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IMPROVEMENT OF REMOTE SETTING OF THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*) ON FRENCH PLASTIC PIPES.

by

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ABSTRACT : The oysters culture in France is based on a natural spatfall, collected on cultches in setting areas. The collectors are then transfered on intertidal parcs for development during 1.5 to 3 years. The subsequent ongrowing stage is performed after separating the oysters and putting them in bags or by off-bottom cultivation. In order to improve the stock turnover and to improve the rearing conditions for the juveniles, experiments were performed to define optimal conditions for remote setting. After considering the previous studies a standard density of 2 000 larvae per cultch (plastic pipe) was selected. This corresponded to an average, theoretical density of 2.3 larvae.cm^{-2}. The resulting density, at the end of the growing stage should average 25 kg of oysters (6 pipes) per metre of trestle. This was considered as equivalent to what was assessed on this area, for the end of the on-growing stage in bags. The spats usually aggregate themselves in a extremity of the cultch, which may results in poor growing conditions. Among the factors which act on this aggregation, two of them were considered on the present work. The first was the horizontal distribution of the cultches in two layers, inside the setting tank and the second one, concerned the timing for the distribution of the larvae into the tank (50 % the first day, and 50 % the second day, just after turning the pipes upside down). The parametres analysed were the setting ratio and the spatial distribution of the spats on the pipes (extremities, centre, upper and lower sides). Best results were obtained with horizontally disposed pipes, in two layers. Distributing separate amounts of larvae at 24 hours intervals and returning the cultch, improved the setting.

RESUME : L'ostréiculture française est basée traditionnellement sur la captage de naissains de gisements naturels. Les collecteurs sont ensuite transférés sur l'estran pour une durée d'élevage de 1,5 à 3 ans. La croissance finale s'effectue généralement après avoir séparé les huîtres de leur support dans des poches posées sur des tables ou à plat sur le sol. Pour améliorer la rotation des cheptels et les conditions d'élevage des juvéniles, des expérimentations ont été réalisées afin de définir les conditions optimales du télécaptage. Après une analyse des différents travaux antérieurs, une densité de 2 000 larves par capteur (tube plastique) a été retenue. Le potentiel théorique de fixation est ainsi estimé à 2,3 larves.cm^{-2}. Le rendement attendu en fin d'élevage devrait être de 25 kg par mètre de table ostréicole, pour 6 tubes au mètre, soit un rendement équivalent à celui de l'élevage en poche. Lors de la fixation, les naissains sont généralement répartis de manière hétérogène sur les collecteurs ce qui engendre une forte variabilité de croissance. L'effort de recherche pour cette étude a essentiellement porté sur l'homogénéisation spatiale des huîtres sur la périphérie des tubes plastiques. Différents plans expérimentaux incluant des replicats ont été réalisés à échelle réduite (tubes de 0,5 m de longueur). Pour améliorer le rendement et l'homogénéisation de la fixation des larves sur toutes la périphérie des collecteurs, les conditions suivantes doivent être respectées :

- Disposition horizontale des collecteurs, en modules empilés selon 2 niveaux dans le bac,
- Fractionnement de la distribution des larves à raison de 50 % le premier jour et 50 % le 2ème jour, l'ensemble des collecteurs étant retourné entre ces deux jours.

INTRODUCTION

With an annual production of 140 000 tonnes of Japanese oysters, France ranked fourth among the world leading producers countries. Its main characteristic lies on the fact that all the production is devoted to the live market, or halfshell oysters. Therefore, attention is paid by oystermen to the form of the shell and the meat quality during the on-growing. The seed is almost entirely obtained from natural spat settlement in the field during Summer, the larvae being produced by protected beds of oyster, and by the

Table 1 : Changes in the average content in proteins (Lowry positive substances) and lipids of the paste of *Skeletonema costatum* kept refrigerated at 4°C. Standard deviations are reported within backets.

