Water quality
Monitoring
Mussel embryolarval bioassay
Fresh, brackish and sea waters

Qualité de l'eau
Surveillance
Embryons et larves de bivalves
Eaux douces, saumâtres et salées

Monitoring fresh and brackish water quality around shellfish farming areas with a bivalve embryo and larva simplified bioassay method

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Received 18/04/95, in revised form 8/09/95, accepted 12/09/95.

ABSTRACT

The standard bivalve embryos-larval bioassay method has been modified for monitoring the water quality in an important mussel culture area, La Rochelle Bay. Fertilised eggs from naturally spawned *Mytilus galloprovincialis* mussels were incubated for 48 h at 18 ± 1 °C, in different water samples (five replicates) from the bay and its surroundings. The mean percentages of abnormalities, with the 95 % confidence limit, of controls and different water samples were compar ed. The method was shown to allow the application of the the bioassay to fresh, brackish and marine waters. The assessment of the water quality of the different tributaries to the Bay and that of freshwaters from intensively cultivated marshes close to it, showed that it varied form toxic to lethal, according to Woelke's classification. In contrast, the seawater of the mussel culture area was unpolluted.

RÉSUMÉ

Surveillance biologique des eaux douces et saumâtres à proximité des zones conchylicoles à l'aide des embryons et des larves de bivalves, par une méthode simplifiée.

Une simplification de la méthode standard des bioessais à l'aide des embryons et des larves de bivalves marins est proposée pour la surveillance de la qualité de l'eau d'une zone mytilicole, la Baie de La Rochelle. Les œufs fécondés d'individus dont le frai a été provoqué ont été mis en incubation pendant 48 h à 18 ± 1 °C dans des échantillons d'eau prélevés dans la baie et sur son pourtour (cinq répliquats par station). Les pourcentages moyens d'anomalies larvaires calculés avec intervalle de confiance au seuil de sécurité de 95 %, sont comparés à celui d'une eau de mer témoin. La méthode peut être appliquée aussi bien aux eaux marines qu'aux eaux saumâtres et douces. L'étude de la qualité des différents cours d'eau qui se déversent dans la baie, et celle des eaux douces de marais récemment mis en culture intensive montre qu'elles sont soit toxiques, soit létales, selon la classification de Woelke. Au contraire, en ce qui concerne la zone mytilicole elle-même, les eaux peuvent être considérées comme non polluées.

Oceanologica Acta, 1995, **18**, 5, 591-595

INTRODUCTION

Estuaries and coastal areas are productive ecosystems that serve as breeding and nursery grounds for a number of economically important shellfish (Phelps and Warner, 1990). Such areas are frequently subjected to changes in salinity; shellfish such as mussels and oysters are adapted to withstand wide fluctuations of this physical factor with no damage.

Apart from natural fertilisers, pollutants from various origins, notably from industry and agriculture are introduced into the coastal marine environment through natural runoff and may constitute a threat to the ecological stability of the marine environment as well as to aquaculture operations (His and Seaman, 1993).

Eggs, embryos and larvae of marine bivalves are among the organisms most commonly used in ecotoxicological bioassays. For almost half a century they have permitted assessment of the toxicity of micro pollutants (heavy metals, pesticides, detergents, industrial effluents) and evaluation of the water quality of coastal areas subjected to anthropogenic effects. The assessment is based on the percentage of abnormal D-larvae after the fertilised eggs have been incubated for one or two days in the water under investigation, as originally proposed by Woelke (1965 and 1972). Besides Japanese oysters, American oysters, hard clams and mussels are also used as test species (ASTM,1989). Mussels have been utilized by many investigators in marine toxicology (Dimick and Breese, 1965 ; Martin *et al.*, 1981; Robert and His, 1981; Mitchell *et al.*, 1985; Lapota *et al.*, 1993).

In the present study of an important mussel rearing area, *Mytilus galloprovincialis,* was used as the test species.

Under suitable nutritional and thermal conditions, salinity has little effect on embryonic and larval development in *M. galloprovincialis* at values between 20 and 35 (His *et al.*, 1989). Therefore, the biological quality of freshwater or brackish water samples can be analysed by mixing them with an unpolluted oceanic sea water (35) to a level adequate to the development of bivalve embryos (20 to 35).

