Recurrent unexplained mortalities in warm water fish species; emergence of new Tilapia virus and epidemiological approach for surveillance

21st Annual Workshop of the National Reference Laboratories for Fish Diseases

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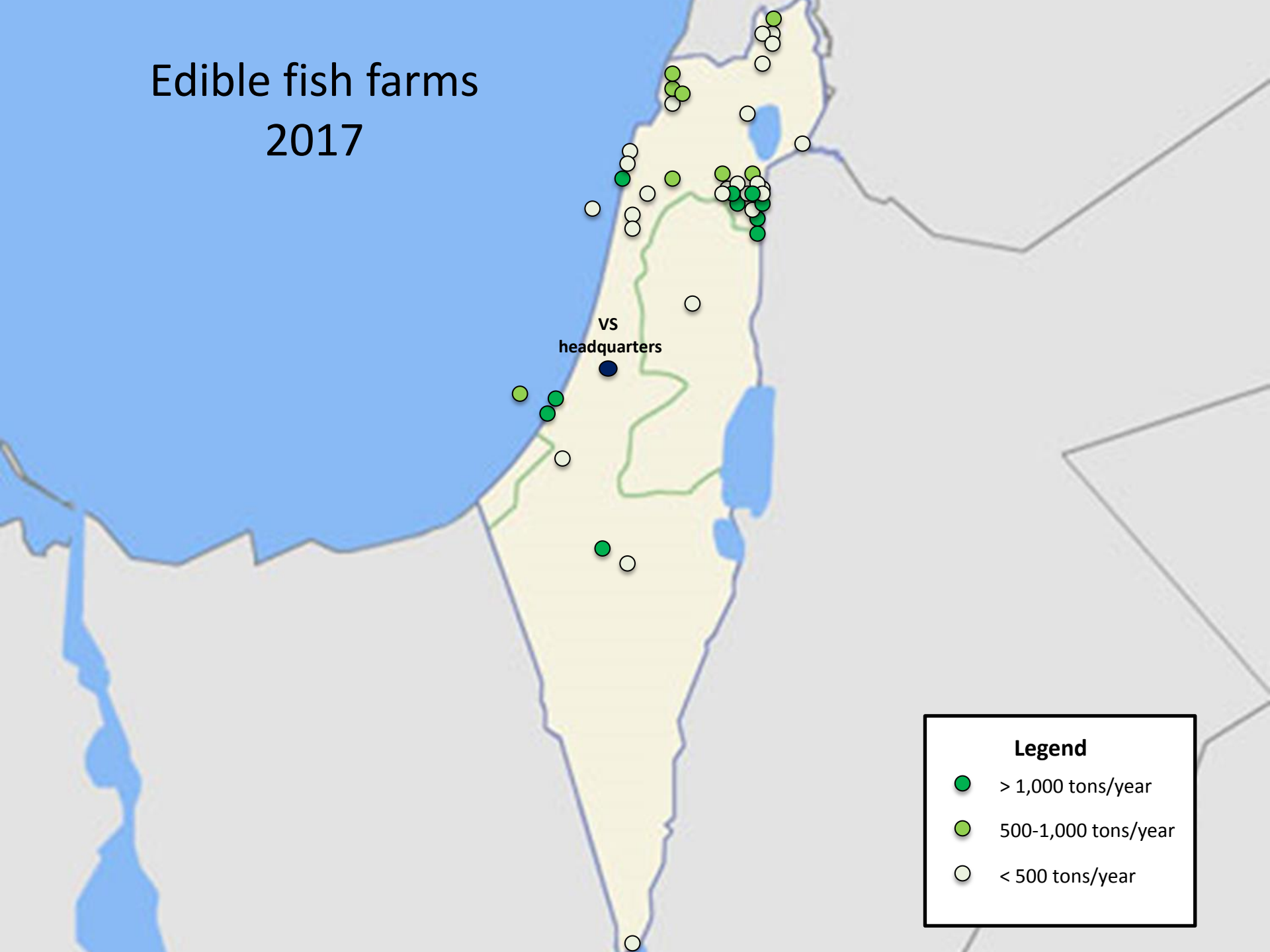
Main topics of the presentation

- Israeli Aquaculture
- Tilapia farming
- A novel RNA virus in Israel and in other countries
- Lake Kinneret
- Epidemiological survey
- Summary

History

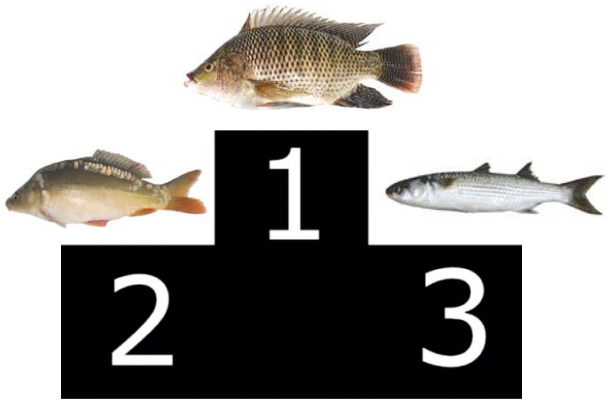
Edible fish farms 2017



Israeli Aquaculture

Inland:

- ~35 edible fish farms.
- ~20 farms rearing Tilapia.
- Total production 2016: 15,000 tons.
- Main species: Tilapia, Common carp, Gray mullet.



Israeli Aquaculture

Mariculture:

- ~10 farms.
  - Sea cages only in The Mediterranean.
  - From 2006, no Sea cages in The Red sea.
- Total production 2016: 2,000 tons.
- Main species: Gilthead seabream.

Israeli Aquaculture

Ornamental:

- ~50 farms.

