2 min at 300*g* then allowed the plankton sample to be separated in three stages: supernatant 2 with a density lower than that of cane syrup, cane syrup containing elements of equal density, and pellet 2 containing elements with a density greater than that of cane syrup and including all bivalve larvae. Light microscopy controls showed no bivalve larvae in supernatants 1 and 2 or the cane syrup cushion, but essentially phytoplankton organisms and many shellfish.

Preparation of anti-larval antibodies

Anti-larval antibodies of *Pecten maximus* were prepared according to the protocol of Paugam et al. (2000), as modified by replacement of the rabbit model with a dwarf goat to obtain greater serum volume. The immunization protocol was the same, except that the amounts of injected antigens were doubled.

Antibody recognition

Pellet 2 (generally 1–2 ml) was stirred in a vortex with 20 ml of phosphate-buffered saline (PBS) for 5 min and then re-concentrated by light centrifugation for 2 min at 300*g*. Repetition (3 times) of this procedure removed the cane syrup in pellet 2 by dilution of the sugar in PBS. The protocol for antibody application (Figure 2) was a simplified version of that used by Paugam et al. (2000). The membranes of the different organisms were permeabilized with Triton X-100 0.2% in PBS for 15 min. The organisms were then washed in three successive 15 min baths in PBS (under vigorous vortex stirring). Non-specific sites were saturated in PBS+gelatin 3% overnight at 4°C. The organisms were washed in PBS before exposure to the action of goat IgG [anti-larvae of *Pecten maximus* diluted 1:100 in PBS for 2 days (minimum of 7 h)]. Three washes in PBS removed unbound antibodies. The organisms were then immersed for 2 h in a PBS-1% gelatin solution containing fluorescent [anti-goat fluorescein isothiocyanate

