Aquatic Animal Health and Role of AAHL in Australia

CSIRO: COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
www.csiro.au

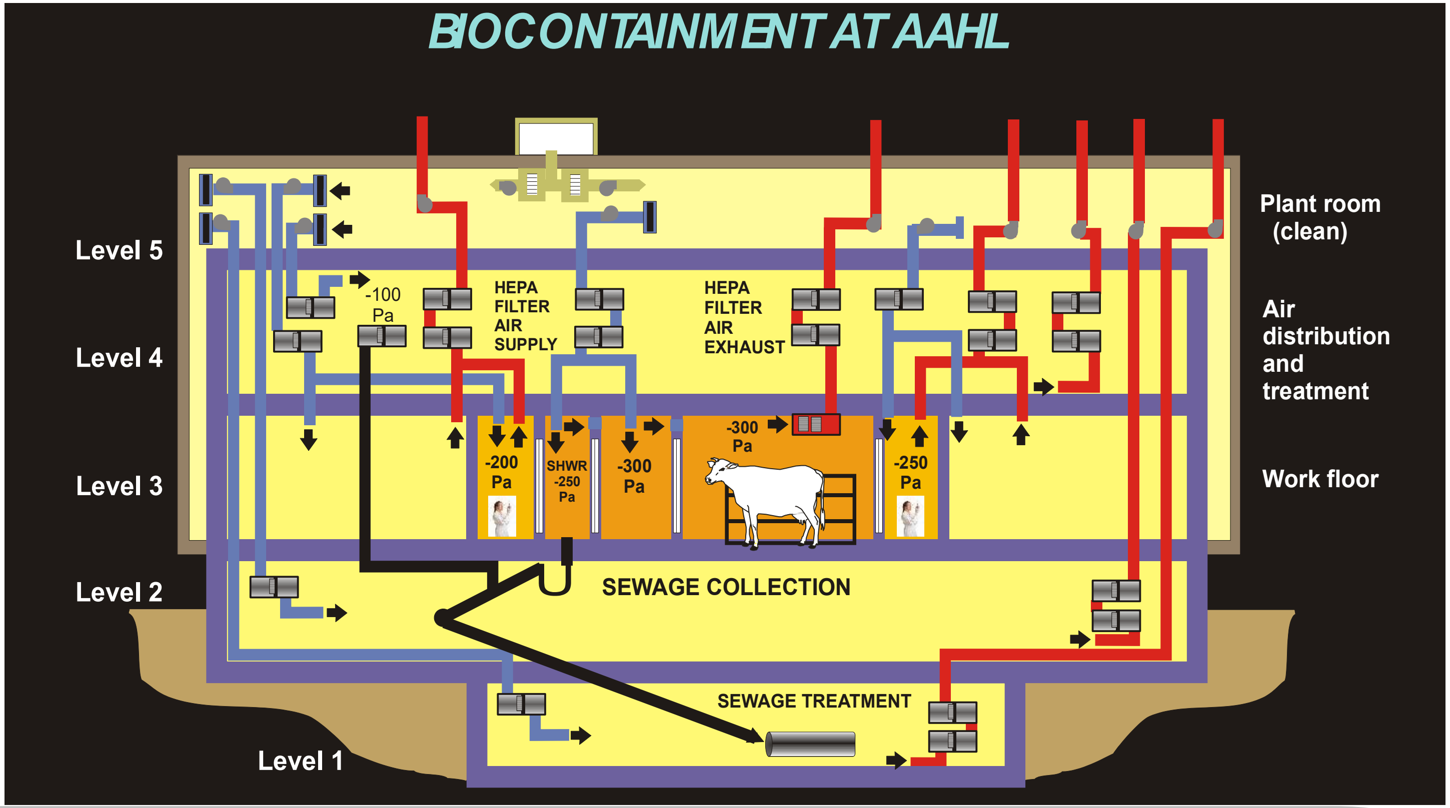
Agus Sunarto, Peter Mohr, Matthew Neave and Serge Corbeil

Outline:

1. Role of CSIRO-AAHL in Australia:
   - AAHL: Australian Animal Health Laboratory
   - AFDL: AAHL Fish Diseases Laboratory
2. Aquatic animal health relevant to Indonesia:
   - Koi herpesvirus (KHV) in carp
   - Abalone herpesvirus (AbHV) in Abalone
   - Tilapia lake virus (TiLV) in tilapia
3. New emerging technologies:
   - Next Generation Sequencing (NGS)
   - Bioinformatics
   - Genome editing (GE)
4. Opportunities - Managing tilapia

CSIRO Australian Animal Health Laboratory (AAHL)

AAHL: 5 levels of technology



Secured lab/HEPA filters/Waste inactivation/Airlock doors

AAHL's functions

Contributes to managing the risks posed by exotic, by new and emerging diseases and by bio-terrorism to the livestock and fishes of Australia through:

- Diagnostic Services: exotic and emerging diseases
- National Emergency Response Capability
- Research and Development
- Training
- Technical Advice
- International Reference Centre e.g. OIE Reference Laboratory for New and Emerging Diseases plus specific diseases e.g. AI, NDV, **YHV1, EHNV, Ranavirus** and **AbHV**
- WHO SARS Collaborating Centre

AAHL's Fast Facts

- Construction began in 1978, opened in 1985.
- It cost $150 million to build and today it's replacement value is $1 billion. Annual Operation: >$40million (DAWR/CSIRO/external funds)
- AAHL employs 300 staff (150 scientists; 150 engineers/support staff)
- Staff working at AAHL must not live on a property with livestock animals.
- Seven day exclusion period.
  - Staff working with aquatic animal diseases cannot keep aquatic animals as pets either. And a seven day exclusion period to visit fish farms.

AAHL Fish Diseases Laboratory: AFDL - Fish Group

- **Group Leader - Dr Mark Crane:**
  1. Aquatic Host-Pathogen Interaction (Team Leader - Dr Mark Crane)
     Dr Ken McColl, Dr Serge Corbeil, Nick Gudkovs, Joanne Slater

  2. Aquatic Diagnostic Capability (Team Leader - Dr Nick Moody)
     Dr Peter Mohr, Dr Dave Cummins, John Hoad, Nette Williams, Stacey Valdeter

NATA

- **Samples referred from states (no direct submissions)**
- **We focus on exotic agents (less competition for the States)**
- **Quality Assurance (ISO 17025, AS/NZ 2243)**

AFDL Submission Categories

Diagnostic submissions from State authorities:

Category 1: Routine samples (surveillance – no disease suspected)

Category 2: Exotic/emergency disease exclusion (low likelihood)

- Test results required within 72 hours

Category 3: Exotic disease exclusion/confirmation (high likelihood)

- Test results required within 24 hours (work overnight)
- Diagnostic test report issued to submitting laboratory, CVO of the submitting state, Australian CVO and Director of AAHL.

AFDL Protocols

- OIE Standards
  - Manual of Diagnostic Tests for Aquatic Animals (OIE Reference Laboratory role)
  - Comment on drafts for Department of Agriculture and Water Resources
  - Participate in international meetings
- European Commission EU Regulations, Directives and Decisions
- SCAHLS ~~SCAHLS~~ ANZSDPs (reviewed through SCAAH)
- Peer-reviewed publications, in-house test development.

