

WORKSHOP REPORT

Workshop: intracellular Bacterial pathogens

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This report presents a resume of the workshop Intracellular bacterial pathogens held at the 22nd international conference on Diseases of Fish and Shellfish, Heraklion, Greece, September 2025.

Obligate intracellular pathogens (mainly Rickettsiales and Chlamydiales) as well as facultative intracellular are important fish pathogens that are responsible for a wide range of diseases affecting farmed fish, with significant variation in severity and economic impact. Their unique ability to invade and survive within host cells raises important issues related to pathogenesis, epidemiology, diagnostics, biosecurity, and therapeutics. Historically, these pathogens have been difficult or impossible to culture, limiting research largely to observations of pathological effects in infected hosts. However, recent advances in molecular biology and genomics have significantly improved our understanding of their biology, life cycles, and transmission. These developments offer promising new avenues for diagnostics, disease prevention, and management in aquaculture.

The workshop explored the latest research and insights on intracellular bacterial infections in aquatic species. Particular attention was given to diseases caused by obligate intracellular pathogens, such as the bacterium causing red mark syndrome and epitheliocystis-causing agents. Epitheliocystis, once thought to have a narrow range of causes, is now known to involve a diverse array of intracellular pathogens, including beta- and gamma-proteobacteria and various chlamydial species.

Intracellular bacterial pathogens have evolved diverse strategies to invade, survive, and replicate within host cells, making them major causes of chronic and persistent diseases in fish. Their complex biology, frequent inability to be cultured, and increasing accessibility to genomic technologies are reshaping our understanding of host–pathogen interactions and providing new opportunities for improved diagnostics and disease control in aquaculture.

Of the facultative intracellular bacteria, a special focus was on *Piscirickettsia salmonis* – a well-known pathogen that has severely impacted the salmon farming industry, particularly in Chile, but is also found in other regions and species.

Increasing evidence suggests that *Midichloria*-like and other *Rickettsia*-like organisms contribute to inflammatory skin diseases in fish, although many aspects of their transmission and pathogenesis remain unresolved. Current findings point to a major role of host immune responses in lesion development

and identify molecular approaches as key tools for clarifying the epidemiology and biology of these emerging diseases.

Epitheliocystis is a globally distributed intracellular gill disease caused by phylogenetically diverse bacteria that independently evolved similar intracellular lifestyles. Comparative genomics and histopathological studies reveal marked differences in virulence mechanisms and host responses among causative agents, emphasizing that epitheliocystis represents a spectrum of distinct host–pathogen interactions rather than a single disease entity.

1. Introduction: intracellular pathogens of fish and their peculiarities

Pantelis Katharios

Intracellular bacterial pathogens occupy a rather unusual position in fish pathology. They are not simply bacteria that infect fish tissues; they are organisms that have evolved the capacity to enter host cells and use them as a biological niche. This lifestyle gives them clear advantages but also imposes important constraints. In many cases, the host cell becomes both a refuge and a battlefield: it offers protection from extracellular immune mechanisms and access to host-derived nutrients, while at the same time exposing the pathogen to intracellular antimicrobial pathways that must be avoided or manipulated (Casadevall 2008; Huang and Brumell 2014).

For this reason, intracellular infections are often more complex than infections caused by extracellular bacteria. The pathogen must first attach to the host cell, enter it, survive the initial antimicrobial response, and then establish a compartment where it can persist or replicate. In many bacterial models, this involves the formation of a bacteria-containing vacuole, which would normally mature towards lysosomal degradation. Successful intracellular bacteria interfere with this process in different ways. Some escape into the cytoplasm, others arrest phagosome maturation, remodel the vacuole, interact with host vesicular trafficking, or exploit autophagy-related pathways. In other cases, the pathogen has adapted to survive in acidic or lysosome-like compartments. Therefore, intracellular survival is not a single strategy, but rather a collection of different solutions that have evolved independently in different bacterial groups (Casadevall 2008).

In fish, intracellular pathogens are particularly important because they are frequently associated with chronic, persistent, and difficult-to-control diseases. Examples include *Piscirickettsia salmonis*, *Renibacterium salmoninarum*, *Mycobacterium* spp., *Francisella* spp., *Edwardsiella* spp., and several bacteria associated with epitheliocystis. These infections can be responsible for mortality, reduced performance, long carrier states, and recurring problems in aquaculture. Their intracellular location makes them harder to detect and study, but also harder to control, since the bacteria may be partly protected from antibodies, complement, and some antimicrobial

treatments. This is one of the main reasons why intracellular pathogens remain a persistent problem in fish health and aquaculture (Munang'andu 2018).