Routine ecotoxicological monitoring requires simple, rapid and inexpensive methods (*e.g.* for use eventually aboard small research vessels with limited space and equipment). We have used a simplification of the recommended standard method (ASTM) with embryos of mussels (*Mytilus galloprovincialis*). Ripe mussels can generally be obtained throughout the winter, spring and summer in Arcachon Bay.

As previously indicated, mussel culture is particularly developed in the La Rochelle area (Atlantic coast of France). Some irregularities in spatting concerning mussels which reproduce in winter have been noticed. This area is subjected to many river inputs which may transfer to the bay toxicants from anthropogenic activities (industrial, agricultural and tourist activities). The quality of fresh and brackish waters pouring into the different parts of the bay as well as that of sea water from the bay itself was monitored in March 1994, that is to say during the main mussel reproductive period.

METHOD

Bioassay procedure

Ripe adult mussels (*Mytilus galloprovincialis*) were collected in the field (Bassin d'Arcachon) on 16 March 1994 and induced to spawn by thermal stimulation (alternating immersion in sea water of 18 °C and 28 °C for 45 min each time). Spawners were individually transferred to beakers with 0.2 µm filtered sea water (FSW) . Eggs were then transferred to a 1 l graduated cylinder with FSW and mixed gently (but thoroughly) by means of a rod with attached perforated disc (temperature 20 °C). Four sub samples of 100 µl were taken under continuous mixing, placed on a slide and the number of eggs counted under the microscope.

Males when spawning were transferred to beakers and just covered with FSW to get a dense sperm solution. Natural spawned mobile spermatozoa were added to the graduated cylinder containing the eggs, after passage through a 32 µm sterile screen (15 ml). Fertilisation was verified by microscopic examination.

Four batches of fertilised eggs each from different females and males were prepared as previously indicated ; one of them, with very round fertilised eggs (100 % as indicated by the presence of the fertilisation membrane) was chosen for the test.

15 min after fertilisation, volumes corresponding to 600 eggs (0.5 ml) were transferred with a sterilised automatic dispensing pipette to Coulter Counter translucent polystyrene cuvettes with 25 ml FSW (five replicates per sample) which were arranged in trays of 50 cuvettes. The trays with capped cuvettes were incubated for 48 h at 18 ± 1 °C.

After incubation, 100 µl of 4 % buffered formalin were added to each cuvette and the percentage of abnormalities determined by direct observation with an inverted microscope. No handling or sieving of larvae was needed. 100 individuals (over the 600) were observed at random in each of the five replicates.

Different abnormalities observed in the larval cultures were noted :

- segmented eggs, and malformed embryos which had not reached the D-larval stage.

- D larvae with either a convex hinge, indented shell margin, incomplete shell, or protruded mantle (Fig. 1).

Mean percentages of abnormalities were calculated for the different water samples with a 95 % confidence limit and compared to the mean percentage in controls.

Water quality of tributaries to the La Rochelle area and of the Bay itself

The biological quality of the water of 10 tributaries to the La Rochelle area and of 10 points from the shoreline or in the central part of the Bay (Fig. 2) were tested with mussel larva bioassays. Water samples of 500 ml were taken by hand or from a boat in polyethylene bottles on 15 March 1994 and transported to the laboratory in the dark at 4 ± 1 °C. The experiment started the day after.

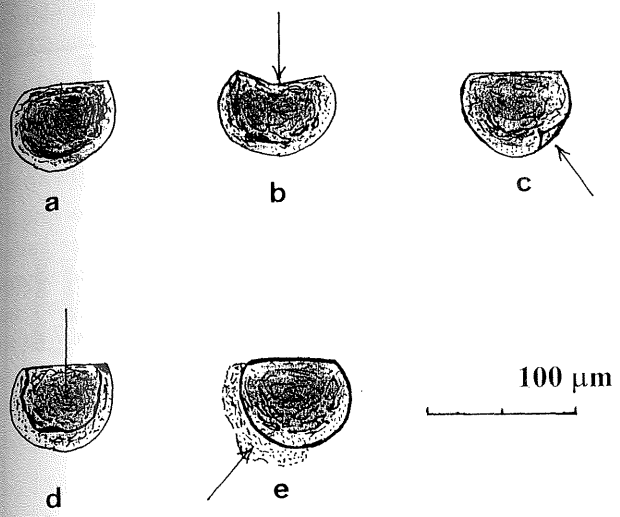


Figure 1

The different abnormalities observed in D-larvae of mussels, Mytilus galloprovincialis *: a : normal larva ; b : convex hinge ; c : indented shell margin ; d : incomplete shell ; e : protruding mantle.*

Sea water taken on the same day in an area known to support a regular naturally reproducing population of *M. galloprovincialis* (Bassin d'Arcachon) was used as a control; its salinity (35) was brought to 25 by mixing this FSW with deionized water to give a final value of 25.

