

RESEARCH ARTICLE

First Documented Outbreaks of *Lactococcus garvieae* in Gilthead sea bream (*Sparus aurata*) in the Gulf of Follonica, Tuscany, Italy

Miriana Coltraro¹ , Alessia D'Alonzo¹, Cristiano Cocumelli¹, Enrica Ricci¹, Amedeo Manfrin², Luana Cortinovis², Andrea Basso², Valeria Paternoster³, Francesca Susini¹

¹ Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, ² Istituto Zooprofilattico Sperimentale delle Venezie, ³ Agroittica Toscana Soc. Agricola S.r.l.

Keywords: *Lactococcus garvieae*, lactococcosis, emerging disease, aquaculture, Gilthead sea bream

<https://doi.org/10.48045/001c.143736>

Bulletin of the European Association of Fish Pathologists

Vol. 45, Issue 3, 2025

Lactococcus garvieae is an emerging pathogen responsible for lactococcosis, a haemorrhagic septicemia affecting fish associated with high mortality and widespread distribution. The disease primarily affects rainbow trout (*Oncorhynchus mykiss*), but *L. garvieae* has also been reported in various freshwater and marine fish species, as well as in mammals, including humans. This study describes the first documented outbreaks of *L. garvieae* in gilthead sea bream (*Sparus aurata*), occurring in the Gulf of Follonica (Tuscany, Italy), following a previous episode in European sea bass (*Dicentrarchus labrax*) in the same area in 2023. Affected fish showed erratic and uncoordinated swimming. Macroscopic lesions included ocular and skin lesions, pericarditis, splenomegaly and haemorrhages in internal organs. Histopathological findings revealed severe fibrinous-necrotic pericarditis and epicarditis associated with bacterial aggregates and widespread granulomatous inflammation in the liver and spleen. Identification of *L. garvieae* was achieved employing multiple techniques: biochemical tests, miniaturized Analytical Profile Index (API rapid ID 32 strep, Biomérieux), MALDI-TOF MS (MicroFlex LT, Bruker), and molecular methods, including two independent end-point PCR assays followed by sequencing of the 16S–23S ITS region and DNA gyrase subunit B (*gyrB*) gene. Antimicrobial susceptibility testing of selected isolates was carried out using disk diffusion and minimum inhibitory concentration (MIC) methods, following standardised guidelines. This study presents new data on the characteristics of lactococcosis in gilthead sea bream (*Sparus aurata*), confirming *Lactococcus garvieae* as a primary pathogen and highlighting its potential impact on Mediterranean aquaculture, as well as the importance of implementing preventive strategies, including vaccination.

INTRODUCTION

Lactococcus garvieae is a Gram-positive, coccus-shaped bacterium and the aetiological agent of lactococcosis, a clinically and economically significant disease of fish. The infection is characterized by hyperacute and haemorrhagic septicaemia and has become a serious problem in aquaculture worldwide (Chuah et al. 2016). Typical signs and gross lesions of lactococcosis include lethargy, anorexia, melanosis, exophthalmos, visceral serositis with haemorrhages, severe enteritis, and splenomegaly (Ghittino et al. 2003).

Up until recently, *L. garvieae* was considered the sole aetiological agent of piscine lactococcosis; however, advanced molecular techniques have revealed that new lactococcal species, *L. petauri* and *L. formosensis*, are also implicated in the disease etiology. Retrospective analysis have demonstrated that numerous outbreaks attributed to *L. garvieae* were actually caused by *L. petauri* or *L. formosensis* (Egger et al. 2023; de Ruyter et al. 2023; Heckman et al. 2024). Due to their close similarity to *L. garvieae* in clinical presentation, phenotype and genetics, *L. petauri* and *L. formosensis* may be misidentified. Isolates of these three species cannot be differentiated by PCR assays targeting the 16S rRNA gene, and currently, MALDI-TOF does not appear to provide sufficient discriminatory power for reliable identification (Heckman et al. 2024). However, sequencing of the 16S–23S internal transcribed spacer (ITS) region and the DNA gyrase subunit B (*gyrB*) gene has been recognized as a reliable molecular approach for differentiating *Lactococcus* species (Ferrario et al. 2013; Stoppani et al. 2023; Barbanti et al. 2024).

Lactococcosis is a major disease in fish, particularly affecting rainbow trout (*Oncorhynchus mykiss*), the most susceptible species, in which the disease manifests acutely with high mortality rates (Pastorino et al. 2019). *L. garvieae* can infect a wide range of wild and farmed freshwater and marine fish species, particularly when water temperatures exceed 15°C (Ghittino et al. 2003). It is considered an emerging opportunistic pathogen due to its expanding host range and adaptability to diverse environmental conditions (Akmal, Yoshida, and Nishiki 2022; Francés-Cuesta et al. 2022).

Since the 2000s, aquaculture in Italy has steadily expanded, focusing on European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), the two most farmed species in the Mediterranean basin. The sector has seen a gradual transition from traditional farming systems to coastal and offshore farms (Zoli et al. 2023). In the summer 2023, an outbreak of lactococcosis caused by *L. garvieae* was reported for the first time in European sea bass (*Dicentrarchus labrax*) in the Gulf of Follonica (Tuscany, Italy) (Salogni et al. 2024). This Gulf is characterised by a high density of intensive in-shore and off-shore fish farms for gilthead sea bream and European sea bass and represents one of the most important production hubs in Tuscany, accounting for over 50% of national fish production (<https://eumofa.eu>).

The study describes the first documented outbreaks of *L. garvieae* in farmed gilthead sea bream (*S. aurata*).

MATERIALS AND METHODS

Fish sampling and anatomopathological examination

From June 2023 to July 2024, a total of 57 gilthead sea bream (*Sparus aurata*) were collected from two neighbouring fish farms located in the Gulf of Follonica (coordinates: NW LAT 42°54.470' – LONG 10°38.000'; NE LAT 42°54.470' – LONG 10°38.717'; SW LAT 42°53.930' – LONG 10°38.000';

SE LAT 42°53.930' – LONG 10°38.717'). The fish were reared both in sea cages (n=53) and in tanks (n=4), with water temperatures ranging from 14,5°C to 25°C. Commercial pellets were used as feed. European sea bass (*Dicentrarchus labrax*) and greater amberjack (*Seriola dumerili*) were also present on the farms.

