WP25 Greater amberjack health

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Tasks within WP25

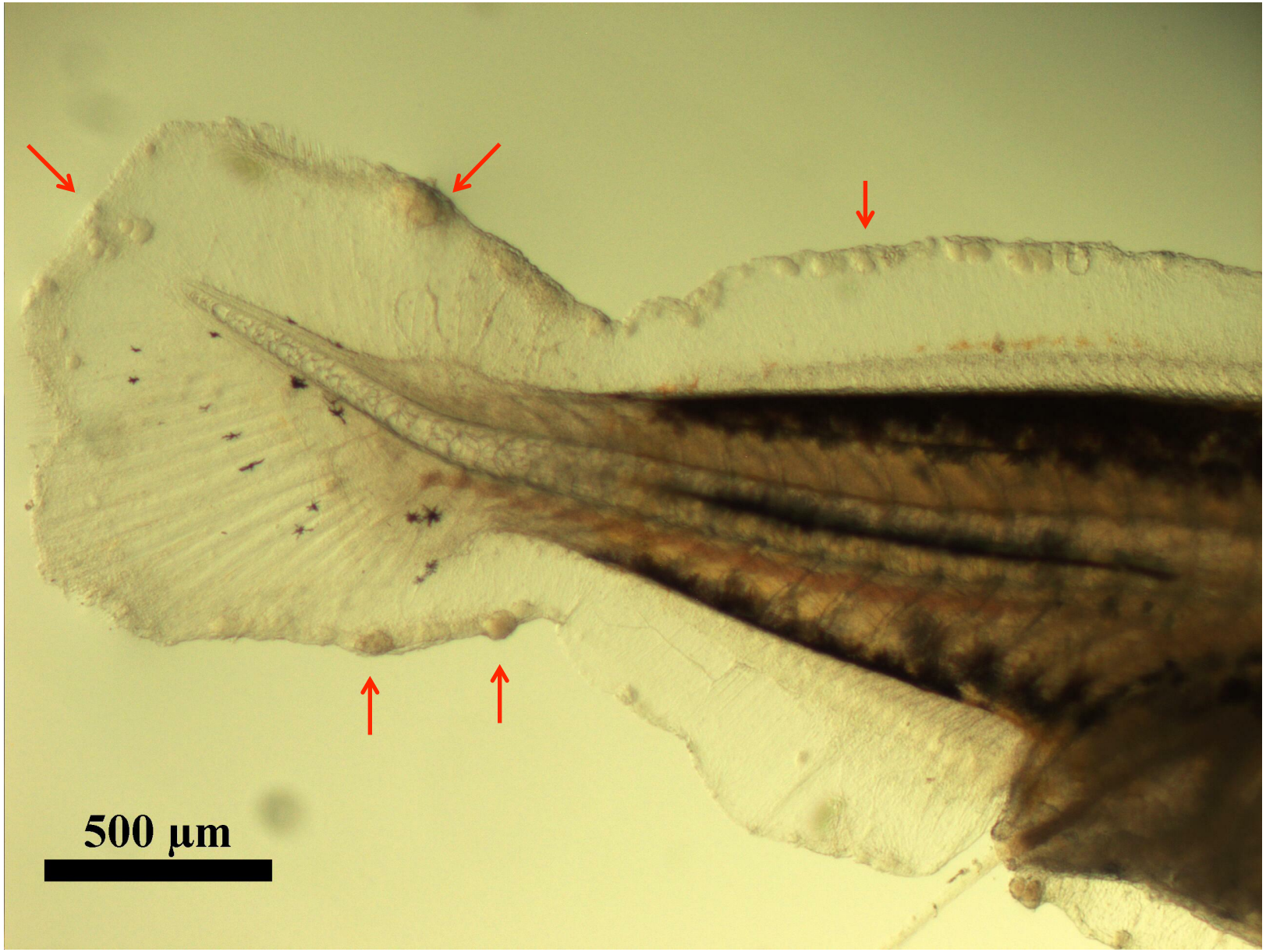
- Study of Epitheliocystis during larval rearing (HCMR)
- Promoting resistance to parasitic incidence on greater amberjack (FCPCT)
- Identification of immune markers (ABDN)
- Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites (IEO)
- Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in amberjack (FCPCT)
- Diagnostic-recommendation manual for greater amberjack health (HCMR)

Epitheliocystis during larval rearing

- Considered benign and chronic disease
- Cysts at the skin and gill epithelium
- Caused by intracellular Chlamydia/Chlamydia-like organisms
- It can be lethal in early life stages
- it has become an emerging problem in Greek Aquaculture causing significant mortalities during transition from hatchery to cages
- It affects greater amberjack

Epitheliocystis and greater amberjack

- Crespo et al. 1990
- Reported as main problem in related species in Japan and Central America
- In HCMR mesocosm facilities epitheliocystis caused 100% mortality overnight in experimental larval cultures of greated amberjack at 15dph


500 µm


500 µm

- Although Chlamydia are considered to be the main cause of epitheliocystis we have seen that β- and γ-proteobacteria are rather the main aetiological agents in Greece
- The disease is caused by a diverse group of pathogens
- The pathogens cannot be cultured

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University of Zurich
Prof. Lloyd Vaughan

Experimental set up 1/2 probe design

Probe	Specificity (sp)	Sequence / fluorophore	Position (E. coli numbering)	Reference
Chls-0523	Phylum Chlamydiae	5'-CCTCCGTATTACCGCAGC-3'Atto488	524-541	{Poppert, 2002 #188}
Pisci-0312	Ca. Piscichlamydia/ Similichlamydia	5'-AGTCCCAGTGTTGGCGATCG -3'Cy3	304-323	This study
Ichthyo-290	Ca. Ichthyocystis genus	5'-CATCCTCTCAGACCAGCTACCGATC-3'Cy3	281-305	This study
Ichthyo-230	Ca. I. hellenicum	5'-GGTCATCGGCCGCTCCTATCGC-3'Cy3	220-241	This study
Endo-474	Endozoicomonas cretensis	5'-AACCTTCAACCTTTCCTCCC-3'Cy3	471-490	This study
E-474	Control: gamma-proteobacteria	5'- AACCTTCAACCTTTCCTCCC-3'Cy3	445-464	This study