One of the most interesting aspects of these organisms is the evolutionary compromise behind the intracellular lifestyle. Living inside a host cell can provide a stable environment, reduced competition, and protection from extracellular defences. However, prolonged adaptation to such a protected niche often leads to dependence on the host. Many intracellular bacteria show signs of genome reduction, including smaller genomes, pseudogenes, and loss of metabolic pathways that are no longer needed because the corresponding metabolites can be obtained from the host cell. At the same time, they tend to retain genes involved in secretion systems, transporters, host interaction, and intracellular survival. In other words, they may lose part of their metabolic independence while investing heavily in mechanisms that allow them to manipulate the host cell (Casadevall 2008; Toft and Andersson 2010).

This trade-off is especially evident in pathogens associated with epitheliocystis. The disease is found in both wild and farmed fish and is commonly linked with gill pathology and respiratory impairment. Interestingly, epitheliocystis-like infections are not caused by a single bacterial lineage. Instead, bacteria from different groups, including Chlamydiae, Beta-proteobacteria, Gamma-proteobacteria, and others, have independently converged towards an intracellular lifestyle in fish gill cells. This makes epitheliocystis a useful example of convergent evolution: unrelated bacteria facing similar ecological conditions have arrived at comparable biological solutions (Seth-Smith et al. 2016; Qi et al. 2016; Blandford et al. 2018).

A major practical difficulty is that many intracellular fish pathogens are difficult, or even impossible, to culture under standard laboratory conditions. This is not just a technical inconvenience; it reflects their biology. Some of these bacteria have lost metabolic functions that would be required for independent growth, while others may depend on specific host-cell factors that are not reproduced in artificial media. The lack of suitable fish cell culture systems further limits experimental work. As a result, much of what we know about several intracellular fish pathogens comes from histology, molecular detection, metagenomics, single-cell genomics, and comparative genome analysis rather than from classical bacteriology (Qi et al. 2016).

New sequencing-based approaches are changing this field. Metagenomics allows recovery of bacterial genomes directly from infected tissues, even when the organism cannot be cultured. Metatranscriptomics and dual host-pathogen RNA sequencing can provide information on both bacterial activity and the host response during infection. Long-read sequencing can help resolve complex genomic regions, plasmids, and mobile elements, while comparative genomics can identify gene losses, retained virulence systems, and possible metabolic dependencies. These tools are gradually moving the

study of intracellular fish pathogens from descriptive pathology towards a more functional understanding of host–pathogen interaction (Westermann et al. 2012; Lanza et al. 2024; Aravena et al. 2026).

Overall, intracellular pathogens of fish are peculiar because their success depends on a delicate balance. They must enter and exploit host cells without being eliminated by intracellular defences, persist long enough to transmit, and often compensate for reduced metabolic capacity through close dependence on the host. This makes them difficult to detect, culture, treat, and prevent. At the same time, they offer an excellent model for understanding how bacteria adapt to host-associated niches and how similar intracellular strategies can evolve independently in different bacterial lineages. For fish health and aquaculture, understanding these pathogens is therefore not only of academic interest, but also essential for improving diagnostics, developing better control strategies, and reducing the impact of chronic bacterial diseases.

2. Piscirickettsiosis

Hamish Rodger

The pathogen *Piscirickettsia salmonis* is a Gram negative, facultative intracellular, coccoid bacterium in the gammaproteobacteria class with 3 main genogroups. The organism causes clinical piscirickettsiosis in marine salmonids (Atlantic, coho, Chinook salmon, and rainbow trout) and a number of other marine finfish species (white sea bass, Mediterranean sea bass, lumpfish and turbot) (Rozas and Enríquez 2013; Schober et al. 2023; Nilsen et al. 2026). However, a related, but different, *Piscirickettsia* species (with 82% average nucleotide identity to *P. salmonis* and different media growth requirements) appears to be involved in many of the recent disease outbreaks in salmon farms in Ireland and Scotland (Rodger et al. 2025).

Clinical signs observed in salmon include lethargy, anorexia, dark skin colour, skin lesions (initially haemorrhagic then ulcerative), exophthalmia and internal gross pathology can be variable but may include ascites, cream-coloured lesions on liver, splenomegaly, petechiae in visceral organs, skeletal muscle, peritoneum and renomegaly. Histopathology includes multifocal necrosis and haemorrhage in kidney and spleen, epicarditis and in some samples presence of basophilic intracellular microorganisms. Diagnosis of the disease is based on a combination of clinical signs and pathology with laboratory investigations which can include histopathology, bacteriology (using selective media), qPCR as well as cell culture and IFAT.