Freshwaters were collected in natural channels (1, Le Lay and 8, Chenal de Lauzière) and at the mouth of the Sèvre Niortaise River (2) each of them opening directly into the Bay near mussel rearing areas. Point 10 (Aytré) and 11 corresponded to the mouth of man-made channels penetrating natural marshes.

Sea water samples were taken in harbours (9, fishing Harbour in La Rochelle; 12 and 17 pleasure harbours of Rochefort on the River La Charente, and Ré Island respectively) or directly on the sea shore (19, Chauveau and 20, Fouras). Points 3 to 7 concerned recently cultivated marshes. Drainage pipes are covered up by 1 m in the ground, leading to recent (4) or more ancient (6) pumps where the marsh waters are pumped into recent (5) or more ancient ponds (7) in relation with different main drainage channels, one of them being the Chenal du Curé (point 3). The sea water at point 18, close to l'Ile de Ré was collected near the output of a fish farming site, whereas points 14, 15 and 16 corresponded to pole mussel culture (Pas de la Tranchée, Aiguillon and Carrelère respectively) point 13 to a hanging mussel culture in deeper waters.

The salinity of the different samples is given in Table 1. For points 9, 10 and 13 to 20, it was between 23.8 and 31.1: the samples were directly 0.2 µm- filtered before starting the experiments, without any salinity change. For the remaining stations, the salinity varied from 7.1 to 0.3. 300 ml of the water to be tested were mixed with 700 ml of SW (35) and 0.2 µm filtered ; the final salinities varied from 26.6 to 24.6 for these samples.

RESULTS AND DISCUSSION

The percentages of normally developed larvae in this experiment are shown in Table 1 and Figure 2.

Controls showed extremely low percentages of abnormalities (0.2 ± 0.4), below the 3 % rate suggested by Woelke (1972) as acceptable for bioassay controls. Fresh and sea water samples gave a great variety in response ranging from highly toxic to non-toxic. All the fresh and brackish waters from natural channels gave values over 70 % abnormalities (from 73.2 ± 3.9, point 8 to 96.8 ± 1.5, point 1).

Concerning the man-made channels, sea water at point 10 (salinity 29.1) showed practically as good results as controls; on the opposite the freshwater at point 11 (salinity 0.4) gave a particularly high value (92.5 ± 2.6).

In the three harbours, a relatively low percentage was observed at Ile de Ré (14.2 ± 3.1, point 17); on the contrary higher values were reached at La Rochelle Harbour