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Dr. Helena Seth-Smith

Experimental design
2/2 larval rearing and samplings

- 5 m^3 mesocosm tank
- Unfiltered sea water
- 5.000 greater amberjack eggs
- Start (25/6/2014)
- Water fractionation through serial filters (0.22-250 μm)
- PCR in fish larvae and filtrates in HCMR
- qPCR at Zurich+FISH

Results

- High mortality in larvae (technical issues)
- Water monitoring for 21 days – no positive signal of any epitheliocystis agent
- Draft genomes from the 3 new pathogens (Univ, Zurich)
- Experiment to be repeated next year

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Promoting resistance to parasitic incidence on greater amberjack

- Mass production of greater Amberjack juveniles for incidence of parasites studies
- Samples of different tissues (spleen, skin, gill, liver, kidney) for identification of immune markers (Task 25.3). Samples sent to partner 5 (University of Aberdeen) by September 2014

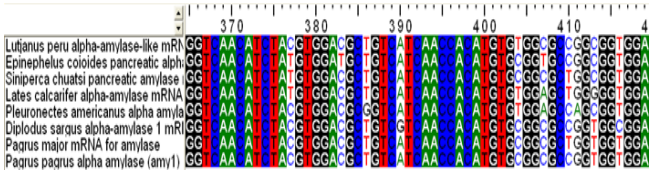
Identification of immune markers

Mucosal Immune Gene Expression

Locate genes from phylogenetically similar studied species in GenBank (NCBI), prepare alignment of gene sequences from all species. From this alignment choose conserved area to design consensus or degenerate primers.

IL-17
IL-22
iNOS
IgT
AMPs

GenBank

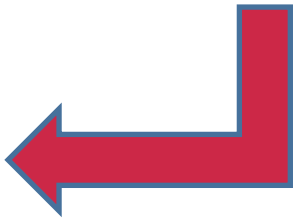


AMPs = antimicrobial peptides, including β-defensins and piscidins

Mucosal Immune Gene Expression

Locate genes from phylogenetically similar studied species in GenBank (NCBI), prepare alignment of gene sequences from all species. From this alignment choose conserved area to design consensus or degenerate primers.

Species - Target	Name	Sequence	bp	G/C	Tm°C
Seriola dumerili IL-17 A/F (sense)	IL-17F	GCGCCAACATGTCTCTGTC	19	57.9	56.5
Seriola dumerili IL-17 A/F (anti-sense)	IL-17R	GACTCTGTGGAGGACCAGGA	20	60	58.1
Seriola dumerili IL-22 (sense)	IL-22F	GCCAACATCCTCGACTTCTA	20	50	54.2
Seriola dumerili IL-22 (anti-sense)	IL-22R	AGTCTTCAGGTCCTCGC	17	58.8	53.9
Seriola dumerili IgM (sense)	IgMF	CGTTTATACGGGAGTCAGTCAAA	23	43.5	54.6
Seriola dumerili IgM (anti-sense)	IgMR	TCAAGGCTGAGCGATAACT	19	47.4	53.5
Seriola dumerili IgT (sense)	IgTF	GGTCACTCTGTTGTGTCTG	19	52.6	52.9
Seriola dumerili IgT (anti-sense)	IgTR	GTGGTGTAAAGACTCGTAAC	20	45	50.5
Seriola dumerili beta –defensin (sense)	DefF	ACATGAAGGGACTGAGCTTG	20	45	52.9
Seriola dumerili beta –defensin (anti-sense)	DefR	ACAGTTACACATCTGCTGCA	18	44.4	49.3
Seriola dumerili piscidin (sense)	PisF	GATGGTCGTCCTCATGGCTG	20	60	57.9
Seriola dumerili piscidin (anti-sense)	PisR	CTTTCAGATGAACCGCCATAGAT	23	43.5	54.9



Greater amberjack
Seriola dumerili

Yellowtail amberjack/ kingfish
Seriola lalandi

Have access to a deep sequencing run of *S. lalandi* immune tissues via a collaborator in New Zealand.