Cold-water main species:
Koi carp, Goldfish

Warm-water main species:
Guppy, Angelfish

Tilapia farming in the world

- Tilapia = common name for nearly 100 species; various Cichlids from 3 distinct genera:
  - *Oreochromis*
  - *Sarotherodon*
  - *Tilapia*

- Dispersion in >135 countries and territories on all continents.

- Main producers: China, Indonesia, Egypt, Bangladesh, Vietnam, Philippines, Brazil, Thailand, Colombia and Uganda.

Source: FAO

Tilapia farming in the world

- In 2014, the production of Nile tilapia (*O. niloticus*) was >3.1 million tons.

- The economic impact of worldwide trade of Tilapia is ~7.5 billion U.S. dollars annually.

- Tilapia farming can be:
  - extensive (ponds or reservoirs).
  - intensive (cages, ponds, raceways) and indoor recirculating systems.

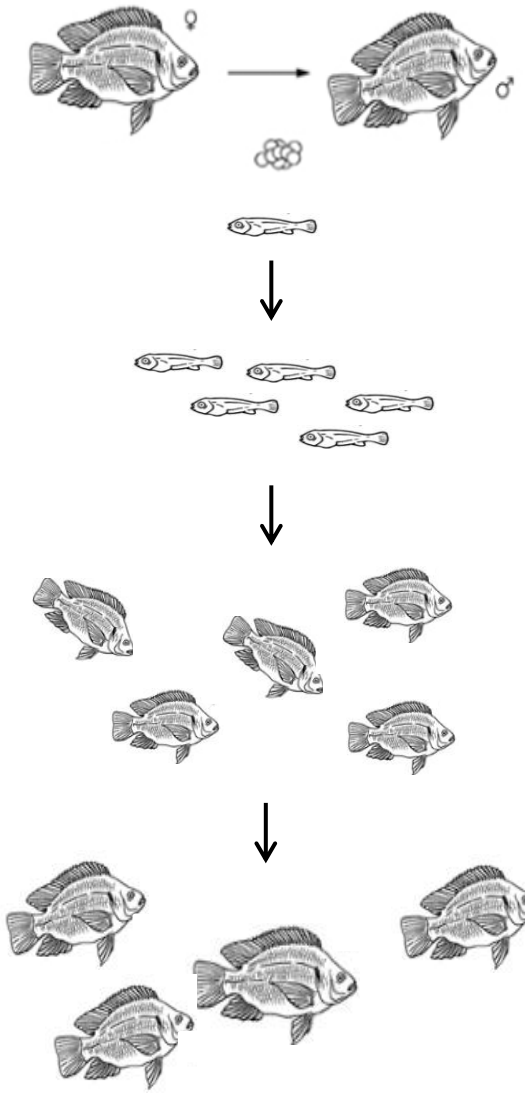
Source: FAO

Tilapia farming in Israel

- The leading species is a Tilapia hybrid (*O. niloticus* X *O. aureus*).
- The hybrid is more resistance to low water temperatures.
- The total production in 2016 reached 7.5 thousand tones.

Production cycle (Israel)

1. Natural breeding
2. Fingerlings
3. Sex-reversal
4. Growout “training”
5. “Winter maintenance”
6. Fattening



Small earth ponds
Mono/poly culture

Large reservoirs
Poly culture

Common Tilapia diseases in Israel - Bacteria

- Streptococcosis, Vibriosis, Aeromoniasis

Common Tilapia diseases in Israel - Parasites

- Monogeneans, Trematodes, Ciliates, Myxosporeans

Common Tilapia diseases in Israel - Fungi / water molds

- Branchiomyces, Saprolegniosis

Other problems – increasing salinity

- *Amyloodinium ocellatum* , Barnacle (infraclass: *Cirripedia*)

Tilapia viral diseases

Virus	Family	DNA/ RNA	Species	Country	Reference
Aquabirnavirus	*Birnaviridae*	RNA	*O. mossambicus*	Taiwan	Hedrick *et al*., 1983
Betanodavirus	*Nodaviridae*	RNA	*O. niloticus*	Western Europe	Bigarré *et al*., 2009
Lymphocystivirus	*Iridoviridae*	DNA	Haplochromine cichlids	Lakes Kitangiri and Victoria	Paperna 1973
Bohle iridovirus, ranavirus	*Iridoviridae*	DNA	*O. mossambicus*	Australia	Ariel & Owens 1997
Iridoviral-like infection	*Iridoviridae*	DNA	*O. niloticus*	USA	Smith *et al*., 1997
Iridoviral-like infection	*Iridoviridae*	DNA	*O. niloticus*	Canada & USA	McGrogan *et al*., 1998
Herpesvirus	*Herpesviridae*	DNA	*O. aureus*	Israel	Shlapobersky *et al*., 2010, Sinyakov *et al*., 2011