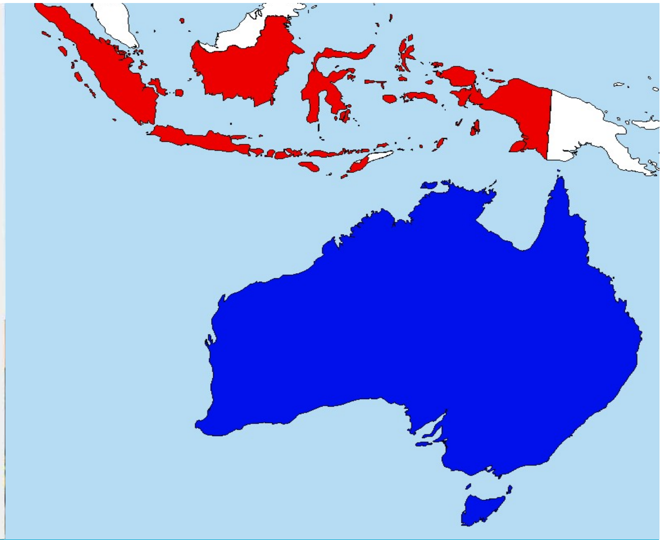
Distribution of Commercially Grown Australian Aquaculture Species

Issues over the past 20 years

1995: **Pilchard mass mortalities (herpesvirus**; multi-state); Haplosporidiosis (pearl oysters; WA)
1996: **GAV/LOV (YHV-2**) and MoV in *P. monodon;* Bennettae baculovirus in *Metapenaeus bennettae* (Qld)
1997: *Uronema nigricans* (tuna; SA); Hepatopancreatic parvo-like virus in *P. japonicas* (Qld)
1998/9: Pilchard herpesvirus; Orthomyxo-like virus (pilchards; SA); IPN-like *Aquabirnavirus* (Tas); *Thelohania* in yabbies (WA)
2000: Barramundi hump-back syndrome; Parvo-like virus in redclaw crayfish (QLD)
2001: Rickettsia-like organism (RLO) in salmonids (Tas), ciliate infection/disease in pearl oysters (WA)
2003: *Megalocytivirus* in Murray cod (Vic), IHHNV (integrated sequence?) confirmed in *P. monodon* (Qld), Herpesviral haematopoietic necrosis in goldfish (WA)
2004: ***Nodavirus* in Australian bass** & other finfish (NSW), Leatherjacket mass mortality (NSW)
2005: Carp mass mortality (Vic), **viral ganglioneuritis in farmed abalone (Vic),** GAV in *Fenneropenaeus merguiensis* (WA)
2006: Eel mortalities – rhabdovirus? (Vic), oedema disease (OOD) in pearl oysters (WA), viral ganglioneuritis in wild abalone (Vic)
2007: Orthomyxo-like virus in salmonids (N. Tas)
2008: Mortalities (Streptococcus sp.) in (wild) grouper (Qld), Kingfish mortalities (WA), abalone viral ganglioneuritis (Tas), white tail disease - Macrobrachium rosenbergii nodavirus (Qld), new strain (previously exotic) of IHHNV (Qld)
2010: Edwardsiella ictaluri in native catfish (NT), barramundi herpesvirus (Vic), Ostreid herpesvirus in Pacific oysters (NSW), Aquabirnavirus in trout (Vic)
2011: AVG in farmed abalone (Tas)
2012: Megalocytivirus in ornamental fish farm (Qld); Orthomyxo-like virus in salmonids (SE. Tas); YHV genotype 7 in farmed prawns (Qld), issues with specificity of OIE YHV assays
2013: YHV genotype 9 and 10 in imported commodity prawns, issues with specificity of OIE YHV assays
2014: *Edwardsiella ictaluri* in wild catfish (Qld)
2015: Turtle mortalities (NSW), Hepatopancreatitis (AHPND-like) in prawns (Qld), *Bonamia exitiosa, Perkinsus olseni, P. beihaiensis* in native flat oysters (Vic)
2016: **Hepatopancreatitis (AHPND-like) in prawns (Qld); Ostreid herpesvirus in Pacific oysters (Tas)**
WSSV in farmed prawns in Queensland

Outline:

1. Role of AAHL in Australia:
   - AAHL: Australian Animal Health Laboratory
   - AFDL: AAHL Fish Diseases Laboratory
2. Aquatic animal health relevant to Indonesia:
   - Koi herpesvirus (KHV) in carp
   - Abalone herpesvirus (AbHV) in Abalone
   - Tilapia lake virus (TiLV) in tilapia
3. New emerging technologies:
   - Next Generation Sequencing (NGS)
   - Bioinformatics
   - Genome editing (GE)
4. Opportunities - Managing tilapia



Koi herpesvirus in carp: The tale of two countries

Agus Sunarto, Matthew J. Neave and Kenneth A. McColl

CSIRO Health and Biosecurity, Australian Animal Health Laboratory (AAHL)
www.csiro.au

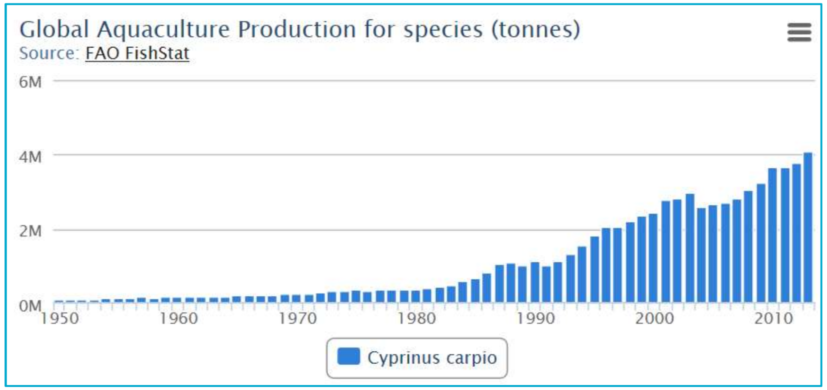
Invasive Animals CRC

NATIONAL CARP CONTROL PLAN
RESTORING NATIVE BIODIVERSITY

CENTRE FOR INVASIVE SPECIES SOLUTIONS

CSIRO

Common carp (*Cyprinus carpio*)



4 million tonnes, value $5 billion.

Main producer countries of **common carp** (FAO 2006)


iSENTIA
INFLUENCE • INFORM • INSIGHT

Copyright Agency Ltd (CAL) licensed copy

Country News insert, Shepparton VIC
17 Jun 2013

General News, page 19 - 350.21 cm²
Regional - circulation 48,668 (-T-----)

ID 199167211

PAGE 1 of 2

Department of Environment and Primary Industries
Victoria

Time to eradicate carp

The intr
has already
eastern
across a
more than
kilometres
Basin alon


Science & Environment

Chicago goes to war with Asian carp

By Pallab Ghosh
Science correspondent, BBC News, Chicago

18 February 2014 | Science & Environment

DEPARTMENT OF PRIMARY INDUSTRIES

Billions of European carp, which make up 90 per cent of fish biomass in the Murray-Darling river system, cause environmental damage on a vast scale

PUBLIC ENEMY NO 1

The Australian, 14 Jan 2016

Carp:

- First introduced in Australia in 1859
- Major pest in 1960:
  - **Damage habitat**
  - **Displace native fish**
- Negative economic impacts: $500M/yr.

CSIRO

Koi herpesvirus: Friend or Foe?

KHV is a lethal virus of koi and carp, devastating these industries in Indonesia and worldwide.
Toba Lake, 2004.

PESTSMART | CONNECT
POWERED BY THE INVASIVE ANIMALS CRC

LEARN ACT CONNECT Pest species Search Animal Welfare News Images Mobile apps

Koi herpesvirus as a biological control for carp

Herpes plan to kill off carp

Kate Wilson, Monday, July 9th, 2012.

Like 0 Tweet 0 Pin it Share

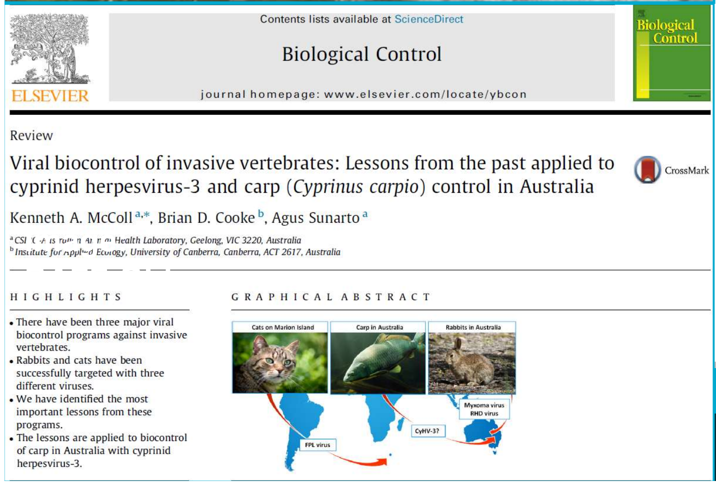
RESEARCHERS are investigating a disease to control European carp, a pest fish which infests Lake Colac.

CSIRO's Dr Ken McColl is researching koi herpes as a bio-control agent.

Researchers are investigating a type of herpes to kill European carp in Australian waterways.

