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Health & Welfare

Emerging epitheliocystis disease in Mediterranean sparids caused by novel bacteria

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New research critical for disease diagnosis and tracing of epidemics

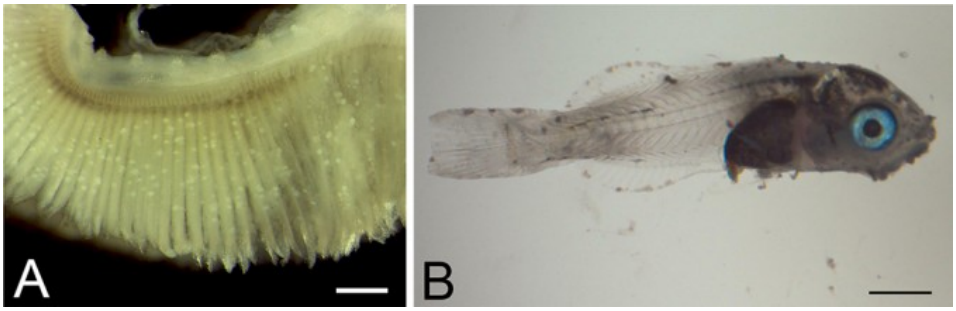

Figure 1A: Gill from infected gilthead seabream, visualised using a dissecting microscope. White spots are epitheliocysts. 1B: Whole infected sharpsnout seabream larva, 28 dph, bright field image. The cysts are readily apparent on the fins and skin. Scale bars 1 mm.

Epitheliocystis is an emerging infection among farmed gilthead seabream and is also lethal in mesocosm cultures of sharpsnout seabream larvae. Two studies of this disease at sites in Greece and Crete have characterized the gill and skin cysts in more detail. Genetic analysis shows that the infections in these fish species are caused by different, novel bacteria, and genome sequencing is giving insights into the diversity of these pathogens and their lifestyle. This research is critical for the diagnosis of epitheliocystis causing agents, and will help to trace epidemics of the disease.

Epitheliocystis in sparids

Epitheliocystis is a disease of fresh- and saltwater fish, causing cysts primarily in the gill and skin epithelia of juvenile and adult fish. Past studies have shown that most infections involve bacteria related to Chlamydia, meaning that most studies only look for these. However, in the last few years, new findings have shown that other, highly unrelated bacteria are also found causing epitheliocystis.

To date there is very little information about this disease in the high-value, aquaculture fish species farmed in the Mediterranean. Some studies from the 1970s describe the disease in gilthead seabream, but with no information on what the cause is. Two novel studies have addressed this information gap, looking at epitheliocystis in farmed gilthead seabream in Greece, and in a mesocosm culture of sharpsnout seabream larvae in Crete.

Epitheliocystis has been found to be an emerging disease in gilthead seabream. Looking at diagnosed cases over the last 15 years within one commercial aquaculture firm, mortality attributed to epitheliocystis has increased from 0-2 percent (as a percent of all fish) with an ongoing upward trend. This corroborates many anecdotal tales of increasing numbers of epitheliocystis cases which are, as yet, unfortunately unrecorded. Our study investigated cohorts of infected fish from 3 sites from November 2012-June 2013, some of which were sampled during epitheliocystis outbreaks. Hosts which are able to clear the infection do not appear to suffer from recurrent infections.

Sharpsnout seabream are being proposed as a new, delicious, candidate species to bring into aquaculture. Using mesocosm methods in which larvae are grown in tanks of supplemented seawater, we have found high mortality rates (up to 25 percent) resulting from epitheliocystis. The surviving juveniles were able to clear the disease and were moved to on-growing tanks.

Diagnosis of epitheliocystis

Sacrificed fish can be diagnosed under the light microscope (Figure 1A and B) with large cysts visible on the gills or on the skin of larvae.

Staining of sections of infected fish using haematoxylin and eosin or methylene blue/azure II/basic fuchsin can also help pathologists with the diagnosis (Figure 2A and B). In both cases we saw cysts which are basophilic, up to 100µm in diameter, surrounded by thick eosinophilic membranes, with

varying degrees of epithelial hyperplasia. In gilthead seabream the secondary gill lamellae can be blunted and fused, causing the gill surface area to be significantly reduced, and mild inflammation is apparent. The cysts within the sharpsnout seabream have a more granular appearance.

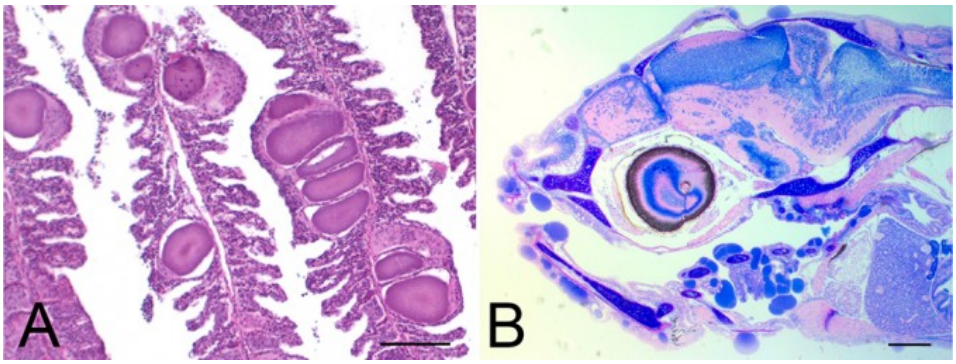

Figure 2A: Gilthead seabream gill section stained with haematoxylin and eosin. 2B: Sharpsnout seabream head section stained with methylene blue/azure II/basic fuchsin. Scale bar A. 100 µm, B. 200 µm.