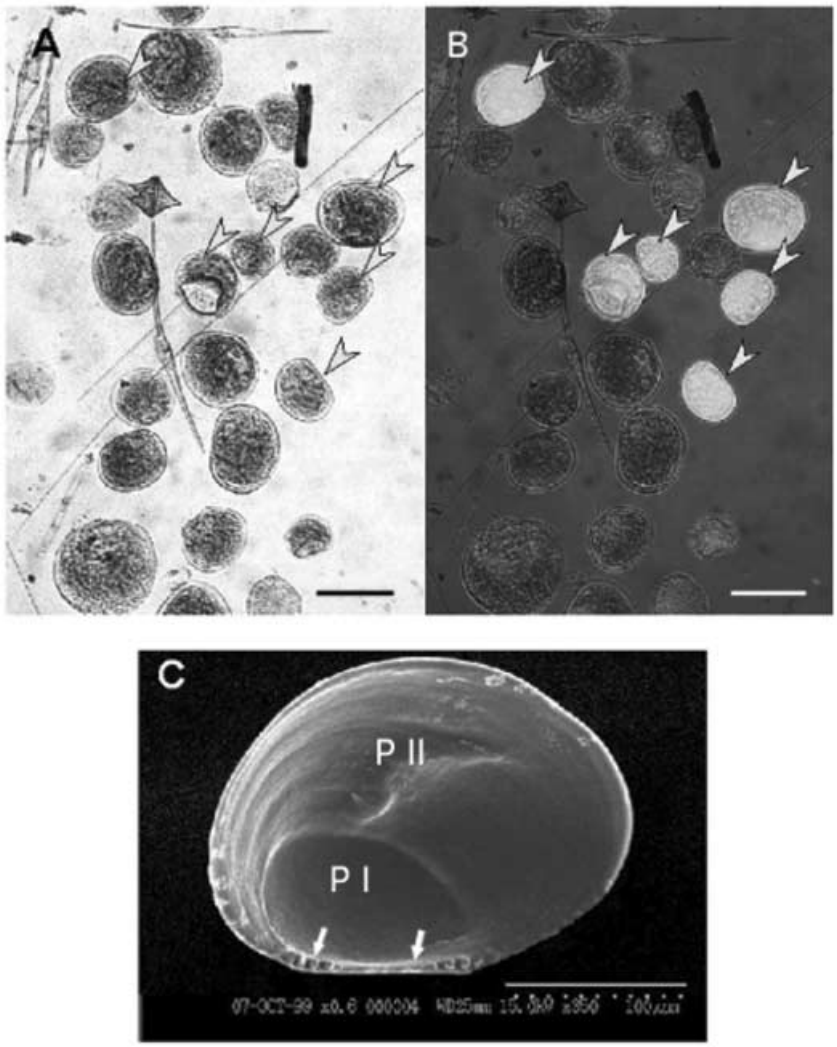

Figure 3. Bivalve larvae collected in Brest Bay. (A) Plankton sample treated with anti-larval antibodies of *Pecten maximus* observed in light microscopy (visible light); (B) same plankton sample as in A, but observed in epifluorescence microscopy under the excitation wavelength of the fluorescent chromophore (FITC) borne by anti-larval antibodies of *P. maximus*. Arrows indicate *P. maximus* larvae; (C) prodissoconch valve of *P. maximus* observed in SEM, showing hinge details (arrows). PI, prodissoconch I of the young veliger; PII, prodissoconch II of older umbonic larva. Scale bars: 100 μm.

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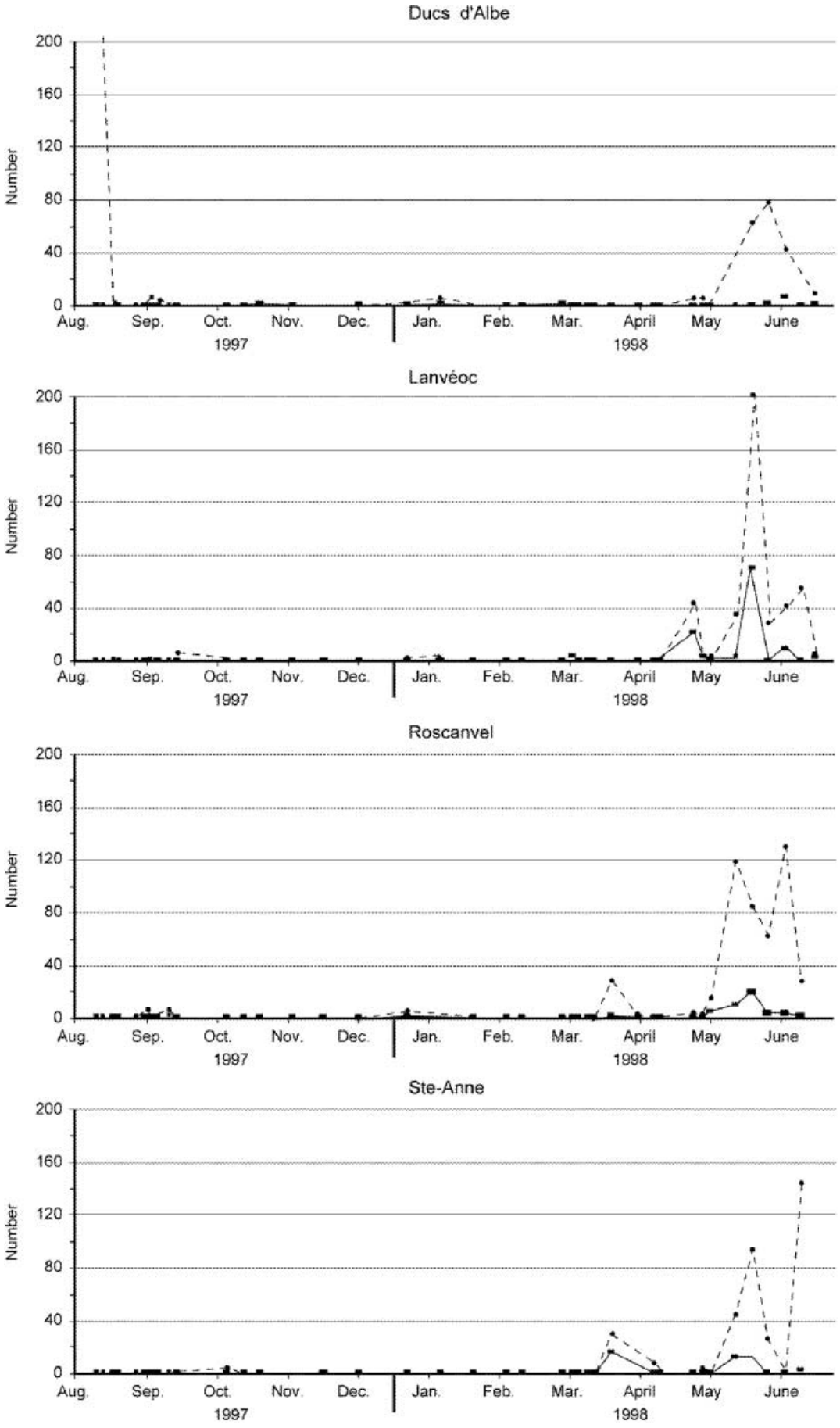

Figure 4. Distribution of larvae on the four sites according to time period. Dotted line: variations in the number of all bivalve larvae; black line: variations in the number of *Pecten maximus* larvae.

