



Food and Agriculture
Organization of the
United Nations



FAO/China Intensive Training Course on Tilapia Lake Virus (TiLV)

Sun Yat Sen University, Guangzhou, China

18-24 June 2018

Session 2

Mona Dverdal Jansen

What is currently known about TiLV

The discovery of a new pathogen



FAO/China Intensive Course on TiLV 18-24 June 2018 Guangzhou China

Tilapia – advantages and disadvantages

- Wide geographical distribution → large market
- Tolerance and adaptability → range of production systems
- Natural hardiness
- Genetic selection and targeted breeding
- **=> lack of focus on potential disease occurrence & thorough disease investigations?**



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The start – farmed stocks



Identification of a Novel RNA Virus Lethal to Tilapia

Marina Eyngor,^a Rachel Zamostiano,^b Japhette Esther Kembou Tsofack,^b Asaf Berkowitz,^a Hillel Bercovier,^c Simon Tinman,^d Menachem Lev,^a Avshalom Hurvitz,^f Marco Galeotti,^g Eran Bacharach,^b Avi Eldar^a

Department of Poultry and Fish Diseases, The Kimron Veterinary Institute, Bet Dagan, Israel^a; Department of Cell Research and Immunology, The George S. Wise Faculty of

- Summer of 2009: massive mortality events in all tilapia fish farming areas
- “Wave of mortality spreading from cage to cage “
- No apparent cause

The start – wild stocks



Identification of a Novel RNA Virus Lethal to Tilapia

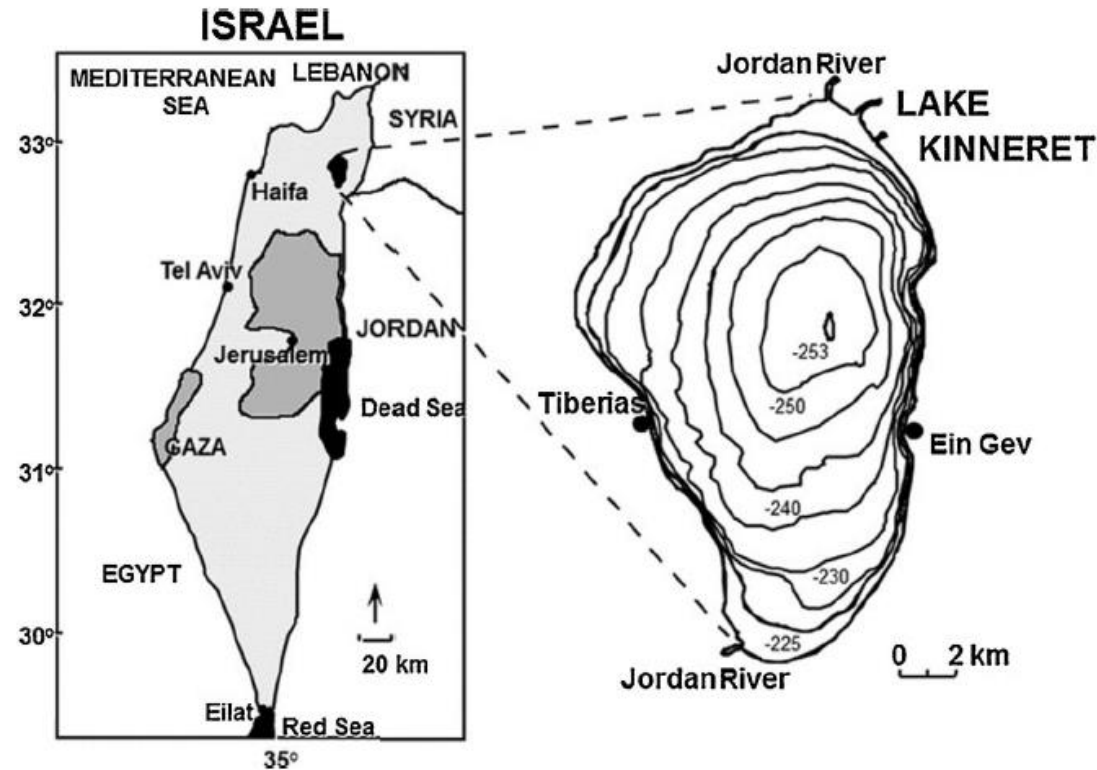
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- *Sarotherodon (Tilapia) galilaeus* (St. Peter's fish): significant decrease in annual catches 2005 – 2010, Lake Kinneret
- No apparent reason for the mortality (known parasites, bacteria, viruses, toxins)

Lake Kinneret (The Sea of Galilee)