Farm veterinarians reported clinical signs including exophthalmos, erratic lateral swimming, swollen abdomen and haemorrhagic skin lesions accompanied by variable mortality rates.

The fish were submitted for anatomopathological examination following standard procedures at the *Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) – Pisa*, a public health institute. Samples from spleen, kidney, heart, liver, brain, eye and representative skin lesions were collected for further analysis.

Bacteriological examination

Bacteriological analyses were performed using Sheep Blood Agar (BA; 5% sheep blood) plates and Marine Broth (MB) cultures from samples of affected eyes, skin lesions, and internal organs (brain, liver, spleen, kidney, and heart). Culture media were incubated at 25°C and bacterial growth evaluated at 24 and 48 hours. Turbid MB cultures were streaked onto solid media to isolate single colonies. Colonies exhibiting distinct morphologies were subcultured to obtain pure cultures.

Isolates were identified based on phenotypic characteristics including colony morphology, hemolytic activity on BA, Gram staining, motility, oxidase and catalase tests, and microscopic morphology under light microscope. Biochemical profiles of relevant isolates were further characterized by macro-method tests and their halotolerance was assessed using Vibrio Broths (BV) with NaCl concentrations ranging from 0% to 10%.

Isolates presumptively identified as, *Lactococcus* spp. were tested using the API rapid ID 32 strep system (bioMérieux, France). The manufacturer's protocol was followed with incubation temperatures adjusted to 25°C and 30°C to ensure accuracy (Vela et al. 2000; Ravelo et al. 2001; Heckman et al. 2024). Resulting identification codes were compared against APIWEB database (bioMérieux, France).

The clindamycin susceptibility test (Elliott and Facklam 1996) was also performed to support phenotypic identification.

Six isolates were further analyzed by MALDI-TOF MS (Microflex LT, Bruker Daltonics; – Library MBT 8468, 2019). Formic acid extraction was applied according to the manufacturer's instructions to improve identification quality. Identification was considered successful with scores above 2.00.

Total genomic DNA was extracted from the isolates using the QIAamp DNA Mini Kit (Qiagen) according to the the manufacturer's protocol. DNA quantity and quality were assessed with a NanoDrop spectrophotometer (Thermo Fisher Scientific), and samples were stored at -20°C until further use.

Because the 16S rRNA gene does not provide sufficient resolution to distinguish a *L. garvieae* from *L. petauri* (Altinok, Ozturk, and Ture 2022), strain identification was confirmed by two independent end-point PCR assays. The first PCR targeted the 16S-23S rRNA ITS region, using the primers 16S: 5'-GCTGGATCACCTCCTTTCT-3' and 23S: 5'-GGTACTTAGATGTTTCAGTTCC-3' (Kabadjova et al. 2002), with protocol and cycling conditions following Stoppani et al. (2023). The alignment of the 16S-23S ITS sequences were analyzed for single-nucleotide polymorphism (SNP) patterns enabling discrimination of *L. garvieae* from closely related species such as *L. petauri*. This SNP pattern is consistently found in Italian field strains of *L. garvieae* (Stoppani et al. 2023) and reference strains of *L. garvieae*: retrieved from NCBI database LMG 9472 (accession number HM241914), LMG 8162 (accession number HM241916), C1 (accession number MZ146920), 108-33 (accession number MZ146924), 106-30 (accession number MZ146925), 168 (accession number MZ146926), 20,684 (accession number AF225967), and L1-5 (accession number AF225968); and Reference strains retrieved from NCBI database of *L. petauri* strains: LG4 (accession number CP086401; region: 1911306-1911836), LG26 (accession number CP086595; region 2048225-2048755), B1726 (accession number CP094882; region 1717542-1718072), NHH01_13 (accession number JANHXCX010000013; region 2696-3716), LG_SAV_20 (accession number SIVY01000041; region 2699-3719).

The second PCR targeted the the *gyrB* gene, encoding the DNA gyrase subunit B, which has proven effective for phylogenetic and multilocus sequence typing (MLST) analyses (Shahin et al. 2022). Primers *gyrB*-F: 5'-CATGCTGGTGGTAAATTTGG-3', *gyrB*-R: 5'-GTCATCCATTCTCCTAAACC-3' were used, following the protocol described by Barbanti et al. (2024). Amplicons were subjected to in-house Sanger sequencing, chromatograms were manually checked, aligned and consensus sequences generated.

The obtained 16S-23S ITS sequences were compared with data from Stoppani et al. (2023), and *gyrB* sequences were analyzed via BLASTn searches against the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Antimicrobial susceptibility test

Phenotypic antimicrobial susceptibility of ten *L. garvieae* strains was determined by disk diffusion and minimum inhibitory concentration (MIC) methods. The Kirby-Bauer (KB) disc diffusion assay was performed against erythromycin (15 µg), oxytetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25 µg/23.75 µg), flumequine (30 µg), florfenicol (30 µg), amoxicillin (25 µg), doxycycline (30 µg), enrofloxacin (5 µg). Testing followed CLSI VET03 and VET04 guidelines. Mueller Hinton agar supplemented with 5% sheep blood was inoculated with the bacterial suspensions standardised to 0.5 McFarland turbidity. Plates were incubated at 22°C for 48 hours.

MICs of the 10 strains were determined using a customized Thermo Scientific micromethod plate. Concentration ranges of antibiotic tested: erythromycin (0.063-32 µg/mL), amoxicillin (0.016-32 µg/mL), florfenicol (0.031-64 µg/mL), flumequine (0.016-32 µg/mL), oxytetracycline (0.008-16 µg/mL), sulfamethoxazole trimethoprim (0.004/0.074-8/152 µg/mL), doxycycline (0.008-16 µg/mL), enrofloxacin (0.004-8 µg/mL). Tests were performed in Cation-Adjusted Mueller Hinton Broth supplemented with 5% lysed horse blood (CAMHB+LHB) and the plates incubated at 22°C for 48 hours, in accordance with CLSI standards.

