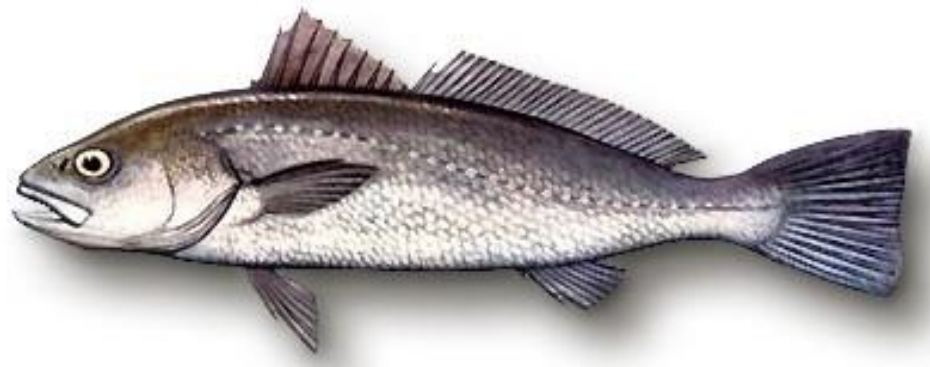


Recent advances in the study of Systemic Granulomatosis in meagre (*Argyrosomus regius*)



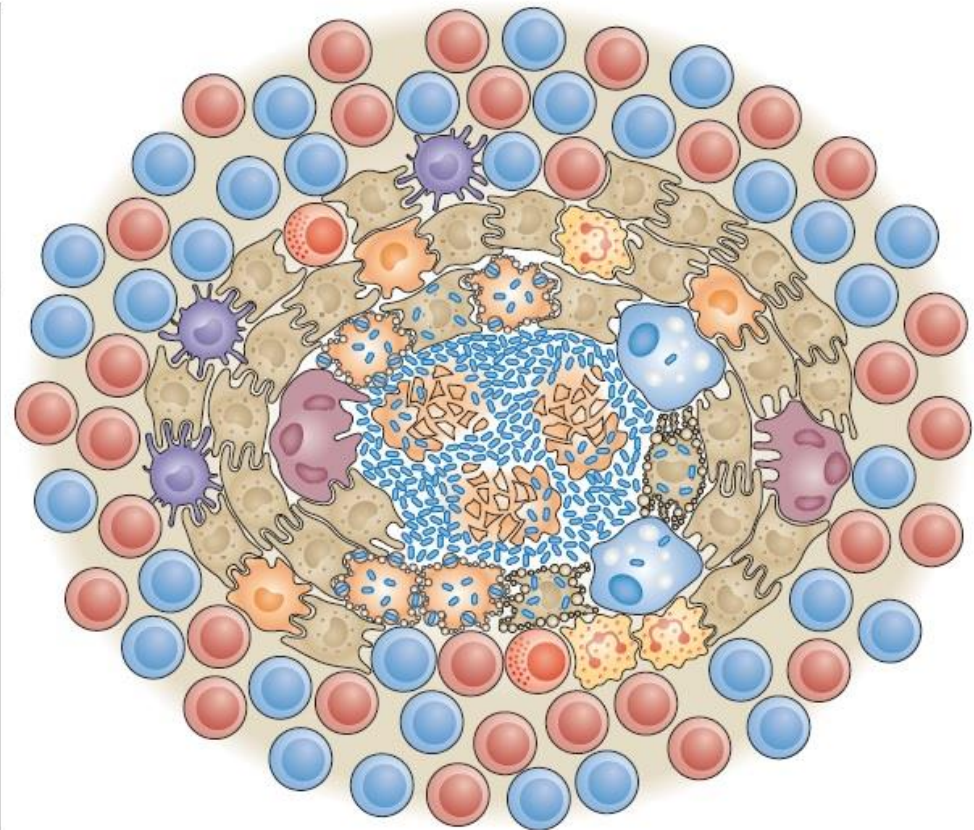
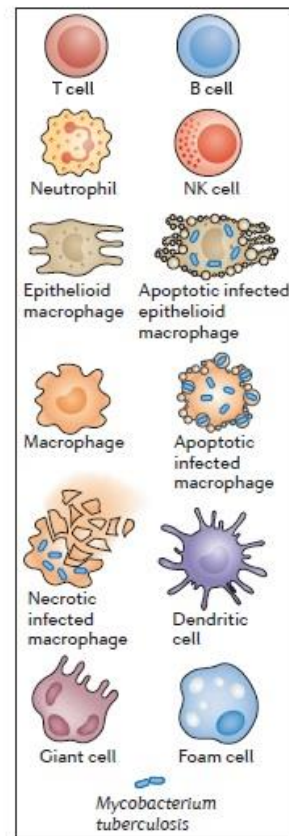
Tsertou M.I., Chatzifotis S., Fontanillas R. , Cotou E.,
Fountoulaki E., Smyrli M., Antonopoulou E., Katharios P.



Definition of the granuloma....

Localized inflammatory reactions

- Bacterial, fungal, and viral infections (Tuberculosis)
- Non- infectious inflammatory diseases (Chron's disease, sarcoidosis)



Systemic granulomatosis in meagre

- Bottleneck for meagre production
- Affects 100% of the population
- Multiple granulomas in all soft tissues
- Aetiology of the disease is unknown









Hypotheses

Metabolic disorder (Katharios et al., 2011)

- Visceral granulomas in seabream
- Visceral granulomas in brook trout
- Renal granulomas in turbot
- Some reports in cichlids and goldfish

Disease caused by pathogens (Elkesh et al., 2014)

- Nocardia/mycobacteria
- Other granuloma-inducing pathogens (fungi, bacteria, intracellular parasites...)

Aim...

Feeding trials to identify potential nutritional causes of SG

Monitoring meagre populations from various locations in Greece to isolate and identify *Nocardia* spp.