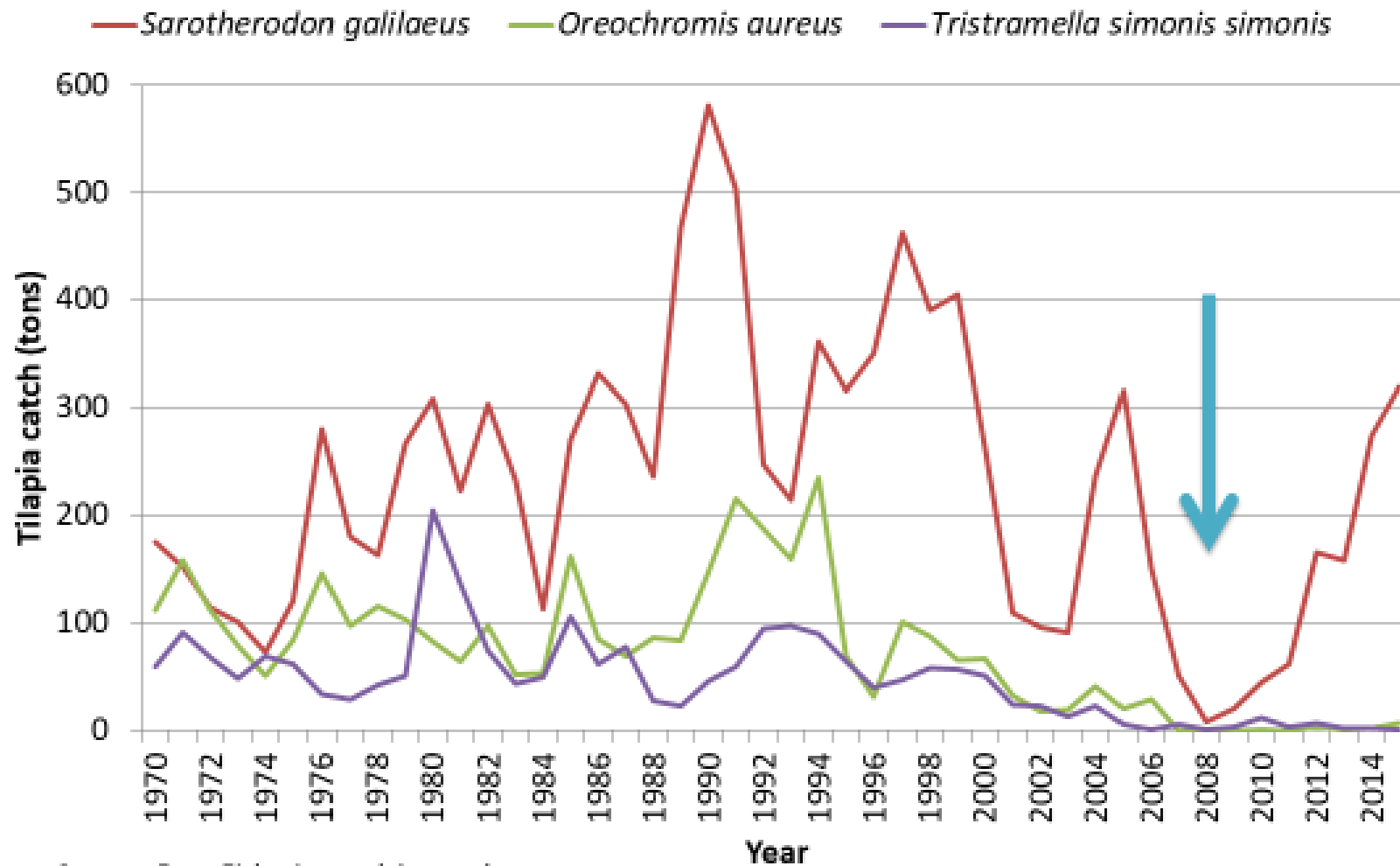
- The only freshwater lake in Israel, located in the central part of the Jordan rift valley.
- Greater fish abundance at the lake periphery than in its pelagic zone.
- More than 27 species of fish (19 are native).



