II

(Acts whose publication is not obligatory)

COMMISSION

COMMISSION DECISION

of 25 July 1995

laying down the animal health conditions and the certification requirements for the importation from third countries of *Crassostrea gigas* for relaying in Community waters

(Text with EEA relevance)

(95/352/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 91/67/EEC of 28 January 1991 concerning the animal health conditions governing the placing on the market of aquaculture animals and products [1], as last amended by the Act of Accession of Austria, Sweden and Finland, and in particular Articles 19 (4), 20 and 21 thereof,

Whereas *Crassostrea gigas* is the main oyster species farmed in the Community thus representing an important economic factor and an important source of income for persons working in the aquaculture sector;

Whereas certain diseases exotic to the Community prevail in third countries which could, when introduced in the Community, have devastating effects for the oyster growing sector;

Whereas the introduction of such diseases must be prevented;

Whereas the risk of introducing diseases is highest when introducing oysters for relaying in Community waters; whereas, in the absence of appropriate disinfection methods for residual water of holding and purification tanks for for oysters, the same risk exists when introducing oysters which are temporarily kept in such facilities before consumption on the territory of the Community;

Whereas therefore the animal health conditions, the veterinary certificates and the third countries from which *Crassostrea gigas* can be introduced for relaying must, awaiting information on the zoosanitary situation and on the organization of the inspection services of third countries not yet listed, be a provisional one;

Whereas the inadvertent importation of other mollusc species in consignments of *Crassostrea gigas* is a considerable disease risk; whereas consignments must be checked before dispatch to avoid this;

Whereas this Decision shall apply without prejudice to the public health conditions established under Directive 91/492/EEC laying down the health conditions for the production and the placing on the market of live bivalve molluscs [2];

Whereas the measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

Article 1

Member States shall authorize imports of molluscs belonging to the species *Carassostrea gigas* for relaying in

[1] OJ No L 46, 19. 2. 1991, p. 1.

[2] OJ No L 268, 24. 9. 1991, p. 1.

Community waters, or for reimmersion in purification centres in contact with Community waters, from the countries listed in Annex I, provided that:

1. the following import conditions are met:

(a) they must have been submitted on the day of loading to a check in order to verify whether they satisfy the requirements laid down in Article 3, point 1 of Directive 91/67/EEC;

(b) they must have been raised during their entire life cycle and have been harvested in the national waters of the country of origin;

(c) they must originate in a zone which has been declared free, by the competent authority, of the diseases *Mikrocytos mackini* and Oyster Velar Velum Disease, in accordance with the sampling procedures and diagnostic methods laid down in Annex II and in which there are no other significant diseases of bivalve molluscs excluding marteiliosis (*Marteilia refringens*) and bonamiosis (*Bonamia ostreae*);

(d) they must, before loading, have been checked for the absence of bivalve molluscs of any other species in accordance with the procedures laid down in Annex III.

2. they are accompanied by an animal health certificate the model of which is laid down in Annex IV.

Article 2

This Decision shall apply from 1 October 1995.

Article 3

This Decision is addressed to the Member States.

Done at Brussels, 25 July 1995.

For the Commission

Franz FISCHLER

Member of the Commission

ANNEX I

Provisional list of third countries from which imports of *Crassostrea gigas* intended to be relaid in the Community are authorized

(AU) Australia
(CA) Canada
(NZ) New Zealand
(US) United States of America

ANNEX II

Sampling procedures and diagnostic methods for declaring a zone free of the diseases *Mikrocytos mackini* and Oyster Velar Disease (iridoviroses)

A. SAMPLING METHODS

1. SAMPLING

1.1. **Sampling points**

For each zone referred to, a number of sampling points must be selected so as to maximize the chances of detecting pathogens. Account must be taken of parameters having an effect on the development of the pathogenic agents, such as stocking density, water flows, and the development cycle of the molluscs.

For a given zone at least three sampling points must be selected. The number of points must be increased for large zones containing several discrete areas of cultivation of the susceptible species.