RESULTS AND DISCUSSION

Of the 57 gilthead sea bream (*Sparus aurata*) collected between June 2023 and July 2024, 41 (71.93%) were found to be infected with *Lactococcus garvieae*, with the first isolation of the pathogen recorded in November 2023. The infected fish measured on average 27cm in length (range 17 - 33 cm) and had a mean weight of 450g (range 90g - 560g). The lactococcosis outbreak was characterised by the rapid onset of general clinical signs, including anorexia, disorientation, and erratic swimming. Typical external signs like exophthalmos also known as “pop-eye” (uni- or bilateral), enucleation of the ocular globe, and diffuse reddening or haemorrhages of skin and in the ocular regions were consistent with observations reported by Salogni et al. (2024) in European sea bass (*Dicentrarchus labrax*) and in previous studies (Vendrell et al. 2006; Meyburgh, Bragg, and Boucher 2017). Furthermore, elevated water temperatures averaging 18°C, reaching a maximum of 25°C, alongside intensive farming conditions, likely contributed as risk factors by increasing fish stress and exacerbating the severity of *L. garvieae* infections (Soltani et al. 2008; Salogni et al. 2024).

Anatomopathological and histological examinations

Anatomopathological examination of infected fish revealed lesions commonly associated with lactococcosis. Ocular alterations included exophthalmos, hyperaemia, corneal opacity, ulcerative keratitis, orbital collapse, and, in advanced cases, orbital collapse or loss of the eye globe ([Fig. 1a-c](#)) in line with

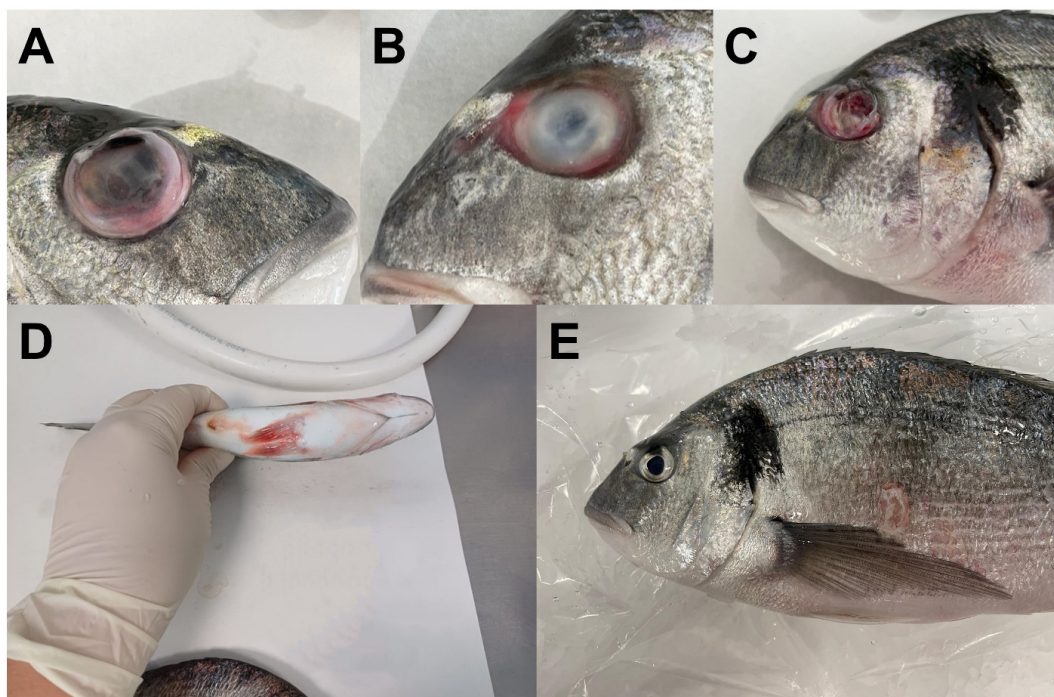


Figure 1. External gross lesions in infected Gilthead sea bream: (a) severe exophthalmos; (b) corneal opacity and periocular redness; (c) orbital collapse and partial loss of globe; (d) redness and haemorrhagic lesions at fins and at abdominal ventral region; (e) erosion at lateral line (red arrow)

previous reports (Vendrell et al. 2006). Other external gross lesions included haemorrhages and diffuse reddening at the base of fins, opercula, and skin (especially in ventral region), as well as skin erosions and pale gills ([Fig. 1d-e](#)).

Internally, affected fish displayed severe pericarditis with grey discoloration and cardiomegaly, hepatomegaly with diffuse petechiae, haemorrhages, and yellow to grey discoloration, splenomegaly with miliary whitish nodules, and bowel dilation ([Fig. 2](#)). These findings are consistent with previous literature identifying spleen, liver, brain, gut, kidneys, and heart as primary target organs affected in *L. garvieae* infections (Vendrell et al. 2006; Meyburgh, Bragg, and Boucher 2017). Similarly, lesions such as splenomegaly, liver haemorrhage, pericarditis and endocarditis have been reported in other marine species affected by lactococcosis, including European sea bass (*Dicentrarchus labrax*) and yellowtail (*Seriola quinqueradiata*), (Kusuda and Salati 1993; Salogni et al. 2024).

Histological analysis of heart, spleen and liver samples revealed severe fibrinous-necrotic pericarditis and epicarditis, characterised by thickened epicardium with abundant fibrin, histiocytic infiltrate, degenerated inflammatory cells, necrotic debris, and intralesional multifocal bacterial aggregates ([Fig. 3](#)). Bacterial aggregates were observed both intermixed within the fibrino-necrotic material and, to lesser extent, intracellularly cytoplasm of histiocytes.