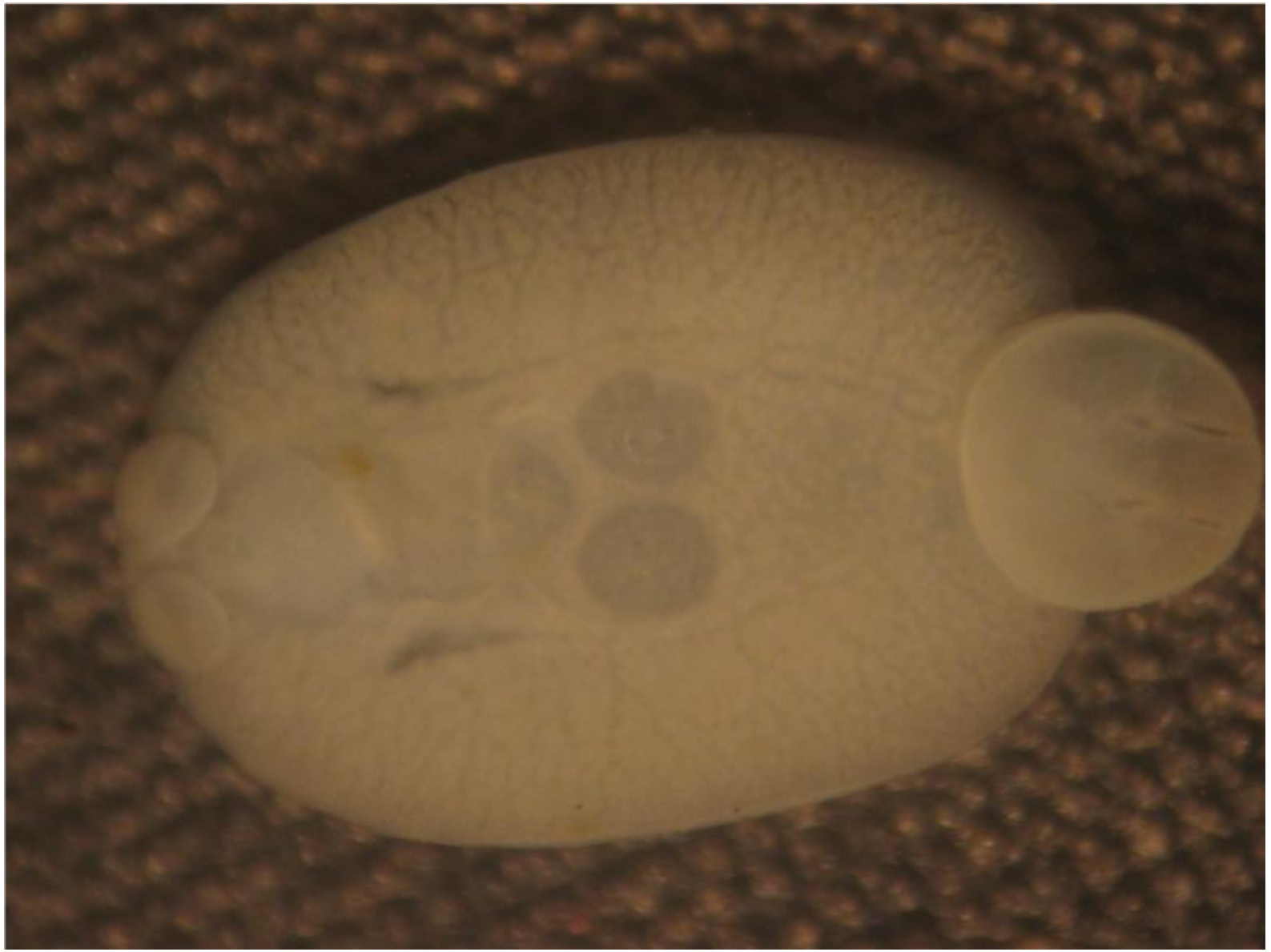
Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites

- ***Design and testing of a collector device as method to detect and quantify the level of infestation of monogenean parasites in the fish rearing tank.***

✓ Counting of the monogenean eggs that are present in the culture tank when laid by the adult parasites that are infesting fish.

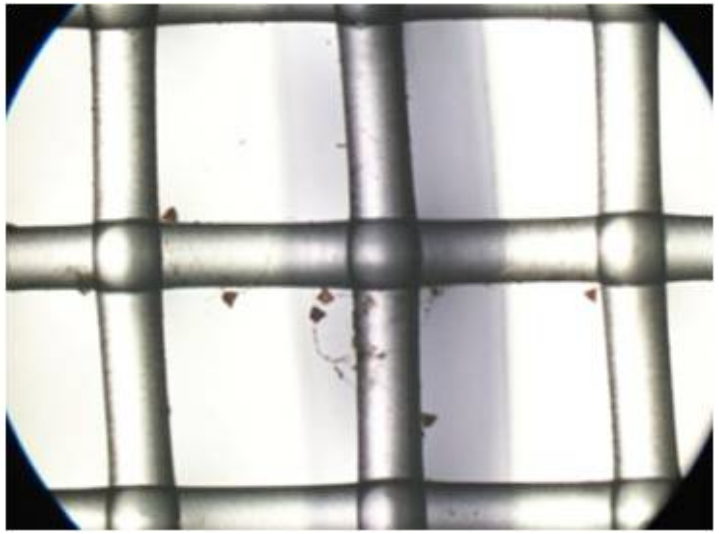
✓ The level of infestation is estimated based on the number of parasite eggs collected by a device that is suspended in the fish tank and removed for observation and counting periodically.

✓ The collector device was designed considering the characteristics of the monogenean eggs, exhibiting filamentous appendages which cause that eggs get entangled in the nylon mesh discs



Rearing site: IEO

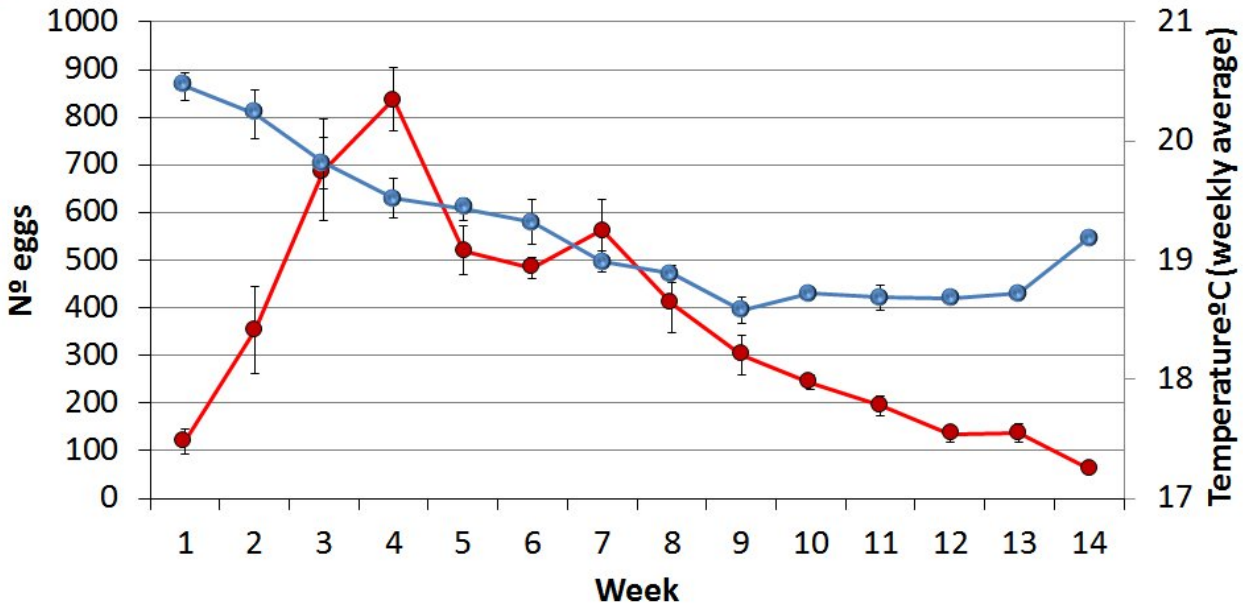
Design and testing of a collector device as method to detect and quantify the level of infestation of monogenean parasites in the fish rearing tank.



