New species for EU aquaculture

Greater amberjack health

Dr. Pantelis Katharios et al, HCMR
Prof. Chris Secombes et al, Aberdeen University
Prof. Daniel Montero et al, FCPCT
Dr. Covadogna Rodriguez et al, ULL
Dr. Salvador Jerez et al, IEO

Athens, 18 September 2018

Objectives within DIVERSIFY project

Identification of immune markers

Mucus as a defense barrier

Diet as a regulator of mucosal defense against parasites

Epitheliocystis and diagnostic tools

Rearing protocol against monogeneans

Major bacterial and viral diseases (+ susceptibility of the species to know

Immune system and immunomodulation

Prevention vs treatment

Innate immune system (first line of defense)

Adaptive immune system (antibodies)

Knowledge of the function of the immune system

Specifics of greater amberjack

Work undertaken by University of Aberdeen and FCPCT

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Universidad de Las Palmas de Gran Canaria

Immune markers

IL-1β, IL-8, IL10, IL-17A/F, IL-17D, IL-22, TNFa, Mx, IFN1, IFNγ, iNOS, IgM, IgT, RAG2

Housekeeping genes EF-1a, β-Actin

Antimicrobial peptides Piscidin, Defencin and Hepcidin

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Testing innate immune response

Focus on fish mucus

Effect of stress on innate immune response as expressed in mucus

Differences in response to various stressors (handling, crowding stress)

Full repertoire of antimicrobial defenses

Great potential for interventions through immunostimulants and additives

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Epitheliocystis

Infectious disease affecting a wide range of wild and cultured fish

Global distribution

First observed in 1920

Described and named in 1969

Caused by intracellular pathogens (Chlamydia?)

Inclusions in gill and skin epithelium of the fish

Despite efforts, no epitheliocystis-related agent has been isolated in culture until today

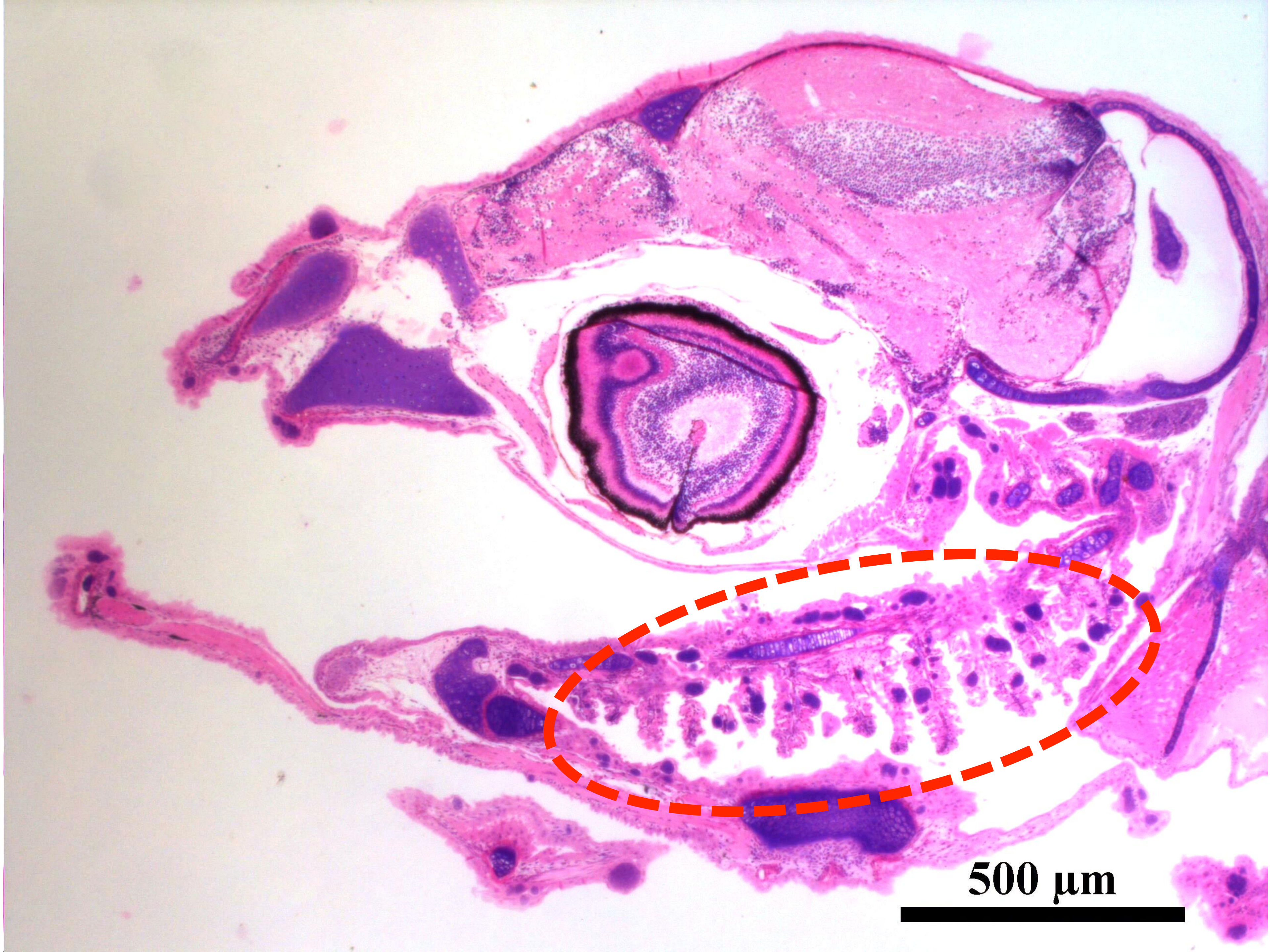
Epitheliocystis and HCMR

100% mortality in greater amberjack (*Seriola dumerili*) larvae

80% mortality in common dentex (*Dentex dentex*) larvae

>50% mortality in sharpsnout seabream (*Diplodus puntazzo*)