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Table 1. *Statistical analysis of inter-site distributions of bivalve larvae.*

	Kolmogorov–Smirnov test			
Bivalves	DN	K-S	P	Answer
Ducs/ Lanvéoc	0.363636	0.887625	0.415532	No difference
Ducs/Roscanvel	0.371212	0.889294	0.413042	No difference
Ducs/Ste-Anne	0.363636	0.852803	0.469984	No difference
Lanvéoc/Roscanvel	0.307692	0.768615	0.59594	No difference
Lanvéoc/Ste-Anne	0.307692	0.751068	0.62537	No difference
Roscanvel/Ste-Anne	0.280303	0.671507	0.757991	No difference

Table 2. *Statistical analysis of inter-site distributions of* Pecten maximus *larvae.*

	Kolmogorov–Smirnov test			
Pecten maximus	DN	K-S	P	Answer
Ducs/Lanvéoc	0.769231	1.96116	0.0009125	Difference
Ducs/Roscanvel	0.769231	1.92154	0.0012415	Difference
Ducs/Ste-Anne	0.769231	1.87767	0.0017326	Difference
Lanvéoc/Roscanvel	0.538462	1.34508	0.0536483	No Difference
Lanvéoc/Ste-Anne	0.636364	1.55334	0.016041	Difference
Roscanvel/Ste-Anne	0.636364	1.5245	0.0191537	Difference

Table 3. *Statistical analysis of intra-site distributions of* Pecten maximus *larvae relative to those of all bivalve larvae.*

	Kolmogorov–Smirnov test			
Pecten/Bivalve	DN	K-S	P	Answer
Ducs	0.769231	1.87767	0.0017326	Difference
Lanvéoc	0.538462	1.37281	0.0461399	No difference
Roscanvel	0.5	1.22474	9.95E-02	No difference
Ste-Anne	0.636364	1.49241	0.0232512	Difference

(FITC)] antiglobulins diluted 1:64. Three successive baths in PBS removed the excessive antiglobulin. The fluorescent antigen–antibody pairs were detectable in epifluorescence microscopy.

Larval counts

Plankton samples previously treated with anti-larval antibodies of *Pecten maximus* were distributed in 5-ml Petri dishes and observed under an epifluorescence stereomicroscope (Leica MZ-FL III). All bivalve larvae displaying fluorescence emission were counted, separated from the rest of the plankton, and observed individually in detail under an epifluorescence microscope (Olympus T70) equipped with a video camera (Sony DXC-107) and a micrometer eyepiece (to approximately 0.005 mm). Measurements were determined for the three dimensions classically used with bivalve larvae: length (L), height (H), and hinge (Hi) length (Rees, 1950; Chanley & Andrews, 1971; Le Pennec, 1978; Salaün, 1994). The number of non-fluorescent larvae was counted exhaustively for 123 samples between 19 September 1997 and 2 July 1998. For the 27 samples, 29 August to 19 September 1997, the number of non-fluorescent larvae was only estimated (and not presented in Figure 4). In each case some of those larvae (chosen at random) were separated from the rest of the plankton for identification under the SEM.

Statistical analysis

As the majority of *Pecten maximus* larvae were found between spring and summer 1998 we reduced our statistical analysis on this restricted period. The homogeneity of larval distributions was evaluated by means of the Kolmogorov–Smirnov non-parametric test, with a 95% threshold. Both inter- and intra-site distributions were analysed. To represent the distribution of measurement values for *P. maximus* larvae, it was necessary to cumulate the numbers for the four sites by date and to assume that all individuals captured were originally from Brest Bay.

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Table 4. *Global analysis of the size distribution of* Pecten maximus *larvae collected in the four sampling sites. Distribution of larvae in 19 groups (g1–g19) are presented, according to three morphological measurements: prodissoconch length and height and hinge length.*

Length (μm)	Height (μm)	Hinge (μm)	No.	
70	70	70	1	g1
80	70	70	4	g2
90	70	70	19	g3
100	80	70	6	g4
100	90	70	12	g5
110	90	70	114	g6
120	90	80	22	g7
120	100	80	3	g8
130	100	90	1	g9
140	100	90	1	g10
150	110	90	7	g11
160	120	90	2	g12
160	150	90	1	g13
210	180	90	1	g14
230	200	90	1	g15
230	220	100	2	g16
240	220	100	3	g17
270	250	100	5	g18
300	220	100	4	g19