(Sunarto et al, JFD 2011)

KHV as a biological control agent for carp in Australia



Contents lists available at ScienceDirect
Biological Control
journal homepage: www.elsevier.com/locate/ybcon
ELSEVIER

Review

Viral biocontrol of invasive vertebrates: Lessons from the past applied to cyprinid herpesvirus-3 and carp (*Cyprinus carpio*) control in Australia

Kenneth A. McColl [a,*], Brian D. Cooke [b], Agus Sunarto [a]

[a] CSI[illegible] Health Laboratory, Geelong, VIC 3220, Australia
[b] Institute for Applied Ecology, University of Canberra, Canberra, ACT 2617, Australia

HIGHLIGHTS

- There have been three major viral biocontrol programs against invasive vertebrates.
- Rabbits and cats have been successfully targeted with three different viruses.
- We have identified the most important lessons from these programs.
- The lessons are applied to biocontrol of carp in Australia with cyprinid herpesvirus-3.

GRAPHICAL ABSTRACT

- **Control measures for carp in Australia:**
  - Poisoning
  - Electro-fishing
  - Physical removal

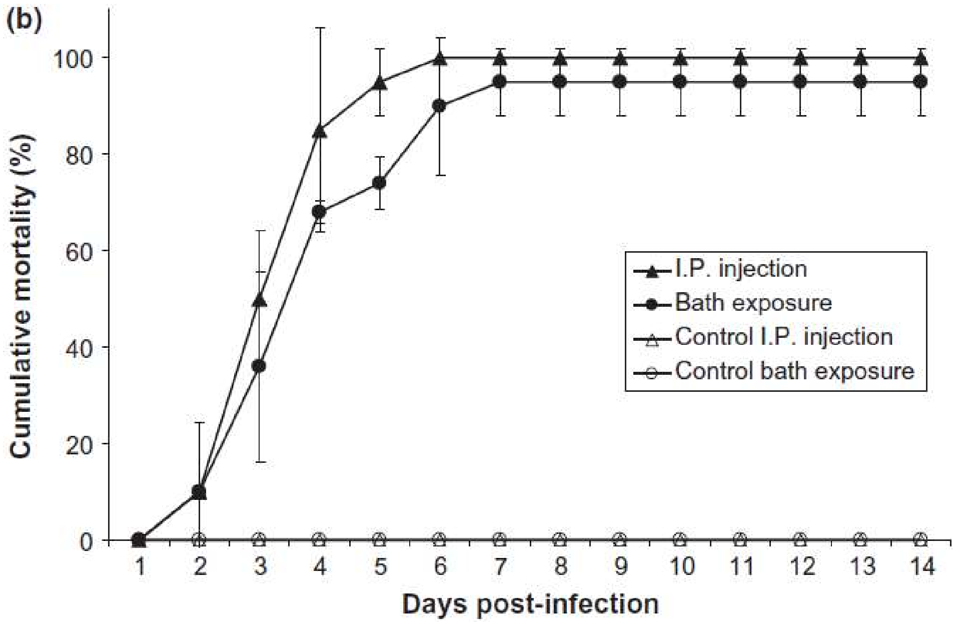
  May be effective at a local level, but not at a national level

- **Viral biocontrol is likely to be a cost-effective and practical solution to managing IAS:**
  - does not require reapplication like poisons
  - once established should be self-sustaining.

KHV as a biological control agent for carp in Australia

Two major concerns for a successful biocontrol virus:

Efficacy



Indonesian KHV C07 is virulent in Australian carp

Safety

Journal of Fish Diseases 2016 doi:10.1111/jfd.12591

Cyprinid herpesvirus 3 as a potential biological control agent for carp (*Cyprinus carpio*) in Australia: susceptibility of non-target species

K A McColl[1], A Sunarto[1], J Slater[1], K Bell[2], M Asmus[3], W Fulton[4], K Hall[4], P Brown[5], D Gilligan[3], J Hoad[1], L M Williams[1] and M St J Crane[1]

1 CSIRO-Australian Animal Health Laboratory, Geelong, Vic., Australia
2 K&C Global Fisheries, Sale, Vic., Australia
3 Department of Primary Industries, Narrandera Fisheries Centre, Narrandera, NSW, Australia
4 Department of Primary Industries, Fisheries Research Branch, Queenscliff, Vic., Australia
5 The Murray-Darling Freshwater Research Centre and La Trobe University, Mildura, Vic., Australia

- Susceptibility of 22 species (fish, frog, turtles, etc).
- More than 1000 animals tested.
- None showed clinical signs of disease.
- **Absence of mRNA by RT-PCR** (Yuasa et al 2012), **indicating no KHV replication in NTS**

RT-PCR to detect viral replication of KHV

Table 2 Results of qPCR and RT-PCR samples tested in CyHV-3 challenge trials on non-target species

	CyHV-3 challenged				Negative control			
	NTS		Carp		NTS		Carp	
Trial	qPCR[a]	RT-PCR[b]	qPCR	RT-PCR	qPCR	RT-PCR	qPCR	RT-PCR
1	36/86[c]	0/36	8/8	5/8	0/27	NT	7/8	0/7
2	27/128	0/27	8/8	5/8	0/33	NT	0/4	0/0
3	2/89	0/2	6/6	4/6	4/15	0/4	0/3	0/0
4	9/164	0/9	4/6	3/4	0/92	NT	1/2	0/1
5	5/158	0/5	3/3	3/3	0/50	NT	0/2	0/0
6	4/66	0/4	3/6	2/3	0/24	NT	0/2	0/0
7	3/147	0/3	5/5	5/5	1/89	0/1	0/2	0/0
8	8/53	0/8	6/6	6/6	0/13	NT	0/2	0/0
9	10/30	0/10	6/6	6/6	0/10	NT	0/2	0/0
Total	104/921	0/104	49/54	39/49	5/353	0/5	8/27	0/8
Percentage	11.3	0	90.7	79.6	0.01	0	29.6	0

NTS, non-target species; NT, not tested.
[a]Protocol of Gilad *et al.* 2004.
[b]Protocol of Yuasa *et al.* 2012.
[c]Data presented as number of positive samples per number of samples tested.

Journal of Fish Diseases 2011, 34, 87–101 doi:10.1111/j.1365-2761.2010.01216.x

Isolation and characterization of koi herpesvirus (KHV) from Indonesia: identification of a new genetic lineage

A Sunarto[1,2,3], K A McColl[1], M St J Crane[1], T Sumiati[3], A D Hyatt[1], A C Barnes[2] and P J Walker[1]

1 CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong, Vic., Australia
2 School of Biological Sciences, The University of Queensland, Brisbane, Qld, Australia
3 Fish Health Research Laboratory, Agency for Marine and Fisheries Research, Jakarta, Indonesia

Virus Research 188 (2014) 45–53

Contents lists available at ScienceDirect

ELSEVIER

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Characteristics of cyprinid herpesvirus 3 in different phases of infection: Implications for disease transmission and control

Agus Sunarto[a,b], Kenneth A. McColl[a,*], Mark St. J. Crane[a], Karel A. Schat[c], Barry Slobedman[d], Andrew C. Barnes[e], Peter J. Walker[a]

[a] CSIRO Animal, Food and Health Sciences, Australian Animal Health Laboratory, Geelong, VIC 3220, Australia
[b] Fish Health Research Laboratory, Centre for Aquaculture Research and Development, Jakarta 12540, Indonesia
[c] Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

Journal of Virology

Koi Herpesvirus Encodes and Expresses a Functional Interleukin-10

Agus Sunarto, Clifford Liongue, Kenneth A. McColl, Mathew M. Adams, Dieter Bulach, Mark St. J. Crane, Karel A. Schat, Barry Slobedman, Andrew C. Barnes, Alister C. Ward and Peter J. Walker

J. Virol. 2012, 86(21):11512. DOI: 10.1128/JVI.00957-12.
Published Ahead of Print 15 August 2012.

Journal of Fish Diseases 2016 doi:10.1111/jfd.12591

Cyprinid herpesvirus 3 as a potential biological control agent for carp (*Cyprinus carpio*) in Australia: susceptibility of non-target species

K A McColl[1], A Sunarto[1], J Slater[1], K Bell[2], M Asmus[3], W Fulton[4], K Hall[4], P Brown[5], D Gilligan[3], J Hoad[1], L M Williams[1] and M St J Crane[1]

1 CSIRO-Australian Animal Health Laboratory, Geelong, Vic., Australia
2 K&C Global Fisheries, Sale, Vic., Australia
3 Department of Primary Industries, Narrandera Fisheries Centre, Narrandera, NSW, Australia

Vol. 113: 127–135, 2015 doi: 10.3354/dao02824 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published March 9

Expression of immune-related genes of common carp during cyprinid herpesvirus 3 infection