In some cases 100% mortality overnight



hcmr
ΕΛΚΕΘΕ

Aquaculture, 90 (1990) 197–207
Elsevier Science Publishers B.V., Amsterdam

Epitheliocystis disease in the cultured amberjack, *Seriola dumerili* Risso (Carangidae)

S. Crespo, A. Grau and F. Padrós
Laboratori de Biologia, Facultat de Veterinària, U.A.B., Bellaterra, Barcelona, Spain

(Accepted 12 March 1990)

Aquaculture, 95 (1991) 1–6
Elsevier Science Publishers B.V., Amsterdam

Epitheliocystis in the wild and cultured amberjack, *Seriola dumerili* Risso: ultrastructural observations

A. Grau and S. Crespo
Laboratori de Biologia, Facultat de Veterinària, U.A.B., Bellaterra, Barcelona, Spain

(Accepted 2 October 1990)

JOURNAL OF THE WORLD AQUACULTURE SOCIETY
Vol. 27, No. 2
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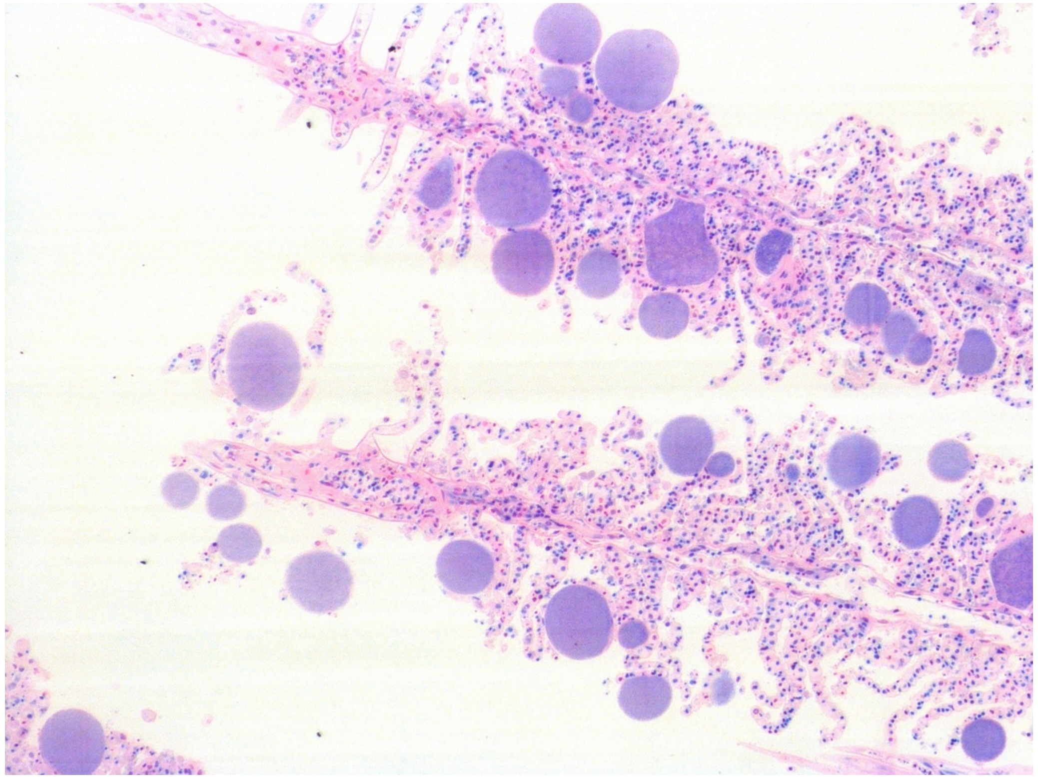
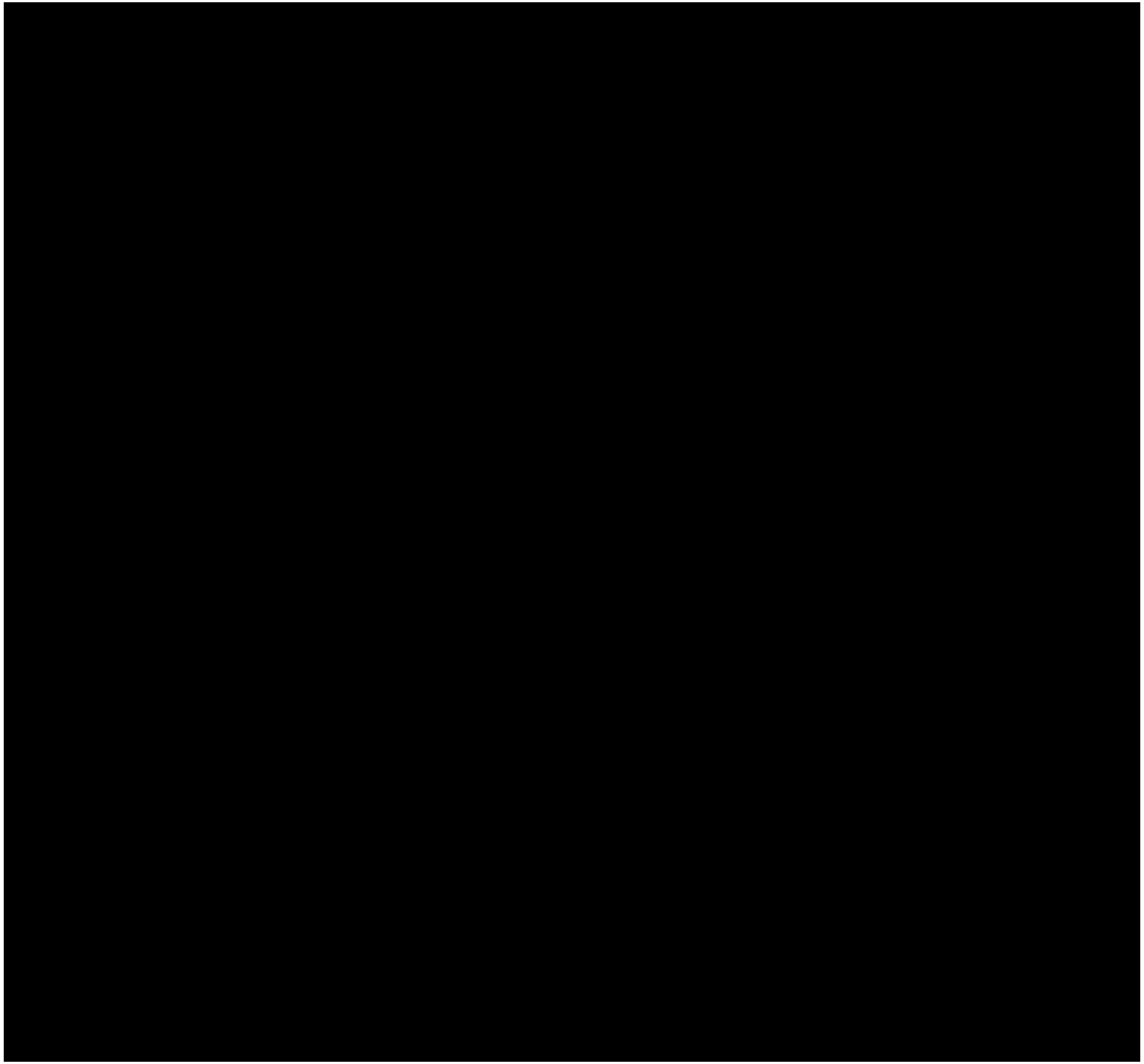
Epitheliocystis Disease in Cultured Yellowtail *Seriola mazatlana* in Ecuador