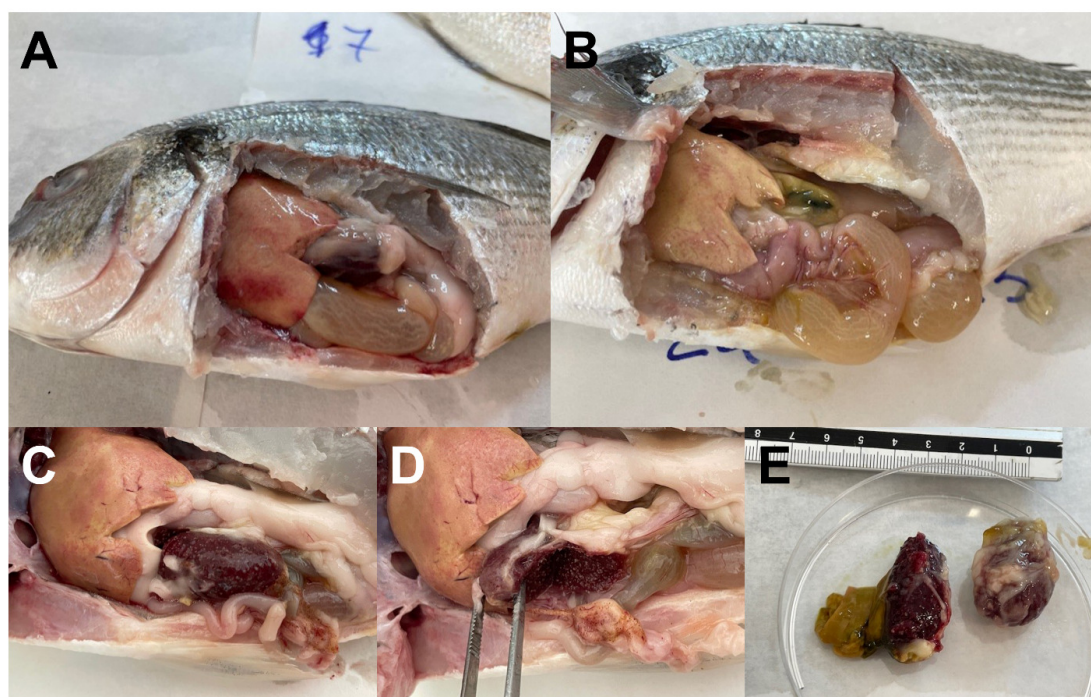


Figure 2. Internal gross lesions in infected Gilthead sea bream: (a) haemorrhagic lesion of liver (white arrow) and dilated bowel; (b) ochre liver and dilated bowel (c-d) enlarged spleen with innumerable miliary nodules on the surface and inside (spleen cut (d)); (e) pericardial lesion (pericarditis) and enlarged spleen with innumerable miliary nodules.

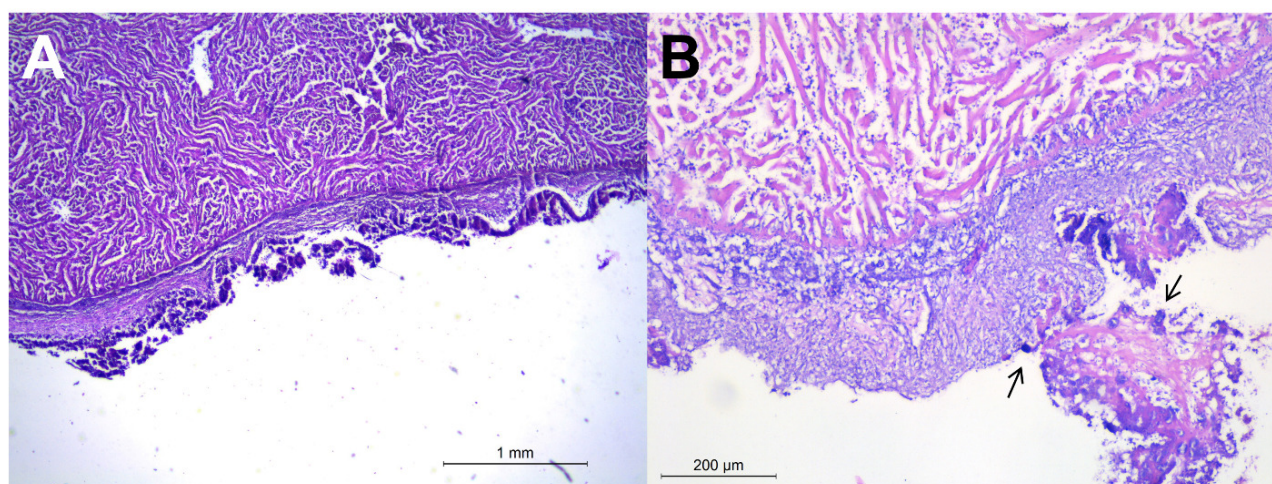


Figure 3. Histological examination of pericardium in infected sea bream, haematoxylin and eosin (H&E) stained sections: (a) severe epicarditis and pericarditis fibrin-necrotic purulent with large numbers of multifocal aggregate bacteria, Bar = 1mm; (b) severe fibrinonecrotic and haemorrhagic epicarditis with multifocal areas of dystrophic mineralization and aggregates of intralesional, intrahistiocytic and extracellular bacteria (black arrows); multifocal haemorrhagic myocarditis, Bar = 200µm

The liver, though grossly lacking nodular lesions, presented multiple necrotic foci and large amount of multifocal to coalescing granulomas with necrotic cores ([Fig. 4a-b](#)). Similar granulomatous lesions were observed in the spleen, with central core of colliquative necrosis ([Fig. 4c-d](#)). Histopathological findings like fibrinous pericarditis, epicarditis, necrotic areas in liver and spleen, are consistent with previous descriptions in lactococcosis-affected fish