Nylon mesh discs removed from the vertical support submerged in the fish tanks are placed in Petri dishes with filtered seawater for observation and egg count under the stereomicroscope.c

Rearing site: IEO

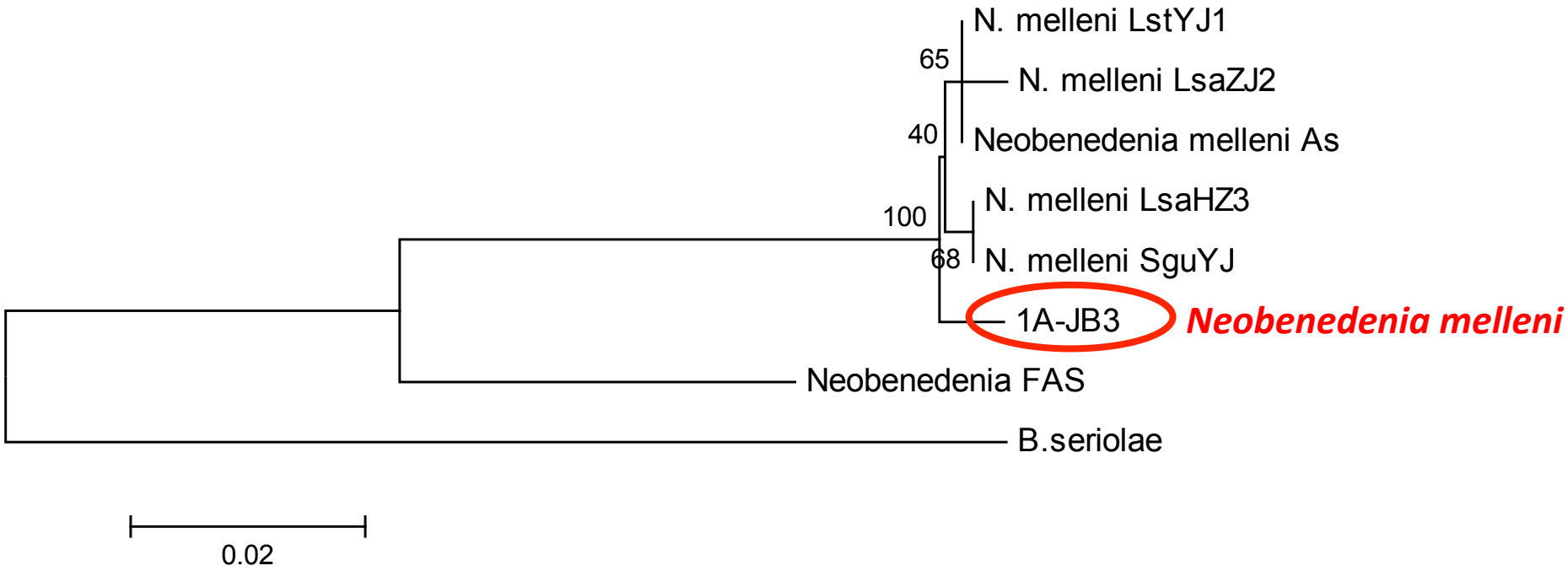
Design and testing of a collector device as method to detect and quantify the level of infestation of monogenean parasites in the fish rearing tank.



Number of monogenean eggs collected in the mesh discs (mean ± standard deviation) (n=3) along 14 weeks in the Seriola dumerili broodstock tank F7.

Rearing site: IEO

Genetic identification of monogenean parasites



Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in amberjack

- Routine samplings for bacterial and viruses of natural occurence in greater amberjack.
- Challenge test nº 1.

Monitoring of bacterial disease

January 2014 . Juvenile greater amberjack
SYMPTOMS Skin ulcers
MICROBIOLOGY
Isolate 3 different strains of bacteria from ulcers and organs. Strains sent to Spanish Type Culture Collection (CECT) for the identification. Identified as bacteria belonging to *Clado Harveyi.*

April 2014 . Juvenile greater amberjack
SYMPTOMS Skin ulcers
MICROBIOLOGY
Isolate 4 different strains of bacteria
Those isolated strains of bacteria have been kept in the lab to check if their incidence is repeated in time and then proceed to their identification

June 2014 . Juvenile greater amberjack
SYMPTOMS Skin ulcers
MICROBIOLOGY
Isolate 2 different strains of bacteria in ulcers and organs
1. Catalase + g + Coco identified as *Staphylococcus epidermidis*
2. Coco + g cat - sent to the CECT identified as *Staphylococcus epidermidis*

CHALLENGE TEST Nº 1.