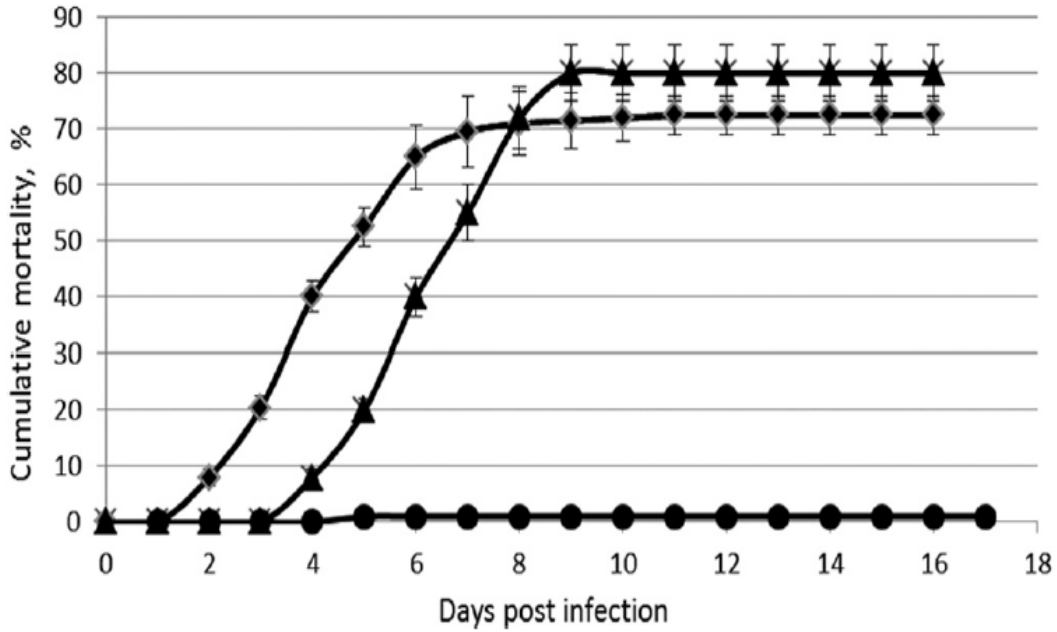
A novel RNA virus in Israel

- Pathogen name: Tilapia Lake Virus (TiLV).
- Family: *Orthomyxoviridae*.
- The virus was isolate from:
  - *S. galilaeus* in Lake Kinneret
  - *O. niloticus X O. aureus* in fish farms
- Later on, the virus has also been attributed to Tilapia mortalities in Ecuador and Colombia.
- Confirmatory test: semi-nested RT-PCR.
- No epidemiological data were presented.

- Eyngor *et al*., 2014
- Bacharach *et al*., 2016
- del-Pozo *et al*., 2016
- Kembou Tsofack *et al*., 2017

Experimental infection with TiLV

- The cohabitation of healthy and injected i.p. Nile Tilapia demonstrated that the disease is contagious (>80% mortalities).



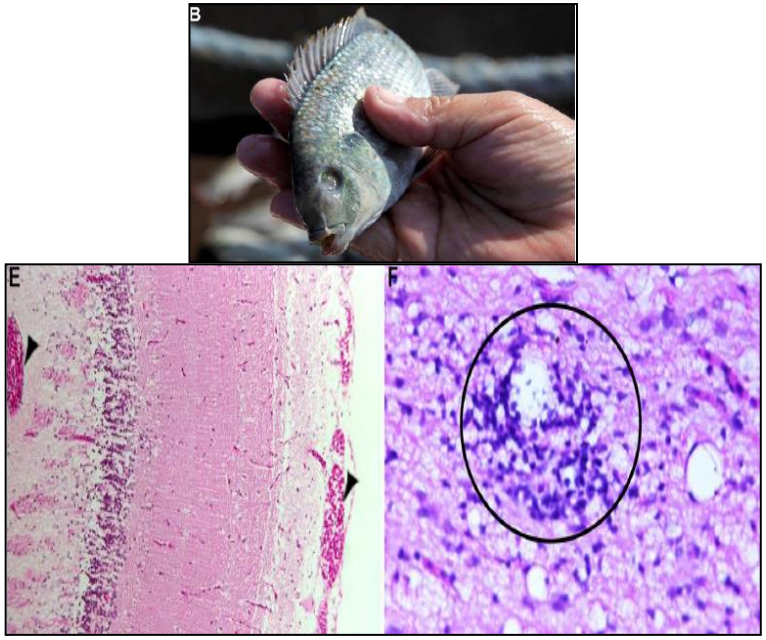
Group1 (◆) i.p. injection.
Group 2 (▲) cohabitation.
Group 3 (●) control group.

Eyngor *et al*., 2014

Histopathology

Tilapia Lake Virus (TiLV)

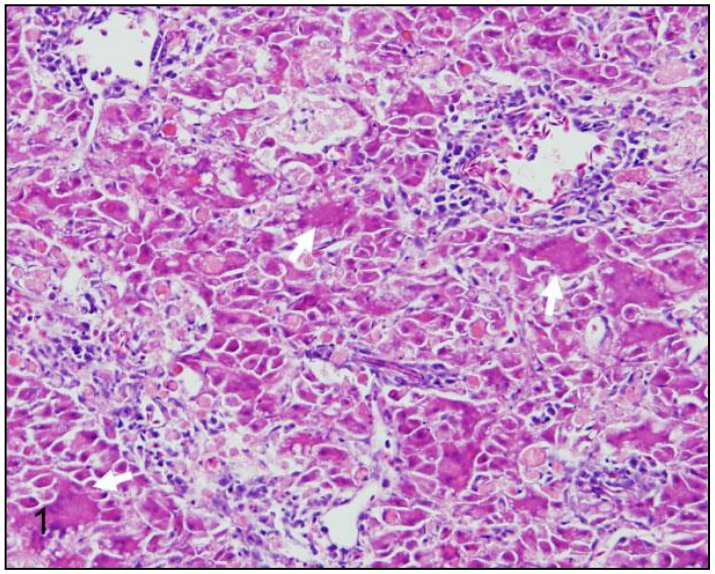
Eyngor et al., 2014



(B) Shrinkage of the eye and loss of ocular functioning. Brain and cortex:
(E) The arrowheadsmark dilated blood vessels packed with large numbers of red blood cells within the leptomeninges and gray and white matter.
(F) Brain and cortex. Perivascular cuffs of lymphocytes.

Syncytial hepatitis of tilapia (SHT)

del-Pozo et al., 2016



Multifocal to coalescing areas of necrosis, frequent single hepatocellular necrosis with dissociation, syncytial cell formation (white arrows), and perivenular, lymphocytic, inflammatory infiltration.