Agus Sunarto[1,2], Kenneth A. McColl[1,*]

[1]CSIRO Biosecurity Flagship, Australian Animal Health Laboratory, Geelong, VIC 3220, Australia
[2]AMFRD Centre for Aquaculture Research and Development, Fish Health Research Laboratory, Jakarta 12540, Indonesia

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Received: 20 October 2016
Accepted: 20 December 2016
Published: 02 February 2017

Transcriptomic analysis of common carp anterior kidney during *Cyprinid herpesvirus 3* infection: Immunoglobulin repertoire and homologue functional divergence

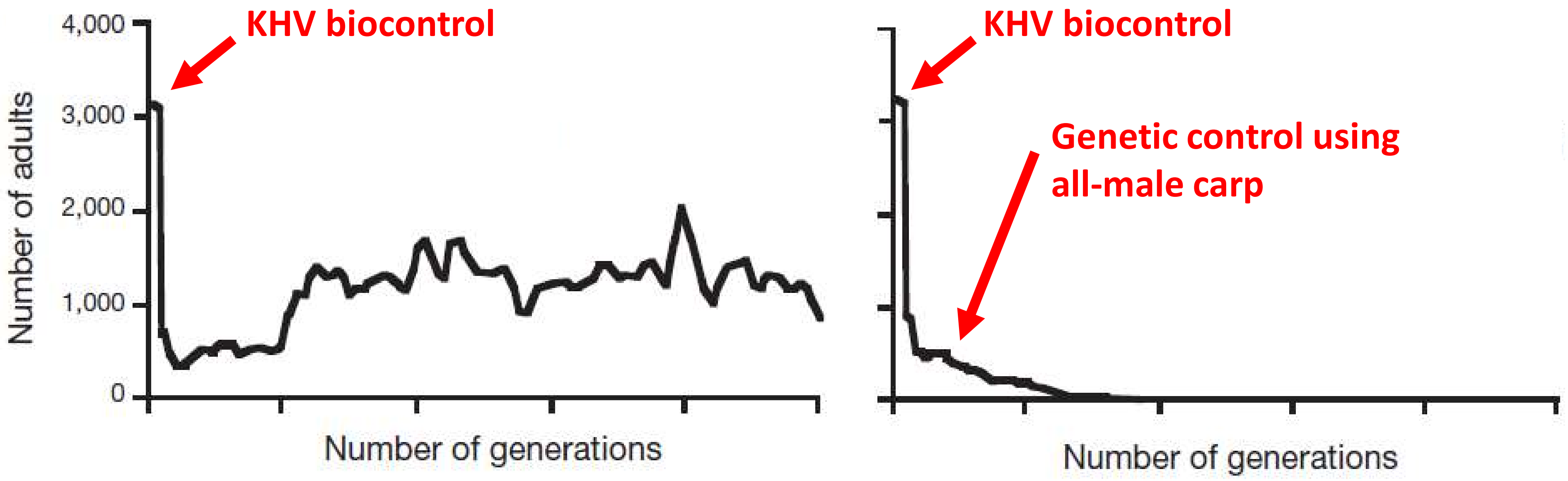
Matthew J. Neave[1], Agus Sunarto[1,2] & Kenneth A. McColl[1]

National Carp Control Plan http://carp.gov.au/

NCCP:

- Risk assessment
- Release strategy
- Public consultation
- Approval

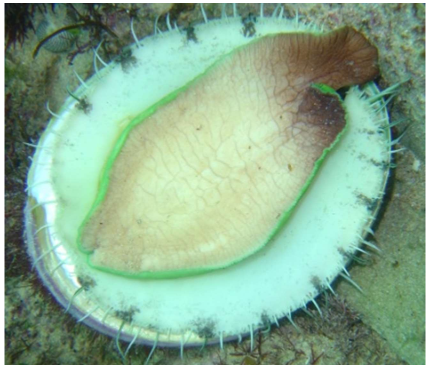
Modelling of carp control and eradication



Thresher et al, Nature Biotech 2014.

Abalone Herpesvirus (AbHV) = Haliotid Herpesvirus (HaHV-1)

Dr Serge Corbeil



- AVG: Abalone Viral Ganglioneuritis
- Clinical signs: swollen mouth and retracted foot

Appeared in Victoria in 2005-2006

Diagnostic Tools & Experimental Model for AbHV

Vol. 92: 1–10, 2010
doi: 10.3354/dao02277
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org
Published October 26

OPEN ACCESS

Development and validation of a TaqMan® PCR assay for the Australian abalone herpes-like virus

Serge Corbeil[1,*], Axel Colling[1], Lynette M. Williams[1], Frank Y. K. Wong[2,6], Keith Savin[3], Simone Warner[2], Bronwyn Murdoch[2], Noel O. I. Cogan[3], Timothy I. Sawbridge[2], Mark Fegan[2], Ilhan Mohammad[2], Agus Sunarto[1], Judith Handlinger[4], Stephen Pyecroft[4], Marianne Douglas[4], Pen H. Chang[5], Mark St. J. Crane[1]

[1]Australian Animal Health Laboratory, CSIRO Livestock Industries, Geelong, Victoria 3220, Australia

Virus Research 165 (2012) 207–213

Contents lists available at SciVerse ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

ELSEVIER

Abalone viral ganglioneuritis: Establishment and use of an experimental immersion challenge system for the study of abalone herpes virus infections in Australian abalone

Serge Corbeil[a,*], Kenneth A. McColl[a], Lynette M. Williams[a], Ilhan Mohammad[b], Alexander D. Hyatt[a], Sandra G. Crameri[a], Mark Fegan[b], Mark St.J. Crane[a]

[a] CSIRO, Livestock Industries, Australian Animal Health Laboratory, 5 Portarlington Road, Geelong East, Victoria 3220, Australia
[b] Biosciences Research Division, Department of Primary Industries, 475 Mickleham Road, Attwood, Victoria 3049, Australia

Susceptibility of Abalone species to AbHV

Vol. 119: 101–106, 2016
doi: 10.3354/dao02989

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published May 3

Australian abalone (*Haliotis laevigata*, *H. rubra* and *H. conicopora*) are susceptible to infection by multiple abalone herpesvirus genotypes

Serge Corbeil*, Lynette M. Williams, Kenneth A. McColl, Mark St. J. Crane

CSIRO Health and Biosecurity, Australian Animal Health Laboratory, 5 Portarlington Road, Geelong East, Victoria 3220, Australia

- **All abalone are susceptible to AbHV.**
- **New Zealand paua (*Haliotis iris*) is resistant to AbHV.**

NGS (RNAseq) to identify the underlying disease resistance mechanisms in paua.

Tilapia lake virus (TiLV): Friend or Foe?

TiLV caused massive mortalities in tilapia, but not in other fish.

Clinical signs:

- **abdominal** distention
- **skin** (darkening & erosions)
- **ocular** alterations (cataract & exophthalmia).

Eyngor et al, 2014; Bacharach et al, 2016.

Confirmative diagnostics

WORLD ORGANISATION FOR ANIMAL HEALTH

Protecting animals, preserving our future

TILAPIA LAKE VIRUS (TiLV) – A NOVEL ORTHOMYXO-LIKE VIRUS

PATHOGEN INFORMATION

http://www.oie.int/international-standard-setting/aquatic-manual/

OIE ELECTRONIC *AD HOC* GROUP ON TILAPIA LAKE VIRUS

Terms of Reference for ongoing work

Feb 2018

8. DIAGNOSTIC METHODS

8.1. Definition of suspicion

High levels of mortality in tilapine species, associated with ocular alterations (opacity of the lens or more severe pathology), should be considered suspicious of TiLV. Skin erosions, haemorrhages in the leptomeninges and moderate congestion of the spleen and kidney may be observed on post-mortem.