	D1	D2	D3	D4	D5
Average contents in protein (mg/g)	285.5 (27.9)	260.2 (14.0)	250.7 (7.1)	257.6 (23.1)	255.2 5.15)
Average contents in lipids (mg/g)	83.9 (7.7)	80.3 (3.9)	79.2 (11.4)	72.5 (9.7)	78.0 (2.0)

Effect of horizontal versus vertical disposal of the pipes.

The water temperature was kept at 25°C during 4 days. It was allowed to decrease down to the sea water temperature (19,5°C) during the last two days (fig. 1).

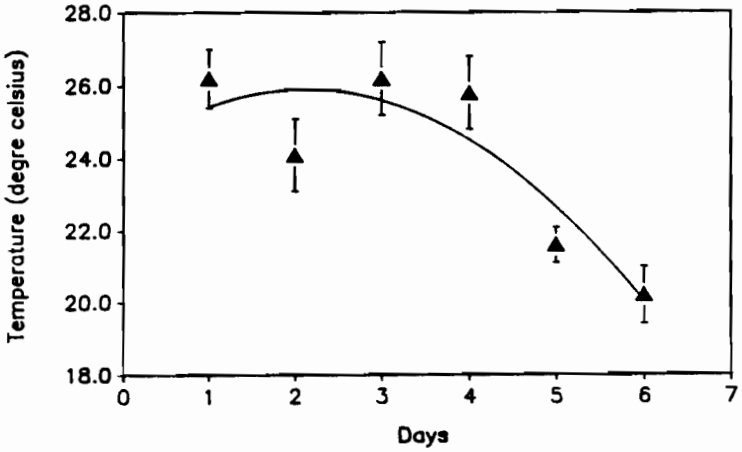

Figure 1 : Changes in the water temperature in the different tanks during the setting experiment. Vertical bars correspond to the confidence intervals (P = 0.95).

The success of setting was higher for the piped disposed horizontally (30 %) then for the pipes disposed vertically (21.4 %) as shown on fig. 2.

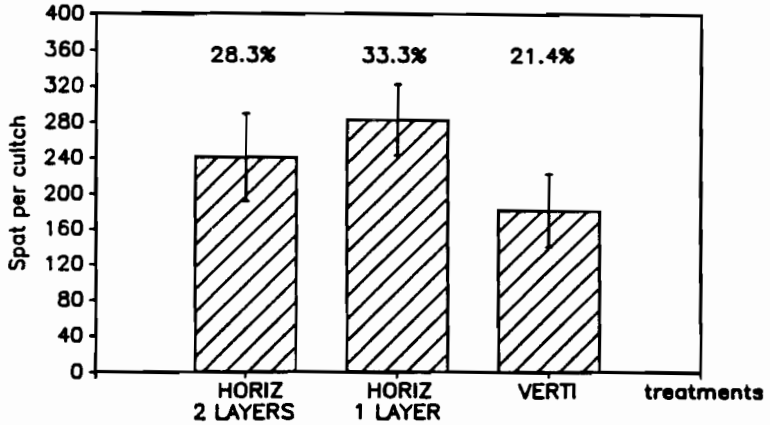

Figure 2 : Numbers of larvae per cultch and setting percentages for the three disposals

The setting was more abundant on the upper part of vertically disposed pipes, and on the lower part of horizontally disposed pipes (fig. 3).

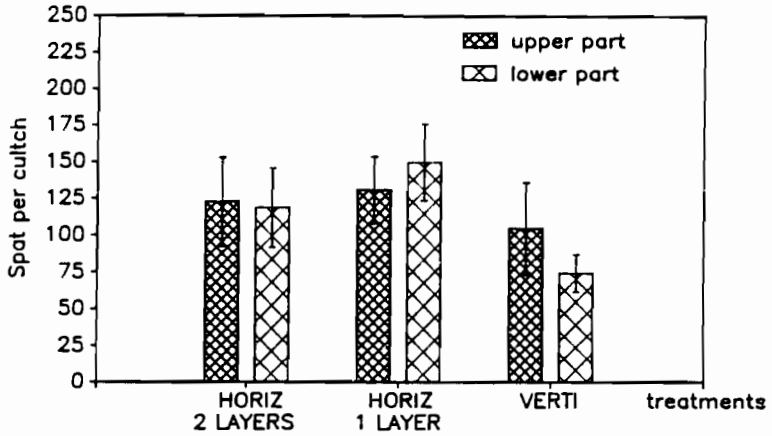

Figure 3 : Number of larvae set on pipes on their upper and lower circonference, for the different disposals. Vertical bars correspond to the confidence intervals (P = 0.95).