Design: Sublethal dose of oportunistic bacteria
INDIVIDUALS. Greater amberjack juveniles (n=30)
BACTERIA/DOSE *Photobacterium demselae* subsp. *piscicida*/10^3cfu/fish

MORTALITY/MICROBIOLOGY

None fish died/no bacteria recovered

Case report

- Four populations of greater amberjack broodfish in Greece in different locations (Souda, Aqualabs-HCMR, Galaxidi and Argosaronikos)
- In total 125 fish examined for gonad maturation by Dinos
- During examination we visually inspected gills for parasites
- All fish were infected by blood flukes and monogenean parasites simultaneously except the fish at HCMR tanks which were infected only by the blood fluke

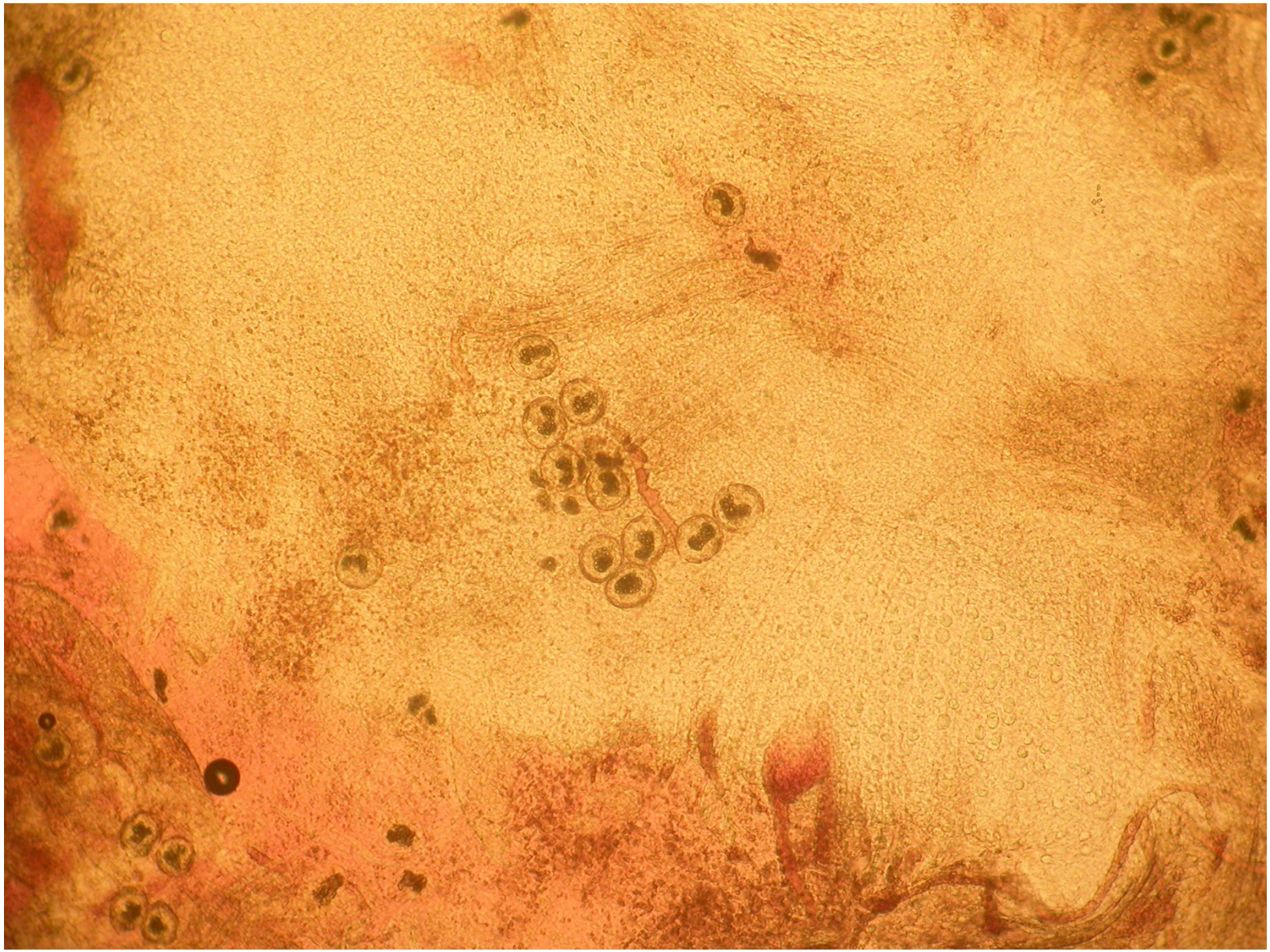
Paradeontacylix sp in amberjack

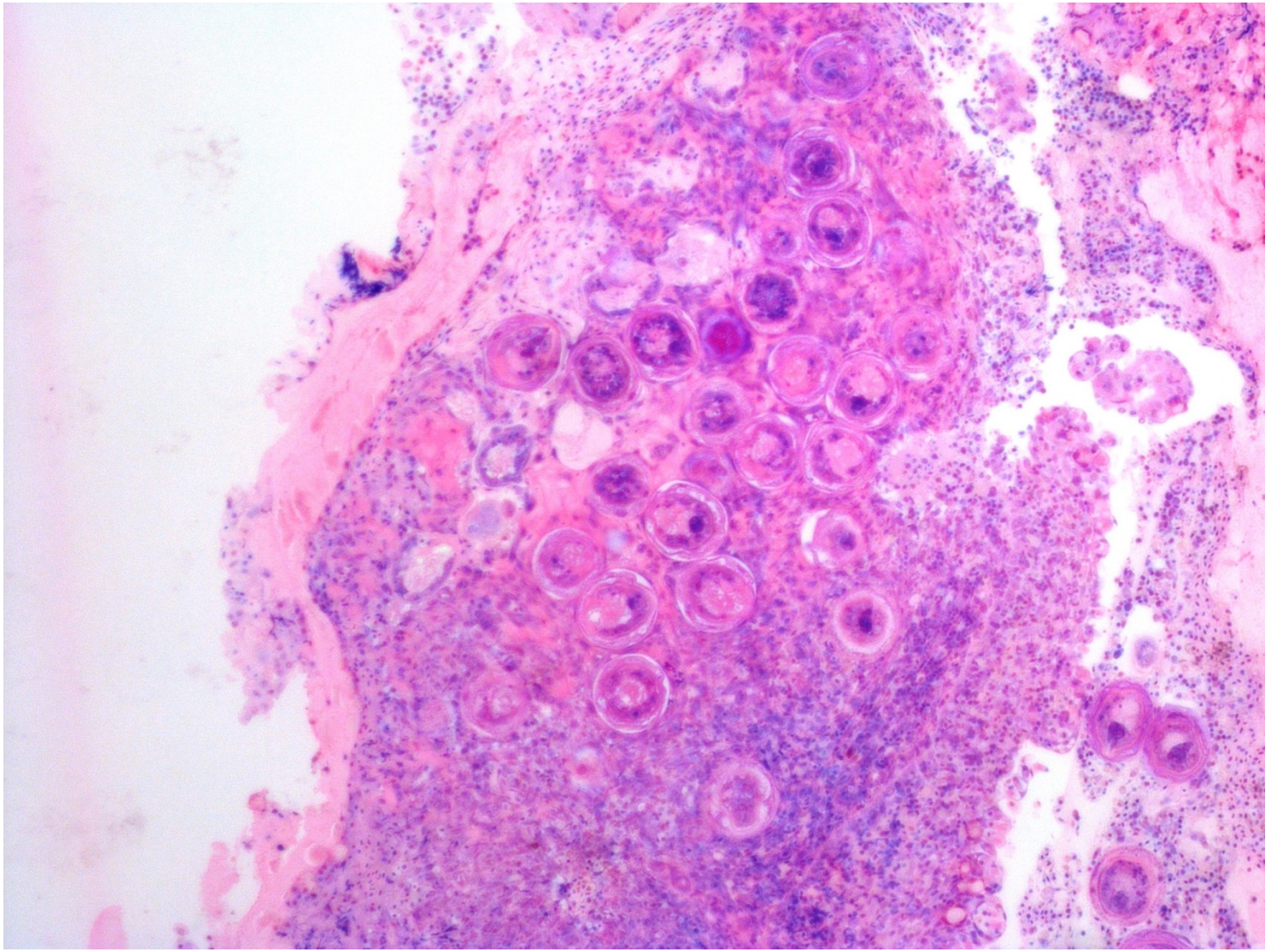
- Only eggs were found- no mature worm
- Eggs were accumulated in the gill lamellae and were seen as white spots blocking gill arteries

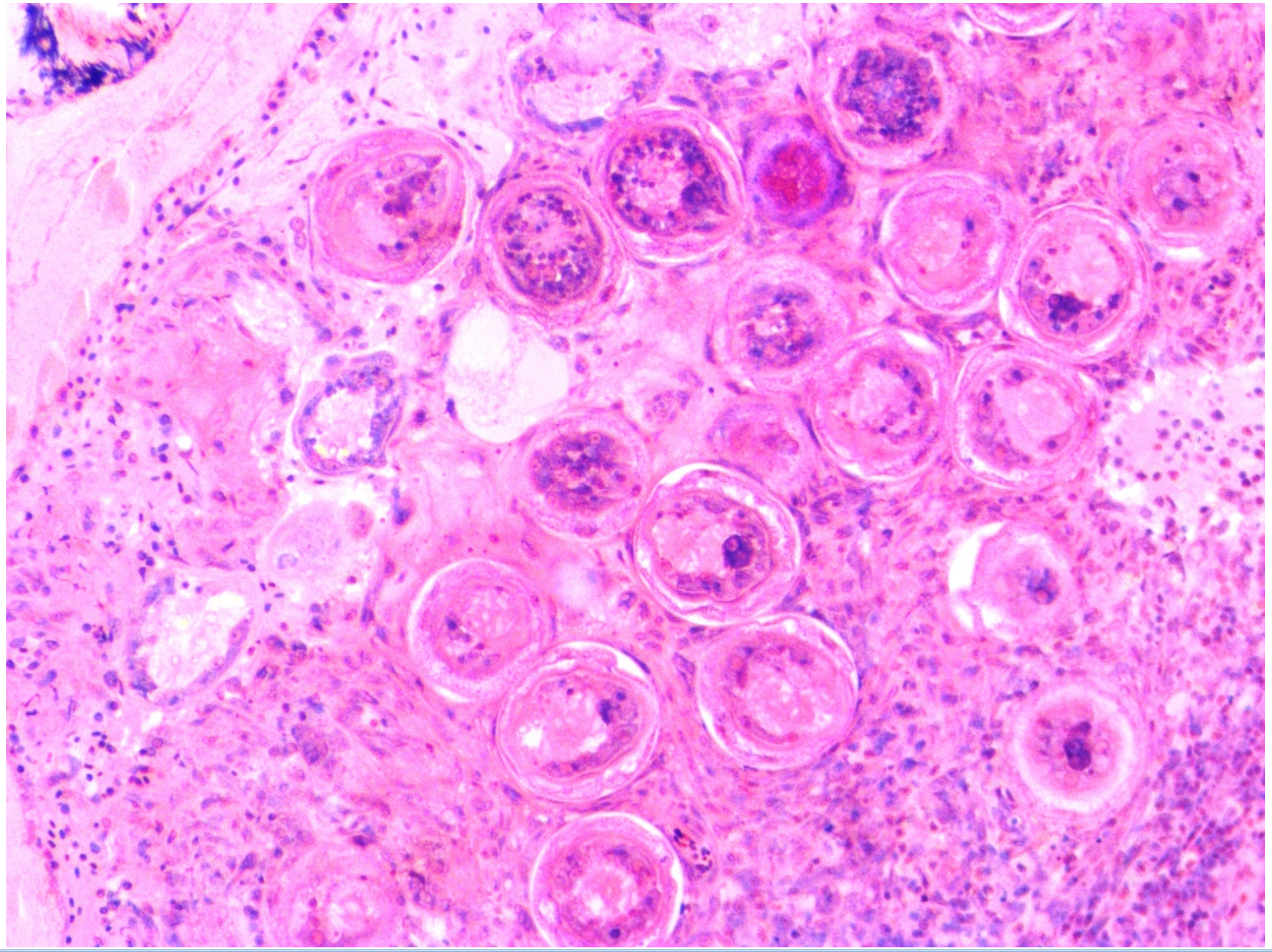

The white spots are accumulated Digenean eggs. Monogenean parasites can also be seen (arrows)