TiLV in Egypt

- The study was conducted due to an unexplained mortalities of Nile tilapia during the summer months.
- Epidemiological data: 68 farms were tested, TiLV was detected in 37% of them.
- Average mortality rate related to TiLV: 9.2%.
- Sequence analysis: 93% homology to the published TiLV sequence described from Israel.
- Hypotheses raised by the researchers:
  - Transmission by migrating birds.
  - Gray mullets may be a potential vector of TiLV.

Fathi *et al.*, 2017

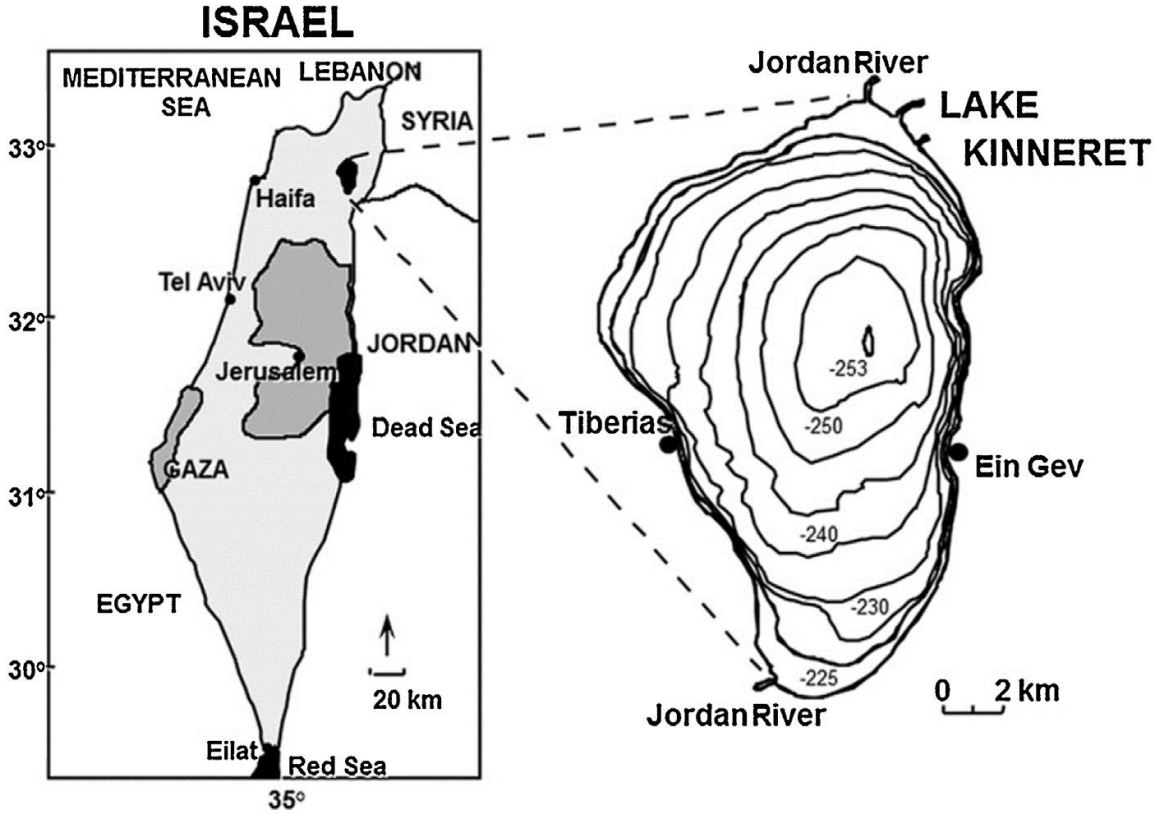
TiLV in Thailand

- TiLV was described in Nile tilapia (*O. niloticus*) and Red tilapia (*Oreochromis* spp.).
- According to fish producers, abnormal cumulative mortalities (20–90%) due to unknown etiological agent(s) have been observed in fingerlings recently.
- Sequence analysis: >96% homology to the published TiLV sequence described from Israel.
- No epidemiological data were presented.

Dong *et al*., 2017

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 then 27 species of fish (19 are native).



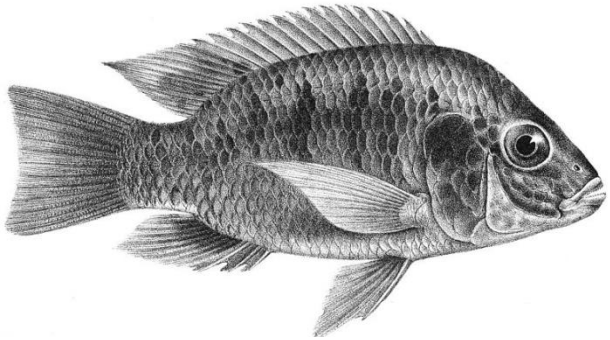
Parparov & Gal, 2012

Lake Kinneret cichlids

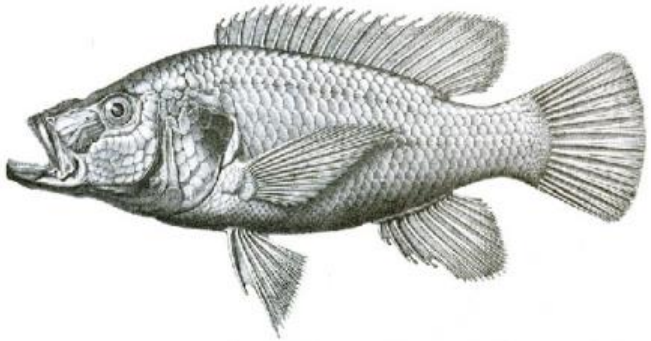
- Tilapia species:

  Sarotherodon galilaeus, *Oreochromis aureus*, *Tristramella simonis simonis*.

  Tilapia zilli, *Astatotilapia flavijosephi*.
- *Tristramella sacra* was endemic to the Lake but has not been recorded since 1990.