An ANOVA on the differential set around the pipes did not reveal any significant differences for the setting in upper and lower parts of the pipes. However, the differences between the treatment were still highly significant (table 2).

Table 2 : Analysis of variance on the number of larvae set per pipe. Controlled factors were the disposal of pipes and upper vs lower part of the pipes. NS = probability > 0.05.

Source of variability	Sum of squares	Degree of freedom	F ratio	Significant level
Main factors	14.94	215		
Factor 1 (treatments)	1.94	2	15.89	< 0.001
Factor 2 (high part/low part)	0.01	1	0.16	NS
Interaction 1, 2	0.15	2	1.24	NS
Residual	12.84	210		

Seeds were unevenly distributed along the pipes (fig. 4). The two extremities were always more intensively covered than the middle of the pipes.

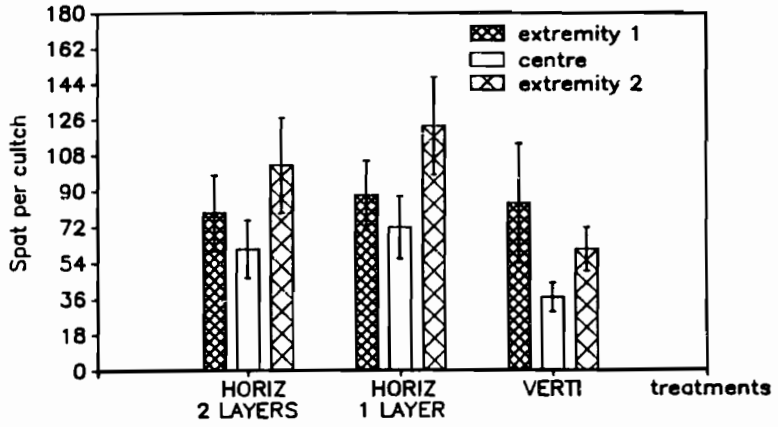

Figure 4 : Number of larvae set on middle and extremities of the cultch. Vertical bars correspond to the confidence intervals (P = 0.95).

The differences observed between the different parts of the pipes (table 3) were highly significant ($p < 0.001$). A significant interaction was also found between the factor disposal and the factor extremities ($P < 0.05$).

Table 3 : Analysis of variance on the number of larvae set per pipes. Controlled factors : disposal of pipes and differential set along the pipes (thirds).

Source of variability	Sum of squares	Degree of freedom	F ratio	Significant level
Main factors	29.05	314		
Factor 1 (treatment)	2.70	2	18.41	< 0.001
Factor 2 (thirds)	3.04	2	20.68	< 0.001
Interaction 1, 2	0.85	4	2.91	< 0.05
Residual	22.46	306		

Separate ANOVA for each modality of the factor disposal were performed, in order to obtain more informations on the differential setting along the pipes, for these modalities (table 4). These ANOVA have shown significant differences for the setting along the pipes for each modalities. These ones were ranked according to the decreasing values of the F-test. Higher differences in setting along the tubes were found for a vertical disposal ($F = 12.39$), then for a horizontal disposal on one layer ($F = 9.49$) and then for a horizontal disposal on two layers ($F = 6.00$).

Table 4 : Analyses of variance performed for each modality of the factor disposal, on the number of larvae set per pipe.