This procedure enabled us to graphically analyse the distribution of all *P. maximus* larvae collected in the four sampling sites, as a function of increases in their measurements the overall distribution of scallop larvae by size category and to perform a modal breakdown as suggested by Comtet (1998). The modes retained as representative of the main size categories among which the larvae were distributed were intended to satisfy a normal law (tested using Statgraphic software). These modes allowed us to distribute all *P. maximus* larvae as a function of morphologic criteria and to plot a graph indicating the distribution of all *P. maximus* larvae captured in the Bay of Brest in terms of their morphology. A qualitative analysis was performed in terms of cohorts on the basis of this data in accordance with following principles. Firstly, each size category was assumed to correspond to a particular biological period of the larval phase of *P. maximus*. Secondly, any presence of *P. maximus* larvae detected after an absence of plankton for 5 days in the case of D larvae and for 10 days in the case of young umbonic larvae was regarded as the emergence of a new larval cohort (after hatchery control from Nicolas, 1999). Thirdly, larvae can remain in the same category for several days, move into the category above or disappear (change in morphological parameters in bivalve larvae with time are always positive or at least equal to 0 as suggested by Salaün, 1994).

Scanning electron microscopy

A sub-sample of 50 larvae was taken at random from all those immuno-identified as belonging to *Pecten maximus* for hinge observation in SEM according to the protocol of Salaün et al. (1991). One valve per larva was glued on the observation block. A second sub-sample of 100 non-immuno-identified larvae was also taken at random from all non-labelled larvae for morphologic identification in light microscopy and hinge observation in SEM.

RESULTS

Observation of bivalve larvae by various microscopic techniques

Among bivalve larvae counted in visible light (Figure 3A), those emitting a strong fluorescence signal were identified as *Pecten maximus* (Figure 3B). In the 150 plankton samples analysed, 227 bivalve larvae were fluorescent. Among all these larvae, a sub-sample of 50 larvae chosen at random was subjected to the preparatory protocol for electron microscopy. In 37 cases, hinge could be analysed and confirmed that they were *P. maximus* (Figure 3C). Larvae not identified by antibodies and analysed in SEM showed no features of *P. maximus*, but essentially those of other families such as mytilids, venerids, and ostreids.

Numbers of bivalve larvae
Variations over the entire sampling period

One thousand eight hundred and thirty-nine individuals were counted. The distribution of larvae according to time period (Figure 4) indicated that the highest numbers ($N > 10$) were found mainly in a minority of samples collected in August and September and between April and July. Ninety-one of the 150 samples contained bivalve larvae, including 35 with *Pecten maximus* larvae.

Bivalve larvae

Bivalve larvae were captured throughout the sampling period (at all sampling sites), but the numbers of larvae obtained were greater during certain periods, mainly in the late spring (Figure 4). Between mid-May and the beginning of July, ten or more individuals were generally collected per site (often simultaneously at the four sites). Between late August and mid-September 1997, many larvae were captured at all four sites (approximately $N > 30$ and not represented in Figure 4). Bivalve larvae appeared and disappeared simultaneously from the four sites. However, the amplitude and tendency of numerical variations during the periods of their presence differed among the sites. Larvae collected on the Lanvéoc, Roscanvel and Sainte-Anne sites between spring and summer 1998 showed three peaks of greatest abundance that were not simultaneous from one site to another.

Pecten maximus *larvae*

Scallop larvae were captured at all sampling sites (Figure 4). The highest numbers were collected during the spring of 1998, mainly in May–June. The counts and variations differed among the four sites. Only samples obtained on 5 June 1998 showed an appreciable simultaneous increase in the number of *P. maximus* larvae at the Lanvéoc, Roscanvel and Sainte-Anne sites. Except for this date, the variations in counts were subject to conditions at each sampling site. For the period between 19 September 1997 and 26 June 1998, isolated *P. maximus* larvae (for a particular site and date) appeared at any time, but were more often captured at Ducs d'Albe and Roscanvel. During the study period, *P. maximus* larvae only rarely constituted

