

XXX

Convegno Nazionale

SIPI

SOCIETÀ ITALIANA DI PATOLOGIA ITTICA

Atti di convegno

ROMA 7-9 ottobre 2026

Sapienza Università di Roma



SAPIENZA
UNIVERSITÀ DI ROMA
Dipartimento di Chimica
e Tecnologie del Farmaco



ISPRA
Istituto Superiore per la Protezione
e la Ricerca Ambientale



Sistema Nazionale
per la Protezione
dell'Ambiente

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Edificio CU034 - Aula ex ingegneria

Piazzale Aldo Moro 5, Roma

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Ordine dei Medici Veterinari



della Provincia di Roma



SAPIENZA
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01

PROGRAMMA

Programma



XXX CONVEGNO NAZIONALE S.I.P.I. - Società Italiana di Patologia Ittica
Roma, 7-9 ottobre 2026
Università La Sapienza - Piazzale Aldo Moro 5, Roma
Dipartimento di Chimica e Tecnologie del Farmaco - Edificio CU034 aula ex ingegneria

Programma
Prima giornata – mercoledì 7 ottobre 2026

- 14:00 – 14:30 Registrazione dei partecipanti
- 14:30 – 15:00 Saluti istituzionali e introduzione
Prof.ssa Antonella Polimenti - Magnifica Rettore Sapienza Università di Roma
Prof.ssa Daniela Secci - Direttrice del Dipartimento di Chimica e Tecnologia del Farmaco
Dott.ssa Nadia Cherif - Aquatic Biosecurity Senior Officer FAO
- 15:00 – 16:45 **Prima sessione lavori scientifici – Malattie infettive**
Moderatore: Dott.ssa Teresa Bossú - Istituto Zooprofilattico Sperimentale Lazio e Toscana
- 15:00 – 15:15 **GAMMA-RAY INACTIVATION OF BETANODAVIRUS: A VALUABLE ALTERNATIVE WAY TO PRODUCE VACCINE AGAINST VNN**
Agrebi H., Buratin A., Berto P., Biasini L., Najahi S., Kangethe R.T., Wijewardana V., Bouhaouala-Zahar B., Cherif N., Sghaier H., Marsella A., Toffan A.
- 15:15 – 15:30 **CUMULATIVE THERMAL STRESS ASSOCIATED WITH BETANODAVIRUS OUTBREAKS IN WILD DUSKY GROUPERS (*EPINEPHELUS MARGINATUS*)**
Volpe E., Mandrioli L., Napolitano R., Garcia Hartmann M., Merotto L., Girons A., Errani F., Brunetti B., Capoccioni F., Ciulli S.
- 15:30 – 15:45 **A POT-POURRI OF CAMBODIAN FRESHWATER FINFISH DISEASES**
Cortinovis L., Boscarato M., Paolini V., Basso A., Menconi V., Pascoli F., Biasini L., Berto P., Mey S., San D., Outh S., Mon K., Kong L., Tey T., Yong C., Rith P., Khan C.D., Pretto T.
- 15:45 – 16:00 **FORMALIN KILLED AUTOGENOUS VACCINE AGAINST AEROMONAS SALMONICIDA SUBSP. SALMONICIDA IN FARMED ARCTIC CHARR (*SALVELINUS ALPINUS*): ELISA-BASED EVALUATION OF SPECIFIC IMMUNITY**
Errani F., Budai J., Volpatti D., Cortinovis L., Leonardi M., Fabris A., Manfrin A.
- 16:00 – 16:15 **PLANT-DERIVED PRODUCTS AS POTENTIAL TOOLS FOR THE SUSTAINABLE CONTROL OF *LACTOCOCCUS GARVIAE* IN ITALIAN TROUT AQUACULTURE**
Fiocchi E., Boscarato M., Cossettini C., Guerrini A., Toson M., Dalla Pozza M., Cortinovis L., Fagnon S., Mattarelli P., Manfrin A.
- 16:15 – 16:30 **ESSENTIAL OILS AND THEIR BLENDS AS POTENTIAL ALTERNATIVES TO ANTIBIOTICS AGAINST *WEISSELLA CETI*: A PRELIMINARY IN VITRO STUDY**
Fiocchi E., Vela Alonso A.I., Fernández-Garayzábal J.F., Caneschi A., Scozzoli M., Boscarato M., Cortinovis L., Toson M., Dalla Pozza M., Mattarelli P., Manfrin A.



- 16:30 – 16:45 **OVERVIEW OF EMERGING AND RE-EMERGING PARASITES IN MEDITERRANEAN MARICULTURE**
Apuzzo F., Caffara M., Tedesco P., Gustinelli A., Čolak S.
- 16:45 – 17:15 **MARINET - INTEGRATED MONITORING AND ARTIFICIAL INTELLIGENCE FOR RISK MANAGEMENT IN AQUACULTURE**
Prof. Marco Galeotti - Università degli Studi di Udine
- 17:15 – 19:00 Aperitivo di benvenuto

Seconda giornata – giovedì 8 ottobre 2026

- 09.30 – 10.00 **IMMUNITÀ DELLA SPIGOLA (*DICENTRARCHUS LABRAX*) IN CORSO DI INFEZIONI BATTERICHE E VIRALI (*BETANODAVIRUS*)**
Prof. Giuseppe Scapigliati - Università degli Studi della Tuscia
- 10.15 – 12.15 **Seconda sessione lavori scientifici – Altre patologie**
Moderatore: Prof. Fabio Marino – Università degli Studi di Messina
- 10:15 – 10:30 **THE DECEPTIVE NATURE OF COLOR CHANGES IN FISHERY PRODUCTS: SIMILAR APPEARANCES AND DIVERGING DIAGNOSES ACROSS PARASITOLOGY, PATHOLOGY, AND ANATOMY**
Bianchi G.B.M., Ghisleni G., Corti I., Grieco E., Cappelleri A.G., Nieddu G., Malandra R., Giudice C.
- 10:30 – 10:45 **PROLIFERATIVE THYROID LESIONS IN MARINE AQUARIUM FISH**
Capparucci F., Martino A., Abbate J.M., Cimino F., Iaria C., Mandrioli L., Pesce G., Anjomanibenisi M., Brunetti B., Marino F.
- 10:45 – 11:00 **HISTOPATHOLOGICAL FINDINGS IN TRANSGENIC AND INBRED LINES OF MEDAKA (*ORYZIAS LATIPES*)**
Falletti R., Capparucci F., Abbate J.M., Vallone D., Loosli F., Lanteri G., Marino F.
- 11:00 – 11:30 Coffee break
- 11:30 – 11:45 **APPLICATION OF PREOPERATIVE PHOTOGRAMMETRY FOR THREE-DIMENSIONAL DOCUMENTATION OF FIN PAPILLOMA SURGERY IN A GOLDFISH (*CARASSIUS AURATUS*, (LINNAEUS, 1758))**
Nieddu G., Bianco C., Rovea C., Ancona N., Volpe E., Ciulli S., Mandrioli L.
- 11:45 – 12:00 **MONITORING THE HEALTH STATUS OF THE MILAN AQUARIUM THROUGH CLINICAL HISTORY ASSESSMENT AND LABORATORY INVESTIGATIONS**
Nieddu G., Ancona N., Salogni C.
- 12:00 – 12:15 **FIRST REPORT OF DERMAL FIBROMYXOMA IN GREATER AMBERJACK (*SERIOLA DUMERILI*): HISTOPATHOLOGICAL AND HISTOCHEMICAL FINDINGS**
Piro M.G., Capparucci F., Martino A., Pesce G., Marino F., Iaria C.
- 12:30 – 14:00 Light lunch



14:00 – 15:30 **Terza sessione lavori scientifici – Miscellanea**

Moderatore: Dott. Francesco Pascoli – Istituto Zooprofilattico Sperimentale delle Venezie

14:00 – 14:15 **EVALUATION FEED SUPPLEMENTS FROM MARINE AND HERBAL ORIGIN CONTAINING BIOACTIVE COMPOUNDS IN FREE AND MICROENCAPSULATED FORMS IN *CYPRINUS CARPIO***

Hafiz S., Natale S., Calinoiu L.F., Vodnar Dan C., Chrysanthos S., Magdalini K., Papadaki S., Moustogianni A., Capparucci F., Iaria C., Marino F.

14:15 – 14:30 **NECTO - THE SHIFT TOWARDS RESILIENCE**

Maurizio A.

14:30 – 14:45 **ASSESSING THE IMPACT OF ENVIRONMENTAL POLLUTANTS AS ENDOCRINE DISRUPTORS: GENE EXPRESSION ANALYSIS IN ZEBRAFISH (*DANIO RERIO*) LARVAE**

Fabrizi F., Ferraù F., Natale S., Capparucci F., Blanca E.S., Bottari T., Giannetto A., Iaria C., Cannavò S.

14:45 – 15:00 **ASSESSMENT OF ANTIMICROBIAL USAGE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM, 1792) FARMS OF TRENTO AND UDINE PROVINCES (ITALY)**

Franzago E., Melotto G., Toson M., Ambrosi A., Donini S., Dalla Pozza M., Bille L., Callegaro A., Mazzucato M., Toffan A., Manfrin A., De Zan G., Dellamaria D., Bortolotti L., Baracetti G., Fabris A., Di Martino G.

15:15 – 15:30 **SUPPORTING AQUATIC ANIMAL HEALTH DATA INTEGRATION IN SALMONID FARMS: A GEOGRAPHIC INFORMATION SYSTEMS APPROACH**

Macario R., Casarotto C., De Conti G., Bonato P., Franzago E., Bille L., Di Martino G., Ferrè N., Mazzucato M.