“Metabolic disorder” hypothesis

Material and methods

- 3 feeding trials
 - Vitamin D₃
(4 diets with increasing levels of vitamin D3)
 - Ca/P levels
(9 nine experimental diets with different levels of Ca and P)
 - Plant ingredients
(4 experimental diets with 60% and 14% FM and increasing levels of P in the diets with 14% FM)

Material and methods

- 3-month old (0.5-2 g) meagre
- Each trial lasted 3 months
- Histology
- Semi-quantitative ordinal-scale scoring system

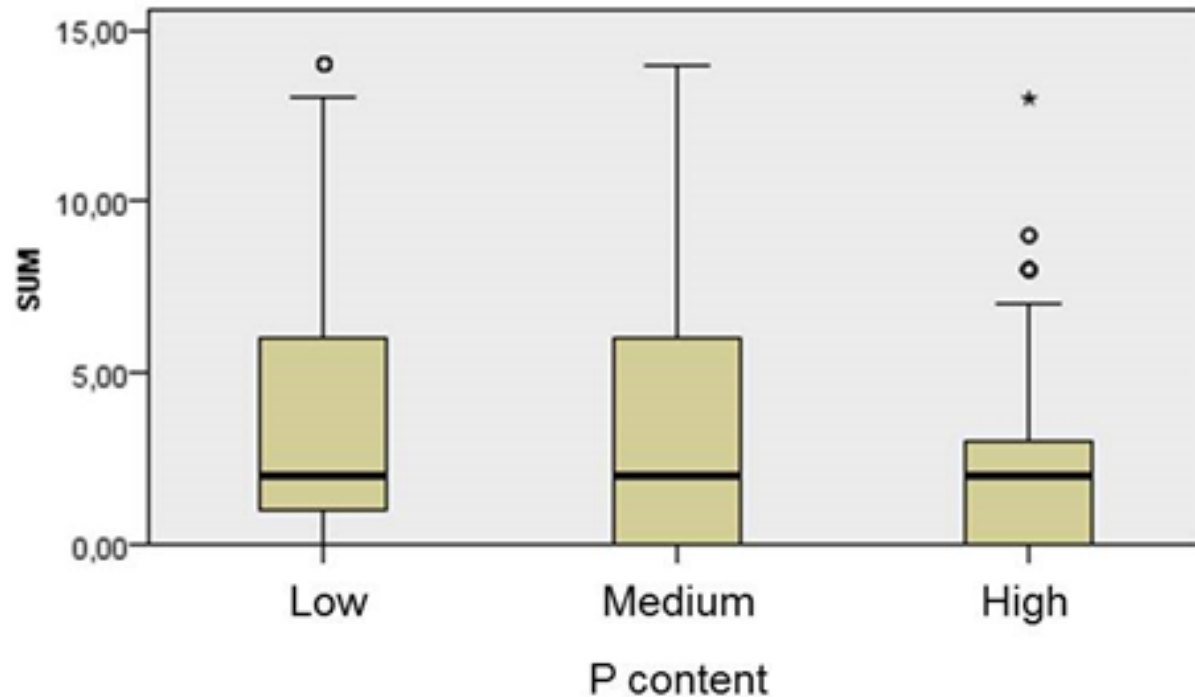
State	Score
No granulomas	0
Granulomas visible only with microscope	1
Granulomas visible macroscopically	2
Tissue calcification	5

Material and methods

Diet												
Stocking												
Sampling												
Individual	Length	Weight	Heart	Liver	Gonads	Intestine	Spleen	Peritoneum	Swimm Bladder	Kidney	SUM	
1	9	13,7	0	0	0	0	1	0	0	0	2	3
2	10,7	21	0	1	0	0	0	0	0	0	0	1
3	11,3	24,7	0	0	0	0	0	0	0	0	0	0
4	10,9	21,7	0	1	0	0	0	0	0	0	0	1
5	9,6	15,8	0	1	0	0	0	0	0	0	1	2
6	10,3	19,25	0	1	0	0	0	0	0	0	0	1
7	10	16,1	0	1	0	1	0	0	0	0	5	7
8	11,3	22,39	0	0	0	0	1	0	0	0	0	1
9	9,8	16,51	2	5	0	0	1	0	0	0	5	13

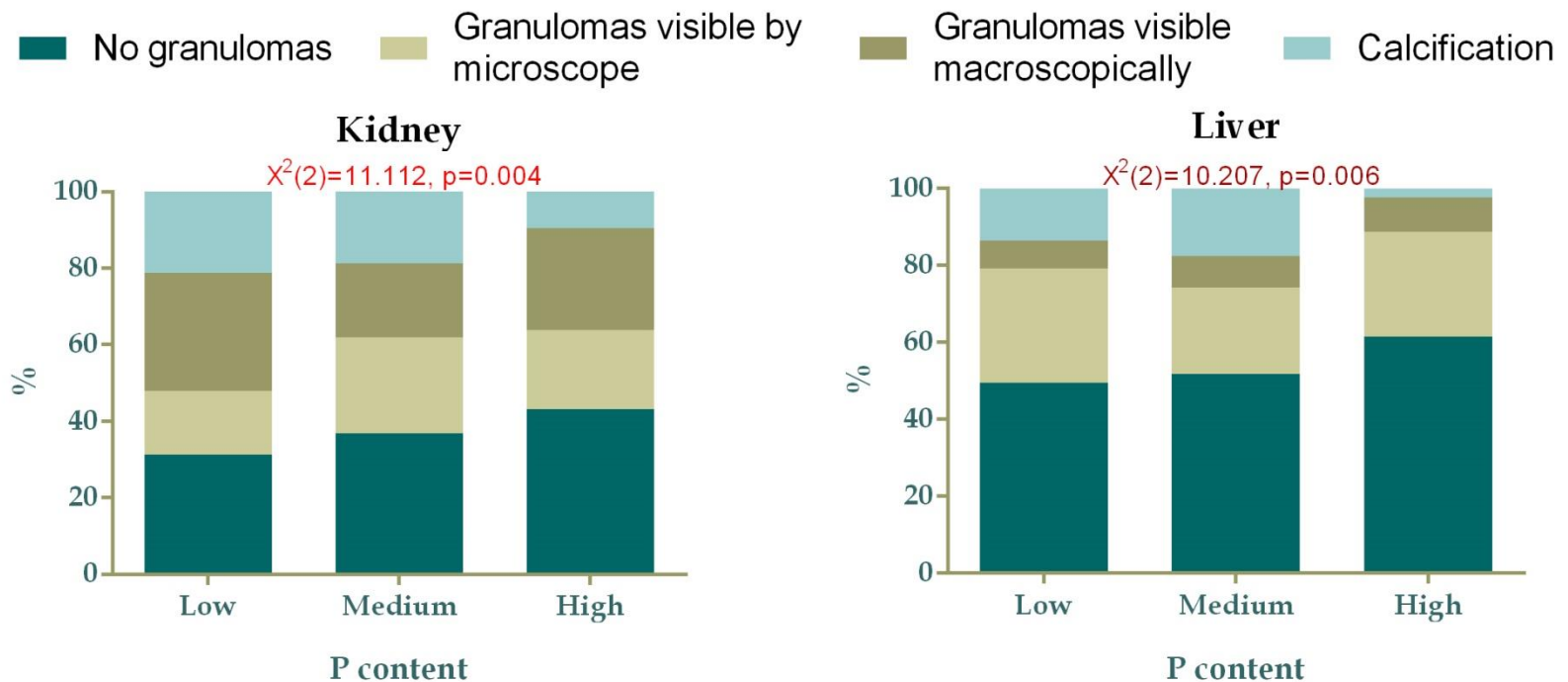
The effect of Phosphorus

- The medians of the groups with high and low P content are significant different