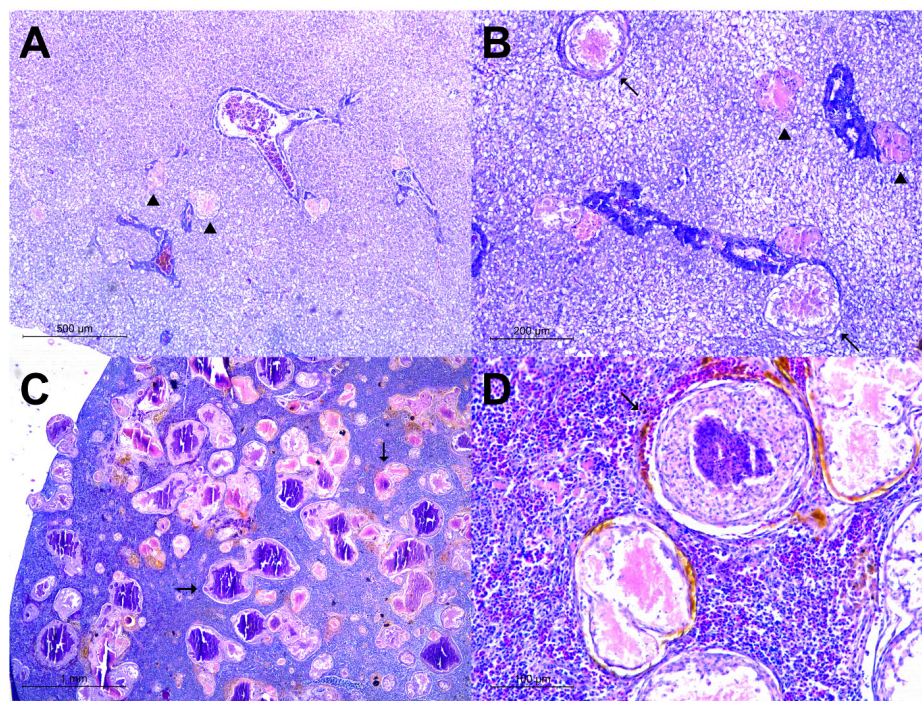


Figure 4. Histological examination of liver and spleen in infected sea bream, haematoxylin and eosin (H&E) stained sections: (a-b) Severe multifocal necrotizing (arrowhead) and granulomatous (arrow) hepatitis characterized frequently by granulomas with necrotic centre, Bar = 500µm (a) and 200µm (b); Severe diffuse granulomatous splenitis frequently characterized by granulomas with necrotic centre (arrow), Bar = 1mm (c), 100µm (d).

(Altun et al. 2005; Avci et al. 2014; Salogni et al. 2024). Notably, this study is the first to report granulomatous lesions in the spleen and liver, the latter only histologically, of gilthead sea bream associated with *L. garvieae*.

Although specific data on feed formulation were not available in this study, the histological evidence of hepatocellular vacuolization in affected fish suggests possible metabolic liver alterations related to dietary imbalance. Similar lesions have been associated with excessive dietary carbohydrate levels in carnivorous marine fish. Gilthead sea bream (*Sparus aurata*) can utilise carbohydrates up to 20%, but such levels may induce hepatic glycogen accumulation and metabolic stress. The pale livers (Fig. 2b) and observed histological changes (Fig. 4a-b) are consistent with these findings and may indicate liver dysfunction, potentially impairing immune competence and predisposing fish to opportunistic infections such as *Lactococcus garvieae* (Enes et al. 2006; Moreira et al. 2012; Peres, Santos, and Oliva-Teles 2014).

Identification of L. garvieae: biochemical and molecular methods

Bacteriological analysis of 41 gilthead sea bream revealed pure cultures of *L. garvieae* from all sampled organs. Colonies on blood agar (BA) were α -hemolytic, small, and grey, tending to become whitish after 48 hours. Isolates were Gram-positive cocci, occurring singly, in pairs or short chains; facultatively anaerobic and non-motile. Salinity tolerance tests, using BV, showed growth at NaCl concentrations of 0-4% for all tested strains, with weak growth observed at 6% NaCl, somewhat differing from Buller's report

of growth at 6 NaCl and weak growth at 6.5% (Buller 2014). These in vitro findings may reflect *L. garvieae*'s ecological adaptation to marine environments.

The bacterial identification via API rapid ID32 STREP galleries, analyzed using the APIWEB software, yielded codes corresponding to *L. garvieae* with their respective percentage of identification: 3033 3301 030 (99.5%); 3032 3301 010 (99.5%); 3023 3201 030 (99.8%); 3032 3201 030 (99.8%); 3022 3001 030 (99.9%); 3002 3000 030 (99.9%). No differences in results were observed at the two incubation temperatures; however, some biochemical reactions exhibited more distinct colour development at 30°C making them easier to interpret on the strip. Biochemical tests in the API rapid ID32, including hippurate hydrolysis and acid production from sucrose and [tagatose](#), differentiated *L. garvieae* from closely related *L. petauri* being negative for the first and positive for the second (Vela et al. 2024). All strains showed resistance to clindamycin, supporting the differentiation from *L. lactis* (Elliott and Facklam 1996; Buller 2014).

All the isolates analyzed by MALDI-TOF MS were identified as *L. garvieae* with scores ranging from 2.01 to 2.36.

The identification of isolates was corroborated using molecular biology approach focusing on the 16S-23S ITS region and the *gyrB* gene. Molecular characterization based on 16S-23S ITS sequences clustered the isolates with *L. garvieae*, showing a characteristic SNP pattern as observed in all Italian field and Reference strains (Stoppani et al. 2023): five SNPs and two indel variations that distinguishing between *L. garvieae* and *L. petauri* adenine (A) insertion at position g.218_219 insA, 5 SNPs at position g.358G>T, g.440T>G, g.442T>A, g.469C>A, g.478G>T, and thymine (T) deletion at position g.443 ([Table 1](#)). BLASTn analysis of *gyrB* gene sequences performed on Genbank, revealed 100% identity with *L. garvieae* strain MS210922A (accession number: AP026069).

Antimicrobial susceptibility test (AST)

Disk diffusion (Kirby-Bauer) results ([Table 2](#)) showed that *L. garvieae* isolates exhibited no zones of inhibition for trimethoprim-sulfamethoxazole (1.25 µg/23.75 µg) and flumequine (30 µg), indicating resistance to these antimicrobials. Note that, could not perform a correct interpretation of obtained AST data for lack of the official internationally harmonized AST interpretive criteria also namely Epidemiological cut-off value and Clinical breakpoint; that are currently not available for *L. garvieae* isolated from aquatic animals.

Table 1. The differences nucleotide composition of *L. garvieae* and *L. petauri* in the 16S-23S rRNA ITS region.