Whenever possible, at least one sample must be taken from natural beds. Any molluscs showing abnormalities (abnormal growth, gaping shells) must be selected.

1.2. **Sampling period and frequency**

The frequency of inspection is based on the infection period, and inspections have to take place thereafter. Inspection periods must also take account of the transfer of molluscs, which generally takes place in spring and autumn. Sampling should therefore be carried out twice a year (spring/autumn) for *Mikrocytos* and for *iridovirus*.

1.3. **Sampling size**

During the initial two-year period which precedes the granting of approved status, the sample size for each sampling point is 150 or a sufficient number to ensure detection at a 95 % confidence level of pathogen carriers at a prevalence of 2 %.

2. SHIPMENT OF SAMPLES

All molluscs sampled must be delivered to the approved laboratory within 24 hours after sampling. They must be packed in accordance with current standards in order to keep them in good condition. A label clearly stating the place of sampling and the health history (if any) must attached to the sample.

3. MACROSCOPIC EXAMINATION

The molluscs must be opened carefully so as not to damage the tissues, in particular the mantle, gills, heart and digestive gland. Anomalies and lesions of the tissues, as well as any shell deformities, shell-boring organisms and conspicuous mantle inhabitants, must be noted.

4. EXAMINATION OF STOCKS WHERE ABNORMAL MORTALITIES OCCUR

Whenever abnormal mortalities occur in stocks of bivalve molluscs an urgent investigation must be carried out to determine the aetiology. In field, an abnormal mortality is a sudden sizeable mortality which occurs in a short time between two observations during low tide cycle. In hatchery a mortality is considered abnormal when the farmer cannot obtain larvae during a period which included successive spawns from different broodstocks. In nursery a mortality is considered abnormal when a sudden sizeable mortality occurs in a short time on many tubes.

The sample taken must consist of 150 individual oysters and must be handled in accordance with the procedure defined for histological analysis. This technique must be used initially, before any other type of examination. The samples are fixed, preferably in Carson's fixative, which allows re-use of the sample for electron microscopy.

B. DIAGNOSTIC METHODS

(a) *Mikrocytos mackini*

1. PREPARATION AND EXAMINATION OF SAMPLES FOR MIKROCYTOSIS

1.1. **Cytological examination**

Cut a section through the abscesses, ulcers or green pustules, remove the excess water by placing the sample on blotting paper, then blot on a slide the sample corrresponding to the section which passes through the infected tissue. The slides are dried in air and then fixed with methanol (2 to 3 minutes).

The slides are stained using any equivalent Wright-Giemsa stain (e.g. Merck's Hemacolor Kit or Baxter's Diff-Quick) in accordance with the manufacturer's instructions. Dip the slides in the first bath for 4 to 5 seconds, then immerse immediately in the second bath (3 seconds). Rinse with tap water, dry completely in cold or warm air, and mount in synthetic resin (Eukitt).

The parasite, 1-3 µm in diameter, appears included in haemocytes or free of the host cells and has blue cytoplasm and a small red nucleus. An observation time of 5 minutes per slide is sufficient.

1.2. **Histological examination**

For histological sections, cut a section through the body of the oyster including pustules, abscesses and ulcers if any are present. Then place the sample in a fixative liquid such as Davidson's, Bouin's or Carsons's. The last-named enables the samples to be reused for electron microscopy if necessary. The ratio of volume of tissue to volume of fixative must be no more than 1 : 10.

The samples are subsequently handled in accordance with the classical histological methods. Several non-specific stains allow *Mikrocytos* to be observed : haemalum-eosin, Masson's trichrome. These examples are not exhaustive. It is recommended that two sections per oyster be examined.

The stages of *Mikrocytos*, intracellular parasite 2/3 µm in diameter can be observed inside cells of the vesicular connective tissue on the periphery of the lesions, in muscle cells and in hemocytes within the lesions.