Sarotherodon galilaeus
(Linnaeus, 1758)



Tristramella sacra
(Günther, 1865)

Crisis?

HAARETZ

The one that got away - Israeli experts' theory on Lake Kinneret's disappearing fish

New study refutes claim made by fishermen, Agriculture Ministry that the great cormorant is responsible for the disappearance of fish in Israel's Kinneret.

By Zafrir Rinat | Mar. 27, 2013 | 9:20 AM | 1

Great cormorants flying over the water of Lake Kinneret. Photo by Gil Eliyahu

GLOBES
ISRAEL'S BUSINESS ARENA

St. Peter's Fish endangered in Sea of Galilee

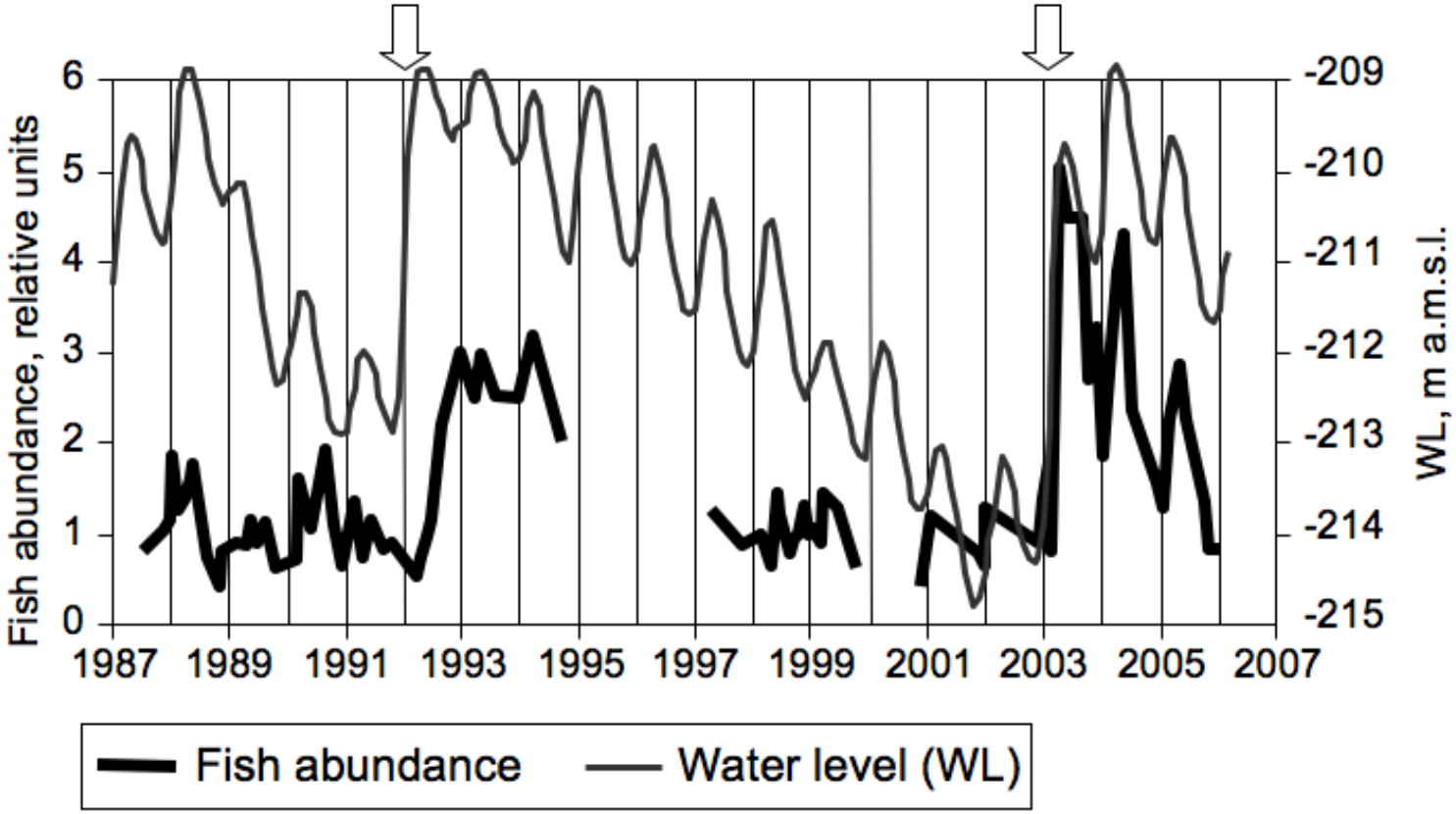
A- A+

An unknown virus is blinding fish in the Kinneret (Sea of Galilee), causing them to starve to death.

When Tiberias fishermen catch St. Peter's Fish (tilapia) without one or both of its eyes, and almost black in color, they know that something is wrong. Nonetheless, these fish still reach market and stores, and from there to customers who have no idea what they are eating.