	16S-23S Region Position						
	219	358	440	442	443	469	478
Reference strains							
<i>L. garvieae</i> LMG 9472*	-	G	T	T	T	C	G
<i>L. garvieae</i> LMG 8162	-	G	T	T	T	C	G
<i>L. garvieae</i> C1	-	G	T	T	T	C	G
<i>L. garvieae</i> 108-33	-	G	T	T	T	C	G
<i>L. garvieae</i> 106-30	-	G	T	T	T	C	G
<i>L. garvieae</i> 168	-	G	T	T	T	C	G
<i>L. garvieae</i> 20,684	-	G	T	T	T	C	G
<i>L. garvieae</i> L1-5	-	G	T	T	T	C	G
<i>L. petauri</i> LG4	A	T	G	A	-	A	T
<i>L. petauri</i> LG26	A	T	G	A	-	A	T
<i>L. petauri</i> B1726	A	T	G	A	-	A	T
<i>L. petauri</i> NHH01_13	A	T	G	A	-	A	T
<i>L. petauri</i> LG_SAV_20	A	T	G	A	-	A	T
Italian field strains**	-	G	T	T	T	C	G
Clinical field strains	-	G	T	T	T	C	G

*The 16S-23S intergenic spacer sequence in the strain *L. garvieae* LMG 9472 (accession number HM241914.1) allowed us to calculate the positions of reported polymorphisms. **The 22 SNPs pattern detected in Stoppani et al (2023)

Table 2. Zone diameters (mm) obtained by Kirby-Bauer disk diffusion method for *Lactococcus garvieae* isolates (N = 10). Dash (–) indicates no growth inhibition. Interpretative criteria were not available for this species; ranges are reported descriptively. Antibiotic disk concentrations are indicated in parentheses.

Zone Diameter (mm)	ERY (15 µg)	OTC (30 µg)	TMP-SMX* (1,25/23,75 µg)	FLU (30 µg)	FFC (30 µg)	AMX (25 µg)	DOX (30 µg)	ENR (5 µg)
6-10	–	–	10	10	–	–	–	–
11-15	–	–	–	–	–	–	–	–
16-20	1	–	–	–	2	1	–	10
21-25	4	7	–	–	7	8	6	–
26-30	5	2	–	–	1	1	3	–
>30	–	1	–	–	–	–	1	–

ERY = erythromycin; OTC = oxytetracycline; TMP-SMX = trimethoprim-sulfamethoxazole (expressed as trimethoprim concentration); FLU = flumequine; FFC = florfenicol; AMX = amoxicillin; DOX = doxycycline; ENR = enrofloxacin.

MIC determinations ([Table 3](#)) revealed limited variation among strains, with elevated MICs for trimethoprim-sulfamethoxazole and flumequine. It is clarified that, some MIC breakpoints are available for *Lactococcus* spp. isolated from humans but the use of these for interpretation may not accurately reflect the in vivo situation for fish.

Erythromycin and oxytetracycline showed the most promising antimicrobial activity based on both MIC and disk diffusion assays.

Table 3. Minimum Inhibitory Concentration (MIC) values ($\mu\text{g/mL}$) for *Lactococcus garvieae* isolates (N = 10), determined by broth microdilution. Values represent the number of isolates inhibited at each concentration. Dash (–) indicates no isolates were inhibited at that concentration.

MIC ($\mu\text{g/mL}$)	ERY	OTC	TMP-SMX*	FLU	FFC	AMX	DOX	ENR
0,004	–	–	–	–	–	–	–	–
0,008	–	–	–	–	–	–	–	–
0,016	–	–	–	–	–	–	–	–
0,031	–	–	–	–	–	–	–	–
0,063	–	–	–	–	–	–	–	–
0,125	2	–	–	–	–	–	2	–
0,25	7	2	–	–	–	–	3	–
0,5	1	2	–	–	–	–	5	–
1	–	6	4	–	–	2	–	1
2	–	–	5	–	3	8	–	6
4	–	–	1	–	7	–	–	1
8	–	–	–	–	–	–	–	1
16	–	–	–	–	–	–	–	1
32	–	–	–	1	–	–	–	–
64	–	–	–	9	–	–	–	–

* TMP-SMX values are expressed as Trimethoprim concentration

Value in grey cell: out-of-scale result

ERY = erythromycin; **OTC** = oxytetracycline; **TMP-SMX** = trimethoprim-sulfamethoxazole (expressed as trimethoprim concentration); **FLU** = flumequine; **FFC** = florfenicol; **AMX** = amoxicillin; **DOX** = doxycycline; **ENR** = enrofloxacin.

Therapy

According to the AST results, infected gilthead sea bream in tanks or cages were treated with erythromycin at 75 mg/kg for 10 days via medicated feed. Erythromycin is commonly used for treating lactococcosis in rainbow trout (Agnetti et al. 2008; Serdoz et al. 2011). Initially, mortality and clinical signs decreased rapidly; however, subsequent treatments showed reduced effectiveness, likely due to challenging field conditions. New outbreaks occurred after 3-4 weeks, suggesting limited long-term benefit, potentially linked to the early anorexia onset in infected fish as previously reported (Salogni et al. 2024).

CONCLUSIONS

The isolation of *L. garvieae* from gilthead sea bream, accompanied by correlated pathological lesions, has not been previously reported in literature. Among the 57 specimens examined between November 2023 and July 2024, 41 showed pure bacterial growth of Gram-positive cocci associated with typical lactococcosis lesions, including ocular and hemorrhagic lesions, pericarditis and endocarditis, splenomegaly and congestion of internal organs. These outbreaks were recorded in the same area where Salogni et al. (2024) described the first case of lactococcosis in European sea bass during summer 2023. At that time, gilthead sea bream within the same farm did not exhibit

clinical signs of lactococcosis or increased mortality. Clinical signs, gross pathological findings, and histological examinations indicate that the disease progression is comparable in both marine species.

The isolation of the bacterium in pure culture from all examined organs is consistent with lactococcosis, which typically results in bacteremia and systemic hyperacute infection. While biochemical and proteomic methods provide useful diagnostic support, molecular identification and sequencing of 16S-23S rRNA ITS region and *gyrB* gene are essential for accurate discrimination of the etiological agent within the *Lactococcus* genus.

Oral treatment with erythromycin demonstrated limited and variable efficacy. Considering that macrolides are classified by the WHO as critically important antibiotics for human health, their use in aquaculture should be restricted. Therefore, antibiotic therapy appears to be an inadequate long-term control strategy for lactococcosis. Rising mortality rates and difficulties in treating the infection with effective antimicrobials represent a significant concern for gilthead sea bream farming, leading to substantial economic losses and animal health and welfare issues.