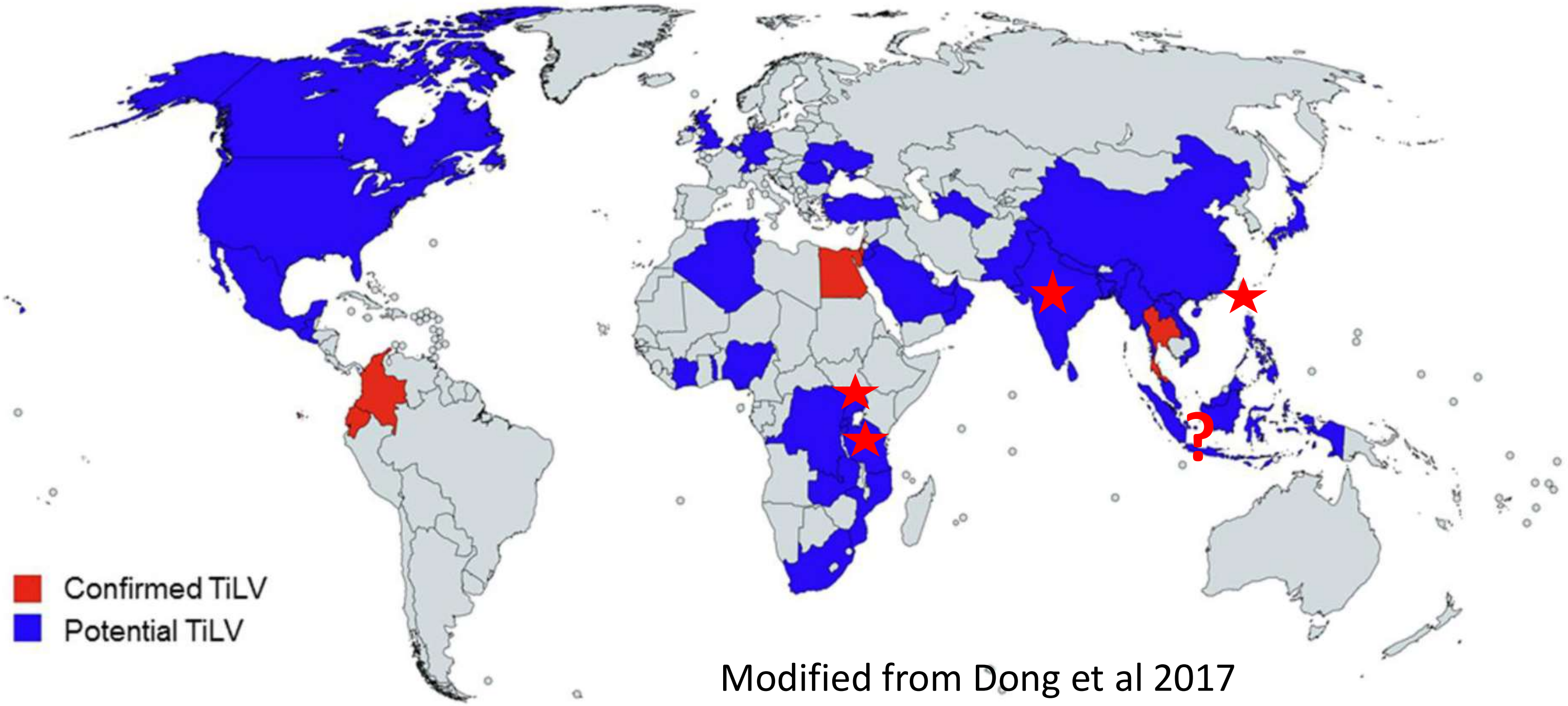
8.2. Presumptive test methods

TiLV can be cultured in primary tilapia brain cells or in an E-11 cell line, inducing a cytopathic effect at 5-10 days (Eyngor *et al.*, 2014). Tsofack *et al.* (2016) describe optimal conditions for culturing TiLV.

8.3. Confirmatory test methods

A PCR primer set has been designed and a reverse transcriptase (RT) PCR has been developed (Eyngor *et al.*, 2014); however, the test was not fully validated. A more highly sensitive, nested RT-PCR has been published and is suitable for the detection of TiLV in clinical cases (Tsofack *et al.*, 2016). Most recently a semi-nest RT-PCR with an improved detection sensitivity (7.5 viral copies per reaction) over the nested RT-PCR, has been published (Dong *et al.*, 2017).

Geographical distribution of TILV



Modified from Dong et al 2017

Jurnal Riset Akuakultur, 13 (1), 2018, 85-92

Tersedia online di: http://ejournal-balitbang.kkp.go.id/index.php/jra

STUDI KASUS INFEKSI *TILAPIA LAKE VIRUS* (TiLV) PADA IKAN NILA (*Oreochromis niloticus*)

Isti Koesharyani[*)#], Lila Gardenia[**)], Zakiyah Widowati[***)], Khun

[*)] Pusat Riset Perikanan
[**)] Balai Riset Perikanan Budidaya Air Tawar dan Penyul
[***)] Balai Uji Standar Karantina Ikan

(Naskah diterima: 11 Desember 2017; Revisi final: 29 Januari 2018; Diset

Fri 13-Apr-18 1:10 AM
Jansen, Mona Dverdal <mona-dverdal.jansen@vetinst.no>
Question relating to a TiLV publication from Indonesia
To Sunarto, Agus (H&B, Geelong AAHL)
You replied to this message on 13-Apr-18 10:47 AM.
If there are problems with how this message is displayed, click here to view it in a web browser.

Message | 6202-18340-2-PB.pdf (177 KB)

Dear Agus,
I hope everything is going well at your end.

I just came across a publication in Indonesian mentioning the detection of TiLV in Lombok. As it's in Indonesian I just wondered whether you could help me by verifying that Google Translate hasn't fooled me completely. If you have time of course!
I only really need to understand the most basic things so here's my understanding of the main points I'm after and a couple of things I'm missing:
They have detected TiLV in Nile tilapia in Lombok, Indonesia. The fish were 8-12 cm in size and clinical signs included skin lesions, discolouration (darkening), abdominal swelling and eye lesions (cataracts and exophthalmia. Sampled in May 2016 from a farm (From one farm or from several farms - is several, any information on how many? And did they sample just one or several farms?) with mass mortality, with 70 – 100% mortality seen at the affected farm(s).
Sequencing of segment 3 showed a 97% homology to the Israeli KU751816 and KJ605619 (as well as Thai and Indian sequences). No information on deposition of Indonesian sequences to GenBank?
Thank you so much in advance!

Regards,

Mona

Kind regards,

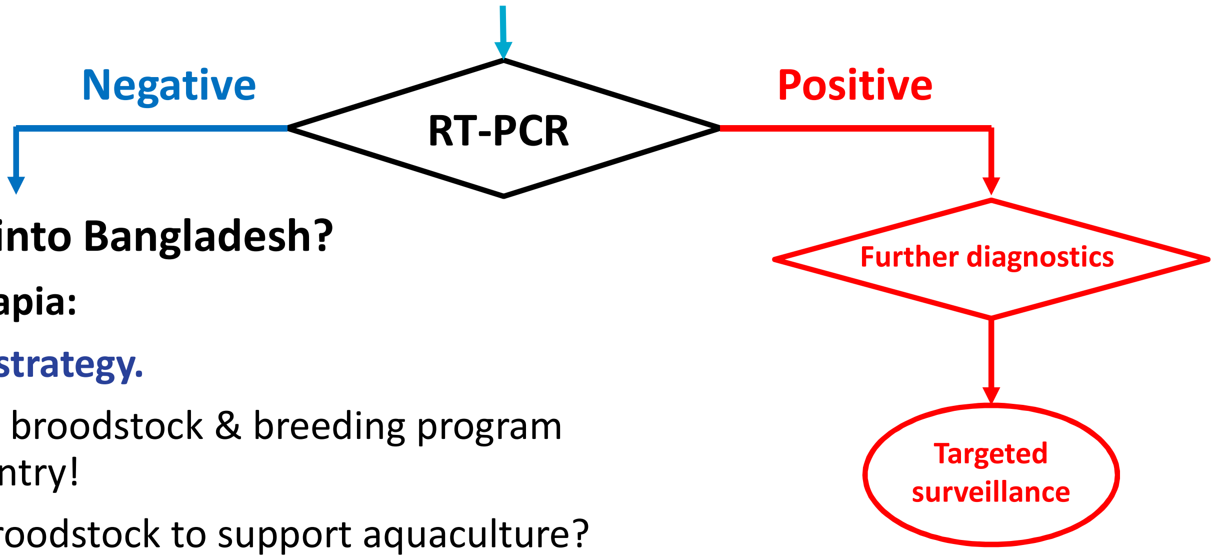
Mona Dverdal Jansen
Researcher

Norwegian Veterinary Institute
PO Box 750 Sentrum
NO-0106 Oslo
Norway

In collaboration with WorldFish Centre

TiLV Mission in Bangladesh

Presence or Absence of TiLV in Bangladesh?



How to prevent TiLV into Bangladesh?