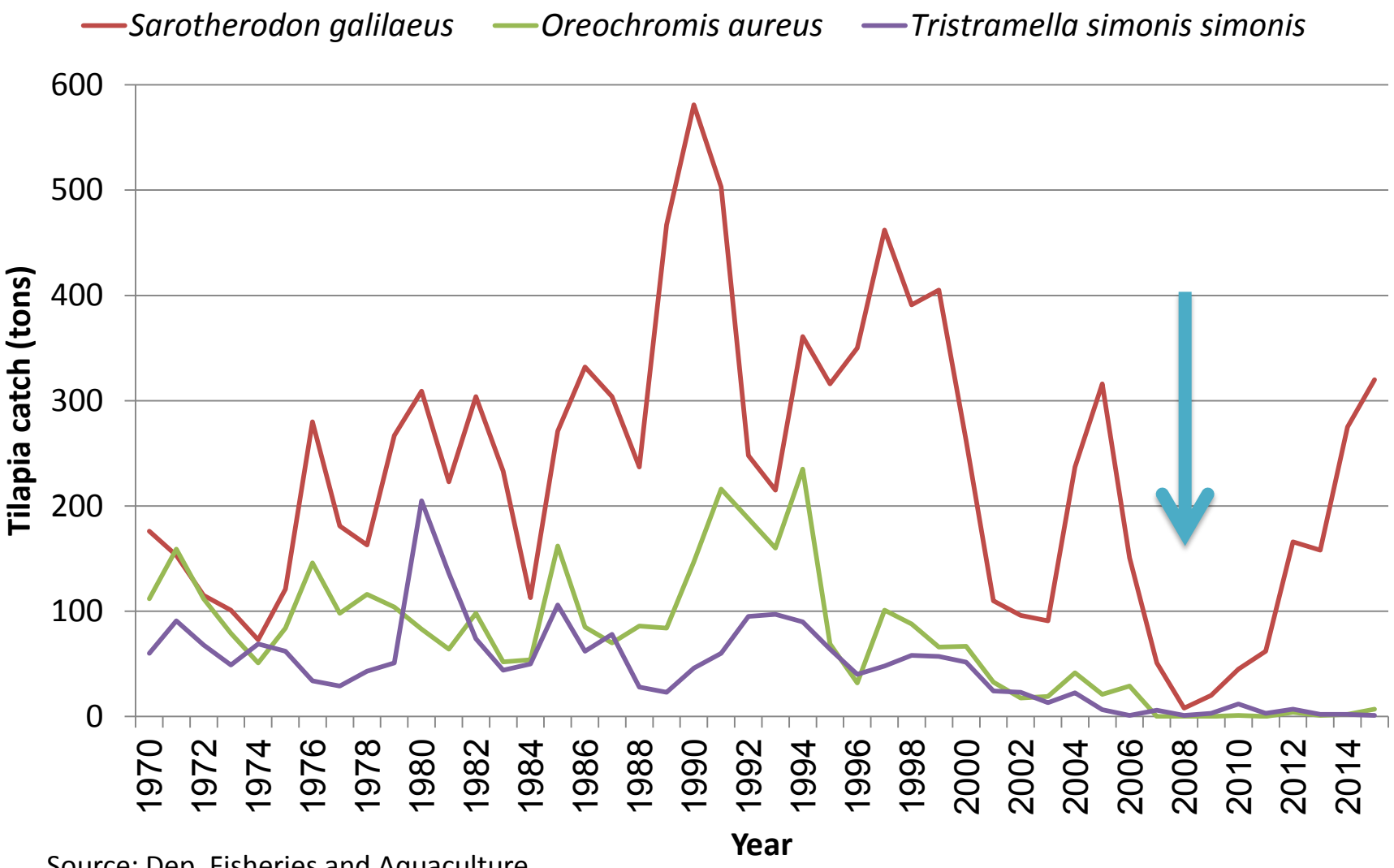
Only Menachem Lev, a veteran fisherman from Kibbutz Ein Gev, separates the diseased fish from the health catch, sending the diseased fish to lab in the hope that a cure can be found.

Water level vs Fish abundance



Zohary & Ostrovsky , 2011

Tilapia catch – Lake Kinneret



Source: Dep. Fisheries and Aquaculture

Changes in the parasitic fauna

Vol. 55: 145–150, 2003 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published July 8

Trematode metacercariae of fishes as sentinels for a changing limnological environment

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ABSTRACT: Trematode metacercaria populations infecting cichlids in Lake Kinneret (Sea of Galilee) were used as sentinels for the changing limnological environment. Parasitological data from 0+ cichlid fingerlings (*Tilapia zillii*, *Oreochromis aureus*, *Sarotherodon galilaeus*) were collected from the northwest shore of the lake during 1999 to 2001 and compared with data obtained from 1982 to

Invasive snail

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

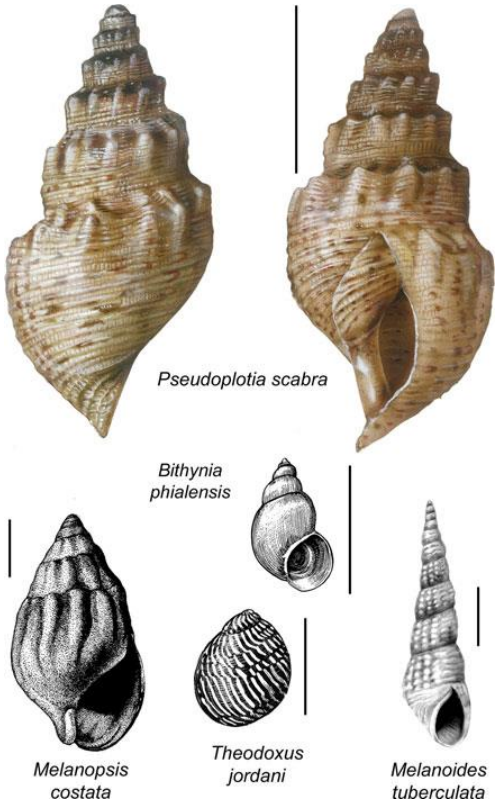
INVASION NOTE

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

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



Thiara scabra (Müller, 1774) → *Pseudoplotia scabra* → *Mieniplotia scabra*

Let’s go back to the fish ponds

What do we know until now?

- Unexplained Tilapia mortalities since 2009.
- Mysterious Tilapia disease / blind Tilapia in Lake kinneret.
- According to fish producers, abnormal mortality events and variable survival rates have been observed.
- Increased production of Tilapia fingerlings.
- Increased number of pygmy cormorant (*Phalacrocorax pygmeus*).
- Detection of TiLV in few Tilapia farms.