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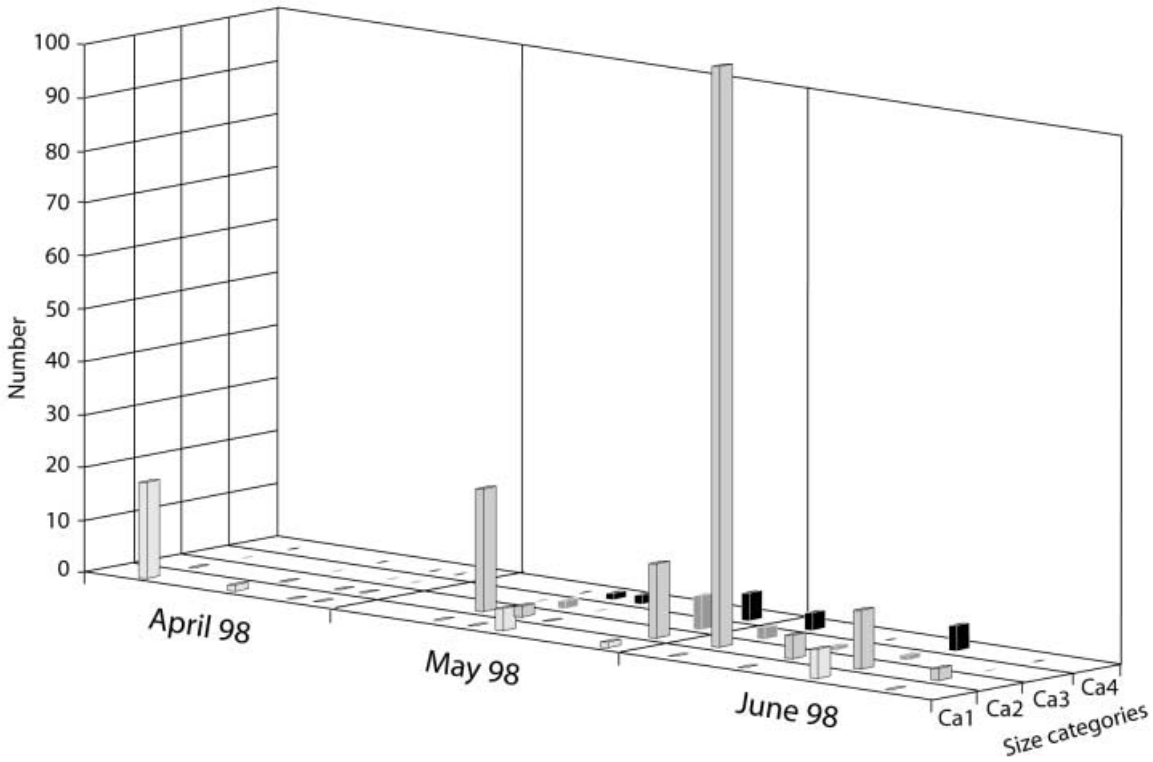

Figure 5. Distribution of *Pecten maximus* larvae by size categories, Ca1, Ca2, Ca3, Ca4, as a function of time. In the 19 groups (g1–g19) represented in Table 5, four size modes have a normal distribution (Statgraphic software) and form the four categories: Ca1, larval number of groups 1–4; Ca2, groups 5–8; Ca3, groups 9–13; Ca4, groups 14–19.

the totality of bivalve larva counts (in winter when few larvae exist).

Variations in larva counts between early spring and early summer

The period from 6 April to 2 July 1998 accounted for 92% (209/227) of the total number of *Pecten maximus* larvae collected during 13 sampling dates at the four sites. The homogeneity of distribution of the larvae within and between sites was analysed statistically.

Inter-site distribution

During this period, the inter-site distributions of bivalve larvae were not statistically different at the 95% threshold (Table 1). The inter-site distributions of *Pecten maximus* larvae were statistically different, except between Roscanvel and Lanvéoc at the 95% threshold (Table 2).

Intra-site distribution of Pecten maximus *larvae compared to that of other bivalves*

The results for the Kolmogorov–Smirnov test are indicated in Table 3. As the distributions of *Pecten maximus* larvae differed statistically at the Ducs d'Albe and Sainte-Anne sites, but not at the Lanvéoc and Roscanvel sites, there was a spatial variability in the specific composition of bivalve larva populations. This analysis confirms the general heterogeneity of the spatial distribution of populations of *P. maximus* larvae throughout the sampling period.