Consequently, vaccination and stringent biosecurity measures represent the most effective strategies to control lactococcosis outbreaks. An autogenous inactivated vaccine targeting gilthead sea bream and European sea bass, developed by the pharmaceutical laboratory of *IZSLT - Siena*, is currently available for Italian fish farmers. However, the feasibility and impact of vaccination programmes will only become appreciable in the medium term.

Submitted: November 28, 2024 CET. Accepted: August 28, 2025 CET. Published: August 29, 2025 CET.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

REFERENCES

- Agnetti, F., M. Latini, E. Di Raimo, and C. Ghittino. 2008. "Il controllo delle malattie dei pesci nel bacino del Mediterraneo." In *Proceedings of the XV Convegno Nazionale SIPI, Workshop "Acquacoltura Mediterranea: Aspetti Normativi E Sanitari a Confronto."*
- Akmal, M., T. Yoshida, and I. Nishiki. 2022. "Complete Genome Sequence of *Lactococcus Garvieae* MS210922A, Isolated from Farmed Greater Amberjack (*Seriola Dumerili*) in Japan." *Microbiology Resource Announcements* 11 (12). <https://doi.org/10.1128/mra.00890-22>.
- Altinok, I., R. C. Ozturk, and M. Ture. 2022. "NGS Analysis Revealed That *Lactococcus garvieae* Lg-Per Was *Lactococcus petauri* in Turkey." *Journal Fish Disease* 45:1839–43. <https://doi.org/10.1111/jfd.13708>.
- Altun, S., A. Diler, Ö. Diler, K. Basak, and B. I. Isikli. 2005. "Histopathology of Streptococcosis in Rainbow Trout (*Oncorhynchus Mykiss* Walbaum)." *Bull. Eur. Ass. Fish Pathol* 25 (3).
- Avci, H., S. S. Birincioglu, T. T. Tanrikul, E. T. Epikmen, N. Metin, and M. L. Avsever. 2014. "Experimental *Lactococcus Garvieae* Infection in Rainbow Trout, *Oncorhynchus Mykiss*, Walbaum 1792: A Comparative Histopathological and Immunohistochemical Study." *Journal of Fish Diseases* 37 (5): 481–95. <https://doi.org/10.1111/jfd.12132>.
- Barbanti, A. C. C., A. E. C. do Rosário, C. R. M. da Silva Maia, V. P. Rocha, H. L. Costa, J. M. Trindade, L. F. F. Nogueira, et al. 2024. "Genetic Characterization of Lactococcosis-Causing Bacteria Isolated from Brazilian Native Fish Species." *Aquaculture* 593. <https://doi.org/10.1016/j.aquaculture.2024.741305>.
- Buller, N. B. 2014. *Bacteria and Fungi from Fish and Other Aquatic Animals: A Practical Identification Manual*. Cabi. <https://doi.org/10.1079/9781845938055.0000>.
- Chuah, L. O., M. E. Effarizah, A. M. Goni, and G. Rusul. 2016. "Antibiotic Application and Emergence of Multiple Antibiotic Resistance (MAR) in Global Catfish Aquaculture." *Current Environmental Health Reports* 3 (2). <https://doi.org/10.1007/s40572-016-0091-2>.
- de Ruyter, T., E. Littman, Z. Yazdi, M. Adkison, A. Camus, S. Yun, T. J. Welch, W. R. Keleher, and E. Soto. 2023. "Comparative Evaluation of Booster Vaccine Efficacy by Intracoelomic Injection and Immersion with a Whole-Cell Killed Vaccine against *Lactococcus Petauri* Infection in Rainbow Trout (*Oncorhynchus Mykiss*)." *Pathogens* 12 (5). <https://doi.org/10.3390/pathogens12050632>.
- Egger, R. C., J. C. C. Rosa, L. F. L. Resende, S. B. de Pádua, F. de Oliveira Barbosa, M. T. Zerbin, G. C. Tavares, and H. C. P. Figueiredo. 2023. "Emerging Fish Pathogens *Lactococcus petauri* and *L. garvieae* in Nile Tilapia (*Oreochromis niloticus*) Farmed in Brazil." *Aquaculture* 565:739093. <https://doi.org/10.1016/j.aquaculture.2022.739093>.
- Elliott, J. A., and R. R. Facklam. 1996. "Antimicrobial Susceptibilities of *Lactococcus Lactis* and *Lactococcus Garvieae* and a Proposed Method to Discriminate between Them." *Journal of Clinical Microbiology* 34 (5). <https://doi.org/10.1128/jcm.34.5.1296-1298.1996>.
- Enes, P., S. Panserat, S. Kaushik, and A. Oliva-Teles. 2006. "Rearing Temperature Enhances the Utilization of Dietary Carbohydrates in Gilthead Sea Bream (*Sparus Aurata*) Juveniles." *British Journal of Nutrition* 95:605–10.
- Ferrario, C., G. Ricci, C. Milani, G. A. Lugli, M. Ventura, G. Eraclio, F. Borgo, and M. G. Fortina. 2013. "*Lactococcus Garvieae*: Where Is It from? A First Approach to Explore the Evolutionary History of This Emerging Pathogen." *PLoS One* 8:e84796. <https://doi.org/10.1371/journal.pone.0084796>.