ARIETTA VENIZELOS[1]
AIC—Aquaculture International Consulting, 228 Seaview Drive, Key Biscayne, Florida 33149 USA

DANIEL D. BENETTI
Rosenstiel School of Marine and Atmospheric Science, Division of Marine Biology and Fisheries, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149 USA

Epitheliocystis





Novel findings regarding epitheliocystis

Caused by various unrelated bacteria

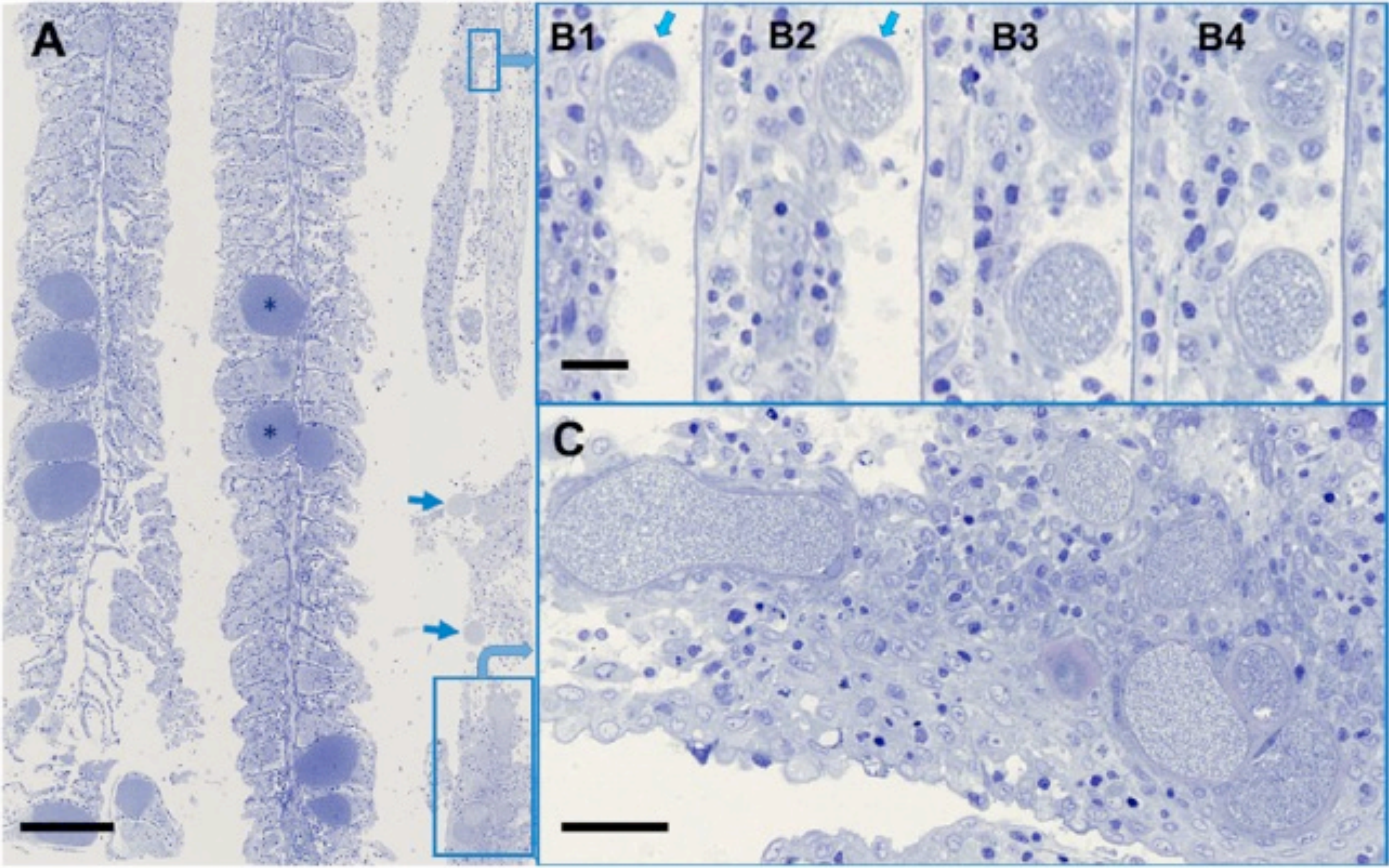
Ca. Endozoicomonas cretensis

Ca. Ichthyocystis sparus

Ca. Ichthyocystis hellenicum

…and various Chlamydia

In most of the times more than one species co-infect the same fish (same filament)



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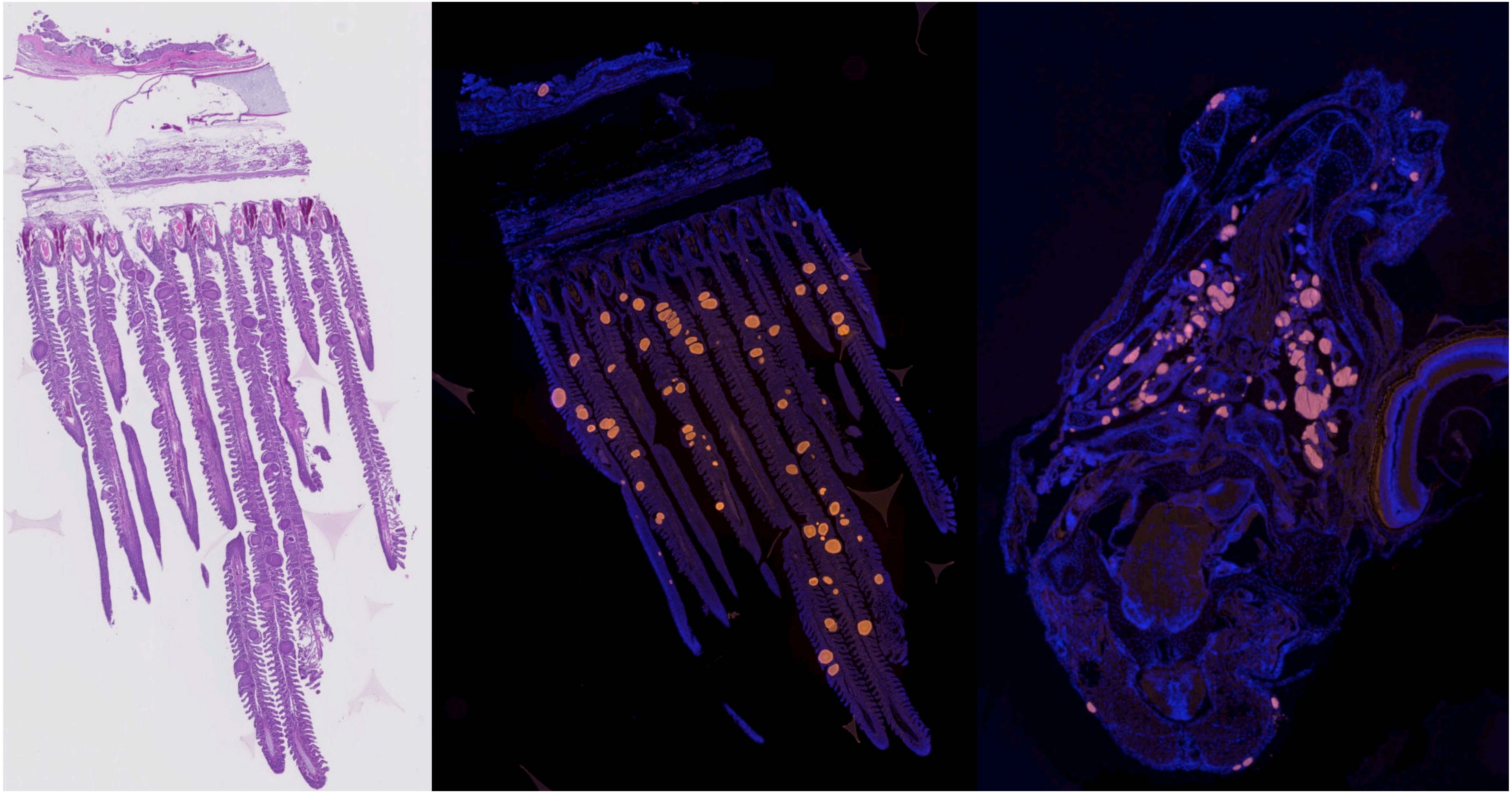
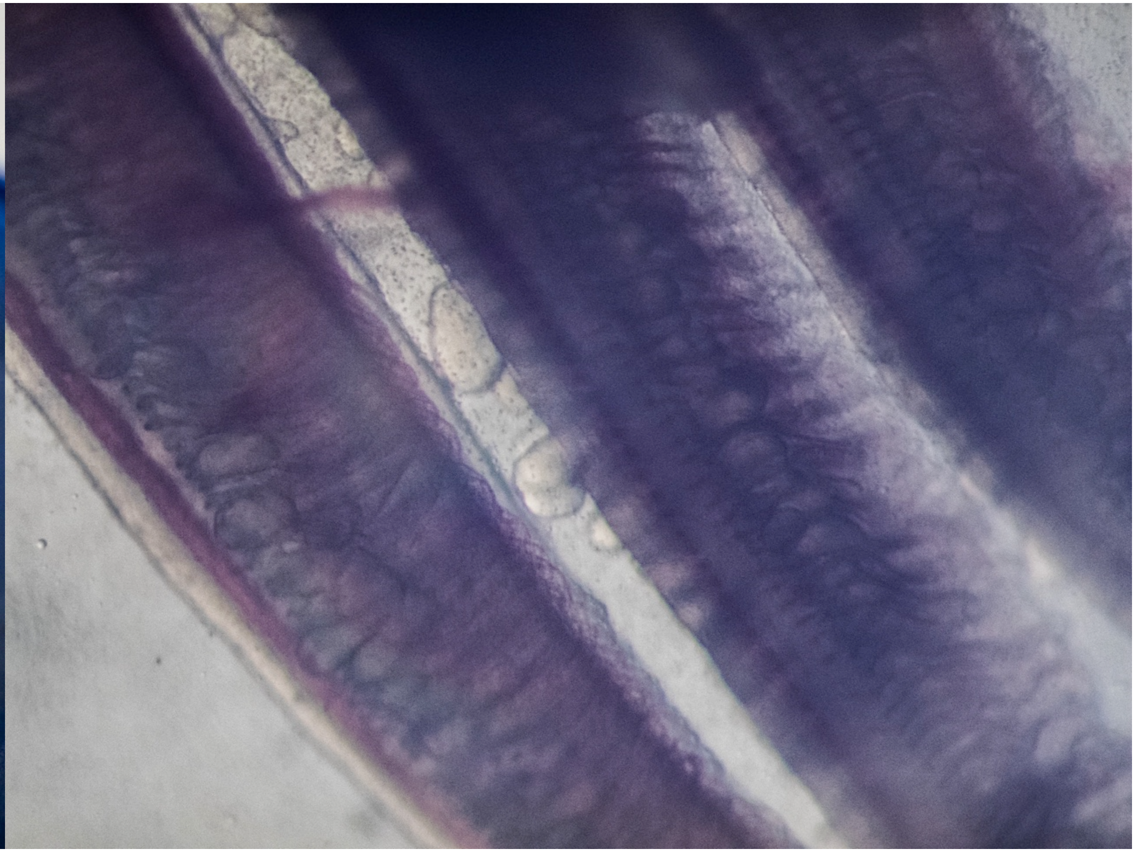