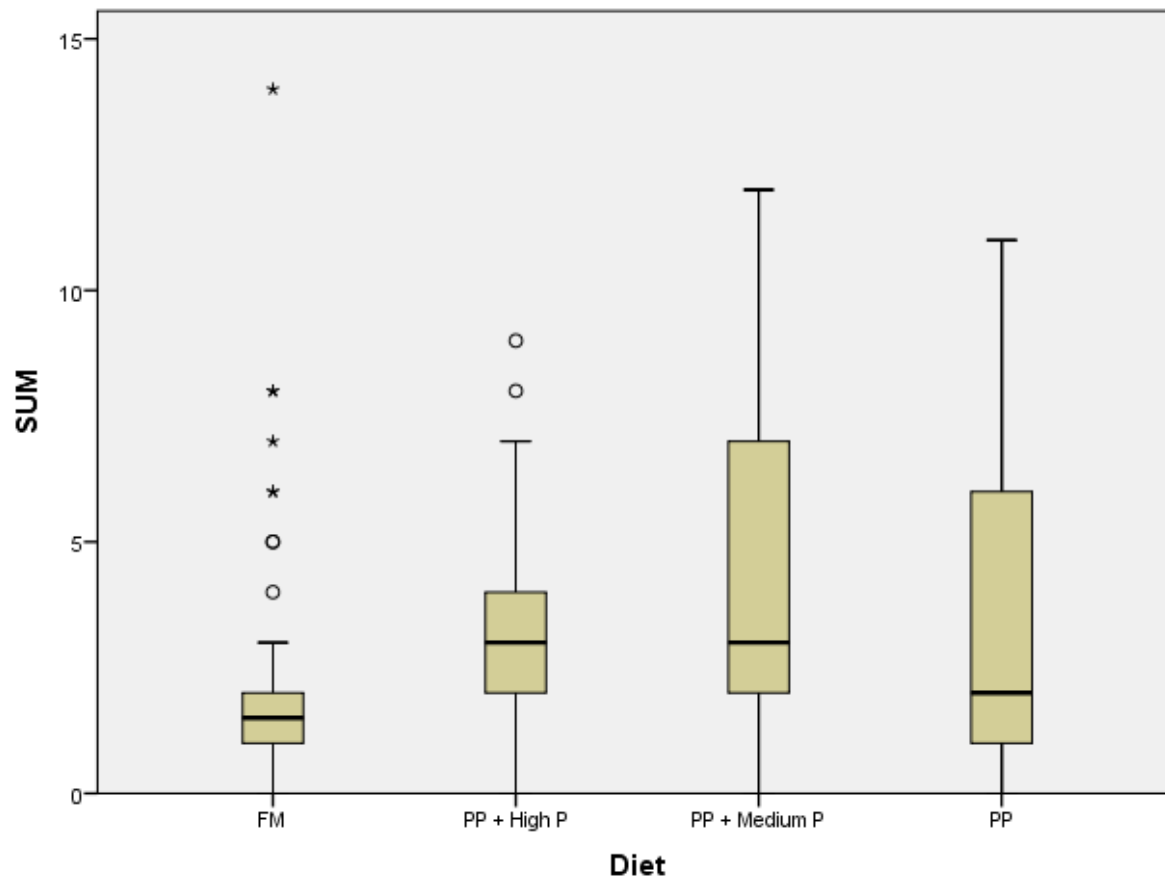
The effect of Phosphorus

- Statistical significant difference exists between the diets and the development of granulomatosis for both tissues