Farmed *O. niloticus* X *O. aureus*

Wild *Sarotherodon galilaeus*

Farmed *Cyprinus carpio*

What we don’t know?

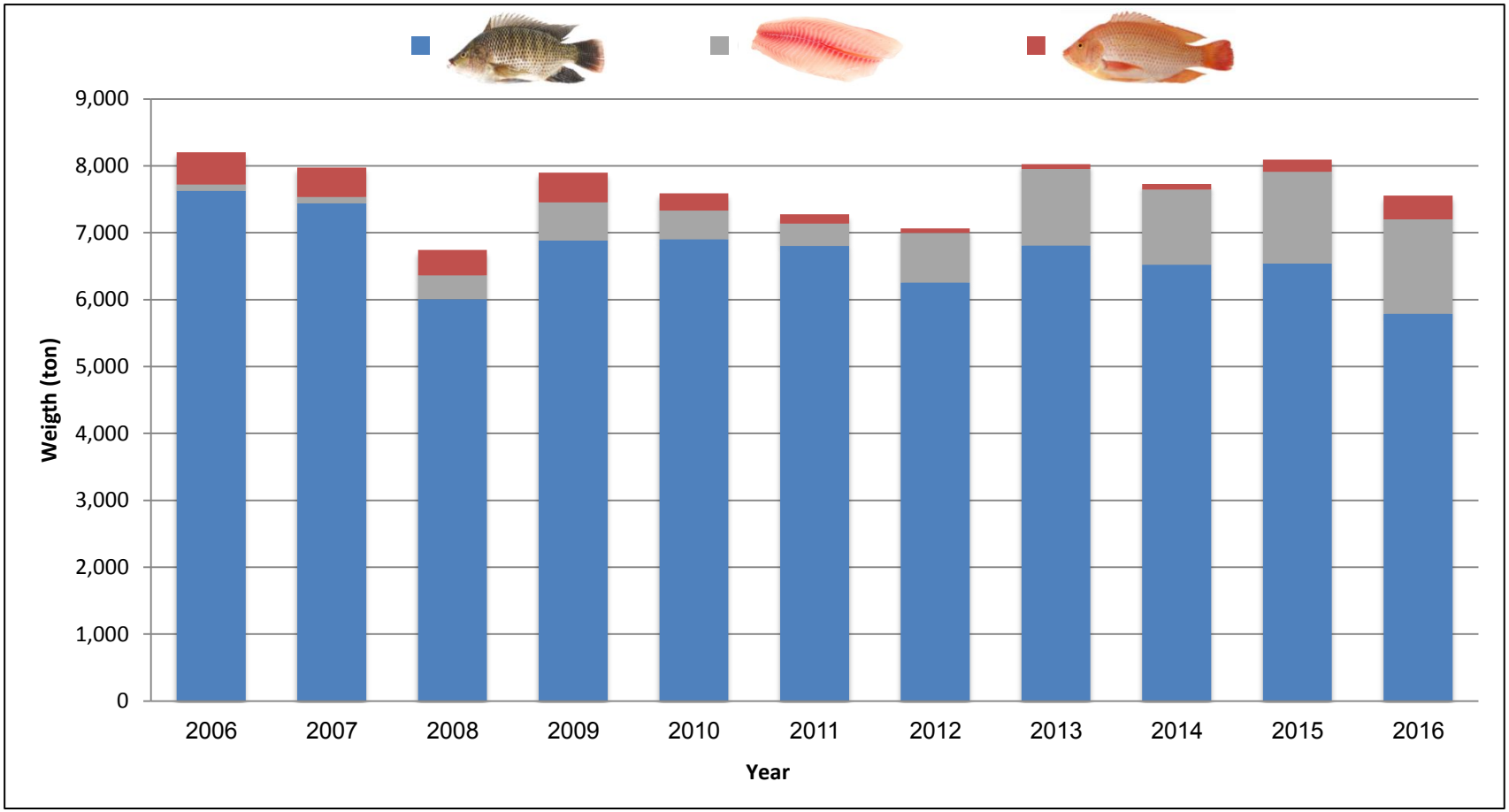
- Infectivity to different Tilapia species, attack rate, fatality rate and clinical manifestation are scarce.
- Environmental factors and the complexity of open system aquaculture make it challenging to estimate the true impact of the new agent.

In aquaculture, disease is the result of complex interactions between pathogens, environmental factors, host condition, husbandry practices, and management practices (Subasinghe 2005).

Epidemiological study

- Collaboration between The Israeli Veterinary Services and Koret School of Veterinary Medicine.
- Prof. Eyal Klement – Epidemiologist.
- 2 years scholarship for MVPH student:
  - Dr. Revital Skornik – DVM, MSc (Aquatic Veterinary Studies).