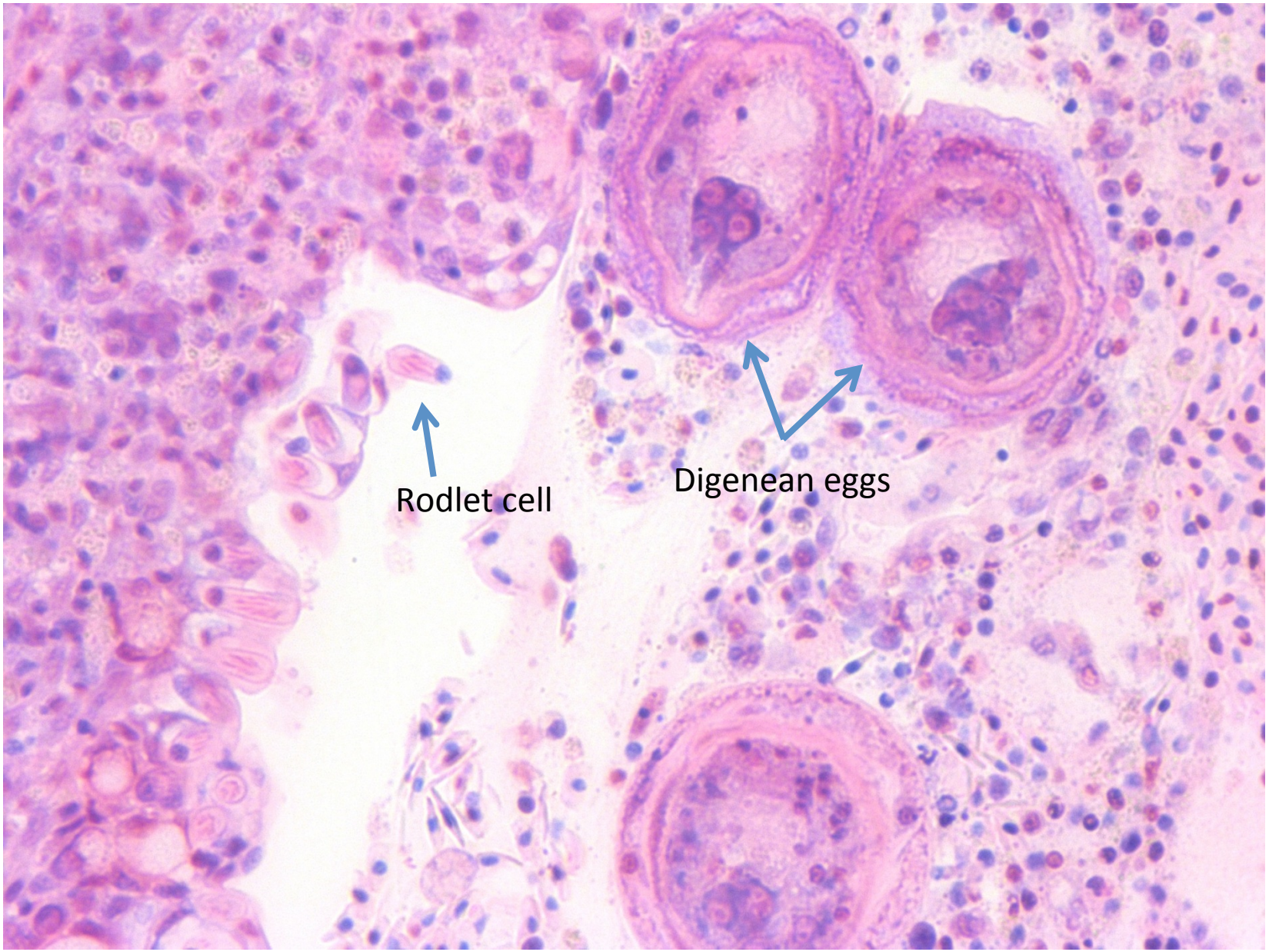
- We took small gill biopsies for further examination under stereoscope on site and for histology
- The cysts were aggregates of Digenean eggs probably from *Paradeontacylix* sp. which is a blood fluke reported in this species.
- The monogenean was *Zeuxapta seriolae*
- Interestingly there was a marked inflammatory reaction characterized by the presence of rodlet cells which formed an "epithelium-like" barrier at the gill lamellae.
- Whether this was elicited by the digenean eggs or the monogenean is not very clear