15:30 – 16:00 Coffee break

16:00 – 17:00 **Assemblea Soci**

17:00 – 17:30 **Premiazioni**

20:00 **Cena sociale** presso il ristorante “Antica Osteria Croce” via della Croce 38 (fermata Metro più vicina: Spagna)



POSTER

- 1. COMPARATIVE PARASITOLOGICAL SURVEY OF WILD AND FARMED SALMONIDS**
Benetti S., Apuzzo F., Gustinelli A., Tedesco P., Caffara M.
- 2. INFECTION TRIAL OF LACTOCOCCUS GARVIEAE INOCULATED IN GILTHEAD SEA BREAM (*SPARUS AURATA*) MAINTAINED UNDER CONTROLLED CONDITIONS IN A CLOSED RECIRCULATION SYSTEM (RAS)**
Bignami G., Gustinelli A., Acosta F.
- 3. SWIM BLADDER FLOODING IN KOI CARP (*CYPRINUS CARPIO*): DIAGNOSTIC WORK-UP AND OUTCOME OF A CLINICAL CASE**
Bonazza Foselli M., Leone V.F., Mandrioli L.
- 4. CO-CIRCULATION OF CYPRINID HERPESVIRUSES (CYHV-1, CYHV-2) AND CARP EDEMA VIRUS (CEV) IN KOI CARP (*CYPRINUS CARPIO*) FROM A PRIVATE POND IN ITALY**
Ciurli G., Coltraro M., D'Alonzo A., Ricci E., Di Blasi L., Cocumelli C., Acri C., Parmigiani R., Bovi E., Bonazza Foselli M., Susini F.
- 5. INTRAHEPATIC INFILTRATIVE LIPOMA IN A GIANT RED-TAILED GOURAMI (*OSPHRONEMUS LATICLAVIUS*): A NOVEL SITE FOR TELEOST ADIPOCYTIC NEOPLASIA**
Coltraro M., Cocumelli C., Nicolosi P., D'Alonzo A., Ricci E., Ciurli G., Di Blasi L., Acri C., Parmigiani R., Bovi E., Susini, F.
- 6. FIRST REPORT OF PROLIFERATIVE KIDNEY DISEASE IN NORTHWESTERN ITALY: OCCURRENCE AND SEASONAL TRENDS**
Cotugno, A., Colussi, S., Bondavalli, F., Gini, M., Milanese, G., Zicarelli, G., Glorio Patrucco, S., Esposito, G., Mossotto, C., Gabetti, A., Stilo, G., Fariano, L., Pastorino, P., Prearo, M.
- 7. PHILOMETRA FILIFORMIS IN PAGELLUS ERYTHRINUS: EPIDEMIOLOGICAL, MORPHOLOGICAL UPDATES AND MOLECULAR CHARACTERIZATION**
De Benedetto G., Riolo K., Perdichizzi A., Armeli Minicante E., Veccari G., Sanchez Tinjaca Y.V., Giannetto A., Gaglio G.
- 8. AQUACULTURE OPERATORS' AWARENESS OF ANIMAL HEALTH THREATS AND PREVENTION IN ITALY, UNDER REGULATION (EU) 2016/429: DESIGN AND IMPLEMENTATION OF AN E-LEARNING TRAINING COURSE**
Donini S., Bissoli F., Ricaldi G., D'Este F., Personeni F., Cassani M., Trevisan S., Nadin A., Lunardi L., Franzago E., Marsella A., Piován G., Manca G., Toffan A., Dalla Pozza M.
- 9. EFFECTS OF SINGLE AND COMBINED POLYSTYRENE NANOPLASTICS AND GLYPHOSATE EXPOSURE ON ZEBRAFISH (*DANIO RERIO*) EMBRYOS**
Falletti R., Capparucci F., Vallone D., Foulkes N.S., Abbate J.M., Lanteri G., Iaria C.



- 10. CONCURRENT EDWARDSIELLOSIS AND POSTHODIPILOSTOMUM ANTEROVARII PARASITISM IN LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*): A CASE STUDY**
Giroto M., Basso A., Boscarato M., Pretto T., Manfrin A., Cortinovis L.
- 11. ISOLATION AND MOLECULAR IDENTIFICATION OF *LACTOCOCCUS GARVIAE* ISOLATED FROM RAINBOW TROUT FROM A SICILIAN FRESHWATER AQUACULTURE FARM**
Iaria C., Lo Monaco D., Krezou K., Carrozzo A., Reale S., Scibetta S., Salogni C., Di Gaudio F., Marino F.
- 12. DESCRIPTION OF WHITE STURGEON, *ACIPENSER TRANSMONTANUS*, MORTALITY POTENTIALLY LINKED TO NOTOCHORD DISPLACEMENT**
Marsella A., Pretto T., Buratin A., Biasini L., Cortinovis L., Maurizio A., Accini A., Toffan A.
- 13. DEFORMIA: DEVELOPMENT OF AN ARTIFICIAL INTELLIGENCE-BASED PREDICTIVE SYSTEM IN AQUACULTURE FOR THE SELECTION OF THE BEST BREEDING CANDIDATES**
Paramatti A., Sciuto S., Tafuro V., Colussi S., Acutis P. L., Pastorino P., Prearo M., Gini M., Milanese G., Vandoni L., Balzano G., Revelli M., Botto D., Milanesi M., Colli L., Tomasoni M., Ajmone Marsan P., Fariano L.
- 14. EURO-PAR-FISH: FROM WILD WATERS TO HUMAN HEALTH - A ONE HEALTH APPROACH TO ZOONOTIC HELMINTHS IN EUROPEAN FRESHWATER FISH**
Menconi V., Guardone L., Arcangeli G., Manfrin A., Tosi F., Gustinelli A., Müller-Graf C., Gay M., Caffara M., Prearo M., Pastorino P., Marucci G., Rubiola S., Armani A., Mateusa M., Vasilev S., Bełcik A., Paździor E., Korpysa-Dzirba W., Angeloni G., Bilska-Zajac E.
- 15. NADI-ACQUA: DEVELOPMENT OF A DECISION SUPPORT SYSTEM AS AN INNOVATIVE NON-INVASIVE APPROACH FOR EARLY DIAGNOSIS IN AQUACULTURE**
Tafuro V., Fariano L., Paramatti A., Colussi S., Pastorino P., Acutis P. L., Tomasoni M., Prearo M., Di Nicola M. R., Vandoni L., Balzano G., Revelli M., Botto D., Pascoli F., Toffan A., Manfredi M., Sciuto S.
- 16. EVIDENCE OF *AEROMONAS EUCRENOPHYLA* INFECTION IN *SALVELINUS FONTINALIS* FROM AN ITALIAN FARM**
Torricelli M., Radicchi E., Marchetti F., Di Vincenzo R., Accini A., Agnetti F.
- 17. ESSENTIAL OILS FOR THE CONTROL OF BACTERIAL DISEASES IN AQUACULTURE: A NATURAL ALTERNATIVE**
Zicarelli, G., Colussi, S., Glorio Patrucco, S., Cotugno, A., Bondavalli, F., Amadori, M., Vercelli, C., Re, G.2, Esposito, G., Maganza, A., Merialdi, A., Gasco, L., Prearo, M., Pastorino, P.

02

ABSTRACT

Comunicazioni orali

GAMMA-RAY INACTIVATION OF *BETANODAVIRUS*: A VALUABLE ALTERNATIVE WAY TO PRODUCE VACCINE AGAINST VNN

Agrebi H.¹, Buratin A.², Berto P.², Biasini L.², Najahi S.³, Kangethe R. T.⁴, Wijewardana V.⁴, Bouhaouala-Zahar B.^{3,5}, Cherif N.¹, Sghaier H.^{6,7}, Marsella A.², Toffan A.²

¹ Disease of Aquatic organism service, Aquaculture Laboratory (LR16INSTM03), National Institute of Marine Sciences and Technologies, 2025 Salammbô, Tunisia.

Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Centre for Fish, Mollusk and Crustacean Diseases, Viale dell'Università 10, 35020 Legnaro (PD), Italy 2

³ Laboratory of Biomolecules, Venoms and Theranostic Molecules (LR20IPT01), Institut Pasteur Tunis, University Tunis El Manar, 13 Place Pasteur, 1002 Tunis, Tunisia.

⁴ Animal Production and Health Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, 2444 Seibersdorf, Austria.

⁵ Medical School of Tunis, 15 rue Djebel Lakhdhar, La Rabta, University Tunis El Manar, 1007 Tunis, Tunisia.

⁶ Laboratory "Energy and Matter for Development of Nuclear Sciences" (LR16CNSTN02), National Center for Nuclear Sciences and Technology (CNSTN), Sidi Thabet Technopark 2020, Tunisia.

⁷ University Manouba, ISBST, BVGBR-LR11ES31, Biotechpole Sidi Thabet, 2020, Ariana, Tunisia.

Conventional inactivation methods for viruses generally compromise viral immunogenicity or can introduce cytotoxic effects, highlighting the need for safer and more effective alternatives for virus inactivation. As an alternative, gamma irradiation offers a safer and more effective substitute to standard inactivation methods avoiding the risk of chemical residues, preserving surface antigens and efficiently inactivating pathogens through targeted damage to nucleic acids (DNA or RNA). This study explores gamma irradiation as an alternative approach to inactivate *Betanodavirus*, the causative agent of the Viral nervous Necrosis (VNN), for producing safe and efficacious vaccine. Viran Nervous Necrosis is the most threatening viral diseases for mediterranean fish species, especially for European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), highly impacting on economy of the marine fish farming.

A reassortant Betanodavirus RGNNV/SJNNV recently isolated in Tunisia, was used as prototype virus in order to determine the optimal irradiation dose in combination with different percentual of trehalose that ensures complete viral inactivation without compromising antigenic properties for vaccine development.

Virus samples were exposed to gamma radiation doses ranging from 5 to 60 kGy. The D₁₀ value (7.55 kGy), the inactivation dose (55.35 kGy), and the gamma radiation dose (70.45 kGy) ensuring a Sterility Assurance Level (SAL) were established. *In vitro* immunological assays, including enzyme-linked immunosorbent assay (ELISA), Immunofluorescent antibody tests, transmission electron microscopy (TEM) were used to assess the that the inactivated virus retained antigenicity post-irradiation. *In vivo* assays proved that irradiated virus administered by intraperitoneal injection in European sea bass juveniles can stimulate specific IgM production further confirming the immunogenicity of the inactivated virus. Finally, the role of trehalose, a polysaccharidic molecule widely used as radioprotector, has been evaluated by comparing throughout the experiment samples including different percentual of trehalose (6% and 12%) to samples without this additive. No significant increase in preservation of the antigen nor immune response stimulation has been observed.

Overall, our findings support gamma irradiation as a promising non-chemical approach for production of inactivated *Betanodavirus* vaccines without the drawbacks of traditional inactivation techniques. Further studies incorporating larger volumes, larger sample sizes, and controlled challenge experiments will be necessary to confirm protective efficacy, to evaluate vaccine potency and finally to optimize vaccine formulations.