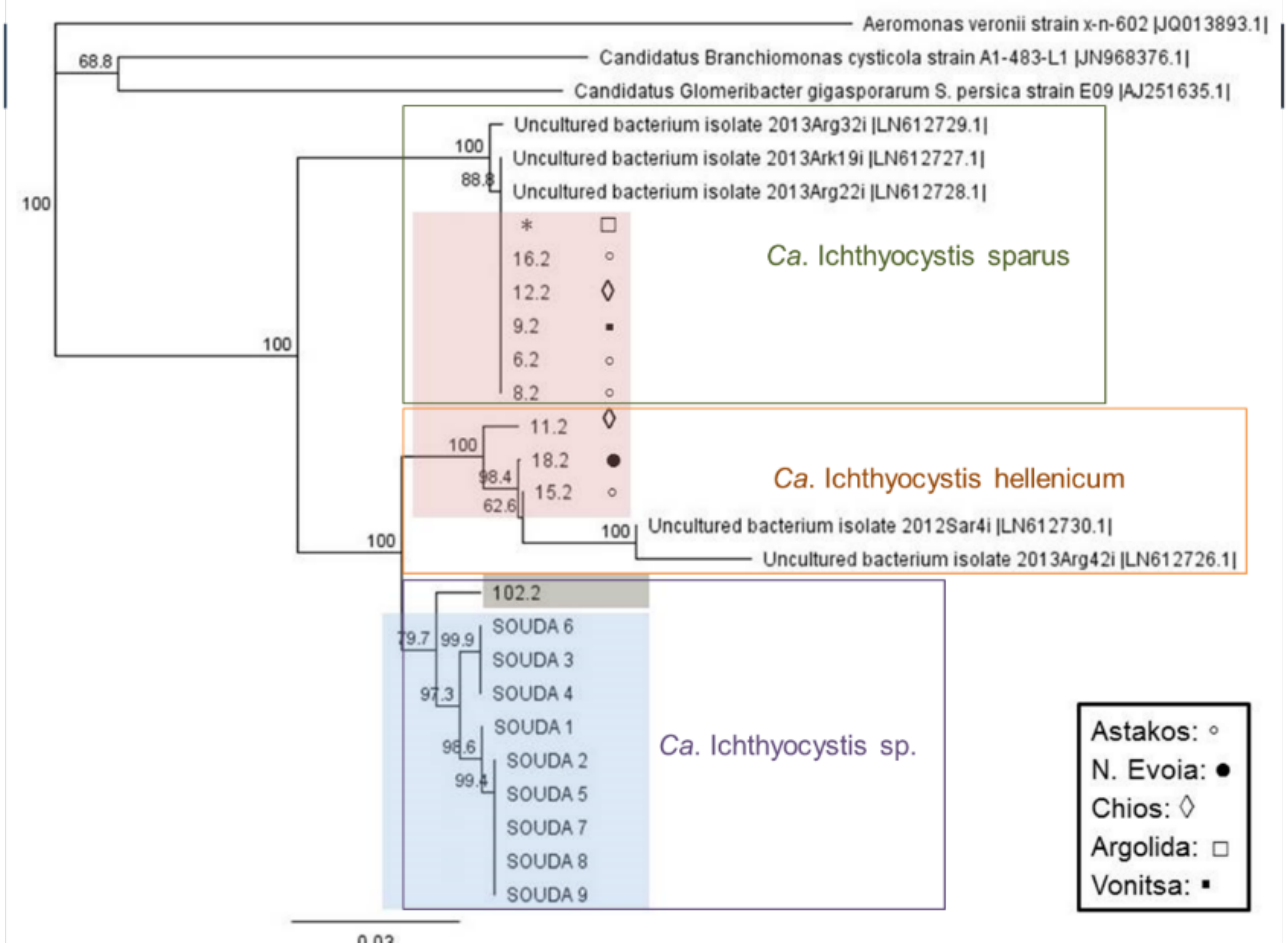
Toolbox for proper diagnosis

- PCR
- qPCR
- histology
- Fluorescence In Situ Hybridization (FISH)
- Electron Microscopy
- µCT

Prof. Lloyd Vaughan
University of Zurich
Functional Genomic Centre
HCMR

Epitheliocystis in greater amberjack in Greece is caused by a novel Ichthyocystis species





Aeromonas veronii strain x-n-602 |JQ013893.1|
Candidatus Branchiomonas cysticola strain A1-483-L1 |JN968376.1|
Candidatus Glomeribacter gigasporarum S. persica strain E09 |AJ251635.1|
Uncultured bacterium isolate 2013Arg32i |LN612729.1|
Uncultured bacterium isolate 2013Ark19i |LN612727.1|
Uncultured bacterium isolate 2013Arg22i |LN612728.1|
Ca. Ichthyocystis sparus
16.2
12.2
9.2
6.2
8.2
11.2
18.2
15.2
Ca. Ichthyocystis hellenicum
Uncultured bacterium isolate 2012Sar4i |LN612730.1|
Uncultured bacterium isolate 2013Arg42i |LN612726.1|
102.2
SOUDA 6
SOUDA 3
SOUDA 4
SOUDA 1
SOUDA 2
SOUDA 5
SOUDA 7
SOUDA 8
SOUDA 9
Ca. Ichthyocystis sp.
Astakos: ◦
N. Evoia: ●
Chios: ◊
Argolida: □
Vonitsa: ▪
0.02

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Epitheliocystis in greater amberjack

At early larval stages can be lethal

Transition from hatchery to sea-cages (first months)

Mortality is generally low and lesions resolve without treatment quite fast

However, epitheliocystis does not come alone !!!

Mortalities can be high when gills are also infected by *Zeuxapta seriolae*

Vibriosis caused by *Vibrio harveyi*

Bacterial infection

Proper identification is rather difficult

Needs high resolution molecular methods (MLST)

Outbreaks with changes in temperature (spring, autumn)

High mortality (>40%)

Difficult to treat

Antibiotic resistance

Doxycycline is the only effective antibiotic

Lack of vaccine (Spain?)