Total Tilapia production in Israel 2006-2016



Source: Fish Breeders Association

TiLV diagnosis by PCR

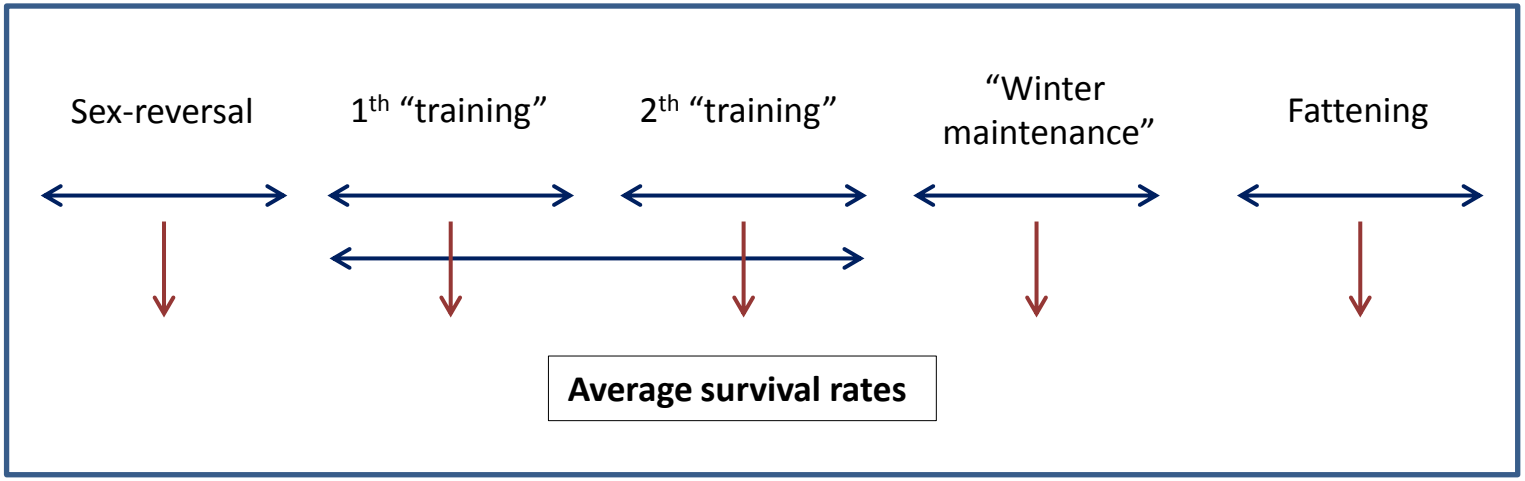
Fish disease lab – Kimron Veterinary Institute

Number	Farm	Species	Date	Clinical signs / mortality	Date	Result
1	A	Tilapia hybrid	Apr-16	Clinical signs	May-16	neg
2	B	Tilapia hybrid	May-16	Mortality	Jun-16	neg
3	C	Tilapia hybrid	Aug-16	Mortality	Aug-16	neg
4	E	Tilapia hybrid	Aug-16	Mortality	Aug-16	neg
5	E	Tilapia hybrid	Aug-16	Mortality	Aug-16	neg
6	D	Tilapia hybrid	Aug-16	Mortality	Sep-16	neg
7	E	Tilapia hybrid	Sep-16	Clinical signs	Sep-16	pos
8	E	Tilapia hybrid	Sep-16	Clinical signs	Sep-16	pos
9	F	Tilapia hybrid	Oct-16	Clinical signs	Nov-16	neg
10	E	Tilapia hybrid	Oct-16	Clinical signs	Nov-16	pos
11	D	Tilapia hybrid	Oct-16	Clinical signs	Nov-16	pos
12	D	Tilapia hybrid	Oct-16	Clinical signs	Nov-16	pos
13	G	Tilapia hybrid	Nov-16	Clinical signs	Nov-16	neg
14	G	Tilapia hybrid	Nov-16	Clinical signs	Nov-16	neg
15	H	Tilapia hybrid	Nov-16	Clinical signs	Nov-16	neg
16	I	Tilapia hybrid	Nov-16	Mortality	Nov-16	neg
17	D	Tilapia hybrid	Nov-16	Mortality	Nov-16	neg
18	E	Tilapia hybrid	Nov-16	Mortality	Nov-16	neg
19	E	Tilapia hybrid	Nov-16	Mortality	Nov-16	neg

2/8 farms are positive for TiLV.
0/9 are positive within mortality cases.

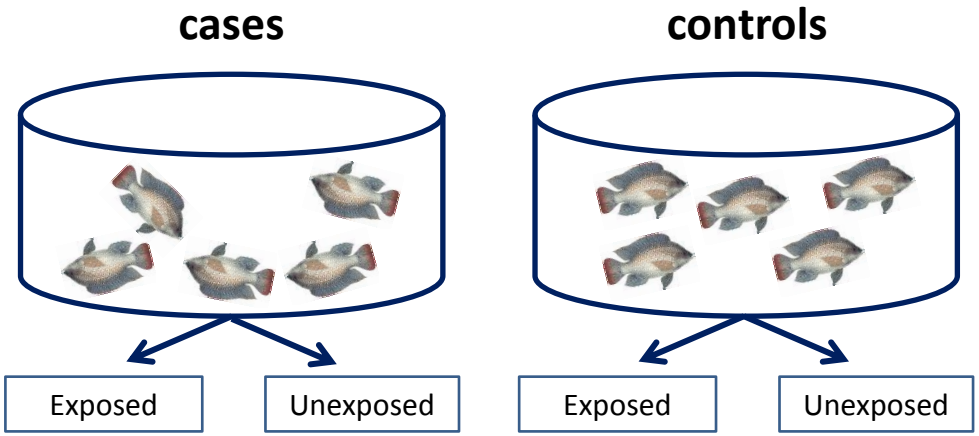
Epidemiological study

- Epidemiological retrospective survey:
  - Questionnaire for Tilapia farms
  - Data collection
  - Multi factorial analysis
  - Secular trends in survival rates in the different age categories



Epidemiological study

- Budget for 300 PCR tests.
- Case control studies:
  - “case” - diseased fish, dead fish.
  - “control” - no clinical signs.
  - Presence of the TiLV.
  - Other data of the fish and ponds (water temp, density etc.).
  - Correlation between TiLV presence and clinical signs.



Summery

- TiLV is an emerging pathogen, in accordance with Article 1.1.4. of the OIE *Aquatic Code*.
- Clinical manifestation and mortality trends along the different production stages need further studies.
- Epidemiology is crucial for better understanding of the pathogen and clinical manifestation.
- Molecular tools for the detection of antibodies may provide further information about exposure and immunization – need to be developed.
- International collaboration can help enhance the understanding TiLV importance in Tilapia industry.

Acknowledgment

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- Kimron Veterinary Institute

Thank you for your attention!

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