- Francés-Cuesta, C., I. Ansari, J. F. Fernández-Garayzábal, A. Gibello, and F. González-Candelas. 2022. "Comparative Genomics and Evolutionary Analysis of *Lactococcus garvieae* Isolated from Human Endocarditis." *Microbial Genomics* 8 (2). <https://doi.org/10.1099/mgen.0.000771>.
- Ghittino, C., M. Latini, F. Agnetti, C. Panzieri, L. Lauro, R. Ciappelloni, and G. Petracca. 2003. "Emerging Pathologies in Aquaculture: Effects on Production and Food Safety." *Veterinary Research Communications* 27 (1): 471–79. <https://doi.org/10.1023/B:VERC.0000014204.37722.b6>.
- Heckman, T. I., Z. Yazdi, C. E. Older, M. J. Griffin, G. C. Waldbieser, A. M. Chow, I. M. Silva, et al. 2024. "Redefining Piscine Lactococcosis." *Applied and Environmental Microbiology* 90 (5). <https://doi.org/10.1128/aem.02349-23>.
- Kabadjova, P., X. Dousset, V. Le Cam, and H. Prevost. 2002. "Differentiation of Closely Related Carnobacterium Food Isolates Based on 16S-23S Ribosomal DNA Intergenic Spacer Region Polymorphism." *Applied and Environmental Microbiology* 68 (11): 5358–66. <https://doi.org/10.1128/AEM.68.11.5358-5366.2002>.
- Kusuda, R., and F. Salati. 1993. "Major Bacterial Diseases Affecting Mariculture in Japan." *Annual Review of Fish Diseases* 3 (1993): 69–85. [https://doi.org/10.1016/0959-8030\(93\)90029-B](https://doi.org/10.1016/0959-8030(93)90029-B).
- Meyburgh, C. M., R. R. Bragg, and C. E. Boucher. 2017. "*Lactococcus Garvieae*: An Emerging Bacterial Pathogen of Fish." *Diseases of Aquatic Organisms* 123 (1): 67–79. <https://doi.org/10.3354/dao03083>.
- Moreira, M., H. Peres, A. Couto, and A. Oliva-Teles. 2012. "Temperature and Dietary Carbohydrate Level Effects on Growth Performance and Metabolic Utilization of Gilthead Sea Bream Juveniles." *Aquaculture* 354–355:64–72.
- Pastorino, P., A. I. V. Alonso, S. Colussi, G. Cavazza, V. Menconi, D. Mugetti, M. Righetti, et al. 2019. "A Summer Mortality Outbreak of Lactococcosis by *Lactococcus garvieae* in a Raceway System Affecting Farmed Rainbow Trout (*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*)." *Animals* 9 (12). <https://doi.org/10.3390/ani9121043>.
- Peres, H., S. Santos, and A. Oliva-Teles. 2014. "Blood Chemistry Profile as Indicator of Nutritional Status in European Sea Bass and Gilthead Sea Bream." *Aquaculture* 420–421:57–63.
- Ravelo, C., B. Magariños, J. L. Romalde, and A. E. Toranzo. 2001. "Conventional versus Miniaturized Systems for the Phenotypic Characterization of *Lactococcus garvieae* Strains." *Bull. Eur. Ass. Fish Pathol* 21 (4).
- Salogni, C., C. Bertasio, A. Accini, L. R. Gibelli, C. Pigoli, F. Susini, E. Podavini, F. Scali, G. Varisco, and G. L. Alborali. 2024. "The Characterisation of *Lactococcus garvieae* Isolated in an Outbreak of Septicaemic Disease in Farmed Sea Bass (*Dicentrarchus labrax*, Linnaeus 1758) in Italy." *Pathogens* 13 (1). <https://doi.org/10.3390/pathogens13010049>.
- Serdoz, F., D. Voinovich, B. Perissutti, I. Grabnar, D. Hasa, R. Ballestrazzi, E. Coni, and E. Pellegrini. 2011. "Development and Pharmacokinetic Evaluation of Erythromycin Lipidic Formulations for Oral Administration in Rainbow Trout (*Oncorhynchus Mykiss*)." *European Journal of Pharmaceutics and Biopharmaceutics* 78:401–7. <https://doi.org/10.1016/j.ejpb.2011.03.007>.
- Shahin, K., K. Mukkatira, Z. Yazdi, C. Richey, K. Kwak, T. I. Heckman, H. H. Mohammed, et al. 2022. "Development of a Quantitative Polymerase Chain Reaction Assay for Detection of the Aetiological Agents of Piscine Lactococcosis." *Journal of Fish Diseases* 45:847–59. <https://doi.org/10.1111/jfd.13610>.
- Soltani, M., G. Nikbakht, H. Mousavi, and N. Ahmadzadeh. 2008. "Epizootic Outbreaks of Lactococcosis Caused by *Lactococcus Garviae* in Farmed Rainbow Trout (*Onchorhynchus Mykiss*) in Iran." *Bulletin of the European Association of Fish Pathologists* 28.

- Stoppani, N., S. Colussi, P. Pastorino, M. Prearo, S. Sciuto, I. Altinok, R. Ç. Öztürk, et al. 2023. "16S-23S rRNA Internal Transcribed Spacer Region (ITS) Sequencing: A Potential Molecular Diagnostic Tool for Differentiating *Lactococcus garvieae* and *Lactococcus petauri*." *Microorganisms* 11 (5). <https://doi.org/10.3390/microorganisms11051320>.
- Vela, A. I., M. del Mar Blanco, S. Colussi, C. Kotzamanidis, M. Prearo, I. Altinok, P. L. Acutis, et al. 2024. "The Association of *Lactococcus petauri* with Lactococcosis Is Older than Expected." *Aquaculture* 578:740057. <https://doi.org/10.1016/j.aquaculture.2023.740057>.
- Vela, A. I., J. Va'zquez, V. Va'zquez, A. Gibello, M. M. Blanco, M. A. Moreno, P. Lié Bana, et al. 2000. "Phenotypic and Genetic Characterization of *Lactococcus garvieae* Isolated in Spain from Lactococcosis Outbreaks and Comparison with Isolates of Other Countries and Sources." *Journal Of Clinical Microbiology* 38 (10). <https://doi.org/10.1128/JCM.38.10.3791-3795.2000>.
- Vendrell, D., J. L. Balcázar, I. Ruiz-Zarzuela, I. de Blas, O. Gironés, and J. L. Múzquiz. 2006. "*Lactococcus Garvieae* in Fish: A Review." *Comparative Immunology, Microbiology and Infectious Diseases* 29 (4): 177–98. <https://doi.org/10.1016/j.cimid.2006.06.003>.
- Zoli, M., L. Rossi, M. Costantini, C. Bibbiani, B. Fronte, F. Brambilla, and J. Bacenetti. 2023. "Quantification and Characterization of the Environmental Impact of Sea Bream and Sea Bass Production in Italy." *Cleaner Environmental Systems* 9:00118. <https://doi.org/10.1016/j.cesys.2023.100118>.

SUPPLEMENTARY MATERIALS

Raw Data Tables

Download: <https://eafpbulletin.scholasticahq.com/article/143736-first-documented-outbreaks-of-lactococcus-garvieae-in-gilthead-sea-bream-sparus-aurata-in-the-gulf-of-follonica-tuscany-italy/attachment/300024.docx>