Parparov & Gal, 2012

Courtesy of N. Davidovich, Ministry of Agriculture and Rural Development

Tilapia catch – Lake Kinneret



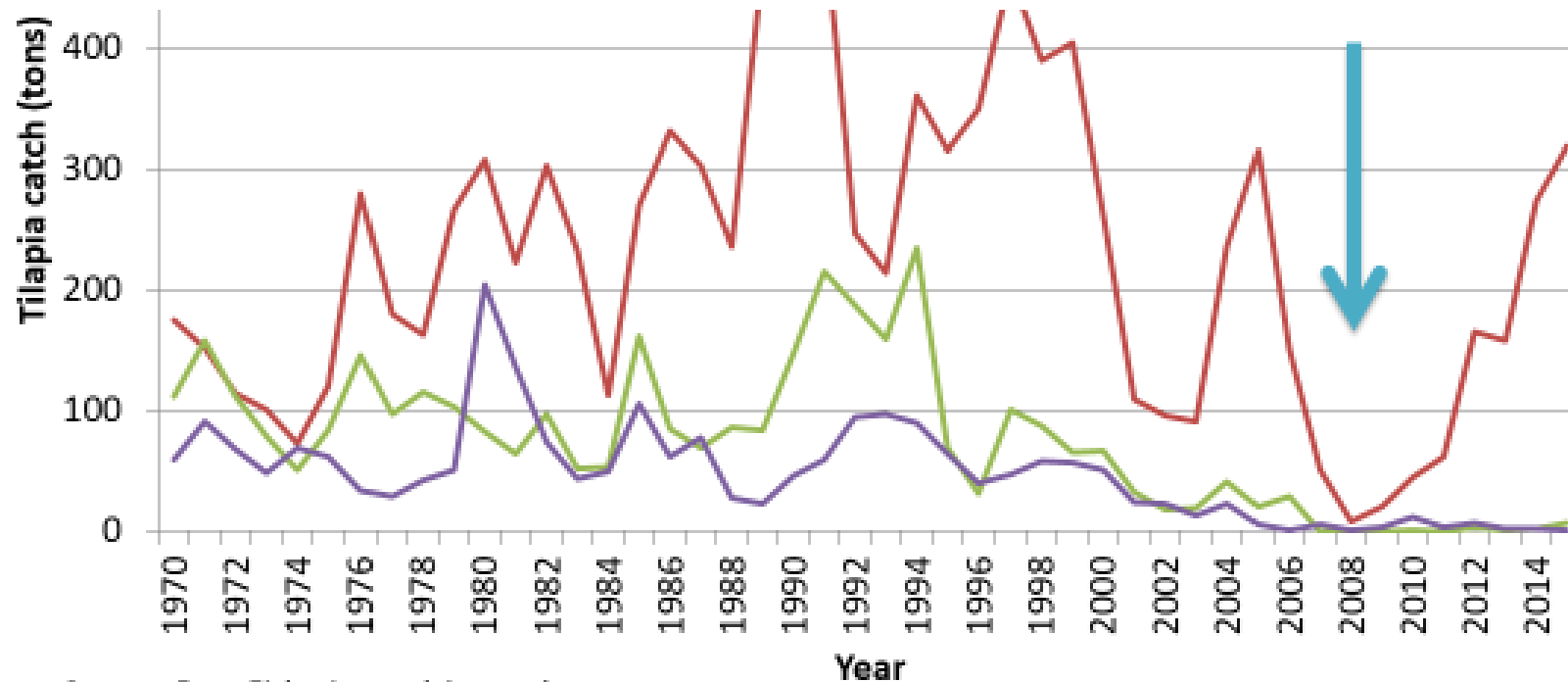
Source: Dep. Fisheries and Aquaculture

Courtesy of: N. Davidovich, Ministry of Agriculture and Rural Development

Tilapia catch – Lake Kinneret

— *Sarotherodon galilaeus* — *Oreochromis aureus* — *Tristramella simonis simonis*