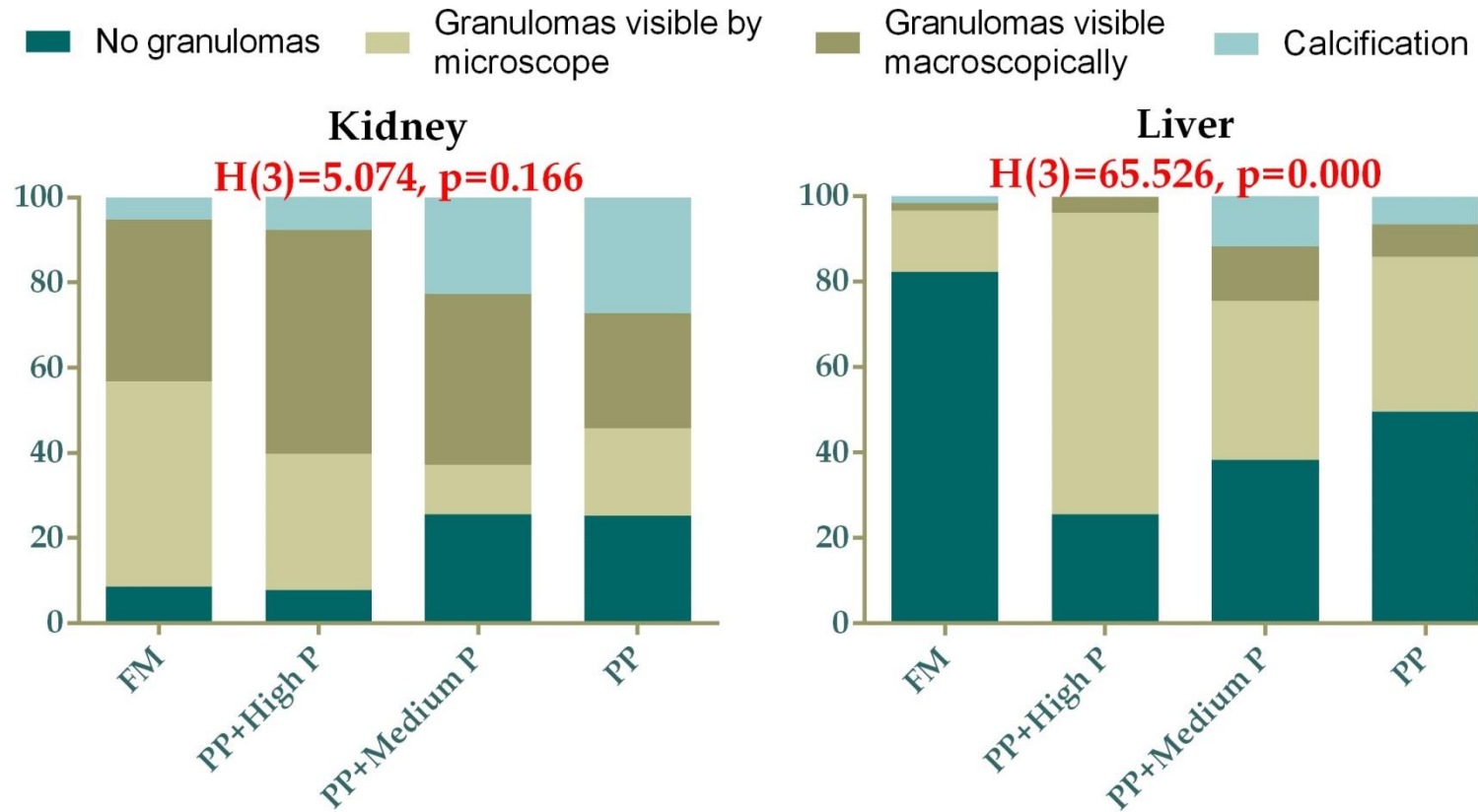
The effect of plant protein

- FM median was significant different from PP median

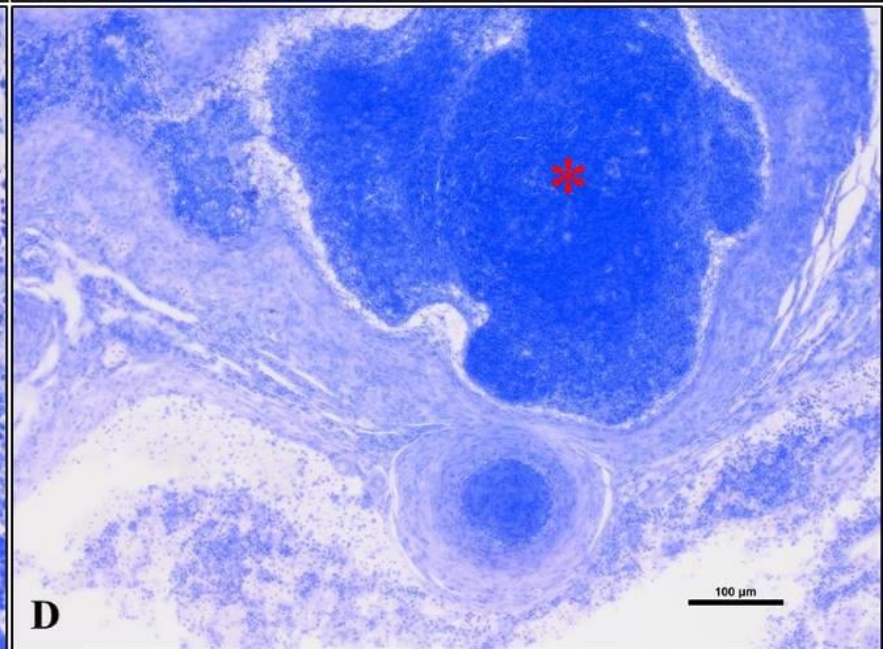
