Protocol for Health Certification of Barramundi Fingerlings for aquaculture, prior to entry into NSW.
Revised May 2007

Introduction

NSW DPI has authorised aquaculture permits for the culture of barramundi in NSW. The industry in NSW is based on barramundi fingerlings imported into NSW.

A major concern with the importation of barramundi fingerlings is the possibility of introducing disease along with the imported barramundi. Barramundi nervous necrosis virus (BNNV) in particular is highly virulent and causes heavy mortalities in infected barramundi at or before three weeks of age. To minimise the risk of this virus being imported, NSW DPI has determined that no barramundi fingerlings for aquaculture will be permitted to enter NSW unless their import complies with a strict protocol.

Detection of BNNV generally relies upon routine laboratory examinations, histology and confirmatory electron microscopy. However, other diagnostic tests, including viral isolation in cell culture, and molecular genetic tests have been developed in recent years, and can be useful in cases where results from routine testing are not definitive.

As laboratory testing procedures alone provide no absolute guarantee that sampled batches of barramundi larvae and fry are disease-free, every effort needs to be made to ensure that the system under which the fish are bred and reared is itself free of disease. Good hygiene and quarantine principles, minimizing stress and regular monitoring can help achieve this.

This protocol is designed to minimise the risk of introducing lymphocystis virus or BNNV, or other disease or parasites associated with barramundi, into NSW in imported fingerlings.

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Background

The protocol relates to all barramundi fingerlings to be imported into NSW for aquaculture. Barramundi fingerlings must only be purchased from a hatchery which adheres to this Protocol for Health Certification of Barramundi Fingerlings, prior to their entry to NSW.

A permit under Section 217(1) of the *Fisheries Management Act 1994* is required to import live fish into NSW. Barramundi fingerlings shall only be imported to aquaculture facilities where permit holders have barramundi listed on their permit.

A copy of the certification of clearance of barramundi nervous necrosis virus (BNNV) with the pathology report must accompany shipments of fingerlings entering NSW issued by a NSW DPI recognised laboratory under the terms of the protocol. This certificate must be retained by the permit holder for at least three years.

To allow time for any mortality caused by the virus to be expressed from BNNV, and to obtain the results of the certification testing required by the protocol, NSW DPI has determined that, unless otherwise approved by NSW DPI, no barramundi larvae or fry less than 42 days (6 weeks) of age will be permitted to enter NSW..

Any barramundi larvae or fry entering NSW under this protocol must be transported directly to a fish farmer holding a current permit to farm barramundi.

Hatchery Hygiene and Quarantine Requirements

All other animals (aquatic and terrestrial animals, but not including food organisms) must be excluded from the hatchery. The hatchery will be secure and capable of being locked up.

The hatchery will be enclosed by materials that prevent the entry of airborne water droplets. Access to the hatchery is to be restricted to trained and authorised staff only. Access to the hatchery and between different parts of the hatchery will be through foot baths containing fresh 2% formalin solution (200ml commercial grade formalin per 10 litres of water) or a 1% solution of sodium hydroxide (10g NaOH per litre of water, replaced daily). An iodophor hand rinse will be required before entry to the hatchery.

Each rearing tank will have its own set of non-transferable equipment, which is disinfected daily using an iodophor solution containing 50-100mg/L free iodine.

A NSW DPI Veterinary Pathologist or the equivalent officer from NSW DPI may inspect the hatchery for compliance to the hygiene requirements at any time.

Hatchery and Pond Recording and Sampling Protocol

A daily log is to be kept for each rearing tank or rearing pond. It will include details of the number of fry/larva, feeding regimes, water quality parameters and any mortality, as well as any other relevant information such as abnormal appearance, behaviour, and cessation of feeding.

This log will be retained by the hatchery for at least three years and the log or copies of the log will be available for inspection by a NSW DPI veterinary pathologist or their representative at any time. A summary of the completed log, recording details of water and fish samples, and other relevant information will be signed by the owner/manager and attached to the Declaration of Origin. The log must show that:

- fingerlings remained clinically normal to 42 days (minimum age of entry); and
- there were no unexplained mortalities in the fingerling batch.

Sampling protocol:

- Each rearing tank will be sampled and larvae/fry preserved in 10% seawater formalin (100ml laboratory grade formalin and 900ml seawater).
- The level of detection required for the test will be 2% prevalence with 95% confidence, assuming 100% test sensitivity.
- The sample for each rearing tank / pond will be 150 effective samples comprising Central Nervous System (CNS) sections plus retina sections of fish aged between 21 and 42 days. The CNS plus retina sections will be subject to a histopathological examination. The result from the histopathological examination must be negative for the BNNV infection

All samples will be collected randomly (by pooling at least four beaker or dip net sub-samples taken from different sites in the rearing tank/pond when the larvae/fry are distributed evenly throughout the water column) unless abnormal or diseased larvae/fry are present, in which case these larvae/fry will be selectively sampled. If requested, further samples must be supplied from specified rearing tanks/ponds in a specified manner as directed by NSW DPI for further laboratory testing. Any fish showing clinical signs consistent with BNNV infection must be examined histologically and shown to be BNNV negative. Further testing may also be required to demonstrate BNNV negative status of such fish and may include, but not be limited to, viral isolation in cell culture, molecular genetic testing and/or examination by electron microscopy.

Pond Hygiene and Quarantine Requirements

Each fry-rearing pond must have its own set of non-transferable equipment, or in the case of harvesting nets, they must be disinfected prior to use in the pond.

The fry-rearing pond should only receive ‘first use’ water not tail water from another section of the farm. Once harvested fingerlings must be kept isolated from other batches of fingerlings and "Hatchery Hygiene and Quarantine Requirements" should be observed.

Laboratory Examination Protocol

All samples must be examined grossly and/or subgrossly with a dissecting microscope for the presence of abnormalities and parasites on external surfaces.

All samples must be processed routinely for histology and examined microscopically for evidence of disease and associated agents.

If any lesions are presumptively associated with viruses further samples must be collected into 3% glutaraldehyde in cacodylate buffer and the lesions examined under the electron microscope.

Copies of Pathology Reports for each batch must be attached to the Veterinary Examination Certificate.

Selection Criteria

As the sensitivity of histological testing is comparatively low, and as there is still an incomplete knowledge on the diseases of barramundi, the following criteria will be used to evaluate a batch's suitability for health certification:

- Are there any lesions present, e.g. nervous system vacuolation, or focal necrosis, which when seen by light microscopy indicate the presence of a virus?

- Are there any protozoan or metazoan parasites present; or fungal or internal bacterial infections associated with an inflammatory or degenerative lesion present; or an organism in these groups which is known to cause disease in other fish species present?

- The presence of any bacteria associated with an internal lesion?

If the answer to <u>all</u> of these questions is "NO" then a batch may be certified as free of disease under the terms of this protocol. If the answer to <u>any</u> of these questions is "YES", then the batch must be rejected.