Piscirickettsiosis remains the most important economic disease challenge in Chilean salmon farming and accounts for 95% of the antibiotic use in that industry. The disease has also recently emerged to be a priority issue for salmon aquaculture in Australia, Ireland and Scotland where it has caused elevated mortalities and increased treatment and vaccine requirements. The

disease has also been detected sporadically in Western Canada, Norway and New Zealand, however, the clinical impacts in these countries has been of a low level. Disease outbreaks have been reported to often occur after high variations in water temperatures or plankton blooms, bath treatments or exposure to other stressors. Reservoirs of the pathogen include infected farmed salmon and the mortalities on a farm, however, the bacteria have also been detected in cleaner fish (ballan wrasse) (UK), at least four wild fish species (Chile), raw seawater, farm work surfaces and work boats and siphonophores (*Apolemia* sp.) (Ireland) (Rodger et al. 2026).

Mitigation of the disease is challenging as the disease is difficult to treat (intracellular bacteria), and disinfect against (biofilm formation), and the pathogen can survive for weeks in the marine environment. In addition, existing vaccines are only partially effective. To improve mitigation of the disease the application of synchronized site fallowing in water bodies, improved biosecurity of work vessels between farms, regions and countries, careful mortality disposal, screening of broodstock for the pathogen, no livestock movement of infected fish and PCR surveillance of sites on a regular basis are all recommended (Gaete-Carrasco et al. 2026; Rees et al. 2014). Early treatment where disease is confirmed as well as bacterial sensitivity, sequencing and application of new vaccine technologies to appropriate isolates will also be important. Genetic selection for *P. salmonis* tolerance, improvements in skin and gill health and staff training will also be vital for future control.

Rickettsia-like organisms, midichlorians and other Star Wars stuff in fish skin syndromes

Francesc Padrós and Jacob Günther Schmidt

Historical evidence of RLOs in fish derives from earlier studies reporting Rickettsia-like organisms in cultured tilapia (Chern and Chao 1994), Plecostomus catfish (Khoo et al. 1995), and European seabass (Comps et al. 1996). However, the identity and pathogenic role of these organisms remained poorly understood, and definitive causal relationships were not established. The Rickettsiales order consists of mainly obligate intracellular bacteria, many of which are symbionts of hematophagous arthropods such as hard ticks and cause disease when transferred to terrestrial vertebrates, e.g. Rocky Mountain spotted fever, rickettsial pox, boutonneuse fever, and typhus (Salje 2021). A central pathogenic mechanism in these diseases is vasculitis caused by infection and damage to endothelial cells, resulting in skin manifestations such as petechiae, haemorrhage, and oedema (Salje 2021). In recent years, inflammatory skin diseases with similar clinics are increasingly being observed in aquaculture (Schmidt et al. 2018), suggesting that Rickettsial diseases may also occur in fish. However, in most of these cases, the ‘Rickettsia-like’ reported previously, either been shown to belong to other groups of bacteria or have remained uncharacterized. One notable

exception to this is red mark syndrome (RMS aka cold-water strawberry disease (CWSD) or in the Americas simply “strawberry disease”), a skin disease of rainbow trout caused by a *Midichloria*-like bacterium.

At the turn of the millennium, the Rickettsiales order consisted of the families *Rickettsiaceae* and *Anaplasmataceae*. Since then, several families have been added to the order (Castelli et al. 2024). One of these is the *Ca. Midichloriaceae*. The original and most well-described member of this family is *Candidatus Midichloria mitochondrii* a bacterium associated with mitochondria in hard ticks and named after the fictional “midichlorians” from the Star Wars universe because of its intramitochondrial symbiotic lifestyle (Sassera et al. 2006). However, since then the family has expanded, and the family *Ca. Midichloriaceae* now consists mainly of bacteria from aquatic environments (Giannotti et al. 2022).

RMS was first observed in the USA in the 1950s and remained enigmatic for many years (Olson et al. 1985), although the condition appeared to respond to treatment with antimicrobials and thus had a bacterial cause. The effect of antimicrobial treatment was since supported experimentally (Schmidt et al. 2021). Since its first description in 2008, DNA from a *Midichloria*-like organism (MLO) has been consistently detected in RMS lesions by PCR (Lloyd et al. 2008; Metselaar et al. 2022), and very recent evidence indicates that this MLO belongs to a novel sister genus to *Ca. Midichloria* (Zarantonello 2025). In support of a causal association between this MLO and RMS, the quantity of MLO DNA has been shown to correlate with lesion severity in several studies (Lloyd et al. 2011; Schmidt et al. 2018; Pardo et al. 2024; Zarantonello et al. 2026).