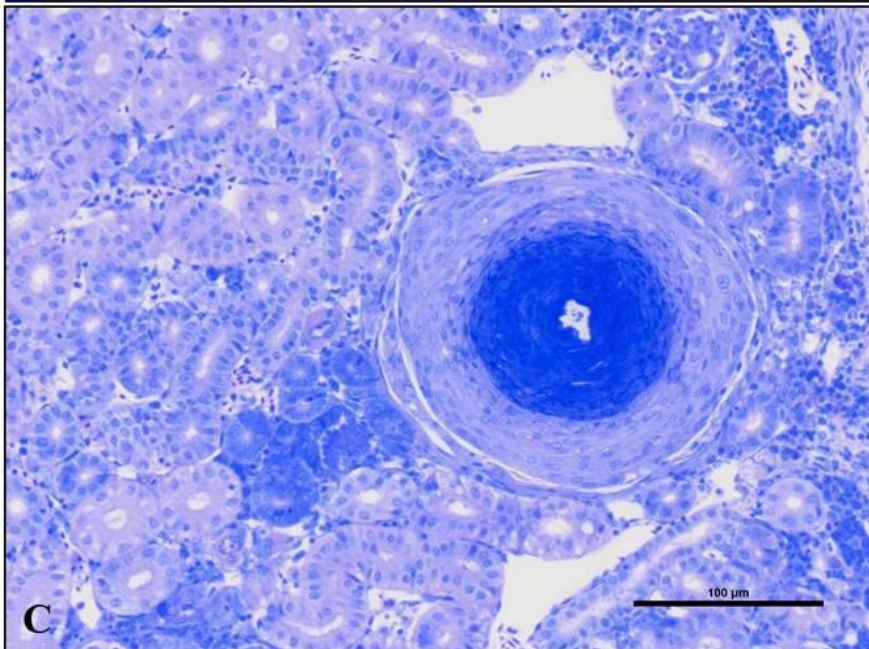
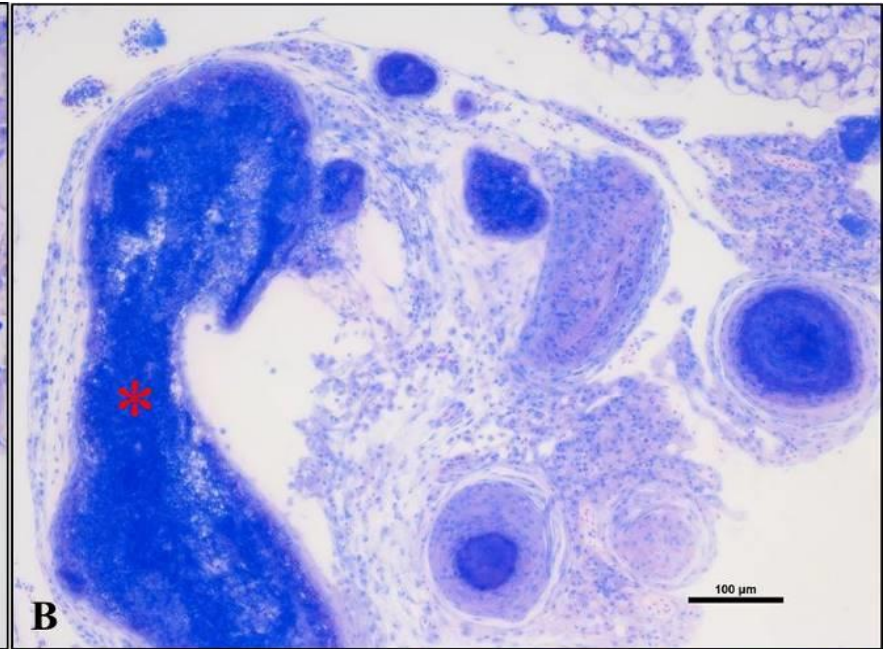
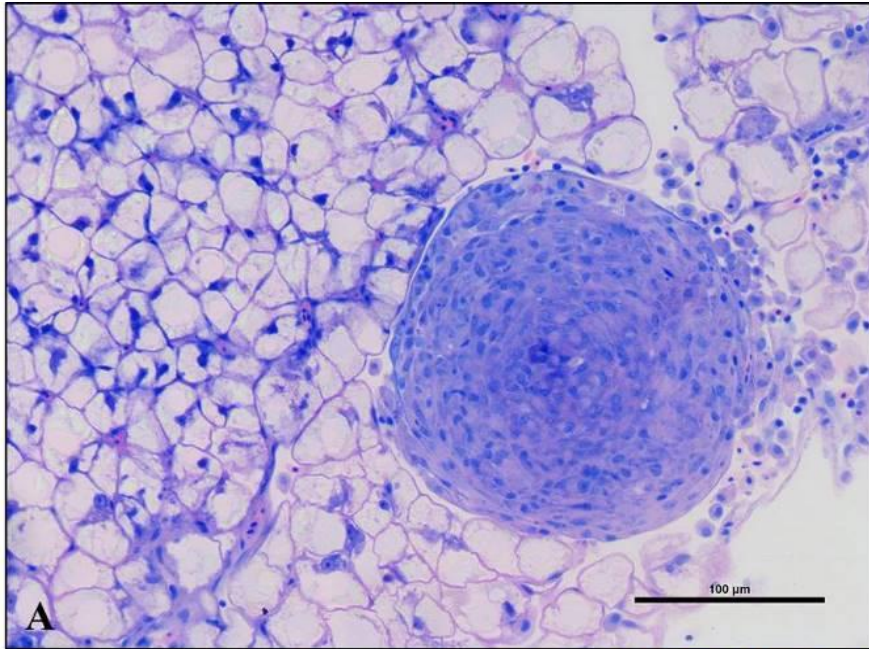