Source of variability	Sum of square	Degree of freedom	F ratio	Significant level
Main factor	9.38	107		
Factor 1 treatment	0.96	2	6.00	< 0.01
Residual	8.42	105		
Main factor	6.40	106		
Factor 2 treatment	0.99	2	9.49	< 0.001
Residual	5.41	104		
Main factor	8.90	106		
Factor 3 treatment	1.71	2	12.39	< 0.001
Residual	7.19	104		

A decrease in the number of larvae set on the central part of the pipes for every modality was revealed by a test of Newman Keuls. At the end of this experiment, disposing the cultch horizontally in one layer, appeared to be more efficient.

The effect of turning the cultch upside down and distributing the larvae in two times

Disposing the pipes in one layer should then have resulted in better setting. However, for all the experiment, the pipes were disposed horizontally in two layers. Economic considerations led to perform the following experiments with pipes disposed on two layers. 24 hours after the beginning of the experiment, the two levels were changed. At the same time, two rotations of the pipes were made, one which turned them upside down for each layer, and another which rotated them horizontally.

Another factor was tested. In spite of distributing all the larvae at the beginning of the experiment, they were given 50 % at the beginning of the experiment and the other 50 %, 24 hours later. The two factors were tested simultaneously and the experiment was repeted in two batches, in order to assess the variability of the results.

Changing the two levels after 24 hours resulted in a lower number of larvae settled than for the control (fig. 5). No clear difference appeared when distributing separate amounts of larvae, but combining the two factors resulted in an higher number of larvae settled per cultch than for the control.

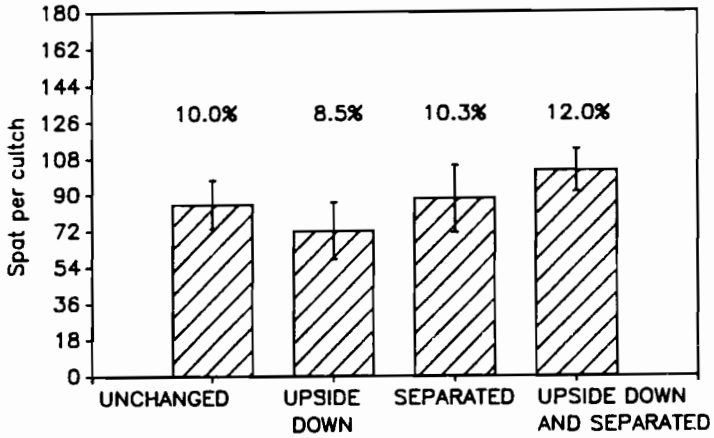

Figure 5 : Number of larvae settled per cultch for the two factors (returning the pipe and separate amount of larvae) performed 24 hours after the begining of the experiment.

However, the percentages of setting were low (10 % in average), probably because of the use of another batch of larvae. The differences between the 2 factors were small but signicant (table 5).

Table 5 : Analysis of variance on the factors affecting the position of the two levels and the distribution of larvae.

Source of variability	Sum of squares	Degree of freedom	F ratio	Significant level
Main factor	3.69	95		
Factor 1 (treatments)	0.42	3	3.98	< 0.05
Residual	3.27	92		

The small differences observed did not allow to rank the modalities in four independent groups. A test of Newman Keuls resulted into the identification of only two different groups, one corresponding to the returning of the pipes (percentage of set = 8.5 %) and the other corresponding to the combination of the two factors (percentage of set = 12 %).

Table 6 : Contingency table computed from the test of Newman-Keuls for the different modalities of the factor returning of pipes and the factor distribution of larvae.

Treatments	Mean	Homogeneous groups	
TR4 (4)	1.99	A	
TR1 (1)	1.91	A	B
TR3 (3)	1.89	A	B
TR2 (2)	1.80		B

The settlement of larvae on the upper and lower parts of the cultch was clearly different for the control and for the distribution of separate amounts of larvae (fig. 6).