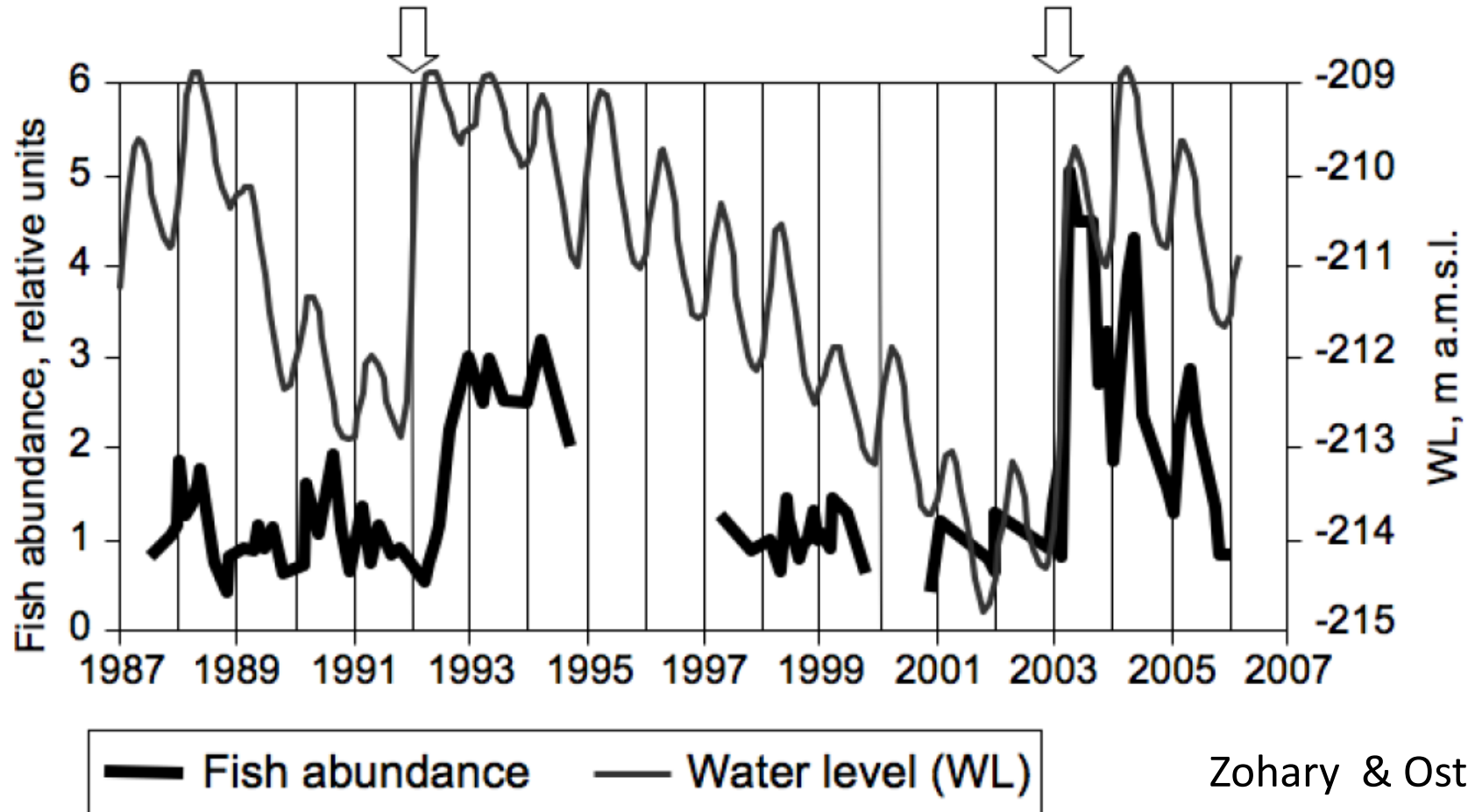
S. galilaeus: 2015: 316 tons, 2007: 51 tons, 2009: 8 tons, 2010: 45 tons (Eyngor *et al.* 2014)



Source: Dep. Fisheries and Aquaculture

Courtesy of: N. Davidovich, Ministry of Agriculture and Rural Development

Fish abundance vs water level – Lake Kinneret



Zohary & Ostrovsky , 2011

Parasitic fauna – Lake Kinneret

Parasite 2014, 21, 32
© M. Caffara et al., published by EDP Sciences, 2014
DOI: 10.1051/parasite/2014034


Available online at:
www.parasite-journal.org

RESEARCH ARTICLE

OPEN ACCESS

Redescription of *Clinostomum phalacrocoracis* metacercariae (Digenea: Clinostomidae) in cichlids from Lake Kinneret, Israel

Monica Caffara^{1,*}, Nadav Davidovich², Rama Falk², Margarita Smirnov², Tamir Ofek², David Cummings³, Andrea Gustinelli¹, and Maria L. Fioravanti¹

¹ Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy

² Central Fish Health Laboratory, Department of Fisheries and Aquaculture, Ministry of Agriculture and Rural Development, 10803 Nir David, Israel

³ Israel Oceanographic and Limnological Research, Kinneret Limnological Laboratory, 14950 Migdal, Israel

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Abstract – Clinostomidae are digeneans characterized by a complex taxonomic history, continuously under revision based on both morphological and molecular analysis. Among the 14 species considered valid so far *Clinostomum phalacrocoracis* has been well described only at the adult stage, whereas the morphology of the metacercarial stage has been reported only once. During a parasitological survey carried out on 262 wild cichlids sampled from Lake Kinneret (Israel) metacercariae referable to *C. phalacrocoracis* were found in 18 fingerlings. In this study, we report this clinostomid species for the first time in wild fish from Israel describing the metacercarial stage of *Clinostomum phalacrocoracis*, coupling its morphological description with molecular analysis carried out on ITS rDNA and COI mtDNA sequences.

Key words: *Clinostomum phalacrocoracis*, Metacercaria, Wild Cichlids, Lake Kinneret, Israel, Molecular Analysis.