The effect of plant protein in combination with P inclusion

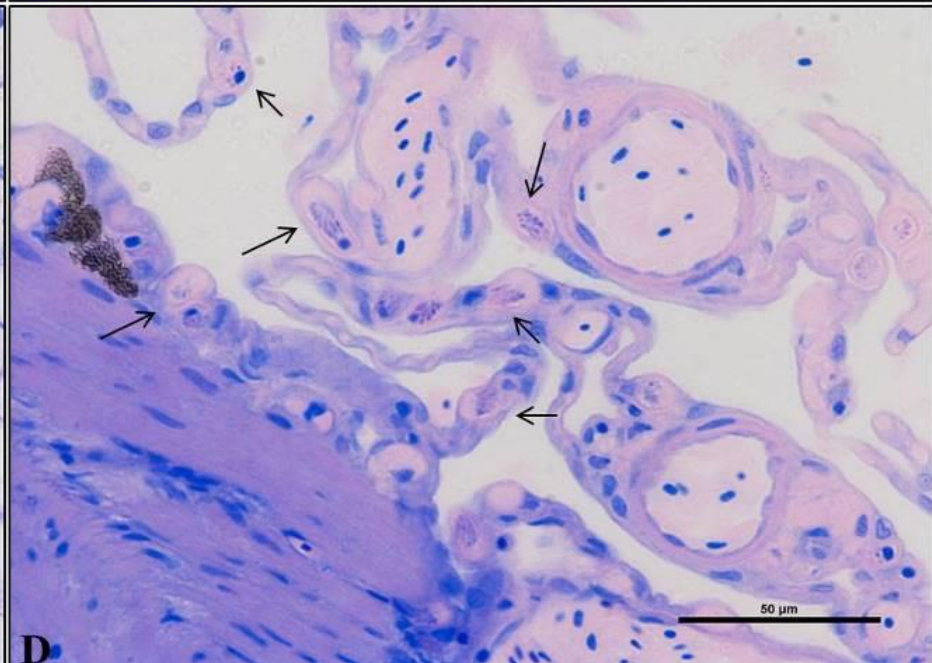
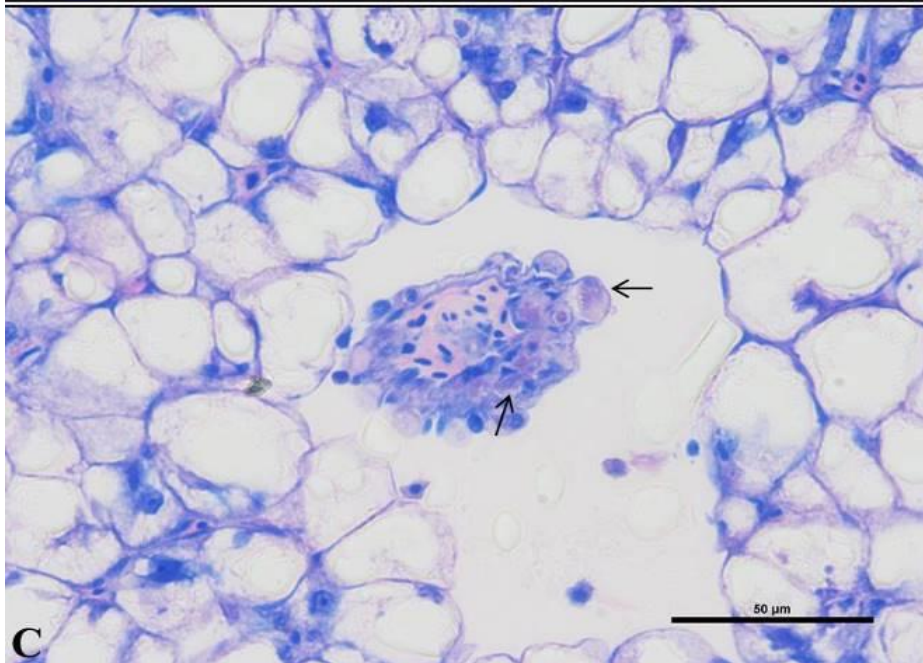
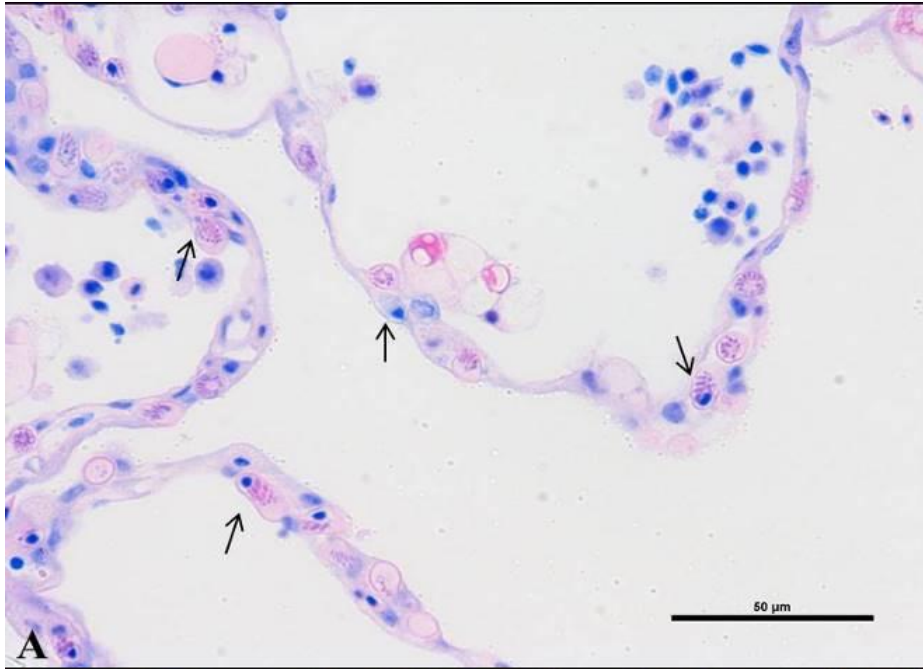
- A statistical significant difference exists only for the liver