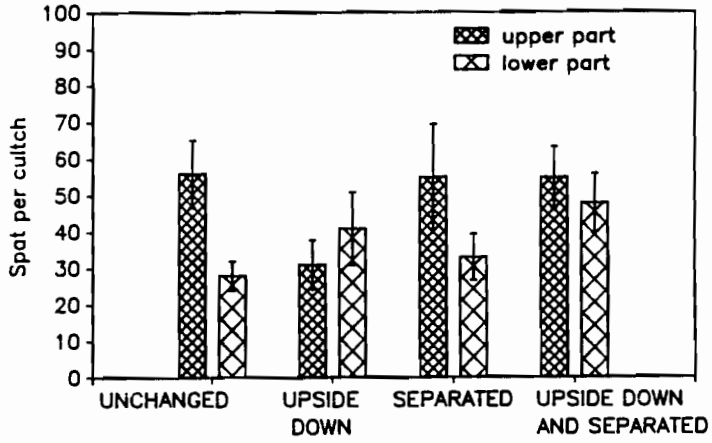

Figure 6 : Number of larvae settled on the upper and lower parts of the cultchs, for the two factors (returning the pipes and separate amounts of larvae) performed 24 hours after the beginning of the experiments.

In this experiment, the setting was better on the upper part, of those pipes which were not turned upside down. If the pipes were turned, the setting was apparently homogenous. The ANOVA performed on these results (table 7) revealed that significant differences were found between the upper and lower part of the pipes ($P < 0.01$) and that significant interactions were observed within the factors.

Table 7 : Analysis of variance on the number of larvae settled. Controlled factors : 1 = returning the pipes and distribution of larvae, 2 = upper and lower parts of the pipes.

Source of variability	Sum of squares	Degree of freedom	F ratio	Significant level
Main factors	11.26	191		
Factor 1 (treatment)	0.89	3	6.28	< 0.001
Factor 2 (high part/low part)	0.52	1	11.03	< 0.01
Interaction 1, 2	1.19	3	8.46	< 0.001
Residual	8.66	184		

In the table 8 are presented separate ANOVA performed for each factor. They confirmed that returning the cultch has resulted in a more homogeneous settlement on the two parts of the pipes ($F = 2.82$; $F = 3.31$ for the two treatments).

During this experiment, the larvae have settled more homogeneously along the pipes. No significant difference was observed between their extremities and center, as shown on figure 7.

Table 8 : Analysis of variance performed on the number of larvae settled in upper or lower part of the cultch. Factor 1 = control. Factor 2 = returning of the cultches. Factor 3 = larvae given in separate amounts. Factor 4 = returning of the cultches and larvae given in separate amounts.

Source of variability	Sum of square	Degree of freedom	F ratio	Significant level
Main factor	2.44	47		
Factor 1 treatment	1.05	1	34.76	< 0.001
Residual	1.39	46		
Main factor	3.23	47		
Factor 2 treatment	0.19	1	2.82	NS
Residual	3.04	46		
Main factor	3.12	47		
Factor 3 treatment	0.44	1	7.55	< 0.01
Residual	2.68	46		
Main factor	1.17	46		
Factor 4 treatment	0.08	1	3.31	NS
Residual	1.09	45		

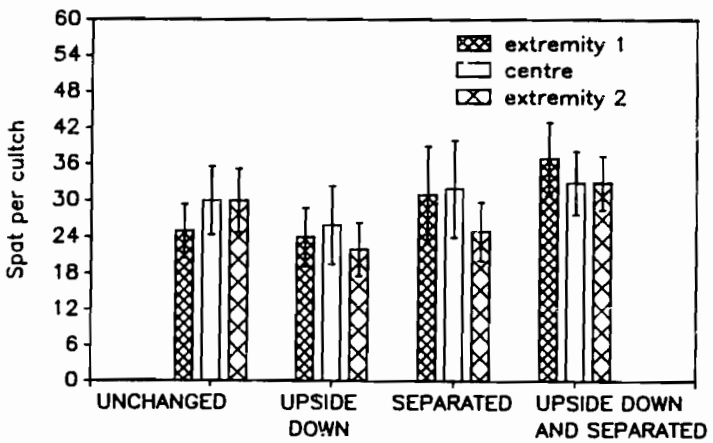

Figure 7 : Number of larvae settled on the differente parts of the cultch for the four controlled factors.