The disease develops slowly and is highly temperature dependent. At approximately 12°C, an incubation period of around 40-45 days occurs before visible lesions appear. Lesions then progressively develop over approximately thirty days before eventually resolving, with complete skin healing occurring afterward. At temperatures above approximately 16°C, lesions are milder and lack much of the hallmark gross pathological signs such as oedema, hyperaemia, haemorrhage, and scale loss (Orioles et al. 2022). Histologically, lesions are characterized by severe histiolymphocytic infiltration extending into the skin and underlying muscle tissues (Galeotti et al. 2017; 2021). B cells and secreted IgM are particularly abundant in advanced lesions (Jørgensen et al. 2019; Herranz-Jusado et al. 2025).

An important concept presented was that RMS pathology may be largely host-driven rather than caused by direct bacterial activity, leading to the unusual observation that pathology is often most severe in large, otherwise healthy fish. The authors discussed the possibility that hypersensitivity reactions, particularly type III immune-complex-mediated responses, contribute significantly to tissue damage. This hypothesis is supported by experimental studies using cortisol as a chronic stressor to suppress immune

function. Fish treated with cortisol developed less severe pathology despite carrying larger amounts of MLO (Schmidt et al. 2026). These findings suggest that host immune responses are responsible for a substantial part of the observed lesions and inflammation.

Although mortality is not considered associated with RMS, the disease creates serious economic problems because lesions commonly appear in fish close to market size. Disease management is complicated by the long incubation period and by difficulties in detecting infected fish before visible lesions appear. Asymptomatic fish may therefore contribute to disease spread between farms. The possible role of vectors such as ciliates, particularly *Ichthyophthirius multifiliis* (Pasqualetti et al. 2021), in transmitting Midichloria-like bacteria was also discussed. Related bacteria have also been identified in ciliates and other protozoa, supporting this possibility (e.g. Zaila et al. 2017).

Other diseases discussed included Warm Water Strawberry Disease (WWSD), Puffy Skin Disease (PSD) in rainbow trout, and Petechial Rash in gilthead seabream. WWSD presents lesions similar to RMS but usually occurs at higher temperatures and during different seasons (Oidtmann et al. 2013). Puffy Skin Disease (PSD), mainly affecting rainbow trout in the United Kingdom, exhibits different histopathological features but also appears transmissible and inflammatory in nature (Maddocks et al. 2015). Petechial Rash in gilthead seabream and European seabass represents another syndrome of interest. In these marine species, skin lesions resemble those observed in RMS and PSD, and transmission electron microscopy has suggested the presence of RLOs (Padrós, personal observation), although definitive evidence remains lacking.

Based on the knowledge currently available, and taking into account what is known about the pathogenesis of RLOs in terrestrial vertebrates, it appears plausible that Midichloria-like organisms may most commonly enter fish via vectors such as ectoparasites or epibiotic organisms such as ciliates or amoebae. The bacteria may subsequently infect endothelial cells or other host cells, replicate intracellularly, induce apoptosis and vascular leakage, and trigger inflammatory cytokine release, although these effects have not yet been demonstrated or visualized in tissues from affected fish. The resulting vasculitis and immune activation could explain the petechiae, oedema, haemorrhage, and inflammatory skin lesions observed in these syndromes.

Much remains unknown regarding these skin diseases, and several important research priorities have been identified. These include metagenomic and proteomic approaches, improved histopathological studies, better characterization of disease progression, identification of potential vectors, and further investigation into intracellular localization and transmission mechanisms.

Epitheliocystis

Maria Chiara Cascarano

Epitheliocystis is a gill and skin disease of teleost fish characterised by the formation of membrane-bound intracellular cysts containing a replicating intracellular bacteria (Nowak and LaPatra 2006). The disease has been reported worldwide in over 90 freshwater and marine species, with severe outbreaks predominantly observed in farmed juveniles (Blandford et al. 2018). Diagnosis relies on the observation of basophilic cysts (10–100 µm) in routine histology, and whilst in many cases the infection remains subclinical with sporadic cysts and no pronounced epithelial reaction (Nowak and LaPatra 2006), severe presentations do involve hyperplasia leading to lamellar fusion, respiratory distress, and mortality, most frequently in larvae and juveniles in aquaculture settings (Katharios et al. 2008). Epitheliocystis can further exacerbate concurrent gill conditions, as demonstrated in complex gill disease in salmonids (Herrero et al. 2018), where *Candidatus* Branchiomonas cysticola has been recognised as a major contributor (Gjessing et al. 2021), and as in seabream, experiencing heavy co-infection with the ectoparasite *Sparicotyle chrysophrii* (Toxqui-Rodriguez et al. 2024).