This research was performed under the Technical Cooperation (TC) Project TUN5032 (AquaVac-ir, <http://www.aquavac-ir.tn/>) and the IAEA CRP 2296 (Project Code: D3.20.37) provided by the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture through the International Atomic Energy Agency (IAEA)

CUMULATIVE THERMAL STRESS ASSOCIATED WITH BETANODAVIRUS OUTBREAKS IN WILD DUSKY GROUPERS (*EPINEPHELUS MARGINATUS*)

Volpe E.¹, Mandrioli L.¹, Napolitano R.^{2,3}, Garcia Hartmann M.⁴, Merotto L.⁵, Girons A.⁶, Errani F.¹, Brunetti B.⁷, Capoccioni F.², Ciulli S.¹

¹ Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna, Cesenatico, Italy

² Research Centre for Animal Production and Aquaculture, Council for Agricultural Research and Economics, Monterotondo, Italy

³ Institute of Aquaculture, University of Stirling, Stirling, United Kingdom

⁴ MarLab, Mougins, France

⁵ Portofino Marine Protected Area, Santa Margherita Ligure, Italy

⁶ ICTIOVET S.L., Barcelona, Spain

⁷ Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna, Ozzano dell'Emilia, Italy

Rising Mediterranean temperatures and increasingly frequent marine heatwaves may alter host-pathogen interactions and promote infectious disease emergence in wild fish. Betanodaviruses, the causative agents of viral encephalopathy and retinopathy (VER), are strongly influenced by temperature. This study investigated the thermal conditions associated with mortality events in wild dusky groupers (*Epinephelus marginatus*) recorded in 2018 and 2019 in the Larvotto Marine Protected Area, Principality of Monaco, and the Portofino Marine Protected Area, Italy.

Virological, molecular and pathological investigations confirmed infection by red-spotted grouper nervous necrosis virus (RGNNV). In parallel, daily sea surface temperature (SST) data from the Copernicus Marine Environment Monitoring Service Mediterranean Sea Physics Reanalysis were examined for 2000-2019. At each site, SST during mortality years was compared with the local climatology using two temporal windows: October, when most mortality events were observed, and July-October, representing the warm season preceding them. Prolonged thermal exposure was also quantified through a cumulative heat index based on daily exceedances of the climatological 90th-percentile threshold.

The October analysis produced heterogeneous results. In 2019, SST anomalies were limited at both Portofino (+0.3 °C) and Larvotto (+0.2 °C), whereas Larvotto showed a +0.7 °C anomaly in October 2018, corresponding to the 90th percentile. A more consistent pattern emerged over July-October. In 2019, anomalies reached +0.9 °C at Portofino and +1.1 °C at Larvotto, corresponding to the 90th and 95th percentiles, respectively. In 2018, Larvotto recorded a +1.53 °C anomaly, the highest value in the analyzed time series. During the same year, the cumulative heat index reached its maximum at both sites, with 37.33 °C·days at Larvotto and 36.2 °C·days at Portofino. Values remained elevated in 2019, although they were lower and ranked at the 80th percentile.

These findings indicate that temperatures recorded close to mortality events may not adequately represent the environmental conditions preceding disease emergence. Indicators integrating the duration and intensity of warm-season exposure provide a more informative description of the thermal stress potentially associated with VER outbreaks than short-term temperature anomalies. Although the limited number of events precludes causal inference, the results support the integration of cumulative thermal indicators with targeted health surveillance of wild fish populations in marine protected areas.

A POT-POURRI OF CAMBODIAN FRESHWATER FINFISH DISEASES

Cortinovis L.¹, Boscarato M.¹, Paolini V.¹, Basso A.¹, Menconi V.¹, Pascoli F.¹, Biasini L.¹, Berto P.¹, Mey S.², San D.², Outh S.², Mon K.², Kong L.², Tey T.³, Yong C.³, Rith P.³, Khan C.D.², Pretto T.¹

¹Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro (PD) Italy

²General Directorate of Fisheries (GDF), Dept. of Aquaculture Development (DAD), Phnom Penh, Kingdom of Cambodia

³Marine Aquaculture Research and Development Centre (MARDeC), Preah Sihanouk, Kingdom of Cambodia

The Kingdom of Cambodia is a water-rich country in Mainland Southeast Asia, where inland fisheries and aquaculture play a fundamental role in the economic and social landscape. Its extensive network of rivers, lakes, wetlands and floodplains, particularly the Mekong and Tonlé Sap systems, provides highly favourable conditions for fish production. While capture fisheries remain of primary importance, aquaculture is steadily expanding to meet the growing domestic demand for fish. Cambodian inland aquaculture is strongly influenced by pronounced seasonal variations, with the alternation of wet and dry seasons driving major changes in water availability, quality and habitat connectivity. During the wet season, flooding of the Tonlé Sap and surrounding floodplains connects pond facilities with natural aquatic environments, allowing interactions between captive and wild stocks. Conversely, during the dry season, declining water levels, rising temperatures and limited water exchange can lead to environmental deterioration, algal blooms and drastic fluctuations in oxygen and temperature. Cambodian fish species are generally well adapted to the country's highly dynamic aquatic conditions. Giant snakehead (*Channa micropeltes*), striped snakehead (*Channa striata*), black ear catfish (*Pangasius larnaudii*) and climbing perch (*Anabas testudineus*) are particularly resilient to high temperatures and hypoxia, being able to obtain oxygen from air. Asian seabass (*Lates calcarifer*) and tilapia species are also important farmed fish, although more sensitive to low oxygen conditions. In the context of the Aquae Strength project “strengthening capacity on aquatic animal health and epidemiological surveillance”, several sampling activities have been carried during field visits in Cambodian farms. The aim of this presentation is to describe some of the most interesting episodes of disease observed during this collaboration with the Cambodian General Directorate of Fisheries (GDF - DAD), and the Marine Aquaculture Research and Development Centre (MARDeC). The presentation will focus in particular on describing a case of epizootic ulcerative syndrome (EUS) caused by *Aphanomyces invadans* in striped snakehead; an outbreak of motile Aeromonas septicaemia (MAS) by *A. schubertii* in juvenile giant snakehead; an episode of streptococcosis caused by *Streptococcus agalactiae* in African catfish (*Clarias gariepinus*); and a case of enteric septicaemia caused by *Edwardsiella ictaluri* in black ear catfish. Sampling, bacterial isolation and preliminary biochemical tests were performed on-site. Bacterial isolates, histological samples fixed in 10% neutral buffered formalin and portions of tissues preserved in RNA later were shipped to IZSVe laboratory for further analysis. Confirmatory bacterial identification was achieved through MALDI-tof MS (Bruker) and molecular techniques (namely amplification and Sanger sequencing either of the 16S rRNA or gyrB genes). *A. invadans* detection was performed directly on fixed muscle tissues through ITS1 specific end point PCR followed by Sanger sequencing. Histological preparations were processed using standard histological techniques and stained with haematoxylin and eosin (H&E). Additionally, Gram and Grocott's methenamine silver (GMS) staining were performed when bacterial or fungal infection was suspected, respectively. The cases presented emphasize the wide range of infectious threats that may affect Cambodian aquaculture. Strengthening local diagnostic expertise and disease surveillance is therefore crucial to support sustainable aquaculture development and improve the health and productivity of farmed fish.

The AQUAE STRENGTH project is financed by the Italian Ministry of Health and supported by the World Organisation for Animal Health (WOAH).

FORMALIN KILLED AUTOGENOUS VACCINE AGAINST *AEROMONAS SALMONICIDA* SUBSP. *SALMONICIDA* IN FARMED ARCTIC CHARR (*SALVELINUS ALPINUS*): ELISA-BASED EVALUATION OF SPECIFIC IMMUNITY

Errani F.¹, Budai J.¹, Volpatti D.¹, Cortinovis L.², Leonardi M.³, Fabris A.⁴, Manfrin A.²

¹Dipartimento di scienze agroalimentari, ambientali e animali - UNIUD, Udine; ²Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova; ³Aquazzurra, San Lorenzo, Trento; ⁴Associazione Piscicoltori Italiani, Verona.

The Arctic charr (*S. alpinus*) is a stenothermic cold-water species naturally occupying the circumpolar aquatic environments. Although a dedicated aquaculture is a relatively small industry, Arctic charr fillets are quite appreciated in niche markets. Italian farms currently reach the annual production of 175 t (FEAP, 2024) and are mainly located in Trentino Alto-Adige (Salmerino del Trentino IGP). As well as other salmonids, Arctic charr is strongly susceptible to furunculosis, a septicemic infection caused by *A. salmonicida* responsible for acute or chronic mortality in the affected populations. In general, vaccination has proven to be an effective means of preventing *A. salmonicida* infections, and some commercial vaccines against this pathogen have been developed and tested also in *S. alpinus*. In Italy there are not specifically targeted authorized commercial vaccines, and autogenous formulations might be a suitable tool to limit the outbreaks. The present trial aimed at evaluating the technical in-field applicability and the effectiveness, in terms of serum conversion, of a novel autogenous formulation with or without the inclusion of a non-mineral oil as adjuvant. Two thousand juveniles weighing 52 ± 8 g, hosted in a commercial facility, were inoculated intraperitoneally with a formalin-inactivated bacterin (autogenous, strain 71/ITT/24) with or without the inclusion of adjuvant Seppic ISA763® (N=1000 fish per group, respectively). The vaccination was carried out by skilled staff equipped with automatic injection guns (0.1 ml/fish). Three weeks post vaccination, both groups of fish were transferred from the hatchery to dedicated on-growing concrete tanks and monitored throughout a year. Specifically, they were submitted to growth/survival rate observation and to serum sampling at defined intervals: T1, 43 days post vaccination (dpv); T2, 75 dpv; T3, 106 dpv; T4, 239 dpv; T5, 284 dpv; T6, 358 dpv. The post-vaccination systemic response (*A. salmonicida* specific IgM in serum) was evaluated by an indirect peroxidase-based ELISA. Comparative analysis between the “bacterin” and “bacterin + adjuvant” treated groups demonstrated the key role of the adjuvant in enhancing and prolonging the humoral immune response in the salmonid species under study. A significantly higher specific response was observed starting at 75 days post-vaccination in the group treated also with the adjuvant, whereas it appeared later-starting at 106 days post-vaccination-in the group treated without the adjuvant. The “bacterin + adjuvant” group exhibited highly interesting long-term kinetics: specific antibody levels remained significantly higher up to the final time point analyzed. Still, the results revealed also an evident individual variability in specific immune responses across both treatment groups. The growth trend of the fish during the post-vaccination annual survey was not negatively affected after the administration of both formulations (bacterin or bacterin + adjuvant). Specifically, fish reached a size of about 700-800 g in correspondence to the last in-field serum sampling, without apparent differences between the groups. The post-vaccination mortality rates, recorded monthly, resulted always less than 1% of the vaccinated fish belonging to both experimental groups. Due to the limited commercial availability of dedicated kits or species-specific antibodies, a “customized” (in-house) ELISA protocol was developed for Arctic char against *A. salmonicida*. This approach has proven to be a valuable tool for monitoring vaccination progress in Arctic charr and for supporting fish farmers wishing to participate in vaccination campaigns using commercial or farm-specific formulations against major bacterial diseases. The results confirmed the efficacy of adjuvanted, intracelomic vaccines, demonstrating significant seroconversion starting from two months post-vaccination, with an antibody response that remained substantial for nearly a year.