Vibrio harveyi

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Zeuxapta seriolae

Monogenean parasite

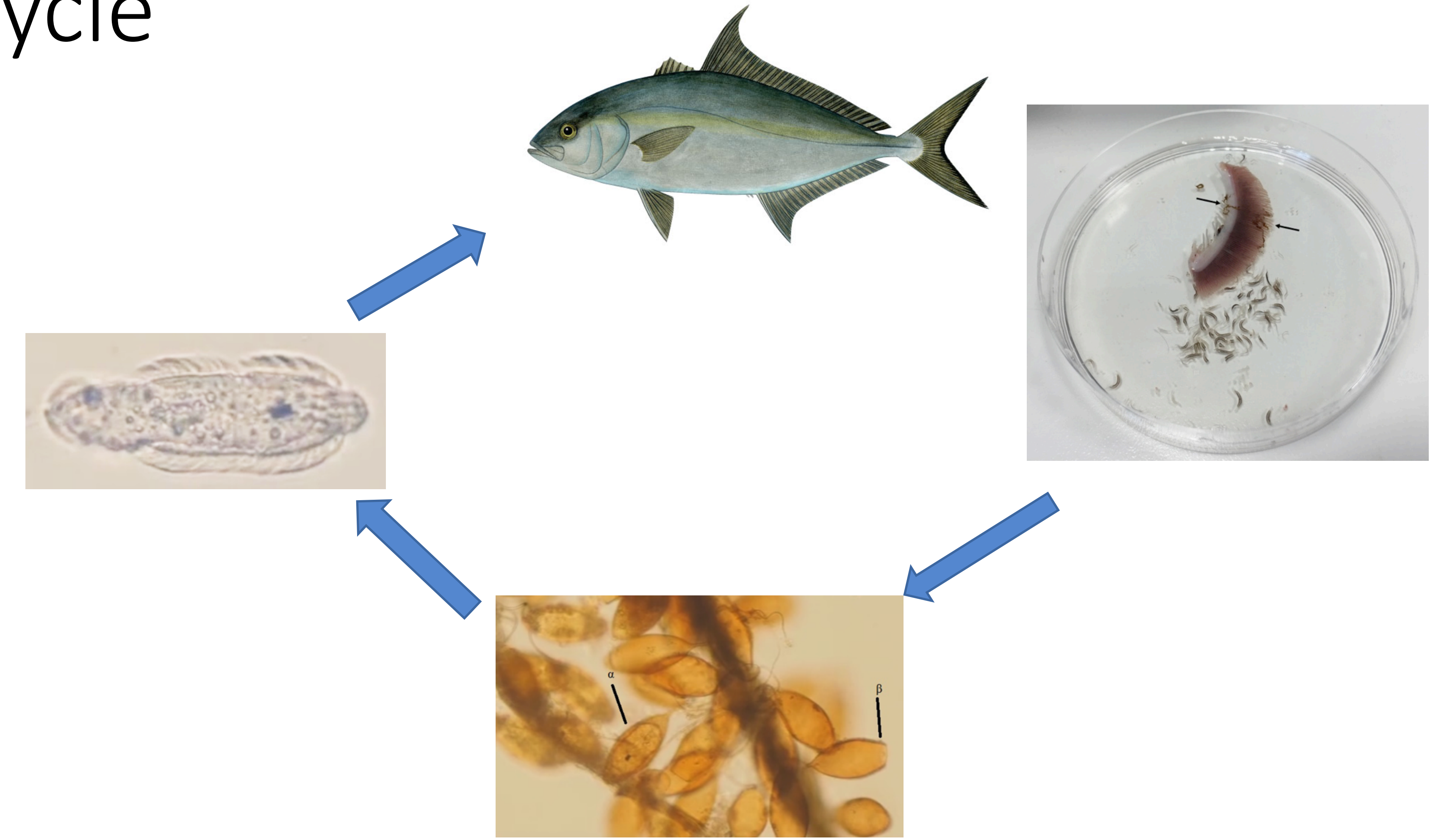
Infects the gills

Causes anemia

High mortalities (~100%)

The most important pathogen of greater amberjack

Life cycle



The monogenean *Zeuxapta seriolae* parasite of greater amberjack, *Seriola dumerili*

Dr. Pantelis Katharios
Institute of Marine Biology, Biotechnology and Aquaculture
Hellenic Centre for Marine Research

Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal for Parasitology 35 (2005) 315–327
www.parasitology-online.com

Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*

L.A. Tubbs[a,*], C.W. Poortenaar[b,1], M.A. Sewell[a], B.K. Diggles[b,2]

[a]School of Biological Sciences, University of Auckland, Private Bag 92019, P.O. Box 109 695, Auckland, New Zealand
[b]National Institute of Water and Atmospheric Research (NIWA), P.O. Box 109 695, Auckland, New Zealand

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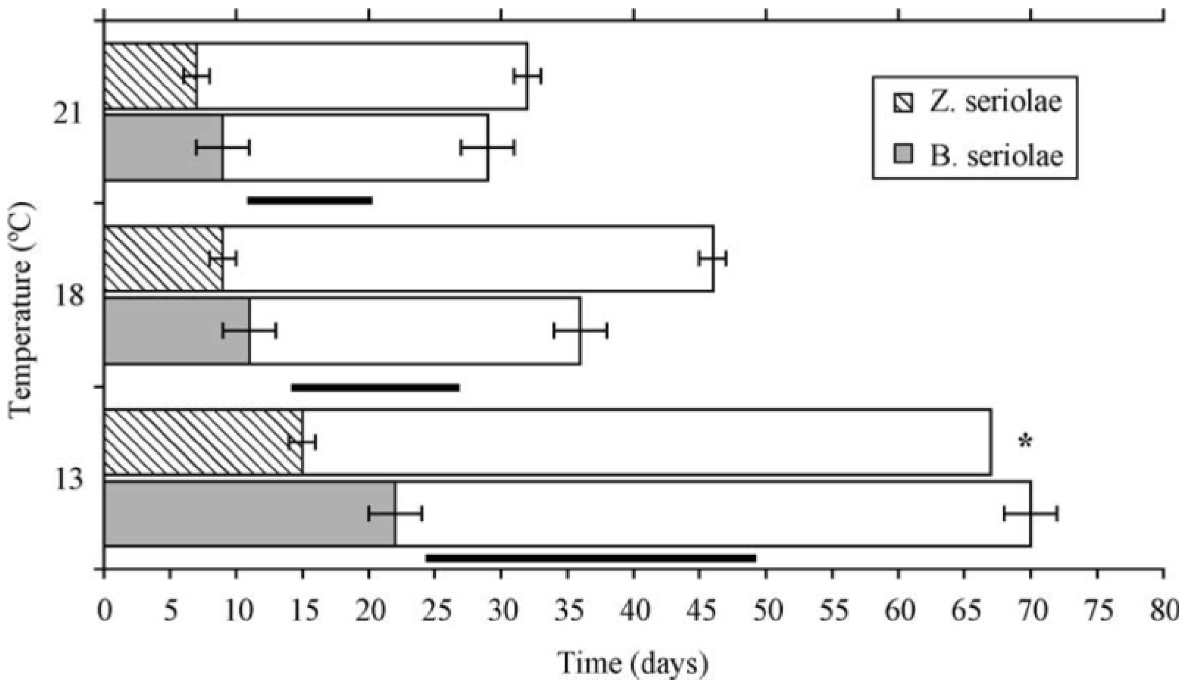

Generation times
Egg hatching times:
13^{o}C: 21d
18^{o}C: 10d
21^{o}C: 7d
From hatching to maturity:
13^{o}C: 45d
18^{o}C: 36d
21^{o}C: 21d

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The influence of different water temperatures on *Neobenedenia girellae* (Monogenea) infection, parasite growth, egg production and emerging second generation on amberjack *Seriola dumerili* (Carangidae) and the histopathological effect of this parasite on fish skin

Noritaka Hirazawa [a,*], Ryoko Takano [b], Hiroko Hagiwara [b], Mitsuyo Noguchi [b], Minoru Narita [b]

[a] Central Research Laboratories, Nippon Suisan Kaisha, Ltd., 559-6 Kitano-machi, Hachioji, Tokyo 192-0906, Japan
[b] Advanced Technology Development Center, Kyoritsu Seiyaku Corporation, 2-9-22 Takamihara, Tsukuba, Ibaragi 300-1252, Japan

Table 1

Neobenedenia girellae infection and parasite growth on amberjack *Seriola dumerili* at 13 days after exposure to oncomiracidia at different water temperatures.