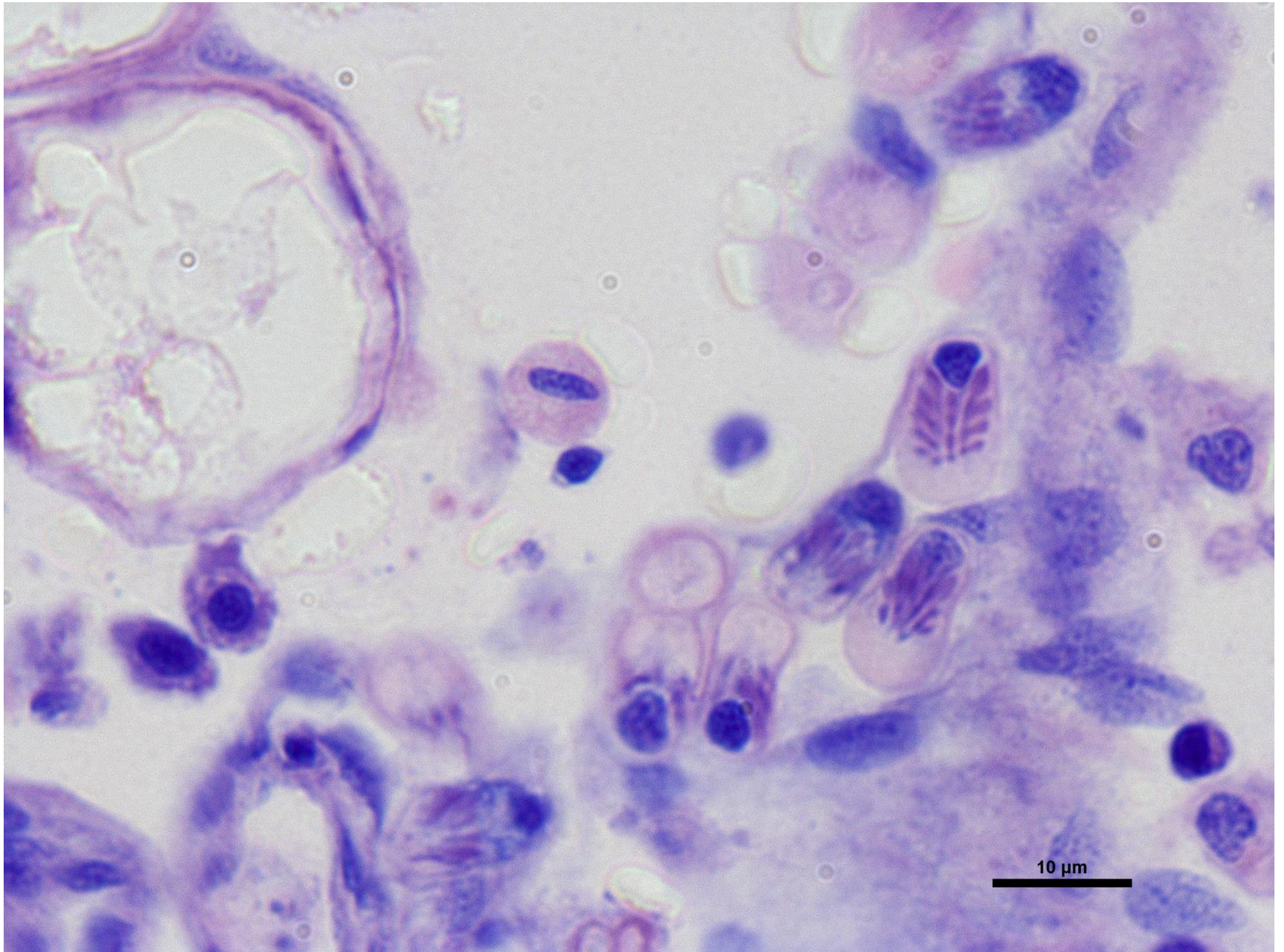
“Metabolic disorder” hypothesis



“Metabolic disorder” hypothesis



“Metabolic disorder” hypothesis

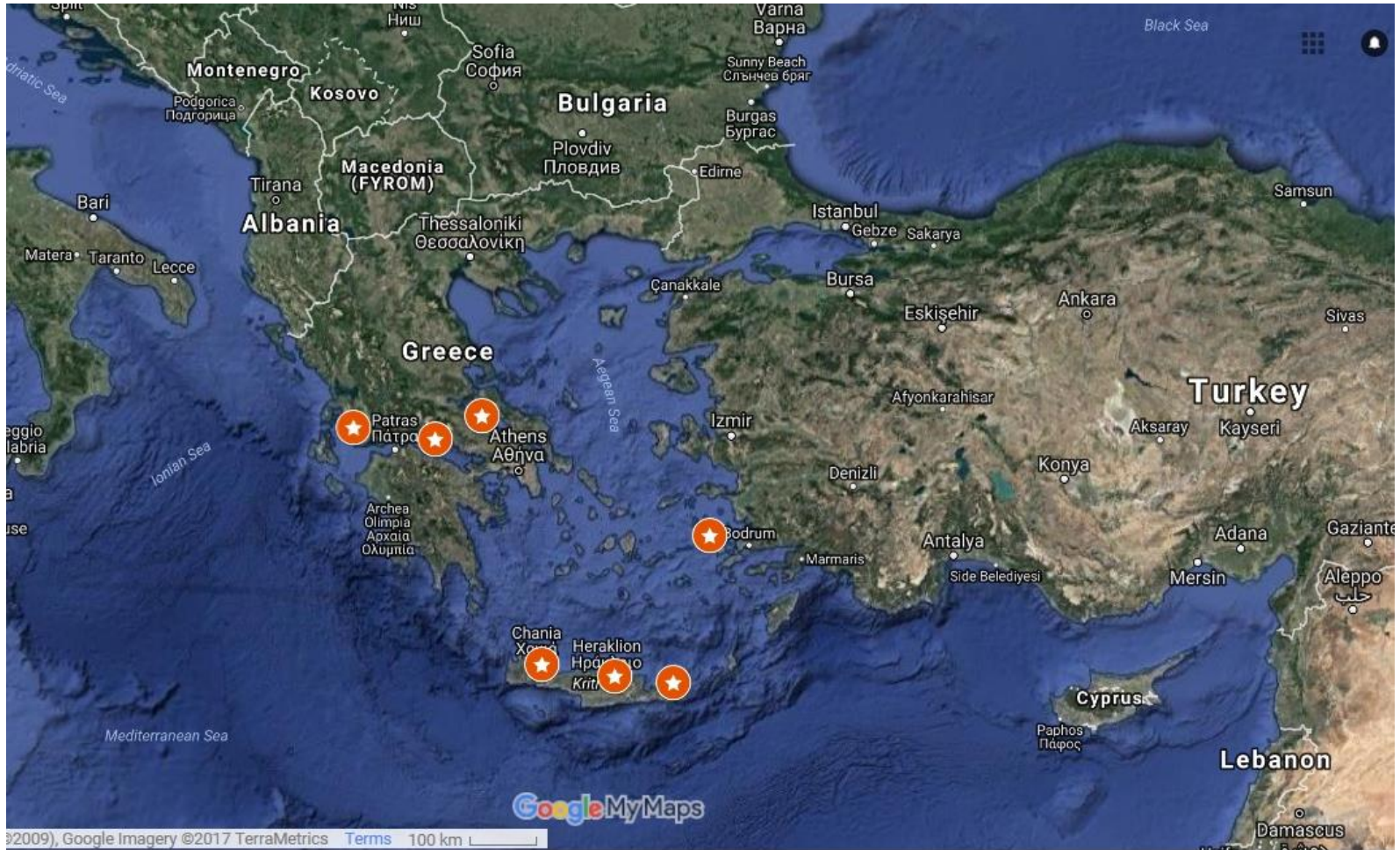


Conclusions

- Vitamin D did not affect the development of the disease
- High P in the diet seems to improve the condition
- Plant protein negatively affects the condition
- The overall pathology was not different to that described by Katharios et al. 2011

“Pathogen” hypothesis

Material and methods



Material and methods

- Microbiological techniques
(General and selective nutrient media)
- Molecular techniques
(PCR for *Nocardia* spp)
- Histological techniques
(standard and special staining techniques)

Results

- All fish examined had granulomas in their tissues
- In most of the cases no bacterial growth was observed
- In total we purified approximately 25 isolates
- None of the isolated bacteria had phenotypes consistent to *Nocardia* spp.
- Sequencing confirmed that none of the isolates belonged to the *Nocardia* genus
- More likely environmental strains

Results

PCR analysis directly on SG-affected tissues using specific primers against *Nocardia* spp. were negative for all samples assayed except....

“Pathogen” hypothesis

- 2 fish from Astakos, West Greece
- Severe dermal lesions and ulceration of the skin
- PCR for *Nocardia* spp. was positive in 4 out of the 6 different organs



BLAST analysis showed a 100% identity with *Nocardia seriolae*

https://blast.ncbi.nlm.nih.gov/Blast.cgi#91204703

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information katharios My NCBI Sign Out

BLAST » blastn suite » RID-32PGBJTJ014 Home Recent Results Saved Strategies Help

BLAST Results

Your search is limited to records matching entrez query: txid37332 [ORGN].

[Edit and Resubmit](#) [Save Search Strategies](#) [Formatting options](#) [Download](#) [YouTube](#) [How to read this page](#) [Blast report description](#)

Nucleotide Sequence (580 letters)

RID 32PGBJTJ014 (Expires on 11-21 15:08 pm)

Query ID |cl|Query_188757
 Description None
 Molecule type nucleic acid
 Query Length 580

Database Name nr
 Description Nucleotide collection (nt)
 Program BLASTN 2.5.1+ [Citation](#)

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#)

[+ Graphic Summary](#)
[- Descriptions](#)