The study has been developed within the project AquaVax (MASAF, Italy) in collaboration with the Italian Fish Farmers Association (API).

PLANT-DERIVED PRODUCTS AS POTENTIAL TOOLS FOR THE SUSTAINABLE CONTROL OF *LACTOCOCCUS GARVIEAE* IN ITALIAN TROUT AQUACULTURE

Fiocchi, E.^{1,2}, Boscarato, M.¹, Cossetti C.³, Guerrini, A.⁴, Toson M.¹, Dalla Pozza M.¹, Cortinovis, L.¹, Fagnon S.⁵, Mattarelli, P.², Manfrin, A.¹

¹ Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD)

² Dipartimento scienze e tecnologie agro-alimentari, Alma Mater Studiorum - Università di Bologna, Bologna (BO)

³ Chemifarma S.p.A., Forlì (FC)

⁴ Valhen S.r.l., Ravenna (RA)

⁵ Phytosynthese, Saint-beauzire (France)

Lactococcus garvieae is an important bacterial pathogen in worldwide aquaculture and represents a significant health concern for Italian trout farming, where numerous summer outbreaks have been reported in rainbow trout (*Oncorhynchus mykiss*) farms since 1990s. Lactococcosis treatment relies on antimicrobials, but their excessive use may contribute to increase antimicrobial resistance and public health concerns. Plant-derived products containing bioactive compounds from medicinal and aromatic plants may represent promising alternative or complementary strategies as preventive or supportive treatment for bacterial infections in sustainable aquaculture.

The present study therefore aims to evaluate the *in vitro* antibacterial activity of two plant-based products against *L. garvieae* strains isolated from Italian trout farms.

Eleven *L. garvieae* strains were investigated to determine the Minimum Inhibitory Concentration (MIC) of two phyto-genic products, Active Fitobaxin and Product Fitobaxin. Both formulations contain a mixture of nature-identical active ingredients authorised for use in animal feed as additives and listed in the EU Feed Additives Register under Regulation (EC) No. 1831/2003: carvacrol, thymol and trans-cinnamaldehyde. Ten *L. garvieae* strains were recovered from trout farms located in northern Italy, while one reference strain (DSMZ 6783) was included in the study. All strains were identified using MALDI-TOF mass spectrometry and molecular biology including end-point PCR and sequencing of the *gyrB* gene and the internal transcribed spacer (ITS) region between 16S rRNA and 23S rRNA genes. MIC assays were performed in 96-well U-bottom microtiter plates using two-fold serial dilutions in Cation Adjusted Mueller-Hinton Broth (CAMHB) supplemented with 5% Lysed Horse Blood (LHB), according to the Clinical and Laboratory Standards Institute (CLSI) VET-03 guidelines for *L. garvieae*. Product Fitobaxin was tested at concentrations ranging from 2.5% to 0.0049%, whereas Active Fitobaxin was tested from 25% to 0.049%. A 0.5 McFarland suspension of each *L. garvieae* strain was used as the inoculum. Plates were incubated for 24 h at 22°C. MIC assays were performed in triplicate for each strain and product. In addition, the Minimum Bactericidal Concentration (MBC) was determined by subculturing aliquots from appropriate test wells onto blood agar plates and assessing the absence of bacterial growth after incubation.

Both MIC and MBC values could be determined for Active Fitobaxin, whereas for Product Fitobaxin only MIC values could be established. Indeed, bacterial growth was observed on blood agar following subculture from all strains at the highest Product Fitobaxin concentration tested (2.5%). All *L. garvieae* strains showed lower MIC values for Active Fitobaxin than for Product Fitobaxin. Statistical analysis confirmed a highly significant difference between the two products (p-value = 0.0010), indicating a very low probability that the observed difference was due to chance. The MIC₇₅ was 0.098% for Active Fitobaxin and 2.5% for Product Fitobaxin. Overall, this study provides an *in vitro* evaluation of the inhibitory and bactericidal activity of two plant-derived formulations against *L. garvieae*. Active Fitobaxin demonstrated markedly greater antibacterial activity than Product Fitobaxin, as reflected by the significantly lower MIC values observed across all tested strains. These findings support further investigation of phyto-genic products as potential complementary tools for the management of Lactococcosis in trout farming.

ESSENTIAL OILS AND THEIR BLENDS AS POTENTIAL ALTERNATIVES TO ANTIBIOTICS AGAINST *WEISSELLA CETI*: A PRELIMINARY IN VITRO STUDY

Fiocchi, E.^{1,2}, Vela Alonso, A.I.³, Fernández-Garayzábal, J.F.³, Caneschi, A.⁴, Scozzoli, M.⁴, Boscarato, M.¹, Cortinovis, L.¹, Toson M.¹, Dalla Pozza M.¹, Mattarelli, P.², Manfrin, A.¹

¹ Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD)

² Dipartimento Scienze e Tecnologie Agro-Alimentari, Alma Mater Studiorum - Università di Bologna, Bologna (BO)

³ Catedrático de Universidad. Dpto. Sanidad Animal. Facultad de Veterinaria - Universidad Complutense de Madrid, Madrid (Spain)

⁴ GreenVet[®], APA-CT Srl, Forlì (FC)

The increasing occurrence of bacterial diseases and the global spread of antimicrobial resistance (AMR) represent major challenges for human and veterinary medicine. In human medicine, essential oils (EOs) and their blends have received increasing attention as promising natural antimicrobial agents, with several studies reporting bacteriostatic and bactericidal activity against a wide range of bacterial pathogens. This interest has recently expanded to veterinary medicine, including aquaculture, where effective alternatives to conventional antibiotics are urgently needed. Reducing antibiotic use in aquaculture may contribute not only to improved disease management but also to limiting the release of antimicrobial residues into aquatic environments and reducing the selective pressure favouring the emergence and dissemination of AMR. The aim of this study was to provide a preliminary evaluation of the in vitro bacteriostatic and bactericidal activity of 15 EOs and two EO blends (provided by GreenVet[®]) against *Weissella ceti*. *W. ceti* is a Gram-positive bacterium associated with disease in aquatic animals, including cetaceans and rainbow trout (*Oncorhynchus mykiss*), but its presence has not yet been reported in Italy.

Three *W. ceti* strains retrieved from beaked whale were obtained from the University of Madrid. The strains were cultured on blood agar at 22°C and identified by MALDI-TOF mass spectrometry and PCR followed by Sanger sequencing of the 16S rRNA gene. For MIC determination in micromethod, Fifteen EOs, including, *Thymus vulgaris*, *Syzygium aromaticum*, *Cymbopogon nardus*, *Lavandula angustifolia*, *Cinnamomum zeylanicum* and other botanical sources. were tested using two-fold serial dilutions ranging from 6.25% to 0.012%. Two EO blends derived from commercial products B-RED and GILL FISH, were additionally tested at concentrations ranging from 12.5% to 0.024%. Cation Adjusted Mueller-Hinton Broth (CAMHB) supplemented with 5% Lysed Horse Blood (LHB) was used as the dilution medium, following the Clinical and Laboratory Standards Institute (CLSI) VET03 recommendations for Gram-positive cocci. Each well was inoculated with a 0.5 McFarland bacterial suspension. Plates were incubated at 22°C for 24 h. Minimum Inhibitory Concentration (MIC) was determined by visual assessment of bacterial growth. Minimum Bactericidal Concentration (MBC) was subsequently determined by subculturing aliquots from all wells showing no visible growth onto blood agar.

A clear antimicrobial effect was observed against all three *W. ceti* strains. Most promising Eos were clove (mean MIC 0.098%; Mean MBC 0.390%), *Cinnamomum zeylanicum* (mean MIC 0.195%; mean MBC 0.390%) and *Thymus vulgaris* (mean MIC 0.390%; mean MBC 0.780%). Importantly MBC values obtained for almost all EO-strain combinations suggest a predominantly bactericidal effect rather than solely growth inhibition at tested concentrations.

The present study provides preliminary but promising evidence of the in vitro antimicrobial potential of EOs and EO blends against *W. ceti*. The observation of both inhibitory and, for almost all combinations, bactericidal activity supports further investigation of these natural compounds as potential tools for reducing antibiotic dependency in aquaculture. Further research involving a larger number of *W. ceti* isolates and, potentially, *W. tructae* strains would strengthen the robustness and generalizability of the findings. Overall, these findings support the need for continued research into EO-based strategies within a One Health approach to sustainable aquatic animal health management.

OVERVIEW OF EMERGING AND RE-EMERGING PARASITES IN MEDITERRANEAN MARICULTURE

Apuzzo F.¹, Caffara M.¹, Tedesco P.¹, Gustinelli A.¹, Čolak S.²

¹ Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy

² Department of Ecology, Agronomy and Aquaculture, University of Zadar, Ulica Mihovila Pavlinovića 1, 23000 Zadar, Croatia