Table 9 : Analysis of variance on the number of larvae settled on the different parts of the cultches for the four treatments (two controlled factors).

Source of variability	Sum of square	Degree of freedom	F ratio	Significant level
Main factors	16.50	287		
Factor 1 (treatments)	1.34	3	8.24	< 0.001
Factor 2 (parts)	0.03	2	0.28	NS
Interaction (1, 2)	0.21	6	0.64	NS
Residual	14.93	276		

The results were confirmed by the ANOVA (table 9). The numbers of larvae settled on the differents parts of the tribe were not significantly different, while the two treatments (returning the pipes ande separate amounts of larvae) were producing significantly different sets on the pipes. No interaction between the parts of the pipes and the two treatments was observed.

DISCUSSION

One of the main characteristics of the remote setting of oysters larvae lies in the control of technical conditions, which remains not possible in field environment. Optimizing some of these conditions has resulted in better results for the remote setting. The percentage of settled larvae is far higher than what has usually been observed for a natural spat settlement on the French coasts (Berthomé et al., 1984). The observance of technical rules given by Roland and Broadley (1990), the optimal condition for the disposal and returning of the cultches described in the present work, and a good quality and competency of the eyed larvae before setting (Gerard et al., 1989) are among the factors which make the remote setting less variable and more efficient than the natural spat settlement.

The fact that the upper parts of the cultch were more intensively covered with spat may result from the behaviour of the larvae before settlement. These are known to exhibit a swimming activity, before starting a search for an adequate substrate, as described by Cole and Knight-Jones (1939) for *Ostrea edulis*. During that time, swimming is not permanent and the larvae may sunk, thus favourhising preferential contact on upper parts of the cultch. However, erractic trajectories during swimming, may lead some larvae to find a substrate inside the pipes. Such settlement will not allow the oysters to grow. The larvae will then have a search activity while crawling on the cultch. When adequate conditions are found by the larvae, these secrete a cement. At the end of the first day, not all the larvae are settled, and returning the cultches may allow the remaining to set on the other side. A separate amount of larvae distributed at that time, will result on a preferential settlement on that side. However, the time during which a swimming larvae keeps the ability to set is not known. In most of the remote setting operation, the percentage of unsettled larvae remain higher than 60 %, thus indicating that this ability will be lost after few days.

The conditioning of the cultches before setting operations (Jones and Jones, 1988) and the attractive nature of some substances have been studied yet (Bonar et al., 1985 ; Coon et al., 1988). Some chemical components, used in the plastic pipes are toxics, and these pipes may be allowed to leach before the first use. The time needed depends on these components, and the pipes currently used by French oysterman may usually only need to be exposed at sea for two months, when they are new. After cleaning, leaving the cultch conditioning for one week in sea water, will result in the development of bacterial biofilms. A bacteria *Alteromonas colwelliana*, was identified as playing a key role for the settlement of larvae (Weiner et al., 1989). Furthermore, chemicals from the family of catecholamines and L. dopa are known to favourish the settlement of larvae (Coon et al., 1985). However, these substances were not characterised yet in setting conditions, and their action is not understood. The larvae utilised in the present experiment were not treated with L. dopa and therefore the preliminary conditioning may have mainly resulted in the development of a bacterial biofilm.

The development of the remote setting in France is slow (Joly et al., 1988), since natural spatfalls are usually sufficient for the demand. However, national reproductive populations are only found in the South Atlantic coasts of France, and other areas of oyster cultivation, depend of the seed produced in these waters. Its economic cost will result in a slow development of the technique, at a national scale, as long as dramatic improvements are not available, such as genetically improved strains, triploids oysters, and oysters resistant to diseases, from selection processes or genes transferts. However,

the actual costs of seed from natural spatfall is not precisely known for the French producers.

On a technical point of view, several parametres need to be optimised. The food given to the larvae represents a large part of the costs, and its utility may be assessed more precisely. On the other hand, the optimal conditions (temperature, salinity) for larval setting given in the litterature, have appeared to depend also on the conditions encountered during the larval rearing, and further work is needed on this subject.

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