Measurement of Pecten maximus *larvae*

Between late spring and early summer, length, size and hinge measurements were performed on a total of 209 *Pecten maximus* larvae collected at the four sites. Table 4 shows the distribution of these larvae according to increasing measurement values. *Pecten maximus* larvae were distributed into 19 groups (1 to 19). The distribution of scallop larvae according to their measurements values was not homogeneous, as some size groups were represented more than others. A modal breakdown (Comtet, 1998) of the distributions by group was performed graphically. Four size modes (groups 1–4, 5–8, 9–13, and 14–19) satisfied a normal law, constituting four categories corresponding respectively to Ca1, Ca2, Ca3, Ca4 and also respectively to those of Nicolas (1999) (D larvae; young umbonic larvae; older umbonic larvae and pediveliger larvae) to describe the larval life of scallop in hatchery. These categories allowed the size distribution of *P. maximus* larvae (Figure 5) in Brest Bay to be represented temporally. The populations determined for each of the four sites were cumulated by sampling date. During the study period, at least one representative of each category was found at each of the four sampling sites. The 30 larvae in Ca1 were found at six different dates: 6 and 17 April, 18 and 29 May, 19 June, and 2 July; the 152 in Ca2 at eight different dates: 11, 15 and 29 May, 6, 12, 19 and 26 June, and 2 July; the 11 in Ca3 at six different dates: 15 and 29 May, 5, 12 and 19 June, and 2 July; and the 16 in Ca4 at five different dates: 15, 18 and 29 May, and 5 and 19 June.

Cohorts

Although interpretation was difficult because of the low numbers of *Pecten maximus* larvae captured, qualitative analysis was performed in terms of cohorts (Table 5) on

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Table 5. *The four categories of captured larvae, Ca1–Ca4, were distributed in terms of cohorts, Co1–Co6, based on the fact that in Ca1 the* Pecten maximus *larval residence time was less than five days, which is the duration observed in hatchery.*

Cohorts	Size categories	06/04/98	17/04/98	24/04/98	27/04/98	11/05/98	15/05/98	18/05/98	29/05/98	05/06/98	12/06/98	19/06/98	26/06/98	02/07/98
Co1	Ca 1	18	1											
	Ca 2													
	Ca 3													
	Ca 4													
Co2	Ca 1													
	Ca 2					22	2							
	Ca 3						1							
	Ca 4						1	2	5					
Co3	Ca 1							4						
	Ca 2								13					
	Ca 3								6	2				
	Ca 4									3		1		
Co4	Ca 1								1					
	Ca 2									98	4			
	Ca 3										1	1		
	Ca 4											4		
Co5	Ca 1											5		
	Ca 2											10	2	1
	Ca 3													1
	Ca 4													
Co6	Ca 1													1
	Ca 2													
	Ca 3													
	Ca 4													

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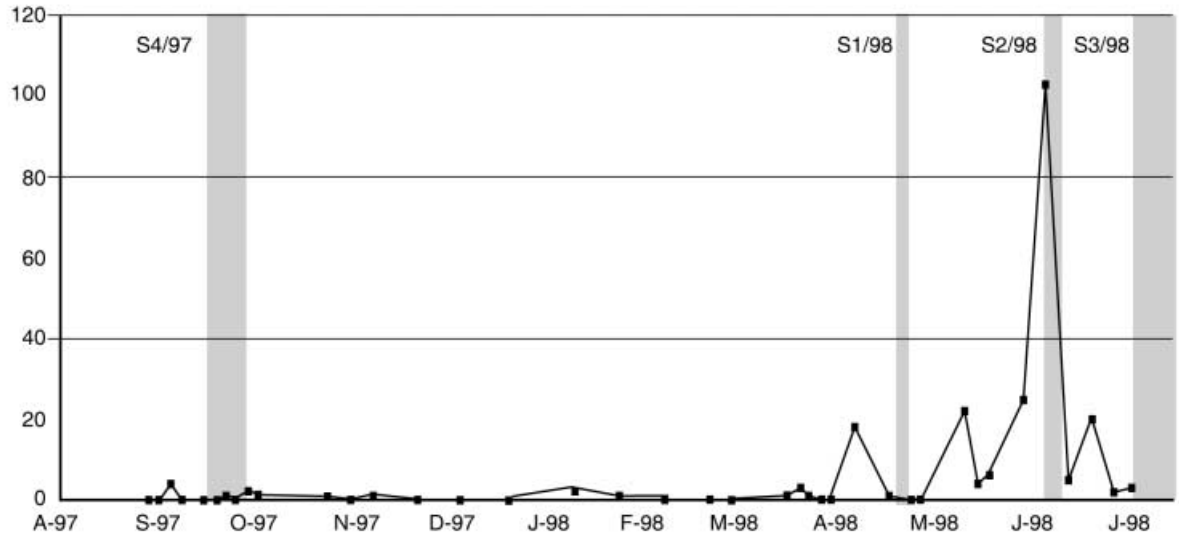