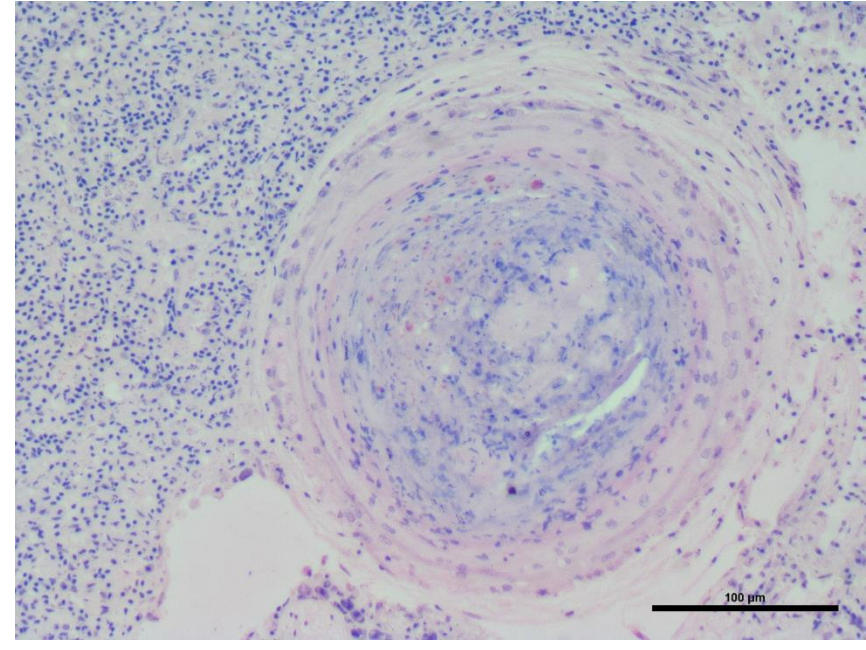
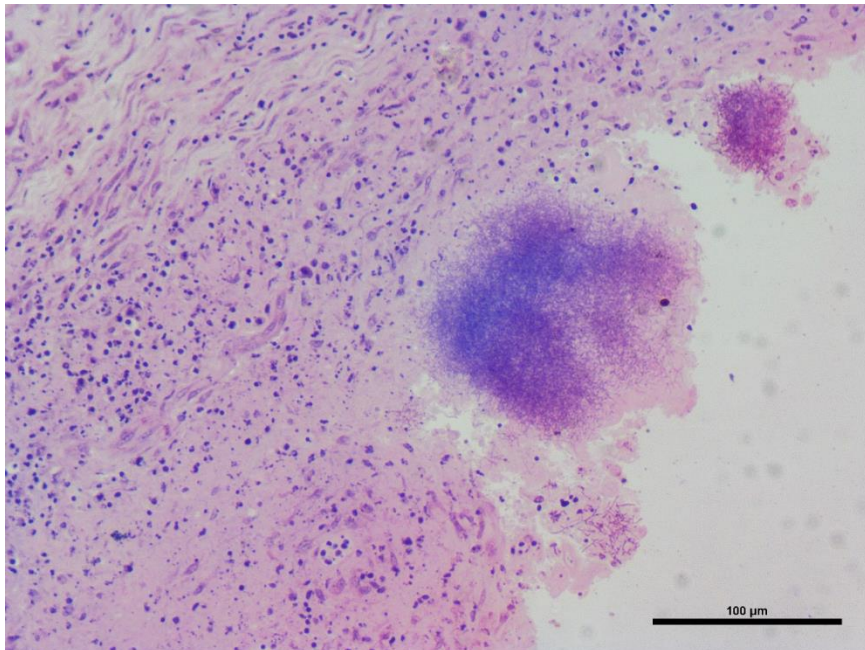
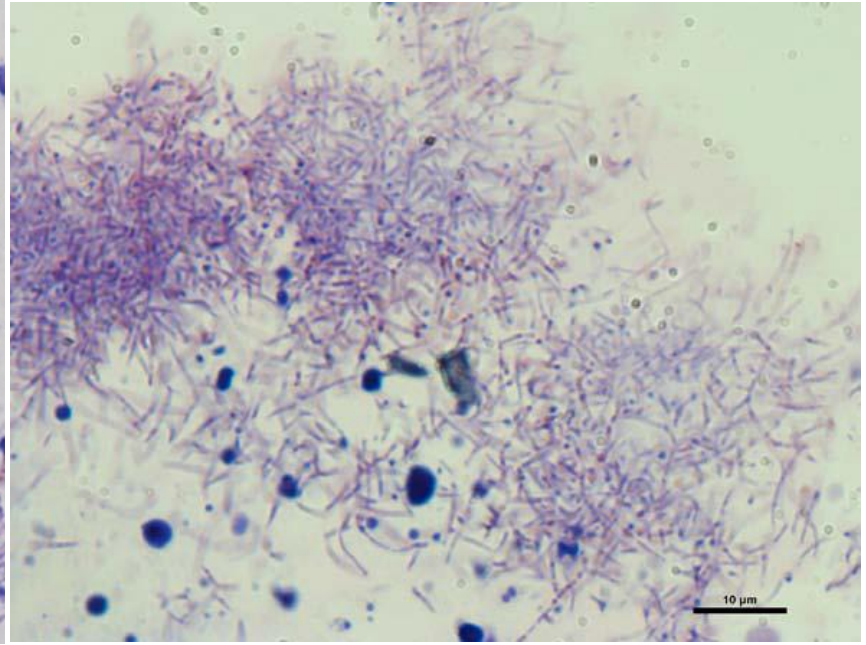
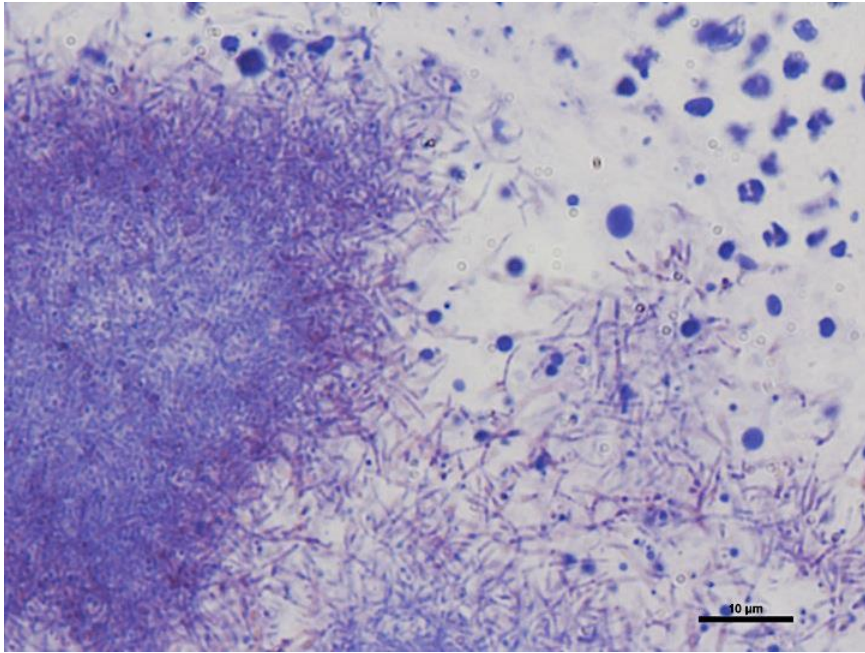
Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Nocardia seriolae strain LH1347 16S ribosomal RNA gene, partial sequence	1072	1072	100%	0.0	100%	KY029027.1
<input type="checkbox"/>	Nocardia seriolae partial 16S rRNA gene, isolate AT3HP14	1072	1072	100%	0.0	100%	LT221194.1
<input type="checkbox"/>	Nocardia seriolae partial 16S rRNA gene, isolate HL4RS10	1072	1072	100%	0.0	100%	LT221160.1
<input type="checkbox"/>	Nocardia seriolae strain WEL_NS01 16S ribosomal RNA gene, partial sequence	1072	1072	100%	0.0	100%	KM387284.1

“Pathogen” hypothesis



Conclusions

- Meagre does not seem to be very sensitive to bacterial infections
- Nocardiosis is present in Greece but... it is not the cause of SG

Conclusions

Metabolic disorder

Improvement of SG by
change in the diet

Disease caused by pathogens

- Absence of pathogens in SG-affected population
- Only a single case of nocardiosis

Thank you
for your attention!!!!

