



Bivalife



Improving European mollusc aquaculture: disease detection and management

Deliverable D5.3

Recommendations for surveillance and diagnosis

and

Deliverable 5.4

Recommendations for surveillance and diagnosis

THEME [KBBE.2010.1.2-08]

[Improving European mollusc aquaculture: disease detection and management - Call: FP7-KBBE-2010-4]

Project acronym: BIVALIFE

Project full title: " Controlling infectious diseases in oysters and mussels in Europe "

Grant agreement no: 266157



WP5. Pathogen control and eradication: development of methods, field tests and recommendations

The general objective of this WP was to define and propose practical methods and general recommendations drawn from knowledge acquired from previous WPs for infectious disease control. This WP included an experimental work in order to evaluate pathogen inactivation and decontamination of system and animals.

WP5 - T2: Recommendations for surveillance and diagnosis (all Participants)

These recommendations follow the aims expressed in the DoW and therefore “focus on pathogens targeted in WP2, Task 2 and potentially new unknown pathogens identified through WP2, Task 4”. As such, the recommendations are established from data obtained in other work packages, especially WP2 Task 2. They aim to contribute to the production of guidelines for sampling and surveillance of pathogens of interest for oyster and mussel culture by identifying: (1) the most suitable diagnostic tools, (2) the nature and size of samples (susceptible species and also other compartments of the field known to carry the pathogens) that should be tested, (3) the most suitable period and sites for sampling.

- OsHV-1 diagnosis recommendations

Techniques in use:

- a) One step PCR targeting fragment C2-C6 (followed by RFLP-Mfel if needed)

Advantages:

Double copy of the gene provides increased sensitivity

The RFLP is well described and useful for distinguishing the μ Var from other genotypes,

SSR region useful for tracking specific strains

Disadvantages:

The fragment is too large to be used in a real-time PCR

Current methodology implies opening the tubes for electrophoresis leading to increased risk of contamination

- b) One step PCR using primer pairs developed by Participant 3 (UCC)

Advantages:

Higher sensitivity

Disadvantages:

Sensitivity needs to be checked using reference material

Current methodology implies opening the tubes for electrophoresis leading to increased risk of contamination



c) Real-time PCR SybrGreen targeting fragment C9-C10

Advantages:

- Can be used as a real-time PCR assay with low risk of contamination
- Useful for confirming results of other assays

Disadvantages:

- Detects single copy and is therefore potentially less sensitive than C2-C6

d) Real-time PCR SybrGreen targeting OsHV-1 DNA polymerase

Advantages:

- Real-time PCR assay with low risk of contamination.

Disadvantages:

- Detects single copy and therefore potentially less sensitive than C2-C6

- Surveillance recommendations

a) Number and type of sample

150 of the susceptible host should be sampled, although not all may need to be processed initially. Shellfish should be processed individually in batches of 30 and further batches only processed if either the first batch was negative (corroborative duplicate) or if there is a need to know the exact prevalence of a batch. A total of 150 negative individuals ensures a 95% confidence that pathogen prevalence is not greater than 2%.

b) Calendar

Should be purpose-specific but for grow-out situations the pre-mortality periods are crucial.

c) Pools vs individuals

Always advisable to process individuals (not pools). Pools can be used when the individuals are so small that they cannot be opened and would not provide sufficient sample from inside the shell. Processing individuals is also useful to estimate prevalence because pools have decreased sensitivity due to a dilution effect.

d) Importance of good records for data related to survival, growth, movements (traceability), and water quality for contributing to better characterisation of disease patterns and proposal of management guidelines that can help to minimise disease outbreaks.



WP5 - T3: Recommendations for pathogen control in controlled culture conditions (hatcheries, nurseries) and in open water on-growing sites

These recommendations follow the aims expressed in the DoW and therefore “focus on pathogens targeted in WP2 - Task 2, as well as from data recorded in the database (WP2 - Task 4)”. As such, they aim to support biosecurity measures for: (1) maintaining a free status regarding pathogens of interest in a controlled area, especially by suggesting water treatments and decontamination of imported animals, as well as materials and equipment used in controlled area containment and eradication in case of an outbreak. The recommendations are linked to WP2 that concerns potential management practices and environmental factors that may influence the impact of diseases (in terms of morbidity and mortality), since eliminating pathogens from open systems is generally not practical.

Thus, WP5 - Task 3 uses the outputs from WP2 (i.e. risk factors for diseases and mortality) to develop recommendations and advice for shellfish farmers in order to minimise the impact of diseases and for their control in open water sites.

Recommendations for biosecurity can be divided in four main areas:

- *Identification and use of reliable sources of stock*

- Source of pathogen free spat tested from clean broodstock, with a history of the hatchery, a supplier's biosecurity plan, and quarantine/isolation of imported lots
- Spat batches should be health tested prior to transport outside an epidemiologic unit for relaying elsewhere in order to avoid potential disease spread
- Avoid mixing spat from different sources at the beginning of the production cycle, even if different batches test negative because there is no absolute guarantee of freedom from disease and because any non-infected batch may be genetically more pathogen susceptible
- Do not accept spat batches which appear ‘unhealthy’ due to mortalities or failure to respond to a percussion test or are easy to open with a fingernail because they may already be infected or will be poor performers and therefore more susceptible to disease and will have a lower chance of reaching harvest size

- *Application of good management practices*

- The spat immersion schedule (size and temperature) is important to reduce the risk of mortality
- Disinfection of equipment and materials is essential. Ropes and nets, if re-used, must be disinfected and dried in the sun for a period of at least one week, recipients used to transport or sample the spat must be perfectly clean and dry before use. The boat deck must be cleaned using a disinfectant after every use



- Limit movements between sites. Although they may be unavoidable they should be evaluated and only performed if no other solution is available
 - Use of boats should be limited to one epidemiological unit
 - Do not mix batches during the production cycle
 - Record all movements of the batches
 - Remove dead individuals as soon as possible and dispose of them away from sites
- *Effective disease recognition*
- Inspect the stock frequently for disease signs or mortalities, and train staff in disease recognition
 - Test on-site batches if infection suspected
- *Identification of effective measures to take in the event of a disease outbreak or unknown mortality*
- Agreed code of practice detailing practical recommendations to follow for obtaining disease-free stock, avoiding disease outbreaks and handling emergency situations taking into account production sector observations and Bivalife conclusions.