Transmission routes remain poorly understood. Horizontal waterborne transmission has been observed in Atlantic salmon (Quezada-Rodriguez et al. 2022), and causative agents have been shown to persist within the host following initial outbreaks in other hosts (Cascarano et al. 2022), suggesting that chronically infected individuals may act as long-term carriers.

A major bottleneck in understanding the disease is the inability to cultivate the causative agents in vitro, which severely limits research on pathogenesis and the development of treatments (Nowak and LaPatra 2006; Blandford et al. 2018). Compounding this, epitheliocystis is caused by phylogenetically diverse obligate intracellular bacteria spanning Chlamydiae, beta- and gammaproteobacteria, the full diversity of which remains largely unknown. Initially described as chlamydia-like organisms (Plehn 1920), early molecular studies employing chlamydial 16S rRNA primers revealed numerous distinct agents across host species (Draghi et al. 2004; Blandford et al. 2018) and demonstrated the possibility of co-infections within a single host (Schmidt-Posthaus et al. 2012), for which FISH with pathogen-specific probes remains the diagnostic gold standard. Non-chlamydial agents were subsequently identified, most notably *Ca. Branchiomonas cysticola* (Toenshoff et al. 2012) and later the *Ca. Ichthyocystis* genus in Mediterranean farmed fish (Seth-Smith et al. 2016).

Given the non-culturable nature of these agents, whole genome sequencing of infected tissue has become the primary investigative strategy, despite the considerable challenge of host DNA contamination limiting bacterial sequence recovery. Seven published genomes spanning all three causative

bacterial groups reveal a gradient of genome reduction that reflects the degree of host dependence of each lineage: the single gammaproteobacterial genome (*Ca. Endozoicomonas cretensis*) shows signs of ongoing pseudogenisation, betaproteobacterial genomes (*Ca. Ichthyocystis* spp., 2.3–2.6 Mb) are characterised by reduced metabolism and large effector protein repertoires (Seth-Smith et al. 2016; Qi et al. 2016), whilst chlamydial genomes (~0.8 Mb) are the most reduced of all, encoding numerous host nutrient scavenging systems and showing striking resemblance to human chlamydial pathogens (Taylor-Brown et al. 2017; 2018). Genomic analysis has also shed light on potential virulence mechanisms: betaproteobacterial genomes encode type II, III, and IV secretion systems and type IV pili, likely involved in host cell invasion and manipulation, whilst gammaproteobacterial genomes retain type II and III secretion systems, T4pili effectors, flagella, and chemotaxis-related genes; chlamydial genomes, despite their extreme reduction, encode numerous transport systems dedicated to nutrient scavenging from the host (Seth-Smith et al. 2016; Qi et al. 2016; Taylor-Brown et al. 2017; 2018).

Gilthead seabream (*Sparus aurata*) and greater amberjack (*Seriola dumerili*) have emerged as well studied models in the Mediterranean aquaculture. Both hosts are presenting co-infection by *Ca. Ichthyocystis* and chlamydial agents, that can be distinguished histologically by staining affinity and lesion characteristics (Seth-Smith et al. 2016; Cascarano et al. 2022). In greater amberjack, the distinction is particularly striking: *Ca. Ichthyocystis* produces large interlamellar cysts associated with pronounced epithelial proliferation and significant mortality, whilst the chlamydial agent establishes a chronic, low-pathology infection of mucus cells at the trailing edge of the filament, the ultrastructure of which has been characterised in detail by Cascarano et al. (2025). The two agents also elicit fundamentally different immune responses: *Ca. Ichthyocystis* triggers acute epithelial proliferation with granulocyte infiltration, whereas the chlamydial infection is characterised by macrophage interaction with infected cells and the presence of granular cells in surrounding tissue, which have been proposed as potential pathogen reservoirs contributing to chronic persistence (Cascarano et al. 2025).

Taken together, these observations underscore that epitheliocystis is not a single host-pathogen system but rather a convergence of independent biological interactions producing a common histopathological outcome, each warranting investigation in its own right.

Author contributions

Pantelis Katharios and Francesc Padrós contributed equally to workshop organisation and all the co-authors contributed equally to manuscript preparation and editing

Competing interests

The authors declare no competing interests.

Ethics statement

No experimental animal procedures were performed as part of this workshop report.

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