A. Ban import of live tilapia:

+ **The most effective strategy.**

+ Strengthen national broodstock & breeding program
  → self reliance country!

- Enough supply of broodstock to support aquaculture?

B. Import of tilapia from TiLV-free countries:

- High risk due to false negative!
- Need careful risk assessments:
  - Which country? Bangladesh imports tilapia from Thailand, Malaysia & Philippines.
- Import must be completed with CoO, HC and quarantine for two weeks! Is it effective?

How to manage TiLV in Bangladesh?

1. National: regulation
2. TBN (broodstock centre): broodstock
3. TMC (hatchery): seed
4. Farm: consumption

Biological Control of Tilapia: A Potential Virus

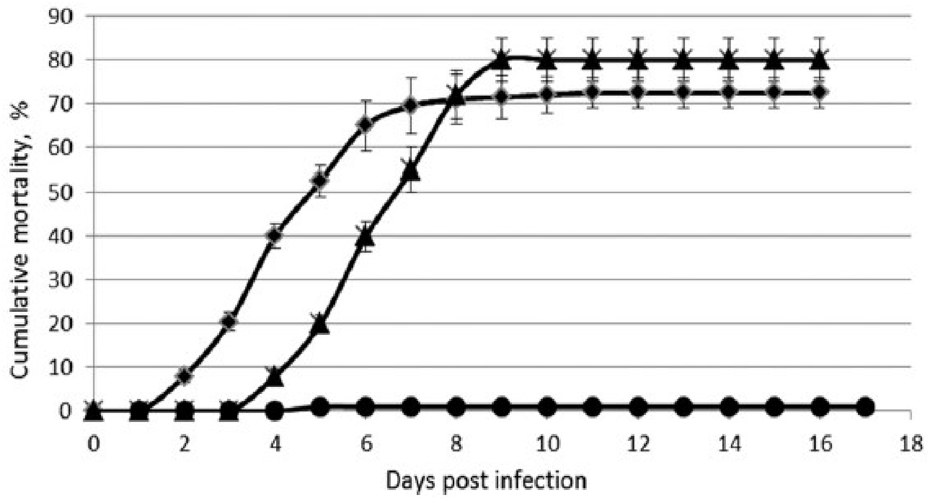
Agus Sunarto and Ken McColl

wsq
Weed Society of Queensland
Pest Animal Branch

Options. Obstacles. Outcomes
5TH QUEENSLAND PEST ANIMAL SYMPOSIUM
7-10 NOVEMBER 2016, RYDGES SOUTHBANK TOWNSVILLE

Efficacy & Safety

TiLV-induced mortality



- 80% mortality, dropped annual prod by 85%.
- TiLV spread through water.

- TiLV causes disease in tilapia, but not in other fish species.
- Kinneret Lake in Israel hosting some 27 species, but TiLV be associated with a decline of tilapia only.

→ **Indicating species-specificity of TiLV**

Outline:

1. Role of AAHL in Australia:
   - AAHL & AFDL
2. Aquatic animal health relevant to Indonesia:
   - KHV, AbHV & TiLV
3. New emerging technologies:
   - Next Generation Sequencing (NGS)
   - Bioinformatics
   - Genome editing (GE)
4. Opportunities - Managing tilapia

Next Generation Sequencing (NGS)

Sanger sequencing

Low throughput: <1000 bp
High cost: $100 M, 20 years.

Conventional NGS

High throughput: genome
HGP: <$1000, days

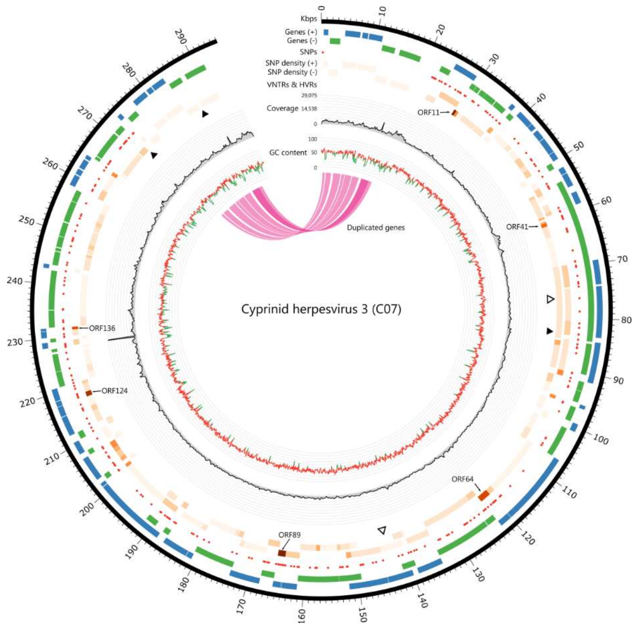
Portable NGS

NGS & Bioinformatics Workflow

Sequencing: **Extract DNA** → **Prepare Libraries** → **Sequence with Illumina MiSeq**

6 million, 150 bp reads

Bioinformatics:



McColl *et al. Virology Journal* (2016) 13:206
DOI 10.1186/s12985-016-0666-4

Virology Journal

COMMENTARY — Open Access

Cyprinid herpesvirus 3 and its evolutionary future as a biological control agent for carp in Australia

Kenneth A. McColl[1,2], Agus Sunarto[1,2] and Edward C. Holmes[3*]

RNAseq of Carp Infected with KHV

Dr Matthew Neave

Sequencing

Extract total RNA → **Enrich for mRNA** → **Sequence with Illumina HiSeq**

- Enrich for mRNA: polyA+ selection
- Sequence with Illumina HiSeq: 400 million, 150 bp reads

Bioinformatics

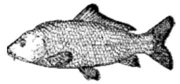
Clean reads → **Map to genome** → **Differentially expressed genes**

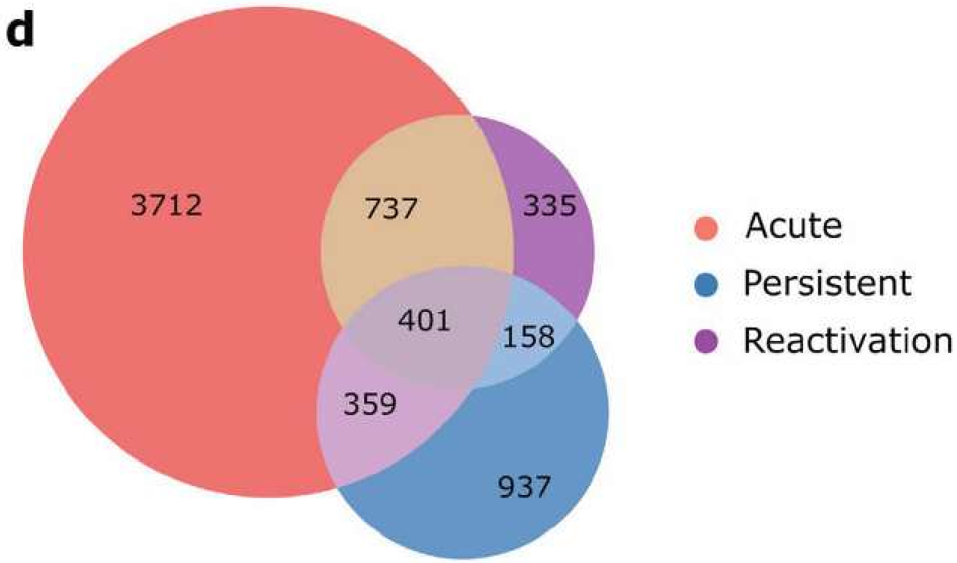
- Clean reads: Quality trim and adapter removal with Trimmomatic
- Map to genome: TopHat for splice-aware alignment
- Differentially expressed genes: HTSeq counts and DESeq2 significance testing

