Susceptibility of Australian native freshwater fish to epizootic haematopoietic necrosis virus

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Epizootic haematopoietic necrosis virus

- Found only in Australia
- Notifiable to the OIE
- Genus: Ranavirus
1985 - mass mortalities
* juvenile redfin perch
* lakes in Victoria

**Origin**
- Unrecognized reservoir?
- Trans-species spread from frogs or reptiles?
- Aquarium fish trade?
1987 - mortalities
* rainbow trout
* a few farms in NSW and ACT
Distribution of reported EHNV outbreaks in Redfin perch (*last reported outbreak)

**Spread**

- **EHNV is very resistant**
- Natural fish movement
- Fishermen
- Water
- Piscivorous birds

Redfin perch *Perca fluviatilis*
Species of the Murray Darling Basin

- Golden perch
- Murray cod
- Silver perch
- Macquarie perch
- Golden perch
- Mountain galaxias

What other species might be susceptible?
Overall Goal:
Assess the impact of EHNV to native Basin fish

Basin Survey
EHN distribution & spread

Experimental Infection Trials
Potential ecological impact

ELISA Development
Tool for Basin Survey

Direct measures to mitigate disease
Immunology

Immune response – blood samples

Positive for antibody

ELISA test

+
ELISA development

FPLC chromatography curve
5 mL Murray cod serum

SDS PAGE gel
Lane 1 Molecular marker,
Lane 2 Murray cod serum (1:20)
Lane 3 flow through (1:5)
Lane 4 elution fraction
Affinity purified immunoglobulin – stage 1 completed

- redfin perch
- silver perch
- Murray cod
- Macquarie perch

*Perca fluviatilis*
*Bidyanus bidyanus*
*Maccullochella peelii peelii*
*Macquaria australasica*
ELISA field survey

Blood samples

- **Lachlan R.**
  - Redfin
  - Murray cod
  - Macquarie Perch

- **Murray R.**
  - Redfin
  - Murray cod
  - Silver perch

- **Darling R.**
  - Murray cod

- **Macquarie R.**
  - Murray cod

- **Barwon R.**
  - Murray cod

- **Murrumbidgee R.**
  - Murray cod

~290 samples

Results to follow next year
Change of driver

Michelle Dennis
Virology Field Survey

Homogenise organs

Inoculate living fish cells

Cytopathic effect
Virology field survey

Tissue samples

Lachlan R.
Redfin

Murray R.
Redfin
Galaxids (3 spp.)
Blackfish

ACT (Cotter Dam Area)
Redfin
Galaxids
Gambusia
Rainbow trout

Burrinjuck Dam
Redfin
Murray cod

Murrumbidgee R.
Blackfish

Buffalo R.
Redfin

~890 fish tested so far…….
Experimental Infection Trials

~30 fish

EHNV Bath Challenge
$10^3 \text{TCID}_{50} \text{ ml}^{-1}$

~30 fish

Non-exposed
Negative Control

• Repeated 2 to 4 times, 20 to 22°C
• Inspected for up to 5 weeks
• Tested for EHNV:
  - **Infection:** Virus isolation, PCR
  - **Disease:** Pathology

Challenged Redfin
Positive Control

Important to have matched samples from the same fish.  
...This requires fish that aren't tiny!
Redfin perch
Liver, Redfin perch
Liver, Redfin perch
Anterior kidney, Redfin perch

Disease is similar in other species!!
Results: Carp gudgeons

- ~50% lived to 35 days
- None infected
  None had disease

EHNV Bath Challenge
$10^3$ TCID$_{50}$ mL

Carp gudgeons are NOT susceptible
(Basin-wide native species)

- None lived to 11 days
- All infected
- Some had disease

Challenged Redfin
Positive Control
Results: Redfin Perch

- ½ die with EHNV-associated disease
- ½ survive, but are infected (with viable virus in organs - VI and PCR +)
- This is in contrast to studies of the 1990’s

This is evidence that:

1. Susceptibility of Murrumbidgee redfin perch has shifted
2. Redfin perch can be carriers of EHNV
<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Status</th>
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<tbody>
<tr>
<td>Silver perch</td>
<td><em>Bidyanus bidyanus</em></td>
<td>Currently in trial</td>
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<tr>
<td>Mountain galaxias</td>
<td><em>Galaxias olidus</em></td>
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<tr>
<td>Macquarie perch</td>
<td><em>Macquaria australasica</em></td>
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<tr>
<td>Murray cod</td>
<td><em>Maccullochella peelii peelii</em></td>
<td>In trial</td>
</tr>
<tr>
<td>Redfin perch</td>
<td><em>Perca fluviatilis</em></td>
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<tr>
<td>Unspecked hardyhead</td>
<td><em>Craterocephalus stercusmuscarum fulvus</em></td>
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<td>River blackfish</td>
<td><em>Gadopsis marmoratus</em></td>
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<tr>
<td>Carp gudgeon</td>
<td><em>Hypseleotris sp.</em></td>
<td>Resistant</td>
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<tr>
<td>Trout cod</td>
<td><em>Maccullochella macquariensis</em></td>
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<tr>
<td>Murray-Darling rainbow fish</td>
<td><em>Melanotaenia fluviatilis</em></td>
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<tr>
<td>Southern purple spotted gudgeon</td>
<td><em>Mogurnda adspersa</em></td>
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<tr>
<td>Southern pygmy perch</td>
<td><em>Nannoperca australis</em></td>
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<tr>
<td>Bony herring</td>
<td><em>Nematalosa erebi</em></td>
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