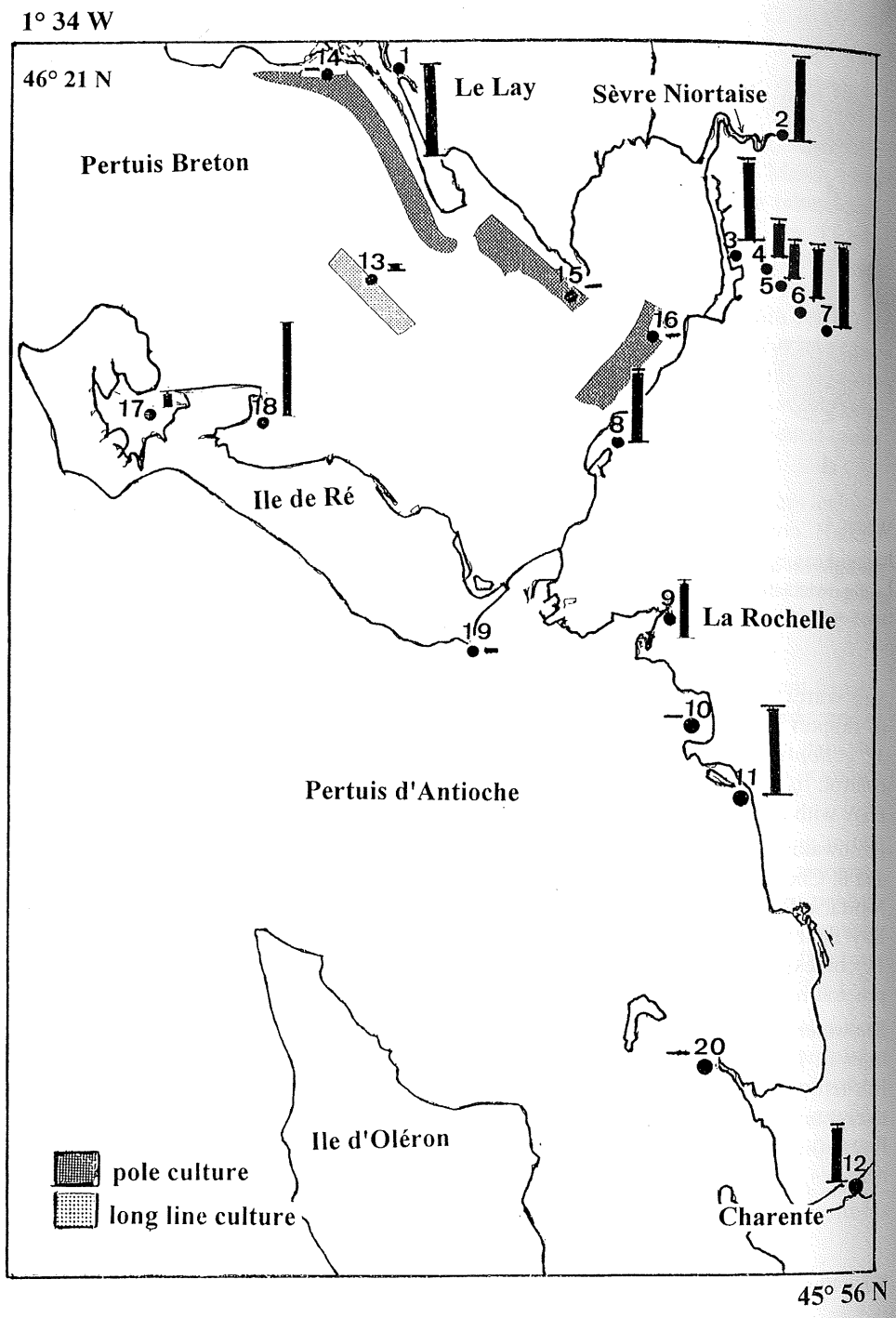
Table 1

Salinities of the different waters tested; and percentages of abnormalities (Mean ± C.I.) after the 2-D incubation period at 18 ± 1 °C of Mytilus galloprovincialis *fertilized eggs in waters at various stations in La Rochelle Bay (numbers 1 to 20). The values* in italics *correspond to samples diluted with 35 ppt FSW.*

	Salinity	**% abnormalities ± I. C**		**Salinity**	**% abnormalities ± I.C.**
Controls	25	0.2 ± 0.4	(11) Chatelaillon	*0.4*	*92.5 ± 2.6*
(1) Le Lay	*0.5*	*96.8 ± 1.5*	(12) Rochefort (harbour)	*0.4*	*56.0± 4.9*
(2) Pont du Braud	*0.3*	*89.2 ± 2.7*	(13) Hanging culture	31.1	3.8 ± 1.7
(3) Chenal du Curé	*0.4*	*83.2 ± 3.3*	(14) Pas de Tranchais (Pole culture)	30.6	1.4 ± 1.1
(4) Esnandes (recent pumps)	*7.1*	*34.8 ± 4.2*	(15) Aiguillon (Pole culture)	29.6	1.2 ± 1.1
(5) Esnandes (recent pond)	*2.3*	*36.6 ± 4.2*	(16) Carrelere (Pole culture)	27.5	1.2 ± 1.1
(6) Esnandes (ancient pumps)	*4.5*	*55.0 ± 4.4*	(17) Ars en Ré	30.8	14.2 ± 3.1
(7)Esnandes (ancient ponds)	*0.6*	*89.4 ± 2.7*	(18) Loix	28.7	99.6 ± 0.5
(8) Chenal de Lauziere	*6.0*	*73.2 ± 3.9*	(19) Chauveau	30.2	2.8 ± 1.4
(9) La Rochelle (bassin)	23.8	57.6 ± 4.3	(20) Fouras (Pointe de la Fumée)	28.1	1.4 ± 1.0
(10) Aytré	29.1	1.0 ± 0.9			

Figure 2

Average percent of abnormal larvae (± 95 % Confidence limit) in bioassayed waters from the La Rochelle area.