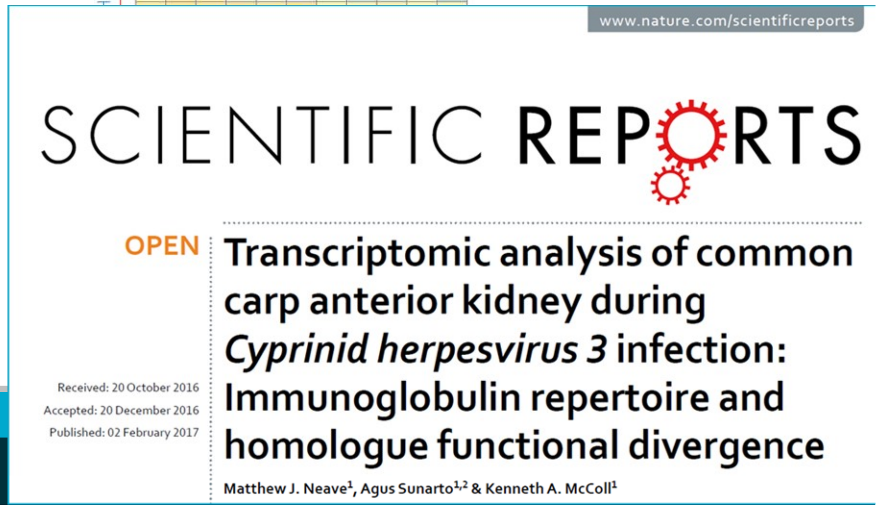
Gene expression for every gene in the host and viral genomes

Neave, Sunarto, McColl (2017) Sci Reports

Carp infected with KHV favour a humoral immune response






www.nature.com/scientificreports

SCIENTIFIC REPORTS

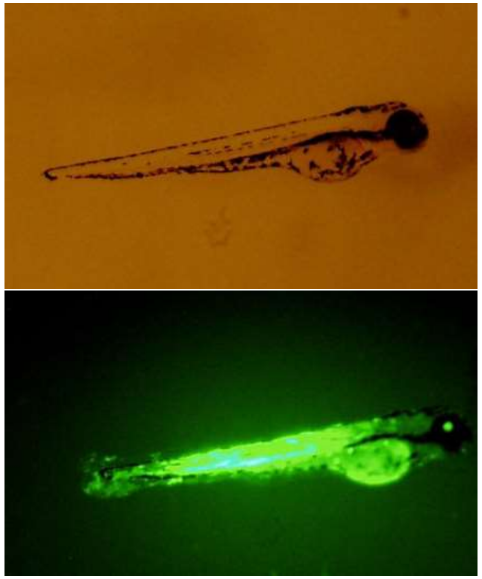
OPEN

Transcriptomic analysis of common carp anterior kidney during *Cyprinid herpesvirus 3* infection: Immunoglobulin repertoire and homologue functional divergence

Received: 20 October 2016
Accepted: 20 December 2016
Published: 02 February 2017

Matthew J. Neave[1], Agus Sunarto[1,2] & Kenneth A. McColl[1]

Tim Doran's genome engineering lab

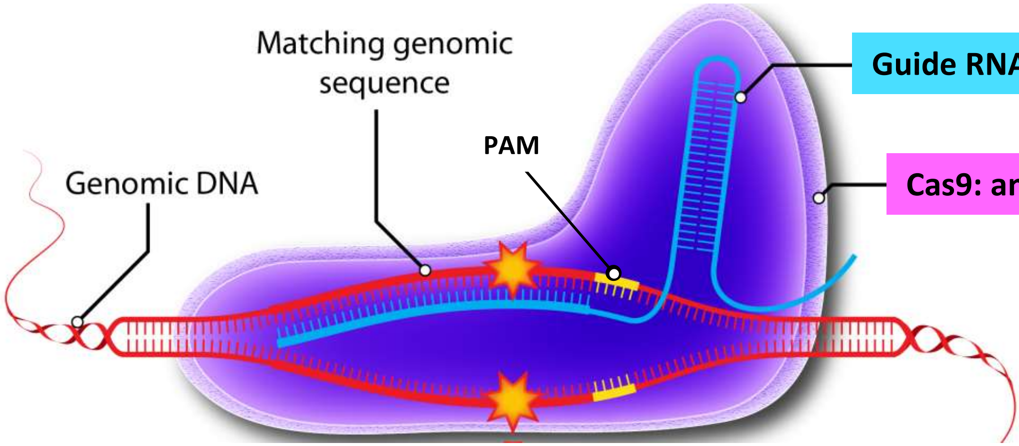


Avian influenza resistant chickens

VHSV resistant zebrafish

Genome Editing Technologies

CRISPR-Cas9 systems:



Male tilapia grows faster than female

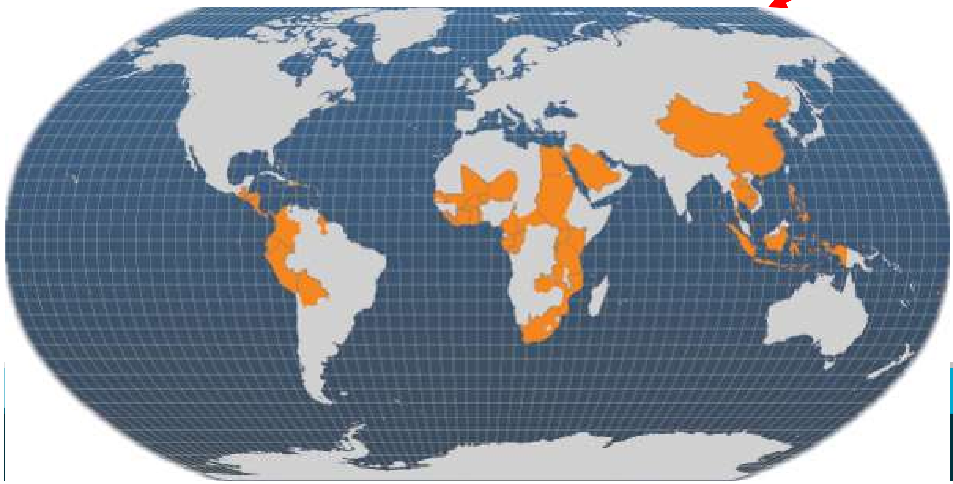
Mutation of *foxl2* or *cyp19a1a* Results in Female to Male Sex Reversal in XX Nile Tilapia

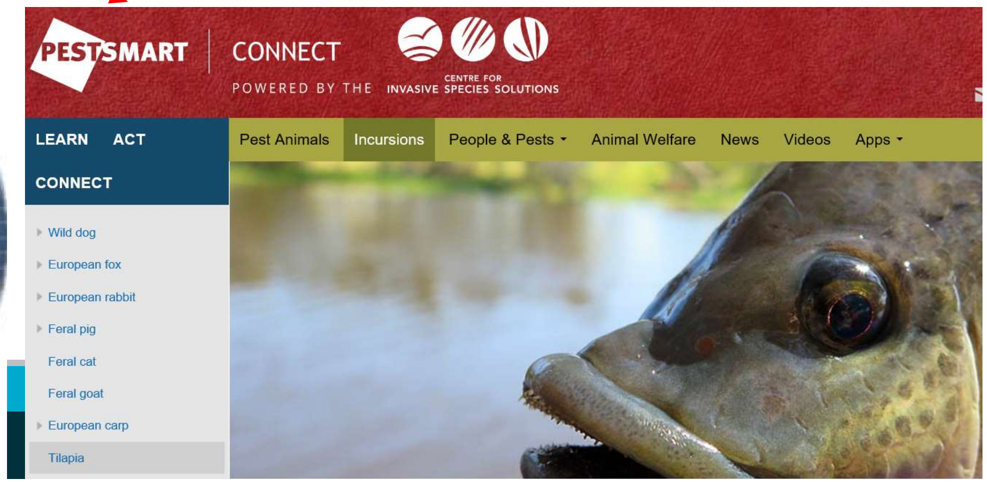
Xianbo Zhang,[1]* Mengru Li,[1]* He Ma,[1] Xingyong Liu,[1] Hongjuan Shi,[1] Minghui Li,[1] and Deshou Wang[1]

Foxl2 - ovarian development

Cyp19 - estrogen production

Genome editing to knock out foxl2 and cyp19 to produce all-male fast growing tilapia





GM vs Non-GM

AquAdvantage Atlantic salmon (at back) grow to twice the size of an normal Atlantic salmon (*Salmo salar*) over the same time.

BIOTECHNOLOGY

Transgenic salmon leaps to the dinner table

Long-awaited decision by US government authorizes the first genetically engineered animal to be sold as food.

DNA recombinant tech (1973)

All white: Arctic apples do not colour in four-hour bruise test. Picture: Okanagan Specialty Fruits

Horticulture

Non-browning apples: CSIRO breakthrough keeps fruit looking fresh

LYNDAL READING, The Weekly Times
October 27, 2017 12:00am

A NON-BROWNING apple, developed using CSIRO technology, will go on sale at some US supermarkets next month.