Figure 6. Simultaneous monitoring of the four gamete emission periods for *Pecten maximus* in Brest Bay, S1/98 (20–22 April 1998), S2/98 (4–11 June 1998), S3/98 (1–15 July 1998), S4/97 (16–30 September 1997), as observed by Paulet, University of Western Brittany (unpublished data), and larval populations of this species (number of larva) between August 1997 (A-97) and July 1998 (J-98).

the basis of data in Figure 5. Interpretation of the successive episodes of larval cohorts allowed residence time in plankton to be calculated for some of these cohorts. Each size category (Cal−Ca4) corresponded to a portion of the larval life of the scallop. In hatchery, the time spent in Cal is less than five days (Nicolas, 1999) and, consequently, each new apparition of Cal larvae *in situ*, after five days of absence, was considered as a new cohort. This allowed differentation of six cohorts in the sampling sites. The first was detected on 6 April, but could not be traced in later samples. The second (Co2), was detected in Ca2 on 11 May and had probably been spawned after 27 April. It disappeared after 18 or 29 May. Cohort 3 (Co3), was first detected in Ca1 on 18 May and disappeared between 5 and 12 June (minimum 18 days, maximum 25 days). Cohort 4 (Co4), appeared on 29 May and disappeared between 19 and 26 June (minimum 21 days, maximum 28 days). Only the appearance dates can be indicated for cohort 5 (Co5), after 12 June and cohort 6 (Co6), after 26 June.

DISCUSSION

Fluorescent immunological labelling

Two hundred and twenty-seven fluorescent *Pecten maximus* larvae were detected by anti-larval antibodies. Verifications by classical and scanning electron microscopy confirmed the specific identification of all of them. Ten of those larvae showed a deep notch on the anteroventral edge of prodissoconch II, they would be automatically separated from the *P. maximus* larvae with the only criteria of morphological speciation. Salaün (1994) reported a similar deformation in hatchery for *P. maximus* larvae. These elements suggest that in natural conditions the abnormal larvae could rarely have been identified specifically with the only criteria of morphological speciation. The main morphological abnormalities for the *P. maximus* larvae were shown between late May and the beginning of June 1998 when the presence of *P. maximus* larvae were most largely represented in the plankton population. This correlation reveals that it is easier to sample abnormal scallop larvae when this species is more abundant in the plankton. Separation of the organisms of the 150 samples by density grading required eight days of work by two persons (8 h/d). The immunological method used (Paugam et al., 2000) then allowed a single person to count the *P. maximus* larvae in the 150 samples within less than 10 days (8 h/d).

Bivalve larvae and their natural environment

Just before summer, the reproduction process intensifies, and a larger number of larvae of all species are found in the water. In these favourable conditions, inter-site distributions were not statistically different. The general, simultaneous detection of bivalves at these sites in spring corresponds to the period at which watercourses entering Brest Bay carry more nutrients, particularly nitrogenous ones conducive to the primary phytoplankton production that feeds numerous benthic species and stimulates the ecology of the bay (Delmas, 1981; Smayda, 1992).

Pecten maximus *larvae*

Numbers

The distribution of *Pecten maximus* larvae within and between sites was not uniform throughout the study, which confirms the general spatial heterogeneity of larval distributions of this species on an annual scale. The spatial heterogeneity of distributions was also apparent during the period in which *P. maximus* larvae were most abundant (between 6 April and 2 July 1998), except for the Lanvéoc and Roscanvel sites where the difference was not statistically significant (at the 95% threshold). A very close relation existed between these two sites, despite the heterogeneity of hydrological conditions, so that the presence and absence

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of *P. maximus* were generally synchronous. As these sites were best situated in terms of the highest densities of adults (Chauvaud, 1998), the simultaneous presence of larvae can be attributed to a sort of generalized, simultaneous breeding behaviour of adults in these two sites. At the same time, it is interesting to find a spatial heterogeneity in the distribution of *P. maximus* and not for the global distribution of all the bivalve larvae. This reality suggests that a steady spatial heterogeneity occurred in the specific richness of bivalve larvae in the Brest Bay scale.