Invasive snail

Biol Invasions
DOI 10.1007/s10530-013-0500-5

INVASION NOTE

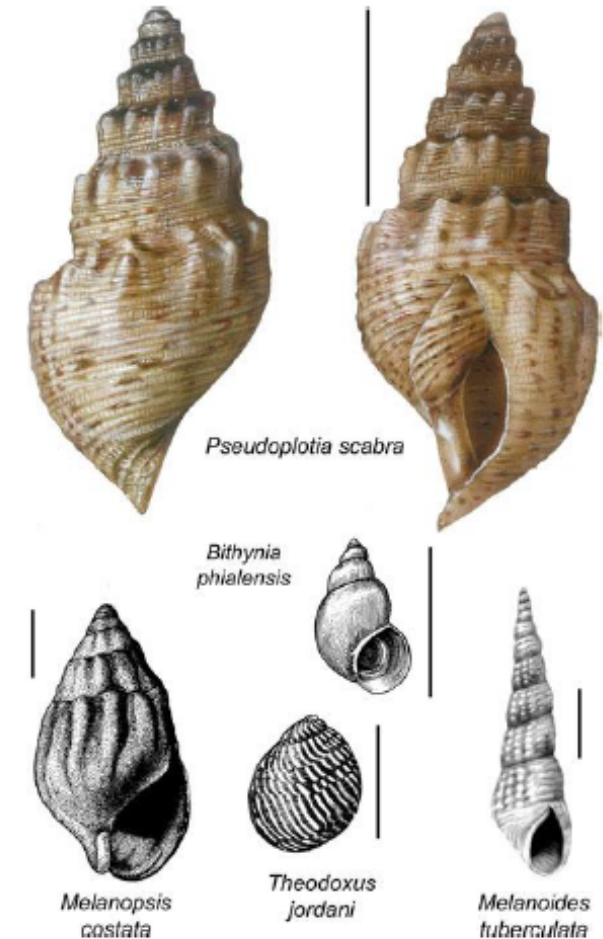
Invasion dynamics of the snail *Pseudoplotia scabra* in Lake Kinneret

Joseph Heller · Adina Dolev · Tamar Zohary · Gideon Gal

Received: 11 October 2012 / Accepted: 16 May 2013
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Abstract The freshwater snail *Pseudoplotia scabra* Müller (Thiaridae) was first spotted in Lake Kinneret, Israel, in the mid 2000s. In a series of field surveys we followed its spread, documenting how by the end of 2010 this invasive mollusc formed >95 % of the snails in Lake Kinneret, nearly eradicating four native species.

2010). Up to about 1990 the first three of these four species were so common in Lake Kinneret that they covered every rock and boulder and every soft substrate, from the waterline to a depth of 15 m, with densities reaching hundreds of individuals m^{-2} (Dagan 1972; Tchernov 1975, J. Heller pers. obs.). By 2004, however, only very few live snails were found during



Courtesy of N. Davidovich, Ministry of Agriculture and Rural Development



A new pathogen – the very short version

- Organs with pathological lesion sampled
- Pooled, homogenized, incubated with 8 different cell lines
- CPE in two cell lines (E-11 cells and primary tilapia brain cells)
- Re-passaged to naïve cultures -> CPE-causing agent recovered from 25 samples
- Electron microscopy -> virion-like structures



Identification of a Novel RNA Virus Lethal to Tilapia

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Department of Poultry and Fish Diseases, The Kimron Veterinary Institute, Bet Dagan, Israel^a; Department of Cell Research and Immunology, The George S. Wise Faculty of

A new pathogen – the very short version

- Sensitive to ether and chloroform
- RNA extracted
- Reverse-transcriptase reaction
- cDNA cloned (shotgun approach)
- Clone 7450 -> identification of 1,326 bases of a putative TiLV sequence (Genbank submission)



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A new pathogen – the very short version

- Blast-search: no significant homologies
- Named “Tilapia lake virus” (TiLV)
- Reproduction of disease through intra-peritoneal injection and cohabitation (Koch’s postulate)
- Development of a PCR assay



Identification of a Novel RNA Virus Lethal to Tilapia

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The first TiLV detections

- Farmed tilapia:
 - 14 TiLV-positive samples
 - Outbreak definition stated
 - 40g – 350g
 - Hot season (May to October), water temperature 22-32°C
- Wild tilapines:
 - 11 TiLV-positive samples
- No further epidemiological information



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Epidemiological investigations on their way

1) Retrospective, descriptive study:

- interview with farmers to collect historical data

2) Prospective study:

- Spatial analyses
- Relationship between morbidity/mortality events and a range of risk factors

Pers. comm. N. Davidovich, Ministry of Agriculture and Rural Development

What about the wild stocks?

- Department of Fisheries and Agriculture repopulate Lake Kinneret with different fish species
 - 80's (decade) - *Oreochromis aureus*
 - Last 30 years - *Sarotherodon galilaeus*
 - Every year - mullets (mainly *Mugil cephalus* and *Liza ramada*)
 - Until two years ago – silver carp (*Hypophthalmichthys molitrix*)
- Every year: a statutory committee decide on fingerling numbers
 - several hundred thousand to few millions
 - water levels and quality, algae population and other considerations

Pers. Comm.: N. Davidovich, Ministry of Agriculture and Rural Development

More details on the virus.....