Detailed imaging of epitheliocysts

Determination of the bacteria involved in these infections was performed by sequencing a signature gene, the 16S ribosomal RNA gene, which is used to identify bacterial species. Once the sequence of this gene was known, probes were designed against the specific sequences and used to confirm that these bacteria are indeed the ones responsible for the infections, as they reside in large numbers within the cysts (Figure 3A and B).

As these bacteria have so far only been found in the fish host, and have not been grown in the laboratory, the methods for investigating them are limited. High resolution transmission electron microscopy (TEM) was therefore used to get more of an insight into the cyst environment, and showed two very different types of bacteria, neither of which looks like the familiar chlamydial type of cyst (Figure 4A and B).

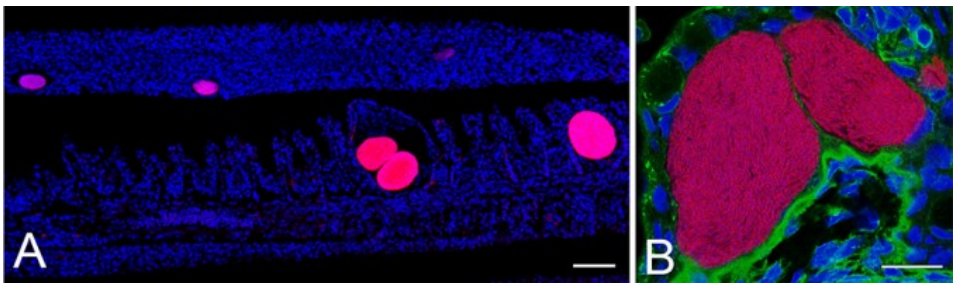

Figure 3. Sections with the respective bacterial 16S rRNA genes labelled fluorescently to show the specific pathogen present in the epitheliocysts. A: Gilthead seabream gill. B: Sharpsnout seabream section. Scale bar A. 50 µm, B. 10 µm.

Identification of novel epitheliocystis pathogens

Using the sequence information, the bacteria responsible for the large cysts have been identified as new fish pathogens. A novel family of bacteria, which we have named *Ichthyocystis*, is found within gilthead seabream, and a species of the widely distributed marine bacterial group *Endozoicomonas* is found in sharpsnout seabream. These are not at all related to chlamydial pathogens previously found in epitheliocystis: the beta-proteobacteria *Ichthyocystis* are related to *Pseudomonas* and *Vibrio* bacteria and the *Endozoicomonas* family belongs to the gamma-proteobacteria, more closely related to agents causing meningitis or melioidosis.

Further work on the genomes of these bacteria showed that the *Ichthyocystis* bacteria are highly diverse, with many strains circulating and co-infecting hosts. These are clearly intracellular bacteria, and are therefore completely dependent on the fish as a host. The *Endozoicomonas* bacteria are related to known symbionts of diverse marine hosts, but appear to be evolving to a more specific niche, perhaps as dedicated fish pathogens.

The emergence of these new epitheliocystis agents should be a cause of concern for those involved in aquaculture and fish welfare. These bacteria can now be added to the many species of *Chlamydiae* which are known to cause epitheliocystis, and which were also found in the gilthead seabream gills, albeit at lower levels. These studies underline the need for the community to use more advanced tools to monitor and diagnose this disease.

Figure 4. Transmission Electron Micrographs. A: Gilthead seabream cyst. B: Sharpsnout seabream cyst. Scale bar A. 10 µm, B. 1 µm.

Perspectives

This work shows that novel fish infections are emerging all the time, resulting in recognized pathologies, but caused by varied bacterial agents. Detailed research needs to continue, particularly as high value fish are involved and incidences are seen to be increasing. Further research is needed on infected fish from more distant sites to investigate how widespread and diverse these bacteria are. More detailed recording of diseases and data sharing is also required to detail the impact that these infections are having on the aquaculture industry.

These studies are a first step towards understanding the bacteria involved in epitheliocystis, and can lead to faster and more accurate diagnosis. This is required to determine whether improvements in animal husbandry can reduce infection rates and improve fish health, and whether vaccines can be developed in the future.

Author notes:

If you are interested in helping our further research in terms of data or samples, please contact Dr. Pantelis Katharios, katharios@hcmr.gr (mailto:katharios@hcmr.gr). All images reprinted by permission from Macmillan Publishers Ltd: Scientific Reports, doi: 10.1038/srep17609 (2015) and The ISME Journal, doi: 10.1038/ismej.2015.223 (2015).

Publications describing this work:

Katharios, P. et al. "Environmental marine pathogens: striking genomic and morphological features of a novel Endozoicomonas sp. causing disease in sharpsnout seabream". Scientific Reports (2015), doi: 10.1038/srep17609

Seth-Smith, H.M.B. et al. "Emerging pathogens of gilthead seabream: characterisation and genomic analysis of novel intracellular β—proteobacteria." The ISME Journal (2015), doi: 10.1038/ismej.2015.223

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