Size categories

In our study, Ca1, D larvae, (N=30) were greatly under-represented as compared to Ca2, young umbonic larvae, (N=152). However, Ca1 larvae, by increasing the size of their valves, augment counts in higher categories. The behaviour of these larvae makes them less sensitive to pumping, and their growth rate is so rapid that the sampling process used was unable to detect them with the same probability as larvae in higher categories. The experiments of Cragg (1980) have shown that veliger larvae constitute the first stage with a true capacity to swim within the water column, whereas Ca1 includes most of the young larvae that have not yet developed maximal displacement speed (Cragg, 1980) and that they stay close to the bottom, thereby escaping the pumping process more easily. Only vertical mixing of the water can facilitate their capture. In hatchery, cultured larvae do not remain in this category for more than five days (Nicolas, 1999), a residence time generally shorter than the period between two samplings. Thus, these factors seem to account for the low numbers of larvae in Ca1 as compared to Ca2. The umbonic larvae of Ca2 (N=152) and Ca3 (N=11) represent the majority of individuals captured. These two categories correspond to larvae with greater swimming capacity, which no longer have nutrient reserves and must use their own means (planktotrophic behaviour) to compensate for daily energy consumption (Lucas et al., 1986; Le Pennec et al., 2002). This active search for food within the water column facilitates their capture. The pediveliger larvae of Ca4 (N=16) search for a substrate for settling and subsequent metamorphosis (Culliney, 1974; Cragg & Crisp, 1991; Dwiono, 1992). These larvae tend to stay close to the bottom because they gradually lose their locomotive velum, which makes them less likely to be captured. These observations, that we attribute to the larval swimming behaviour should be put together with the spatial heterogeneity in the specific richness of bivalve larvae, suggest that it is important to integrate the larval swimming behaviour and the turbulent flow in larval displacement in water to improve the accuracy of the meroplanktonic larvae dispersion model, as mentioned by Metaxas (2001).

Correspondence with the gonadosomatic index (GI)

The breeding periods for *Pecten maximus* in Brest Bay were determined by analysing variations in the gonadosomatic index (Paulet et al., 1997). Four significant breeding events were detected (Figure 6) during the study period (Y.M. Paulet, IUEM/UBO, personal communication): between 16 and 30 September 1997 (S4/97), 20 and 22 April 1998 (S1/98), 4 and 11 June 1998 (S2/98), and 1 and 15 July 1998 (S3/98). Spawning periods were ascertained for the entire bay, and the synchronization of spawning with the presence of *P. maximus* larvae in samples was checked. This analysis was performed by cumulating the counts at the four sites by sampling date. *Pecten maximus* larvae were captured mainly during 1998 between the first two periods of gamete emission (S1=20 to 22 April; S2=4 to 11 June). Between these two estimated spawning periods, five different cohorts were detected (Co 2 to Co 6) and seemed to indicate multi successive spawning events. The sampling procedure used allowed more spawning episodes to be detected than GI analysis would suggest. The few *P. maximus* larvae captured in winter corresponded to spawning events too inconspicuous to be identified by GI monitoring and indicates that reproduction takes place throughout the year and not only in the periods of massive gamete emission identified by monitoring the gonadosomatic indices of adults. Even if they were always sampled as D forms or young umbonic individuals (Ca1 and Ca2), we could question ourselves about the importance of those larvae on the final recruitment of the species. Only Co 2 can be roughly related to S1, in which case the approximate period of its presence in plankton (from the first gamete emission to the disappearance of the last larva in Ca4) was 28 to 40 days. This time scale is comparable to those estimated by Thouzeau (1989), Boucher & Dao (1990) and Chauvaud (1998) for the same species.

CONCLUSION

In the early 1990s, Demers et al. (1993) were the first to suggest the use of monoclonal antibodies to identify *Placopecten magellanicus* larvae. To our knowledge, this method was subsequently applied only once (Raby et al., 1994). The immunological approach is not the only conceivable choice for solving problems related to identification of the larvae of different bivalves. Research on the genetic identification of bivalve molluscs (Patwary et al., 1994; Claxton & Boulding, 1998; Hare et al., 2000) has shown that molecular markers can also provide specific recognition of the larvae of a given bivalve species. However, this approach, unlike the immunological tool, allows global determination (percentage of representation), which is inadequate to study the ecology of larvae in their natural environment. Paugam et al. (2000) suggested another immunological approach based on a principle of identification involving the recognition of specific proteins of *Pecten maximus* larvae. The feasibility of this new tool was demonstrated by the identification of *P. maximus* larvae in plankton samples from Brest Bay. Specific individual labelling of bivalve larvae allows monitoring of the individual growth and the identification of larval cohorts. The present study showed that the sera developed make these applications possible. It has allowed us to find some *P. maximus* larvae throughout the year, even in the middle of winter and though without notifying detectable spawning thanks to the help of the gonadosomatic index.

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