TiLV properties

- Enveloped, -ve sense ssRNA virus
 - 55 to 100nm diameter
 - 10 segments encoding 10 proteins
 - Segment 1 ORF – weak sequence homology to Influenza C virus PB1 subunit
 - Conserved sequences at 5' and 3' termini – resemble Orthomyxoviruses
- > “orthomyxo-like virus”
- > ICTV proposal: *Tilapia tilapinevirus*

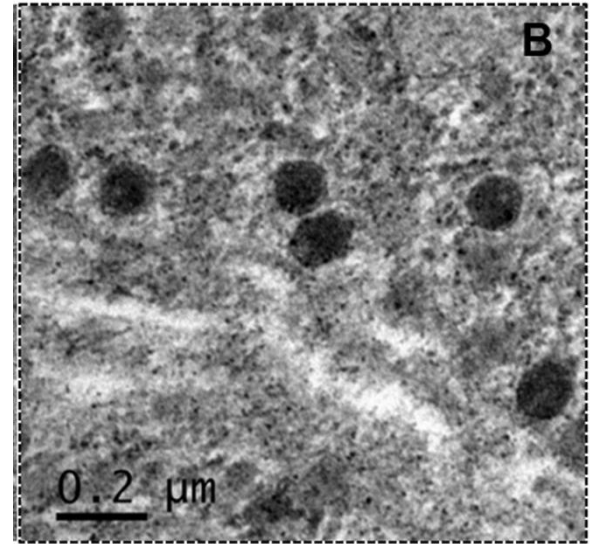


Image by H. T. Dong

Reference list: see Jansen *et al* (2018)

Genetic variation

Source (non-Israeli sources)	GenBank accession no.	Identity to TiLV from Israel (prototype strain)		References
		GenBank accession no. of Israeli TiLV	% nt identity	
Chinese Taipei	Not available	Segment 3 (Accession number not specified)	93%	OIE (2017b)
Ecuador	Not available	Full genome sequences KU751814–KU751823	97.2–99.0%	Bacharach <i>et al.</i> (2016a)
Ecuador	Not available	KJ605629 (ORF)	98% to 100%	del-Pozo <i>et al.</i> (2017)
Egypt	Not available	KU751816 (segment 3)	93%	Fathi <i>et al.</i> (2017)
Egypt	KY817381–KY817390	Segments 3, 4 and 9 (Accession numbers not specified)	93%	Nicholson <i>et al.</i> (2017)
India	MF502419, MF574205 and MF582636	KJ605629 (segment 3)	96.4–97.2%	Behera <i>et al.</i> (2018)
Indonesia	Not available	KU751816 and KJ605629 (segment 3)	97%	Koesharyani <i>et al.</i> (2018)
Malaysia	MF685337	KU751822 (segment 9)	97%	Amal <i>et al.</i> (2018)
Philippines	Not available	Segment 3 (Accession number not specified)	94–95%	OIE (2017f)
Tanzania (Lake Victoria)	MF526980–MF526996	KU552132 (contig 7 = segment 2) KU751815 (= NC029921, segment 2)	Not given†	Mugimba <i>et al.</i> (2018)
Thailand	KY615742	KU751814 (segment 1)	96.3–97.5%	Dong <i>et al.</i> (2017a)
Thailand	KY615743	KU751818 (segment 5)		
Thailand	KY615744 to KY615745	KU751822 (segment 9)		
Thailand	KX631921 KX631930–KX631936	Full genome sequences KU751814–KU751823	95.6–99.1%	Surachetpong <i>et al.</i> (2017)
Uganda (Lake Victoria)	MF536423–MF536432	KU552132 (contig 7 = segment 2) KU751815 (= NC029921, segment 2)	Not given†	Mugimba <i>et al.</i> (2018)

†Authors state that sequences were 'identical with' or 'closely related to' the Israeli sequences.

Effects of the new discovery





Food and Agriculture Organization
of the United Nations

GLOBAL INFORMATION AND EARLY WARNING SYSTEM ON
FOOD AND AGRICULTURE (GIEWS)

SPECIAL ALERT

No. 338

REGION: Global

DATE: 26 May 2017

Outbreaks of Tilapia lake virus (TiLV) threaten the livelihoods and food security of millions of people dependent on tilapia farming

Highlights

- Tilapia lake virus (TiLV) poses a great threat to the tilapia sector. Tilapias are farmed globally and are the second most important aquaculture species in terms of volumes produced, providing a key source of affordable animal protein, income to fishfarmers and fishers, and domestic and export earnings.
- TiLV has been confirmed in some countries in Asia, Africa and Latin America. It is likely that TiLV may have a wider distribution than is known today and its threat to tilapia farming at the global level is significant.
- While there is no public health concern for this pathogen, there is a significant risk of TiLV being translocated both inter- and intra-continentially through the movement of infected live tilapias in the absence of appropriate biosecurity measures.
- Tilapia producing countries need to be vigilant and take appropriate risk management measures (e.g. enhanced diagnostic testing of imported stocks and unexplained tilapia mortalities and reporting to biosecurity authorities, active surveillance, public information campaigns and contingency plans) to reduce the further spread and potential socio-economic impacts of this emerging disease.