Mediterranean mariculture supports a wide diversity of cultured fish species, reared under conditions that may favour parasitic disease outbreaks. In recent decades, the sector has grown considerably, with species diversification becoming increasingly common alongside the traditional gilthead sea bream and European sea bass. This diversification, together with water exchange and the proximity of interconnected production units, may increase opportunities for parasite introduction and transmission. Fish and water movement between farms can facilitate the spread of parasites beyond their usual host or geographic range, while rising sea temperatures are increasingly recognized as affecting parasite life cycles. Given this shifting scenario, ongoing surveillance is essential for fish health management in the region. This study presents an updated overview of parasitic infections diagnosed in farmed Mediterranean fish over a multi-year period and discusses their implications for fish health, zootechnical performance, and pathogen interactions. Diagnostic cases received by the Fish Pathology Unit of the University of Bologna between 2020 and 2025 were reviewed. The cases originated from several Mediterranean mariculture facilities and included both well-established and newly diversified farmed species (e.g. greater amberjack, meagre and red drum). Each specimen underwent complete parasitological examination of the gills, skin, fins, and internal organs, while histological evaluation was carried out when indicated to characterize tissue lesions or investigate concurrent infections. A wide taxonomic range of parasites was identified. Monogeneans were the most recurrent and clinically important ectoparasites: *Neobenedenia girellae* affected gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), often causing skin and eye lesions, while *Sparicotyle chrysophrii* was widely found on the gills of gilthead sea bream, in which heavy infections impaired respiration. In greater amberjack (*Seriola dumerili*), *Zeuxapta seriolae* and *Benedenia seriolae* were significant gill and skin pathogens, while meagre (*Argyrosomus regius*) was affected by *Ktariella polyorchis* and *Sciaenacotyle panceri*. The caligid copepod *Caligus* sp. was also recorded on gilthead sea bream. Among endoparasites, the myxozoan *Enteromyxum leei* was confirmed in gilthead sea bream and, notably, in red drum (*Sciaenops ocellatus*), consistent with the only previous report of this parasite in the species. Clinical presentation varied widely, from subclinical infections to tissue damage, reduced growth, or, in severe cases, increased mortality. A relevant proportion of parasitized fish also presented concurrent bacterial infections, pointing to a bidirectional relationship: parasite-induced tissue damage and impaired host defences may favour secondary bacterial infections, while pre-existing bacterial disease may increase susceptibility to parasites. This highlights the importance of considering parasitic diseases within a broader multi-pathogen context. Overall, these results describe a parasitic disease landscape in Mediterranean mariculture that remains dynamic. Species diversification appears to be facilitating new host-parasite relationships, as suggested by the detection of *E. leei* in red drum, while the frequent overlap between parasitic and bacterial infections shows that disease often cannot be explained by a single pathogen alone. Rising water temperatures are likely to continue reshaping parasite distribution and transmission, potentially putting new areas or species at risk. These findings support the need for continuous surveillance combining parasitological and histopathological diagnosis, with attention to multi-pathogen interactions, to enable early detection and sustainable disease management.

MARINET - Monitoraggio integrato e intelligenza artificiale per la gestione del rischio in acquacoltura**MARINET - Integrated Monitoring and Artificial Intelligence for Risk Management in Aquaculture**

Galeotti Marco*, Tomè Paolo*, Portelli Beatrice*, Serra Giuseppe*, Zrnčić Snjezana**

**Dipartimento Di Scienze Agroalimentari Ambientali e Animali, University of Udine, Udine, Italy*

*** Dipartimento Di Scienze Matematiche, Informatiche e Fisiche, University of Udine, Udine, Italy Laboratory for Aquatic Animal Diseases, Croatian Veterinary Institute, Zagreb, Croatia*

The MARINET - Mariculture Network project, funded by the Interreg Italy-Croatia 2021-2027 Programme, aims to contribute to the development of a more sustainable and climate-resilient marine aquaculture sector by introducing innovative tools for health and welfare management. In the farming of species such as European seabream and European seabass, environmental conditions, animal health and welfare, and farm management practices are closely interconnected, highlighting the need for integrated approaches capable of collecting and combining information from multiple sources.

Within the MARINET project, an integrated digital system has been developed, combining a Progressive Web Application (PWA), a centralized data management infrastructure, and Artificial Intelligence (AI) models. The PWA enables farm operators to systematically record information on daily farming activities, feeding practices, and selected fish welfare indicators. These data are integrated with measurements automatically collected by sensors installed at the farming sites, meteorological and environmental data, and information derived from laboratory diagnostic activities.

The integration and standardization of these heterogeneous data sources provide structured datasets that can be used by AI-based models to identify relationships between environmental conditions, observed welfare and health indicators, and disease occurrence. The system is therefore designed not only as a data collection tool, but also as a decision-support platform, providing farmers and other operators with information for disease risk assessment and, ultimately, the development of early-warning tools.

Preliminary results highlight the value of the PWA in making farm-level data collection more systematic, standardized, and traceable, while progressively improving the quality and consistency of the information available for AI model development. Initial evaluations of the predictive models have also shown promising results in disease risk prediction and in the identification of disease-associated symptoms from fish images. Overall, the integration of real-time environmental monitoring, structured farm data collection, and Artificial Intelligence represents a promising approach to support more proactive, data-driven health and risk management in marine aquaculture.

THE DECEPTIVE NATURE OF COLOR CHANGES IN FISHERY PRODUCTS: SIMILAR APPEARANCES AND DIVERGING DIAGNOSES ACROSS PARASITOLOGY, PATHOLOGY, AND ANATOMY

Bianchi, G.B.M.¹; Ghisleni, G.¹; Corti, I.²; Grieco, E.³; Cappelleri, A.¹; G., Nieddu G.⁴; Malandra, R.¹; Giudice, C.¹

¹ Università degli studi di Milano, DIVAS, Lodi (LO); ² ATS Insubria, Costa Masnaga (LC); ³ ASL Salerno (SA); Direttore sanitario Acquario Civico di Milano, Milano (MI); ⁴ Università di Bologna, DIMIVET, Ozzano nell'Emilia (BO)

Under the legislation of the European "Hygiene Package", the drafting of the 2015 "Guidelines on the hygiene of fishery products" renewed the focus of veterinary inspectors and food business operators on visual inspection. Within this context, many colour alteration could be associated with the presence of parasites; however, upon closer examination this interpretation may prove incorrect. The aim of this study is to investigate such cases, specifically to determine when multifocal colour alterations, initially interpreted as parasitic lesions, require pathological and anatomical expertise for correct interpretation.

In the first phase of this study, the legislation regarding the inspection of fishery products was reviewed to contextualize colour alterations not attributable to parasites. Based on the available scientific literature, these alterations were subsequently categorized according to their macroscopic features. Finally, a retrospective study (2016-2026) was conducted using records from the Milan Fish Market, with the selection of corresponding photographic material and histological/cytological findings to verify their distribution across the aforementioned categories. A further classification assessed agreement or discrepancy between the initial parasitic hypothesis and the outcome of the in-depth diagnostic investigation.

At the legislative level, colour alterations in fishery products are not specifically regulated, and although they may fall within the broader category of organoleptic alterations, the assessment of fitness for human consumption is primarily entrusted to the judgement of the official veterinarian, in accordance with Regulation (EC) N° 178/2002, as subsequently reflected in the 2015 Guidelines on the Hygiene of Fishery Products. Photographic documentation was available for 16 of the 71 collected cases, allowing classification, based on the scientific literature, into six main gross morphological patterns: 1. red spot/focus (n°1 case); 2. black spot (n°5); 3. white/opaque spot; (n°2)

4. nodule/cyst-like lesion (n°4); 5. linear/dark structure (n°2); and 6. altered texture/discoloration (n°2). The cases were then further classified according to their final diagnostic interpretation into four main categories: 1) confirmed parasitic aetiology (n°7); 2) parasitic aetiology different from that initially hypothesized (n°2); non-parasitic pathological processes (n°5); and 4) normal anatomical structures (n°2).

This study demonstrates that the correct interpretation of colour alterations in fish flesh should be supported by histological or cytological confirmation whenever possible. These findings support a multidisciplinary approach to inspection activities, in which not only parasitological expertise but also in-depth knowledge of the pathology and anatomy of aquatic species may be essential for determining the origin of discoloration. By addressing the issue from a cross-cutting perspective within one of the most representative Italian contexts, and by providing extensive photographic documentation, this study offers a practical illustration of the practical relevance of such an integrated diagnostic approach, supporting the decision-making role of the official veterinarian.

PROLIFERATIVE THYROID LESIONS IN MARINE AQUARIUM FISH

Capparucci F.¹, Martino A.^{2,3}, Abbate J.M.¹, Cimino F.⁶, Iaria C.¹, Mandrioli L.⁴, Pesce G.⁵, Anjomanibenisi M.⁴, Brunetti B.⁴, Marino F.¹

¹Institute for Comparative, Experimental, Forensic and Aquatic Pathology (ICEFAP) "Slavko Bambir", Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;

²Aquarium of Genoa, Tropical Department, Costa Edutainment S.p.A., Area Porto Antico, Ponte Spinola, Genoa, 16128, Italy

³Aquaculture Research Group (IU-ECOQUA), University of Las Palmas de Gran Canaria, Carretera de Taliarte, s/n, Telde, Las Palmas, Canary Islands, 35200, Spain

⁴Department of Veterinary Medical Sciences, University of Bologna, Italy

⁵Aquarium of Genoa, Veterinary Department, Costa Edutainment S.p.A., Area Porto Antico, Ponte Spinola, Genoa, 16128, Italy

⁶Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;

Proliferative lesions of thyroid follicular cells in fish include hyperplasia, adenoma and carcinoma. Although already documented in many species, the description of these lesions provides valuable information for improving the management of the animals and their health. The aim of the study was to characterise proliferative thyroid lesions in aquarium fish and to provide further insights into the role of environmental factors in the pathogenesis. Tissue samples were collected from three individual fish belonging to three fish species: *Chelmon rostratus*, *Diplodus vulgaris* and *Apolectichthys xanthurus* reared at the Aquarium of Genoa and submitted for diagnostic evaluation. In *C. rostratus*, the mass was located in the oropharyngeal cavity, protruding from the right operculum, whereas in the other two fish, the lesions were observed on the left side. Macroscopically, masses appeared irregular, lobulated, firm, and reddish in colour. Histopathological examination revealed diffuse hyperplastic thyroid tissue in *C. rostratus* and *D. vulgaris*, characterized by variably sized and distended follicles containing colloid and lined by hyperplastic to hypertrophic follicular epithelial cells. In *A. xanthurus*, the lesion was predominantly characterized by a solid cellular proliferation, with complete loss of the normal thyroid follicular architecture. Based on the histopathological features, the lesions were diagnosed as nodular thyroid hyperplasia in *C. rostratus* and *D. vulgaris*, and thyroid solid carcinoma in *A. xanthurus*. All thyroid proliferations exhibited positive immunoreactivity for pan-cytokeratin (panCK), E-cadherin, and claudin-1, with variable expression of PCNA. In contrast, none of the lesions showed immunoreactivity for TTF-1. Furthermore, ion chromatography results from control seawater reported an iodide value of 98 µg/L, while iodide concentration in samples from affected tanks were below the method's LOQ (< 50 µg/L). Although these findings do not establish a causal relationship, reduced iodide availability was considered a potential contributing factor warranting further investigation. In recirculating or partially recirculating aquatic systems, iodine availability may be influenced by multiple interacting factors, including water-treatment processes, water-renewal regimes, diet and supplementation practices. These conditions support further investigation of iodine dynamics as one of the potential factors associated with thyroid proliferative disorders in RAS. These findings highlight the value of regular water quality and nutritional monitoring and of further studies aimed at defining optimal management strategies for different fish species and aquatic systems to prevent iodine depletion, including supplementation through both water and diet.

