





GENETIC BROODSTOCK CHARACTERISATION FOR PEDIGREE MANAGEMENT IN WHITELEG SHRIMP - GENAEUS

SAMPLING PROTOCOL

MATERIALS

- 1. Sampling box and kit [we can ship if necessary]
- 2. Cryotubes of 1.5 ml (with screw cap) [we can ship if necessary]
- 3. Surgical scissors and tweezers
- 4. Analytical grade (pro analysis) ethanol 95% (absolutely don't use technical grade ethanol)
- 5. Ruler (up to 1 mm) and balance (up to 1 g)
- 6. Gloves

SAMPLES

- 1. Collect preferably live/freshly caught shrimp. Frozen animals are also possible if sampled correctly and kept at -20 °C at all times.
- 2. The aim is to sample if possible 3 different groups (batches/shipments/families)
- 3. A representative sample from a group consists of either 30 individual postlarvae or (up to) 30 broodstock (in which case the second pleopod is sampled).
- 4. Particularly interesting are groups of related animals (for example two parents and 20 of their progeny).
- 5. Shrimp need to be sampled individually and stored in separate vials; do not pool shrimp!
- 6. Make use of the sampling sheet attached to document the samples.

PROCEDURE

- 7. Before sampling, fill the cryotubes (one for each shrimp) with at least 1 ml of analytical grade ethanol 95% (see photo). It is crucial that high quality ethanol is used because other products contain substances which degrade DNA. Each cryotube must be labelled with a sample ID (use pencil, tracing paper and transparent tape).
- 8. The operator has to wear clean gloves.
- 9. For adult shrimp, measure length (up to 1 mm) and weight (up to 1 g).
- 10. Additional phenotypic information is most welcome (reproductive stage, maturity, etc).
- 11. For adult shrimp, it will be very helpful to take a picture of the shrimp on a white, non-reflective, background (e.g. a polystyrene board) with a ruler clearly visible under the shrimp. Place the shrimp on its right side, in a natural body posture and kept in position by surgical needles. Picture should be taken from a vertical point of view in order to avoid parallax bias. A tag with the sample's ID should be placed beside the shrimp in order to avoid confusion between samples' pictures.

12. Store sample (individual postlarva or individual pleopod in case of broodstock) in 1.5 ml plastic (screw cap) vials (cryotubes) filled with 95% analytical ethanol (1 part of tissue should be soaked in 20 parts of liquid). Label properly (pencil or Indian ink on tracing paper or vial). **Make sure the sample codes allow to link family relations** (for example parents and offspring). Put the vials in the special box provided.





- 13. Store the cryotubes at a temperature of $+4^{\circ}$ or -20° in the dark.
- 14. Ship as a regular parcel by air mail. We refund shipping costs.

This task should take no more than a few hours provided that the shrimp are available.

THANK YOU FOR SHARING YOUR TIME AND EXPERTISE.

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SAMPLING SHEET

Metadata on the collector

Family and First Name	
Company/Institution Name	
Address 1	
Address 2	
City and postal code	

Genaeus – Invitation to contribute samples

Country	
Phone	
Fax	
Email	
Comments	

Metadata on the sample

Shipment date	
(dd/mm/yyyy)	
Name of production farm	
Address of production farm	
Sampling date	
(dd/mm/yyyy)	
Estimated time between	
death of animal and	
collection time	
Sample tissue	whole postlarva – 2nd pleopod of adult
Sample code	From
Ex. Pva-#farm#-001	То
Sample liquid	Analytical ethanol 95%
Other	
Comments on the sampling	
Comments on the sampling conditions	
1	
1	

SAMPLING SHEET

NAME OF FARM: SHIPMENT:

SAMPLING DATE:

Sample #	Sample code	Postlarva	Adult	Adult body length (cm)	Adult body weight (g)	Sex (M/F)	Remark
01	Pva-#farm#-001						
02	Pva-#farm#-002						
03							
04							
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Genaeus – Invitation to contribute samples

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				THANK you so much!

#farm# = your farm acronym