An outbreak of fish disease Tilapia lake virus (TiLV), an orthomyxo-like virus belonging to the family Orthomyxoviridae, threatens to compromise the livelihoods and food security of millions of people, particularly of highly vulnerable subsistence fishers and small fish farms. The disease is highly pathogenic, with

mortalities of tilapia, but have not yet identified the cause. A reliable diagnostic test for TiLV is available and it should be applied to rule out TiLV as the causative agent of unexplained mortalities. As of today, the TiLV has been confirmed in some countries in Africa, Asia and Latin America. There is a significant risk of TiLV



DISEASE ADVISORY



Asia Regional Aquatic Animal
Health Programme

**Tilapia Lake Virus (TiLV) – an Emerging Threat to Farmed Tilapia
in the Asia-Pacific Region**

Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand

- *TiLV (an Orthomyxo-like RNA virus) is an emerging disease of cultured tilapia in the Asia-Pacific region;*
- *Originally observed and reported in Israel, Ecuador, Colombia and Egypt, TiLV is now confirmed in cultured tilapia in Thailand causing mass mortalities;*
- *At risk is here is the US\$7.5 billion global industry per annum, especially among the top tilapia-producing countries in the region including China, the Philippines, Thailand, Indonesia, Lao PDR and Bangladesh.*

Tilapias are highly important (and inexpensive) source of fish protein in the world and are one of the most popular species for aquaculture in several regions including the Asia-Pacific. The top 10 producing countries include China, Egypt, Philippines, Thailand, Indonesia, Lao PDR, Costa Rica, Ecuador, Colombia and Honduras. Since 2009, tilapia aquaculture has been threatened by mass die-offs of farmed fish in Israel and Ecuador (Bacharach et al., 2016). The aetiological agent

OIE definition of an emerging disease

“A new infection resulting from the evolution or change of an existing pathogen or parasite resulting in a change of host range, vector, pathogenicity or strain; or the occurrence of a previously unrecognised infection or disease.”



PATHOGEN INFORMATION

1. CAUSATIVE AGENT

1.1. Pathogen type

Virus.

1.2. Disease name and synonyms

Tilapia lake virus (TiLV) disease.

1.3. Pathogen common names and synonyms

Tilapia lake virus (TiLV).

1.4. Taxonomic affiliation

The taxonomic affiliation has not been definitively concluded; however, TiLV has been described as a novel virus in the Family *Orthomyxoviridae* (Eyngor *et al.*, 2014).

1.5. Authority (first scientific description, reference)

3. HOST RANGE

3.1. Susceptible species

Mortalities attributed to TiLV have been observed in wild tilapia *Sarotherodon (Tilapia) galilaeus*, farmed tilapia *Oreochromis niloticus* and commercial hybrid tilapia (*O. niloticus* X *O. aureus*) (Bacharach *et al.*, 2016; Ferguson *et al.*, 2014; Eyngor *et al.*, 2014). To date only tilapines have been shown to be susceptible. It is possible that other species will be found to be susceptible.

3.2. Affected life stage

In the outbreak reported by Ferguson *et al.* (2014) and Dong *et al.* (2017) fingerlings were mainly affected. Dong *et al.* (2017) reported approximately 90% mortality in red tilapia fingerlings within one month of stocking into cages. Mortality just over 9% in medium to large sized Nile perch was noted by Fathi *et al.* (2017). Other reports have not