Outline:

1. Role of AAHL in Australia:
   - AHHL & AFDL
2. Diseases relevant to Indonesia:
   - KHV, AbHV, TiLV
3. New emerging technologies:
   - NGS, Bioinformatics, GE
4. Opportunity: Managing tilapia

Dead fish in one of the Haranggaol's floating net cages in May. Photo by Ayat S. Karokaro/Mongabay

The fish carcasses are hauled away in plastic bags. Photo by Ayat S. Karokaro/Mongabay

Mongabay Series: Indonesian Fisheries, Jokowi Commitments

Military sent to clear fish farms in Indonesia's Lake Toba

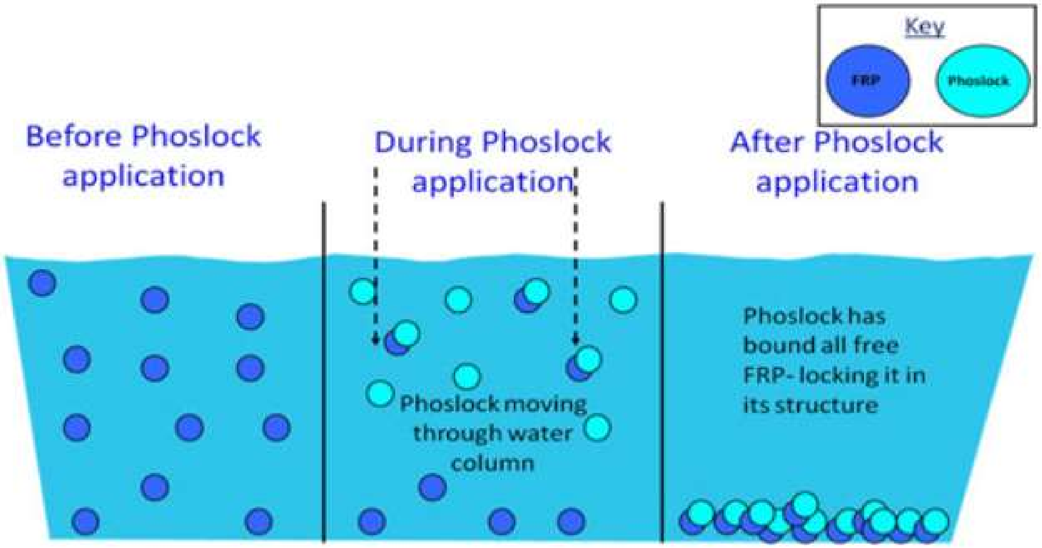
by Aria Danaparamita on 22 July 2016

Hyper-eutrophic

Mongabay Series: Indonesian Fisheries

Why did millions of fish turn up dead in Indonesia's giant Lake Toba?

by Aria Danaparamita on 30 August 2016



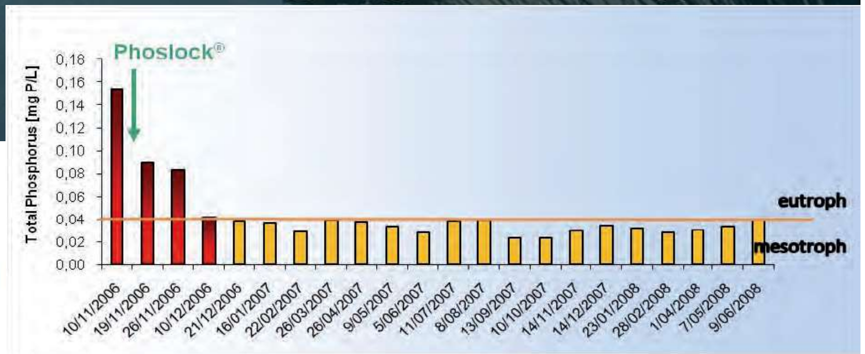


Figure 1: By using Phoslock, the overall phosphorus level was reduced from 160 µg to 36 µg in an application on the Silbersee (Germany).

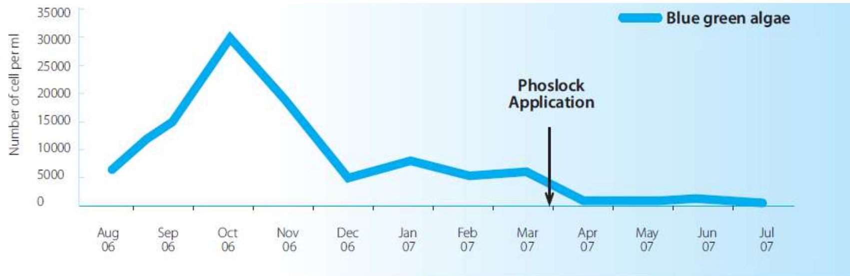


Figure 2: Concentrations (cells/ml) of blue green algae in Australian drinking water reservoir (0 – 8 m) before and after application of Phoslock (August 2006 to July 2007). n = 3 (Sampling points)

Managing tilapia as the aquatic chicken and an invasive species

Agus Sunarto, Ken McColl and Tim Doran

CSIRO Biosecurity Flagship, Australian Animal Health Laboratory (AAHL)
www.csiro.au

CENTRE FOR INVASIVE SPECIES SOLUTIONS

WorldFish

ACIAR
Research that works for developing countries and Australia
aciar.gov.au

CSIRO

Pathway to Impact

Potential funding:

ACIAR
Research that works for developing countries and Australia
aciar.gov.au

Managing tilapia as the aquatic chicken and an invasive species.

CENTRE FOR INVASIVE SPECIES SOLUTIONS

CSIRO **R+ PDF**

Gene editing in aquaculture: development of all-male fast growing tilapia

Public-Private Partnerships:

GenoMar SAFE SUSTAINABLE SEA HARVEST

AQUABEL

EW Group acquired two global tilapia breeding companies, Aquabel & GenoMar

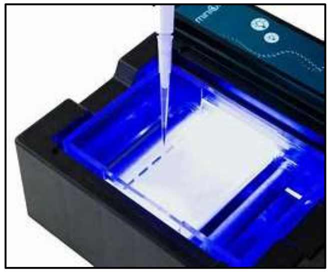
Scaling up:

WorldFish

DFAT-funded scheme

Take home messages

Diagnostics:



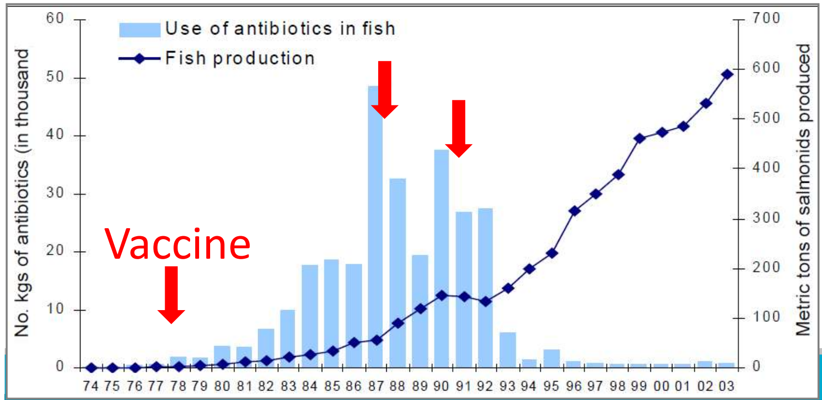
Conventional PCR

Real-time PCR

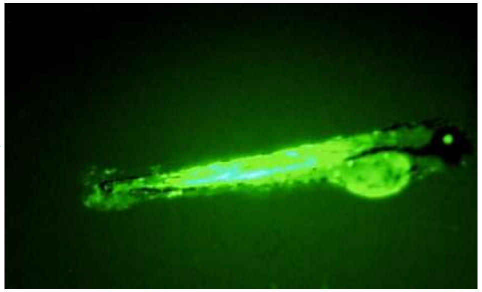
Conventional NGS

Portable NGS

Control:



Vaccine



Virus resistant fish

Acknowledgements

- **CSIRO:**
  - **AAHL Fish Diseases Lab**: Mark Crane, Nick Moody, Nick Gudkovs, Serge Corbeil, Peter Mohr, David Cummins, John Hoad, Jo Slater, Nette Williams, Stacey Valdeter, Matthew Neave & Ken McColl.
  - **Genome Engineering Team**: Tim Doran, Mark Tizard, Kristie Jenkins & Arjun Challagulla
  - **Managing Invasive Species & Diseases**: Andy Sheppard
- **Funding:** DAWR, FRDC, IA-CRC, NCCP, CISS.
- **Collaborators:**