HISTOPATHOLOGICAL FINDINGS IN TRANSGENIC AND INBRED LINES OF MEDAKA (*ORYZIAS LATIPES*)

Falletti R.¹, Capparucci F.¹, Abbate J.M.¹, Vallone D.², Loosli, F.², Lanteri G.¹, Marino F.¹

¹Institute for Comparative, Experimental, Forensic and Aquatic Pathology "Slavko Bambir", Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy. ²Institute of Biological and Chemical Systems (IBCS-BIP), Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany.

The Japanese medaka (*Oryzias latipes*) offers complementary genetic resources for disease research, including targeted knockout lines and highly homozygous inbred lines such as those of the MIKK panel (Fitzgerald et al., 2022). This study aimed to characterise tissue lesions in two medaka knockout lines targeting two DNA repair factors, as well as in one MIKK inbred line at the ages examined, and to assess their potential for investigating genotype-phenotype relationships. Samples from knockout lines lacking genes encoding the DNA repair factors, together with samples from a selected MIKK inbred line, were submitted to the "Slavko Bambir" Institute for histopathological examination. Knockout fish were sampled at 6 and 12 months of age, although no 12-month specimens were available for one of the DNA repair factors knockout lines. MIKK fish were sampled at 6 and 18 months. Controls comprised two wild-type strains: iCab for the knockout lines and Kiyosu for MIKK line. Each group ($n = 8$) consisted of at least six adult fish. All animals were processed for histopathology using standard protocols. At 6 months of age, both DNA repair factor knockout lines exhibited a similar histopathological profile, characterised by moderate, multifocal neuropil vacuolation within the brain, and the underlying mechanism still remains undetermined. Intestinal lesions, including atrophy of the mucosal folds and multifocal inflammatory foci, were observed exclusively in one of the knockout lines, in which undigested *Artemia* cysts were found throughout the intestinal tract, including the distal segment. In the other knockout line, intestinal architecture remained well preserved despite the presence of *Artemia* cysts. At 12 months, fish from one DNA repair factor knockout line displayed severe brain vacuolation, likely representing a progression from the moderate spongiform changes observed at 6 months. In addition, a severe, chronic, multifocal myositis affecting the paravertebral musculature was present, a lesion not observed in younger specimens. Renal architecture was generally maintained, aside from the presence in all fish of a parasite within the collecting ducts of both the head and caudal kidneys, histologically consistent with *Trichodina* spp. Fish from the MIKK panel line were sampled following the observation of bilateral abdominal distension and impaired swimming. Grossly, enlarged kidneys caused the displacement of abdominal organs, such as liver, intestines, and gonads. Polycystic kidney disease was diagnosed in all specimens examined at 18 months and in two-thirds of those examined at 6 months. Cystic tubular dilatation and expansion of Bowman's spaces markedly disrupted renal architecture; dilated tubules contained abundant eosinophilic, protein-rich material admixed with necrotic cellular debris with occasional intraluminal dystrophic mineralization. Overall, distinct tissue phenotypes were identified across the lines examined: progressive neuropil vacuolation in both DNA repair factor knockout lines; late-onset myositis and intestinal lesions occurring in one of the knockout line; and polycystic renal disease in the MIKK panel line. These findings provide a basis for complementary investigations: testing the contribution of targeted genes to the lesions in knockout lines and identifying genetic determinants of the renal phenotype in MIKK panel line through forward genetics. The use of these models and their histopathological characterization provide valuable insights into the link between molecular alterations and the phenotypic expression of the disease at the tissue level. Thus, the medaka serves as an essential model for the study of complex diseases and as an important resource for the analysis of pathological mechanisms *in vivo*.

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APPLICATION OF PREOPERATIVE PHOTOGRAMMETRY FOR THREE-DIMENSIONAL DOCUMENTATION OF FIN PAPILLOMA SURGERY IN A GOLDFISH (*Carassius auratus*, (Linnaeus, 1758))

Nieddu G.¹, Bianco C.², Rovea C.³, Ancona N.¹, Volpe E.³, Ciulli S.³, Mandrioli L.³

1. Acquario e Civica Stazione Idrobiologica di Milano, Milano, Italy

2. Independent Researcher, Derbyshire, United Kingdom

3. Dipartimento di Scienze Mediche Veterinarie Alma Mater Studiorum, Università di Bologna, Bologna, Italy

Photogrammetry is an affordable three-dimensional (3D) imaging method that creates models from overlapping photographs by matching surface features across multiple views. In biological specimens, model accuracy depends on surface texture, color variation, and shape complexity; although it is increasingly used in veterinary teaching, diagnostics, and research, its application in veterinary surgery-especially fish pathology and aquatic medicine-remains largely unexplored. This report describes the application of preoperative photogrammetry in a goldfish (*Carassius auratus*) presenting bilaterally symmetrical exophytic masses affecting both pectoral fins, each measuring approximately $1 \times 1.5 \times 0.5$ cm. The case was selected for photogrammetry because of the gross characteristics of the masses, in terms of geometric complexity and texture. At presentation, the fish, approximately 5 years old and approximately 25 cm in length, was in excellent overall condition, with normal swimming behaviour and appetite, and showed no clinical signs attributable to the pectoral-fin masses. After sedation of the patient with tricaine methanesulfonate, anaesthesia was maintained by continuous oral perfusion of oxygenated anaesthetic water to ensure continuous gill irrigation and ventilation during the procedure. The overall procedure lasted approximately 40 min, including about 10 min for induction to a surgical plane of anaesthesia, followed by photogrammetric acquisition and laser surgical excision of the mass. Recovery was uneventful, and the fish resumed normal swimming within approximately 5 min. Cytological and histopathological findings were consistent with a cutaneous squamous papilloma. Virological investigations to detect possible viral agents are ongoing. Photogrammetry is a practical and portable tool for morphological and quantitative documentation of lesions in pathology but also potentially in surgical settings and can be used for teaching, research and diagnostic purposes. Furthermore, photogrammetry allows the generation and 3D printing of patient-specific models enhancing surgical planning, procedural decision-making and postoperative assessment. Its accuracy depends on high-quality image acquisition and can be compromised by poor lighting, reflections, blood contamination and motion artefacts; moreover, it reconstructs only external surfaces and does not reveal internal tissue architecture. To the authors' knowledge, this represents the first reported application of photogrammetry in the surgical management and three-dimensional documentation of a fin papilloma in a fish. Further studies involving a larger number of cases are warranted to evaluate the diagnostic and clinical utility and performance of this technique in veterinary surgery and aquatic animal pathology.

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MONITORING THE HEALTH STATUS OF THE MILAN AQUARIUM THROUGH CLINICAL HISTORY ASSESSMENT AND LABORATORY INVESTIGATIONS.

Nieddu, G¹. Ancona N.¹, Salogni C.²

¹ *Acquario e Civica Stazione Idrobiologica di Milano, Milano (MI)*

² *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia (BS)*

In ornamental aquaculture fish medicine, environmental conditions are integral to the patient; water quality, stocking density, feeding and social interactions must therefore be included in case assessment. Within the agreement between Milan Civic Aquarium and the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER), In this study, the term “disturbed-habitat” was considered a multifactorial etiopathogenic category, identified when environmental data, medical history, management records, and pathological findings indicated that an alteration in the housing system had contributed directly or indirectly to mortality, even in the presence of opportunistic infectious agents. Over one year, diagnostic activity addressed routine health assessments, “disturbed-habitat” events and abnormal mortalities in aquarium fish. A standardized environmental anamnesis form comprising 61 variables was integrated with clinical, necropsy and histopathological records. Sixty-one fish belonging to 20 species, including 14 marine and 45 freshwater specimens, were examined through 28 laboratory submissions. Standard laboratory bacteriological, parasitological and virological investigations were performed using standard culture, microscopic, phenotypic and molecular methods. “Disturbed-habitat” represented a major mortality category, together with physiological causes, undetermined causes and diseases associated with obligate or opportunistic pathogens. The most common related findings were acute or chronic gill damage (n=17), gas disease (n=17), asphyxia (n=8), inadequate feeding (n=7), ovarian retention (n=4), trauma (n=2) and conflict-related injuries (n=3). These conditions were associated with suboptimal water chemistry, overcrowding, nutritional imbalance and inadequate environmental enrichment. As a minor percentage isolation relevance, the isolation of such pathogens such as *Aeromonas salmonicida* ssp. *salmonicida* highlights the importance of the improvements of biosecurity system as quarantine procedure protocols for newly introduced fish. Integrating detailed environmental anamnesis with pathological examination improved interpretation of clinical signs and lesions, supported attribution of mortality causes and guided interventions on welfare, habitat management and biosecurity. This approach may provide a practical surveillance model for public aquaria and intensive aquaculture systems such as RAS (Recirculating Aquaculture System).

FIRST REPORT OF DERMAL FIBROMYXOMA IN GREATER AMBERJACK (*SERIOLA DUMERILI*): HISTOPATHOLOGICAL AND HISTOCHEMICAL FINDINGS

Piro, M.G.¹, Capparucci, F.¹, Martino, A.^{2,3}, Pesce, G.⁴, Marino, F.¹, Iaria, C.¹

¹ Institute for Comparative, Experimental, Forensic and Aquatic Pathology (ICEFAP) "Slavko Bambir", Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;

² Aquarium of Genoa, Tropical Department, Costa Edutainment S.p.A., Area Porto Antico, Ponte Spinola, Genoa, 16128, Italy;

³ Aquaculture Research Group (IU-ECOQUA), University of Las Palmas de Gran Canaria, Carretera de Taliarte, s/n, Telde, Las Palmas, Canary Islands, 35200, Spain;

⁴ Aquarium of Genoa, Veterinary Department, Costa Edutainment S.p.A., Area Porto Antico, Ponte Spinola, Genoa, 16128, Italy.

Fibromyxoma is a rare benign mesenchymal neoplasm originating from dermal fibroblasts, characterized by a dual fibrous and myxoid stromal component. In fish, its occurrence has been documented in a limited number of species, including mullet (*Mugil cephalus*), tench (*Tinca tinca*), flower horn cichlid, and turquoise killifish (*Nothobranchius furzeri*).