WAHIS Interface

Animal Health Information
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 [Disease control measures](#) |
 [Data between 1996 and 2004](#) |
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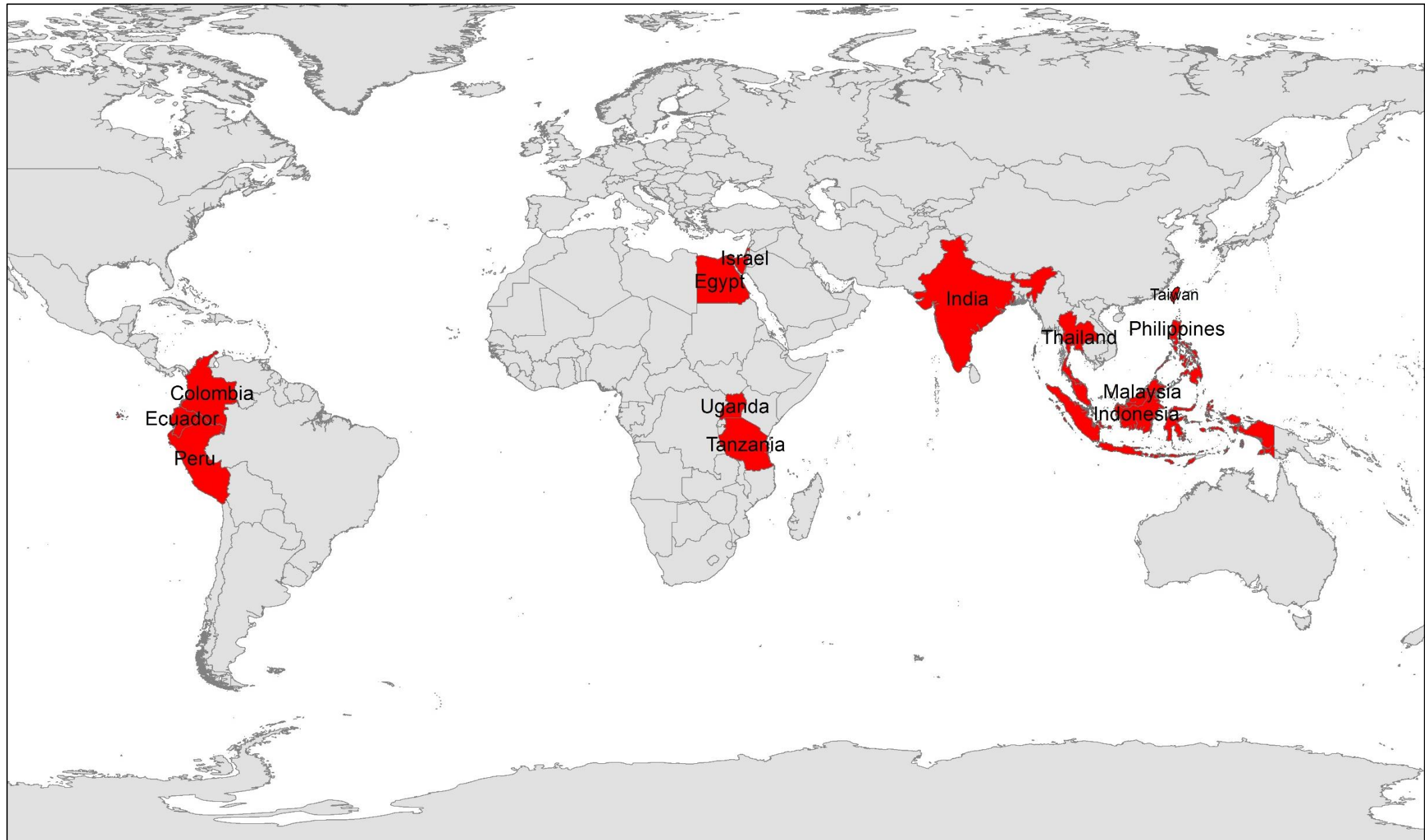
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Exceptional epidemiological events

Year:

These countries have submitted the following immediate notifications to OIE in response to epidemiologically significant events. Click on an event to find more information

Summary	Report	Country	Date of Notification	Disease	Reason for notification	Disease manifestation	Outbreaks	Date resolved
		Afghanistan	23/01/2018	Highly path. avian influenza	Recurrence	Clinical disease	2	Continuing
		Afghanistan	07/02/2018	Highly path. avian influenza	First occurrence	Clinical disease	1	Continuing
		Afghanistan	06/01/2018	Highly pathogenic influenza A viruses (infection with) (non-poultry including wild birds)	Recurrence	Clinical disease	1	Continuing
		Argentina	05/04/2018	Equine influenza	Unexpected change or increase	Clinical disease		
		Armenia	01/02/2018	Bovine tuberculosis	Recurrence	Clinical disease	1	23/01/2018
		Bhutan	13/03/2018	Highly path. avian influenza	Recurrence	Clinical disease	2	Continuing
		Bhutan	06/04/2018	Peste des petits ruminants	Recurrence	Clinical disease	1	Continuing
		Brazil	06/06/2018	West Nile Fever	First occurrence	Clinical disease	1	Continuing



Retrospective analyses of samples

- Earlier detections
- Origin



Image: depositphotos[®]

Potential consequences of disease occurrence



Welfare
Environmental
impact



Economic
loss



Consumer
confidence



Market access
Reputation

Photo: S. Tavoranpanich (1); Colourbox (2-4)

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