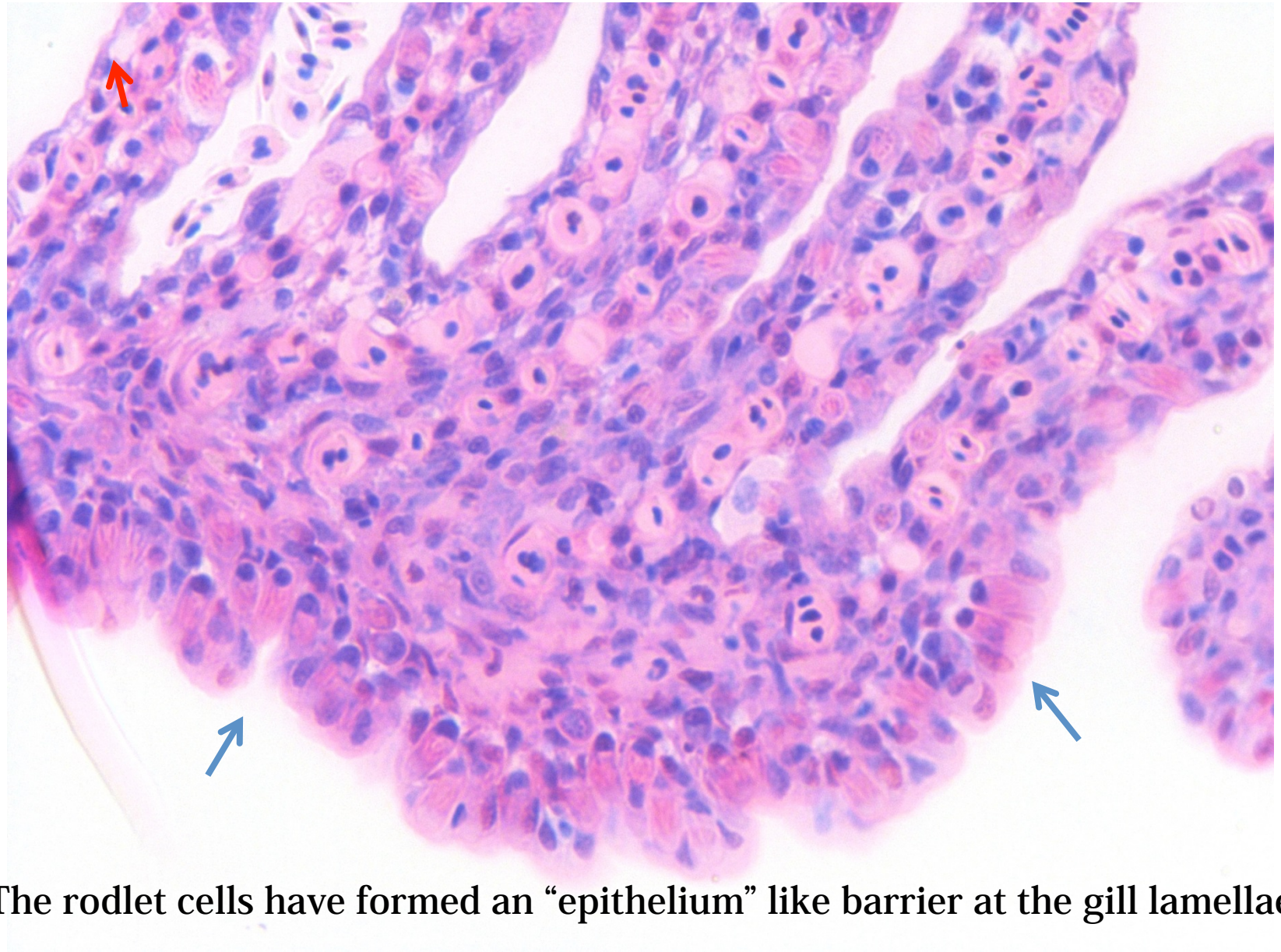
Squash-prep of a fresh gill tissue with digenean eggs








Rodlet cell
Digenean eggs

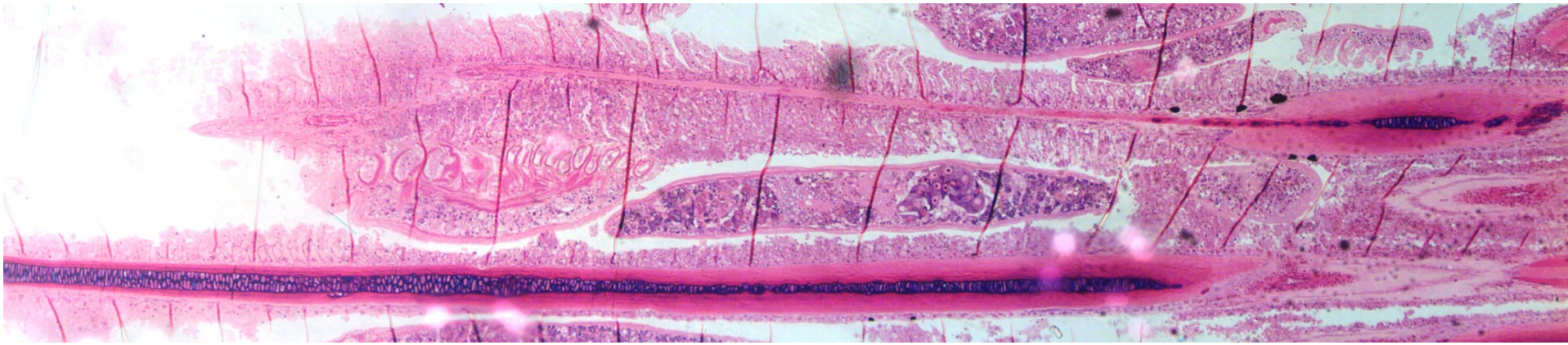


The rodlet cells have formed an “epithelium” like barrier at the gill lamellae

Zeuxapta seriolae

- It is a polyopisthocotylean monogenean worm
- It is a blood feeding parasite of the gills of greater amberjack and it is quite common
- It can reach very high numbers on the gills and causes significant problems

This is a dead amberjack from Argosaronikos. The fish was sent with ice at the Institute. Freshwater of the ice killed the parasites and made them visible



Specimen of the parasite and histological section of a greater amberjack gill (this is from a previous case from my collection)

Treatment

- We tested praziquantel treatment in the fish of Souda
- The treatment was performed in two rounds
- Initially we used injectable Droncit (unfortunately this is no longer available in Greece)
- We injected the fish intramuscularly with 5 mg/kg PZQ
- After a week we gave orally PZQ (Cestocur-Bayer) at 100 mg/kg for 5 consecutive days
- We re-examined the fish after ten days to assess therapeutic efficacy

IM injection of DRONCIT

Necrotic areas at the gill lamellae following treatment.

Efficacy

- After visual inspection of the 12 fish received the PZQ treatment we found that none had visible signs of any parasite
- There were necrotic areas in some gill lamellae (presumably due to blockage of the blood flow from digenean eggs) and mucous overproduction
- **PZQ was very effective against both parasites**

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Thank you for your attention