This study describes the first case of fibromyxoma in a greater amberjack (*Seriola dumerili*) reared at the Aquarium of Genoa. An adult female, measuring 150 cm in length and weighing 37 kg, presented with a slow-growing submandibular mass that had been present for at least five years. The mass was collected during routine health monitoring, fixed in 10% neutral buffered formalin, and submitted for histopathological examination. Macroscopically, it was solid and greyish-white, with a focal haemorrhagic area, and appeared homogeneous, whitish, and highly vascularized on sectioning. No evidence of necrosis or infiltration into deeper tissues was observed.

Histologically, the neoplasm was confined to the dermis and showed alternating fibrous and myxoid areas. The fibrous component consisted of spindle-shaped cells embedded in abundant collagenous stroma, whereas the hypocellular myxoid areas contained scattered stellate cells within an abundant extracellular matrix. Masson's trichrome confirmed the collagen-rich nature of the fibrous component, while Toluidine Blue demonstrated metachromasia of the myxoid matrix, consistent with the presence of acidic mucopolysaccharides and glycosaminoglycans. Alcian Blue-PAS (pH 2.5) further supported the mucinous nature of the matrix.

The absence of cellular atypia, mitotic figures, necrosis, and infiltrative growth supported a benign biological behaviour. The coexistence of well-defined fibrous and myxoid components, together with the dermal localization and histochemical findings, supported the diagnosis of fibromyxoma and its distinction from fibroma, myxoma, fibrosarcoma, and myxosarcoma. To our knowledge, this represents the first report of fibromyxoma in *S. dumerili*, expanding the spectrum of mesenchymal neoplasms described in teleosts and providing additional morphological and histochemical criteria for the recognition of this rare tumour.

Evaluation Feed Supplements From Marine and Herbal Origin Containing Bioactive Compounds in Free and Microencapsulated forms in *Cyprinus carpio*

Hafiz Shahryar^{1&2}, Natale Sabrina², Calinoiu Lavinia Florina⁴, Vodnar Dan Cristian⁴, Chrysanthos Stergiopoulos⁵, Magdalini Krokida⁵, Papadaki Sofia⁶, Moustogianni Anna⁷, Capparucci Fabiano², Iaria Carmelo², Marino Fabio².

¹University School for Advanced Studies IUSS Pavia, Palazzo del Broletto, Piazza della Vittoria 15, 27100 Pavia, Italy

²Institute for Comparative, Experimental, Forensic and Aquatic Pathology (ICEFAP) "Slavko Bambir", Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy, Messina, Italy.

³Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy, Messina, Italy.

⁴Department of Food Science, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 400372, Cluj, Romania.

⁵National Technical University of Athens, School of Chemical Engineering, Laboratory of Process Analysis and Design, Zografou Campus, GR-15700 Athens, Greece.

⁶Dignity Private Company, 30-32 Leoforos Alexandrou Papagou, Zografou 157 71, Athens, Greece.

⁷Zoonomi S.A., Perigiali Korinthias, Greece.

Use of feed supplements in aquafeed is considered a beneficial strategy to enhance the overall health status of farm fish. Bioactive compounds are considered sensitive and unstable. Efficient delivery of bioactive compounds could enhance their stability. Microencapsulation could be of paramount importance to fully exploit their potential. Main objective of this research was to evaluate the first screening of microencapsulation of feed supplements containing bioactive compounds from marine and herbal origins on the growth performance, intestinal and hepatic tissue histomorphology as well as antioxidant gene expression analysis of common carp (*Cyprinus carpio*)

Three 60-day-feeding trials were conducted to evaluate the effects of microencapsulation of spirulina (SP, *Arthrospira platensis*), olive leaf extract (OLE, *Olea europaea*), and lemon peel extract (LE, *Citrus limon*). Trial 1 evaluated SP, trial 2 evaluated OLE, and trial 3 evaluated LE. At the end of trials animals were sacrificed and organs were collected to perform growth, histological and antioxidant gene expression analysis. In vivo procedures have been performed according to D.Lgs. 26/2014 and Italian Ministry of Health authorization n. 421/2025-PR.

Trial 1 showed that final weight is significantly higher in microencapsulated SP(SP-EC) as compared with other treatments. Mucosal fold height showed significant improvement in control and SP-EC. Number of goblet cells were significantly higher in both SP treatments as compared with control (CTRL). No steatosis and any damage were found in the hepatocytes among all treatments. SOD genes showed significant difference in the SP as compared with CTRL and SP-EC. CAT and GPx did not show any significant difference among treatments. Trial 2 revealed that final weight, mucosal fold height, mucosal width and number of goblet cells were statistically significant in microencapsulated OLE (OLE-EC) as compared with other treatments. Hepatic tissue did not show any alteration, steatosis and nucleus displacement among all treatments. CAT showed significant difference in the OLE-EC as compared to both OLE and CTRL. SOD and GPx revealed no significant differences among treatments. Trial 3 showed different trends. Final weight is significantly higher in both LE and LE-EC as compared with control treatment. No intestinal and hepatic tissue damage were observed among treatments. CAT showed significant upregulation in LE-EC as compared with LE. However, CAT did not show any significant difference between LE-EC and CTRL or LE and CTRL. No significant difference was found for other genes including SOD and GPx in this trial.

It can be concluded from the first screening of microencapsulation that SP and OLE exerted positive effects when inserted with microencapsulation in *Cyprinus carpio* fed. While LE is showed positive effects in both forms.

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NECTO - THE SHIFT TOWARDS RESILIENCE

Maurizio A.

Skretting Italia SpA, Mozzecane (VR)

Improving fish survival is deemed a top priority by the finfish industry, to improve productivity, maintain license to operate, secure reputation and drive sustainable practices. Survival can be impacted by infectious diseases, compromised gills, parasite management, environmental issues, handling, stress and extreme water temperatures. This multitude of factors has steered the industry towards preventive and proactive health management (contra reactive management) to mitigate health challenges before they impact productivity and profitability.

Survival rates in the finfish industry are not increasing to any significant degree. In recent years, the incidence and prevalence of chronic inflammatory conditions (associated with the heart, gills, gut and muscles) have increased, which can induce a substantial metabolic burden on the fish and is both a welfare and productivity concern. In addition, the spotlight is rightly being more firmly placed on fish welfare and well-being, with greater emphasis toward fish resilience and robustness throughout production.

Overall, these factors and developments constitute a level of complexity in finfish health management that requires novel, innovative and effective solutions to turn the tide and improve the well-being of farmed finfish. Complex challenges require “complex” solutions that target multiple pathways and mechanisms, and a holistic approach is needed where functional feeds become commonplace and an integral tool in proactive health management strategies.

Necto is the innovative Skretting functional feed based on proprietary phytotechnology which provide enhanced support towards key physiological processes in finfish. Necto therefore offers a broad functional support toward fish health, improving fish resilience specifically towards complex inflammatory issues. This synergistic action is aimed at mitigating health risks, empowering the fish to meet challenges through a stronger defence.

Assessing the impact of environmental pollutants as endocrine disruptors: gene expression analysis in zebrafish (*Danio rerio*) larvae

Fabrizi F.^{1,2}, Ferrau F.³, Natale S.⁴, Capparucci F.⁴, Blanca E.S.³, Bottari T.^{2,5}, Giannetto A.⁶, Iaria C.⁴, Cannavò S.³, Marino F.⁴

¹*Scuola Universitaria Superiore IUSS Pavia, Piazza della Vittoria 15, Pavia, 27100, Italy*

²*Institute for Marine Biological Resources and Biotechnology (IRBIM-CNR), San Raineri 86, 98122, Messina, Italy*

³*Department of Human Pathology of Adulthood and Childhood "G. Barresi", University of Messina, Messina, Italy*

⁴*Institute for Comparative, Experimental, Forensic and Aquatic Pathology (ICEFAP) "Slavko Bambir", Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy, Messina, Italy.*

⁵*National Biodiversity Future Center (NBFC), Piazza Marina, 61, 90133, Palermo, Italy*

⁶*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy, Messina, Italy.*

Environmental pollutants acting as endocrine-disrupting chemicals (EDCs) interfere with the endocrine system by mimicking, blocking, or altering hormone functions, leading to developmental disorders, immune dysfunction, and increased cancer risk. Global warming exacerbates this issue by increasing the release, persistence, and bioaccumulation of harmful chemicals such as polychlorinated biphenyls, pesticides, and phthalates, which bind to the aryl hydrocarbon receptor (AhR), disrupting hormonal balance and potentially promoting tumorigenic pathways, including pituitary tumor formation (Cui et al., 2023). The zebrafish (*Danio rerio*) is a valuable in vivo model for investigating the toxicological effects of environmental pollutants due to its genetic homology with humans, rapid development, and transparent embryos (Bhagat et al., 2020).

This study aimed to evaluate the effects of three environmental pollutants on gene expression in zebrafish larvae: Benzene (BZ), Aroclor 1254 (AR), and Di(2-ethylhexyl) phthalate (DEHP).

A preliminary zebrafish embryo toxicity test was conducted to determine suitable exposure ranges. Based on embryo survival, hatching success, and developmental abnormalities, three concentrations were selected for the definitive experiment: BZ (90, 120, and 150 mg/L), AR (0.25, 0.75, and 1.25 mg/L), and DEHP (100, 200, and 500 mg/L). Embryos were exposed until 120 h post-fertilization, and gene expression was assessed by RT-qPCR in pools of six larvae, with five independent biological replicates per condition. The target genes included AHR1a, AHR2, AIP, SSTR2a, SSTR2b, SSTR5, CY1a, GH1, Plag1, Plag2, and PlagX. Untreated controls and compound-specific methanol vehicle controls were included, and expression values were corrected when a significant solvent effect was detected.

BZ significantly affected AHR2 expression after correction for the methanol contribution (Kruskal-Wallis, $H = 13.3$, $P < 0.01$), with increases at 120 and 150 mg/L compared with CTRL ($P < 0.01$ and $P < 0.001$). A similar pattern was observed for Plag1 (ANOVA, $F = 8.9$, $P < 0.01$), with significant increases at 120 and 150 mg/L ($P < 0.01$ and $P < 0.001$). AR significantly affected AHR2 expression (Kruskal-Wallis, $H = 11.3$, $P < 0.05$), with increases at 0.25, 0.75, and 1.25 mg/L ($P < 0.05$, $P < 0.05$, and $P < 0.001$). Plag1 showed a similar response (ANOVA, $F = 15.4$, $P < 0.001$), with significant increases at all concentrations ($P < 0.001$). DEHP produced a more restricted transcriptional response, mainly affecting the somatostatin-growth hormone axis. SSTR2a expression was significantly altered (Kruskal-Wallis, $H = 8.1$, $P < 0.05$), with an increase at 100 mg/L ($P < 0.05$). GH1 expression was also significantly affected among groups (ANOVA, $F = 5.9$, $P < 0.01$), with an increase at 100 mg/L ($P < 0.01$).