(57.6 ± 4.3) and Rochefort Harbour (56.0 ± 4.9), the last sample having been diluted with sea water.

Along the shoreline the sea water for points 19 and 20, most of the larvae were normal with respectively 2.8 ± 1.4 and 1.4 ± 1.0 abnormalities.

Concerning the recently cultivated marshes all the fresh or practically freshwaters tested after dilution with sea water, exhibited abnormalities close to or over the 40 % limit, with values as high as 83.2 ± 3.4 (point 3, corresponding to waters of a main channel, pouring directly into the bay) and 89.4 ± 2.7 (point 7).

In the bay itself, the sea water at point 18, close to the output of a fish farming site, was highly toxic to mussel embryos and larvae (99.6 ± 0.5). Nevertheless, both the pole and hanging culture zones in the mussel culture area exhibited good results (abnormalities between 3.8 ± 1.7 at point 13 and 1.2 ± 1.1 at points 15 and 16).

The method used in this study is a simplification of the ASTM Recommended Method for monitoring the quality in marine waters (ASTM, 1989). The measurements by direct observation in the cupped cuvettes improve both speed and accuracy. This time-saving technique avoids the destruction of the biological material either by sieving (Woelke, 1972) or by shaking and pipetting sub-samples (ASTM, 1989, Thain, 1991). On the other hand, high numbers of small test chambers improve the experimental design and it is also better from a statistical point of view to increase the number of incubation vessel for each

sample rather than sub-sample as proposed by Woelke (1972) and Thain (1991).

The levels of significance of the differences, in percentages of abnormalities, compared with the controls can be directly calculated from the 95 % confidence intervals of the means, without need for more complex calculations.

As previouly indicated, *Mytilus galloprovincialis* is a euryhaline species. So the biological quality of freshwater samples can be monitored by making up their salinity with sea water of proven good quality to a level adequate for the development of mussel embryos.

Woelke (1965) proposes the following criteria for toxicity evaluation:

< 5 % abnormal larvae : non toxic.

5 to 15 % abnormal larvae : slightly toxic

> 15 % abnormal larvae : toxic.

When more than 50 % of the larvae are abnormal, the lethal threshold is reached and over 90 % it is lethal. In the La Rochelle area, in spite of their dilution with FSW, all the fresh or nearly freshwaters tested (points 13, 14, 15 and 16) as well as the waters of the main inputs into the bay (1, 2 and 12) ranged from toxic to lethal. Sea water close to the sea shore is non toxic (10, 19, 20) or slightly toxic (17) but the samples from near the fish-farming area (18) are lethal and in La Rochelle Harbour sample (9), the lethal level was also reached. As concerns the mussel culture area, (points13, 14, 15 and 16), the samples were non toxic.

Shellfish farming is very important in the coastal waters of the La Rochelle area. The Pertuis Breton is the second largest French *Mytilus edulis* production centre and it is the principal centre for mussel spat production; providing Brittany and Normandy with young mussels. This juvenile production has recently been depressed (Dardignac-Corbeil and Prou, 1995). On the other hand, natural swamps and marshes adjacent to this shellfish farming region have been recently planned for intensive agriculture (Chevalier and Masson, 1988). The principal pesticides used in these areas can be highly toxic to embryos and larvae of the Japanese Oyster, *Crassostrea gigas* (His and Seaman, 1993).

The present study shows that the sea water quality on both pole and hanging culture areas was not seriously affected. However, mussel recruitment, which needs particularly good quality water could be hampered by an intensification of anthropogenic activities. These results also suggest that the mussel bioassay will have useful applications in monitoring human activities near mussel culture installations.

Acknowledgments

We thank Christian Cantin (IFREMER Arcachon) for his technical assistance.

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