	Seawater temperature					
	20 °C		25 °C		30 °C	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Number of mature parasites						
Pre fish	124.4 ± 30.6^{a}	123.2 ± 9.0^{a}	118.2 ± 40.0^{a}	128.8 ± 23.4^{a}	83.6 ± 16.6^{ab}	34.0 ± 47.8^{b}
Per cm^2 per fish surface	0.44 ± 0.09^{a}	0.40 ± 0.05^{a}	0.43 ± 0.12^{a}	0.41 ± 0.07^{a}	0.28 ± 0.04^{ab}	0.12 ± 0.16^{b}
Mature parasite body length (mm)	2.53 ± 0.21^{a}	2.52 ± 0.23^{a}	4.43 ± 0.21^{b}	4.51 ± 0.30^{b}	4.94 ± 0.22^{c}	4.81 ± 0.29^{c}
First laid eggs-recorded days after exposure	13	13	8	8	6	6
Number of laid eggs per net	0.3 ± 0.5^{a}	2.3 ± 3.9^{a}	$1{,}369{,}000 \pm 1{,}036{,}000^{b}$	$4{,}903{,}000 \pm 2{,}183{,}000^{bc}$	$5{,}550{,}000 \pm 4{,}725{,}000^{bc}$	$9{,}698{,}000 \pm 5{,}491{,}000^{c}$
Number of larval parasites						
Per fish	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.8 ± 1.8^{a}	4.0 ± 1.9^{a}	4223.9 ± 2026.3^{b}	2980.5 ± 1017.0b
Per cm^2 per fish surface	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.002 ± 0.005^{a}	0.012 ± 0.005^{a}	14.618 ± 8.704^{b}	10.175 ± 3.458^{b}
Larval parasite body length (mm)	—	—	0.28 ± 0.03^{ab}	0.24 ± 0.04^{b}	0.47 ± 0.16^{ac}	0.54 ± 0.15^{c}

Values are mean and standard deviations.

Values with different superscripts are significantly different at $P < 0.05$.

Treatment

Formalin ineffective even at very high doses

Hydrogen peroxide extremely effective

75 - 100 ppm for 20-30 min

Toxic to fish at higher temperature

It kills adults and juvenile parasites but not eggs

It needs a second (and third) treatment

Praziquantel in feed (and as a bath)

Recurrent infections throughout the year

Nikos Papandroulakis question from the morning session:

Will greater amberjack become the Med salmon?

Integrated Pest Management

- Applied in advanced countries
- Salmon aquaculture in Canada and Norway
- Sea lice
- Sustainability
- Use of chemicals
- Protection of the environment and the consumer
- Protection of the wild stocks

Developing an Integrated Pest Management approach for marine finfish aquaculture activities in B.C.

Aquaculture Management Advisory Committee

March 15 2016

- Multifactorial approach to pest management;
- Series of evaluations, decisions and controls;
- Take advantage of all pest management control options;
- Strategies to achieve long-terms solutions.

- Prevention
- Monitoring
- Threshold for action
- Medicinal and non-medicinal controls

Prevention

- Prevention is fundamental to IPM – reduces the likelihood and severity of sea lice infestations.
- Location of sites – sources of infection and water quality.
- Year class separation – probably the most effective husbandry technique; slowing down acquisition of sea lice.
- Fallowing of sites – reduce or eliminate self-sustaining lice popn’s.
- Husbandry – minimize stress, stocking densities, nutrition, hygiene, regular removal of mortalities, predator control.
- Innovative technologies – cleaner fish, vaccines, immunostimulants.

Monitoring of pest issue

- Decisions about when to conduct treatment should be based on a program of monitoring lice numbers.
- Sampling programs (frequency and sample size) should be conducted continuously following transfer to sea water.
- Selection of appropriate treatment should be based on sea lice population dynamics.
- Monitoring is necessary to ensure interventions are carried out at the correct time with appropriate product.
- Monitoring also allows the site operator to build up a picture of the dynamics of sea lice populations and make predictions around optimal management/treatment approaches.

Threshold for action

- Treatment triggers should be low enough to protect the salmon and reduce risks associated with the transfer of sea lice from farmed to wild fish.
- Too low a trigger can lead to unnecessary therapeutant use, which can be difficult, costly and environmentally unsound.
- Current regulatory approach in Pacific Region requires:
  - March 1 to June 30; if the sea lice abundance exceeds 3 motile lice (Lep spp.) per fish then implement a plan to reduce absolute sea lice inventory within 15 days.
  - July 1 to February 28; if the sea lice abundance exceeds 3 motile lice (Lep spp.) per fish then provide a plan to address exceedance to Department within 30 days.

Integrated Pest Management

- Collective decision
- Regulatory authorities
- Veterinary authorities
- Research centers
- The most important future direction for sustainability


Thank you very much