(b) *Oyster Velar Disease*

1. PREPARATION AND EXAMINATION OF SAMPLES FOR *IRIDOVIRUS*

1.1. **Cytological examination**

Cut the digestive gland and gills along a sagittal plane, soak up excess water by applying absorbent paper, then press that part of the cut sample which passes through affected organs against a glass slide. The slides are dried in air and then fixed with methanol (2 to 3 minutes).

The slides are stained using Merck's Hemacolor Kit (with reagent solution 2 (ref. 11956) for red staining and reagent solution 3 (ref. 11957) for blue staining). Dip the slides in the first bath for 4 to 5 seconds, then immerse immediately in the second bath (3 seconds). Rinse with tap water, dry completely in cold or warm air, and mount in synthetic resin (Eukitt).

It is sufficient to examine each slide for 5 minutes.

The cytoplasm of infected cells stains blue, and it contains a weakly-staining red nucleus and an inclusion body, variable in size, stained bright red.

1.2. **Histological examination**

Cut the visceral mass and the gills with small scissors along a sagittal plane, and place the sample in a fixative (Davidson's, Bouin's or Carson fluid); the last-named is suitable for samples which may be examined later by electron microscopy if necessary. There should be at least 10 parts of fluid to every part of sample by volume.

The samples are then treated by conventional histological procedures.

Many non-specific stains show up iridoviral inclusion bodies : haemalun-eosin, Masson's trichrome and others. Two sections from each oyster should be examined.

Ciliated velar epithelium with intracytoplasmic inclusion bodies (1,2 to 2,4 µm diameter) which are spherical, dense and basophilic in early stage infections but become irregular and less basophilic as virion form. Inclusions bodies occasionally occur in velar supporting esophageal and oral epithelia and rarely in mantle epithelium.

1.3 **Electron microscopy**

Velar epithelial cells with viroplasm (observed as inclusion bodies in histology) that form the viral particles. Viral particles with icosahedral symmetry (228 ± 7 nm diameter) and with a capsid consisting of two bilayered membranes. Complete viral particles have a dense inner core separated from the capsid by moderately dense zone.

ANNEX III

1. For each consignment a visual check of at least 1 000 oysters randomly selected for each place of origin shall be carried out by the competent authority.
2. The batch will pass the test if the check referred to in point 1 does not reveal the presence of molluscs other than *Crassostreas gigas.*

ANNEX IV

MODEL

ANIMAL HEALTH CERTIFICATE

for the importation of *Crassostrea gigas* into the European Community

Note for the importer: this certificate is only for veterinary purposes and the original must accompany the consignment until the border inspection post of entry into the Community.

Reference No: .....

Exporting country: .....

Official service (Ministry, Department): .....

I. **Origin of the oysters**

Part of the country of origin: .....

Farm of origin:

name: .....

address: .....

Consignor: .....

II. **Description of the consignment**

Net weight: .....

No of units: .....

Medium size of the oysters: .....

III. **Destination of the consignment**

Member State of destination: .....

Place of destination: .....

Consignee:

name: .....

address: .....

IV. **Means of transport** (description and identification)

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V. **Health attestation**

I, the undersigned representative of the official authority, hereby certify that the oysters belonging to the present consignment:

1. — have been examined today and do not show clinical signs of disease,
   — are not intended for destruction under a scheme for the eradication of a disease,
   — do not come from a farm which is subject to a prohibition for animal health reasons and must not have been in contact with animals from such a farm;
2. have been raised and harvested in the national waters of the country of origin;
3. originate in a zone which has been declared free of the diseases *Mikrocytos mackini* and Oyster Velar Velum Disease, in accordance with the diagnostic methods and procedures laid down in Annex II of Commission Decision 95/352/EC and in which there are no other significant diseases of bivalve molluscs other than marteiliosis *(Marteilia refringens)* and bonamiosis *(Bonamia ostreae)*;
4. are only of the species *Crassostrea gigas*. The consignment has been subjected to the examination set out in Annex III of Commission Decision 95/352/EC.

Done at ..... Date .....
(day of loading)

Signature: .....

Name in capital letters, qualification and official title: .....

.....

Seal