Overall, these findings demonstrate that environmental pollutants significantly alter endocrine-related and pituitary-associated gene expression in zebrafish larvae. This supports the use of the zebrafish larval model for translational research on pollutant-induced endocrine disruption and provides preliminary insight into molecular pathways potentially involved in pituitary dysfunction.

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This study was funded by PRIN 2017 - "Identification of new biomarkers and clinical determinants for management improvement of patients with pituitary tumor related syndromes" (2017S55RXB)

ASSESSMENT OF ANTIMICROBIAL USAGE IN RAINBOW TROUT (*Oncorhynchus mykiss*, Walbaum, 1792) FARMS OF TRENTO AND UDINE PROVINCES (ITALY)

Franzago E.¹, Melotto G.¹, Toson M.¹, Ambrosi A.¹, Donini S.¹, Dalla Pozza M.¹, Bille L.¹, Callegaro A.¹, Mazzucato M.¹, Toffan A.¹, Manfrin A.¹, De Zan G.¹, Dellamaria D.¹, Bortolotti L.², Baracetti G.³, Fabris A.⁴, Di Martino G.¹

¹Istituto Zooprofilattico Sperimentale della Venezia, Legnaro, PD; ²Azienda Sanitaria Universitaria Integrata del Trentino (ASUIT), Trento, TN; ³Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine, UD; ⁴Azienda Sanitaria Friuli Occidentale Pordenone PN

Aquaculture is considered a particularly critical sector amongst animal production concerning antimicrobial usage (AMU) and its potential impacts on animal health and the surrounding environment. In Italy, official AMU data are not systematically collected, therefore this research project aims to quantify AMU, through the development of a standardised methodology potentially applicable at national level, and to estimate any trend and influencing factors of antimicrobial consumption in the provinces of Trento and Udine, the main areas of rainbow trout farming in the North-East of Italy.

A list of rainbow trout grow-out farms in Udine and Trento was compiled using the National Database of Livestock Establishments, it was then validated by local veterinary authorities (ASUIT/ASUFC) and the Italian Fish Farmers' Association (API). ASUIT and ASUFC provided data on pharmacological treatments carried out in the selected establishments over the three-year period 2022-2024. Production data of each farm and year, expressed in kilograms of slaughtered and/or marketed fish, were provided by API. AMU was calculated using a dose-based methodology, which makes it possible to take into account the potency of each commercial formulation in relation to a specific treatable biomass, known as the Population Correction Unit (PCU).

Overall, data were collected from 57 farms. AMU values can be interpreted as the estimated number of treatment-days per animal equivalent, based on the treated biomass and the DDDAit (Defines Daily Dose tailored for Italy) assigned to each antimicrobial. The results show high variability between different farms as well as within the same farm over the years: there are indeed years in which no pharmacological treatments were administered and others in which the AMU value dramatically increased. Due to this variability, the median is the most appropriate summary measure to describe the data. For both Udine and Trento, the annual provincial median AMU corresponded to no more than 3 treatment-days per animal equivalent. When evaluating the presence of a trend, the data revealed an increasing trend in median values for the province of Udine (which, however, was not significant according to the median regression test applied on only AMU values >0, p-value = 0.341). In contrast, the province of Trento showed an initially increasing and subsequently decreasing trend, which however was not statistically significant (p-value = 0.132).

In the statistical analysis carried out to identify potential factors associated with AMU, none of the variables considered (water source, risk level, biosecurity level, technological level, vaccine usage, or presence of multiple salmonid species) showed a significant association. Assessments incorporating environmental (rainfall and temperature) and geographic (distance from other establishments and altitude) variables are currently ongoing.

In conclusion, this study highlighted the feasibility of applying a dose-based approach to calculate AMU in trout farms. Overall, AMU remained low in both provinces, although substantial variability between farms and years was observed, with no identified variable explaining these differences. Further studies are required to investigate these differences in depth.

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SUPPORTING AQUATIC ANIMAL HEALTH DATA INTEGRATION IN SALMONID FARMS: A GEOGRAPHIC INFORMATION SYSTEMS APPROACH

Macario R.¹, Casarotto C.¹, De Conti G.¹, Bonato P.¹, Franzago E.¹, Bille L.¹, Di Martino G.¹, Ferrè N.¹, Mazzucato M.¹

¹*Istituto Zooprofilattico Sperimentale delle Venezie - GIS Unit. Viale dell'Università 10, 35020 Legnaro (PD) - Italy.*

Geographic Information Systems (GIS) provide a useful way to bring together farm-level biosecurity measures, antimicrobial usage, and the hydrological context in which salmonid farms are located. In this study, GIS was used to examine how farms are positioned within the hydrographic network and how these positions may be related to possible patterns of water connectivity between establishments. This kind of spatial approach can support a better understanding of issues that are relevant for aquatic animal health (AAH) surveillance.

The study refers to salmonid farms located in north-eastern Italy, focusing on the Autonomous Province of Trento and the Province of Udine. It integrates data on hydrological network, farm locations, farm-level points of interest (such as water inlets and outlets), biosecurity data, and antimicrobial usage information were integrated into a dedicated geodatabase. PostgreSQL Relational Database Management System with PostGIS and pgRouting extensions were used. GIS-based tools developed in R were applied to analyse water connectivity, exploring hydrological pathways linking farms and their characteristics across the study area.

The application of GIS made it possible to generate maps and spatial outputs that organised biosecurity scores and pharmacological information in relation to farm location and hydrological connectivity, offering useful material for further evaluation by local health authorities (LHA) and decision-makers. The output from the GIS-based tools is provided in GeoPackage, a widely supported file format that allows users to access and use them in commonly available GIS software for further analysis and map production.

This work highlights the role of GIS not only in mapping aquaculture systems, but also in combining and analysing heterogeneous information in a more structured way. Bringing together biosecurity, pharmacological and spatial data within the same framework, helped to read farm-level differences in relation to the surrounding hydrological setting. This system is very useful for conducting epidemiological investigations following an outbreak. Although the study was developed for salmonid farms, the same approach could also be adapted to other aquaculture contexts where water connectivity is an important factor.

This GIS-based framework offers a practical way to integrate and analyse farm-level information within a hydrological context. It supports a clearer spatial understanding of farm distribution, hydrological relationships, and data organisation, and may be useful for surveillance-related applications in AAH.

This research is funded by the Ministry of Health, RC 06/24 "Assessment of environmental, management, and structural factors for reducing antibiotic consumption in farming and improving the health and welfare of rainbow trout (TROUTment)" (CUP: B23C24000880001).

03

ABSTRACT

Poster

COMPARATIVE PARASITOLOGICAL SURVEY OF WILD AND FARMED SALMONIDS

Benetti S.¹, Apuzzo F.¹, Gustinelli A.¹, Tedesco P.¹, Caffara M.¹

¹Department of Veterinary Medical Sciences (DIMEVET), Alma Mater Studiorum University of Bologna, via Tolara di Sopra, 50, Ozzano dell'Emilia (BO) 40064, Italy

A parasitological survey was carried out on wild and farmed salmonids in the Brenta River drainage basin to evaluate potential epidemiological correlations between the two groups.

From September 2025 to May 2026, a total of 52 trout were examined, including 15 wild and 37 farmed specimens. All farmed fish were rainbow trout (*Oncorhynchus mykiss*), whereas the wild fish comprised 12 *Oncorhynchus mykiss* and 3 brown trout (*Salmo trutta*). Wild trout were captured by rod fishing at sites upstream of the fish farm, with the aim of investigating their potential role as a reservoir of parasitic infections affecting farmed fish. All fish underwent necropsy and parasitological examination. Skin and gill scrapings were collected and examined under a light microscope to detect ectoparasites. Kidney samples were also collected for molecular detection of *Tetracapsuloides bryosalmonae*. Samples from specimens showing skin lesions suggestive of fungal infections were collected for subsequent culture and molecular analysis. Macroparasites specimens detected during the examination were also collected and preserved in 10% buffered formalin or 70% ethanol for subsequent analysis and identification. Parasite identification was performed using different diagnostic approaches. *Gyrodactylus* sp. and *Piscicola geometra* were identified by light microscopy, while *Cystidicola farionis*, *Pomphorhynchus laevis*, and *Cyathocephalus truncatus* were examined using both light microscopy and scanning electron microscopy (SEM). For *Tetracapsuloides bryosalmonae*, DNA was extracted from kidney samples, followed by PCR amplification and agarose gel electrophoresis. Suspected *Saprolegnia* spp. infections were isolated in culture, followed by DNA extraction, amplification, and sequencing for species identification. The results showed clear differences in parasite prevalence between wild and farmed salmonids. Wild fish were mainly characterized by *Cystidicola farionis* (93.3%), *Pomphorhynchus laevis* (86.7%), and *Cyathocephalus truncatus* (53.3%), while *Gyrodactylus* sp. was the most prevalent parasite in farmed fish (63.0%). *Cystidicola farionis* was detected in both groups, with a prevalence of 93.3% in wild and 8.1% in farmed fish, and in these last ones the positive results concerned the larger specimens. *Tetracapsuloides bryosalmonae* was also detected in both groups (46.7% and 21.7%, respectively), as was *Saprolegnia parasitica* (6.7% and 3.7%, respectively). *Piscicola geometra* was detected only in farmed fish, with a prevalence of 3.7%. The results revealed marked differences in parasite community composition. Wild salmonids showed greater parasite diversity, dominated by endoparasites with indirect life cycles, likely associated with feeding habits and the predation of aquatic invertebrates. In contrast, farmed specimens were mainly infested by ectoparasites with direct life cycles, particularly *Gyrodactylus* sp., whose high prevalence appears to be associated with the high stocking densities typical of intensive farming systems. The occurrence of *Tetracapsuloides bryosalmonae*, *Saprolegnia parasitica*, and *Cystidicola farionis* in both groups suggests a possible epidemiological connection between wild and farmed fish, with wild populations potentially contributing to the maintenance of parasite life cycles. These findings highlight the importance of integrated monitoring of both wild populations and aquaculture facilities to improve biosecurity and health management in trout farming.

Infection trial of *Lactococcus garvieae* inoculated in gilthead sea bream (*Sparus aurata*) maintained under controlled conditions in a closed recirculation system (RAS)

Bignami G.¹, Gustinelli A.¹, Acosta F.²

¹ Department of Veterinary Medical Sciences (DIMEVET), Alma Mater Studiorum - Università di Bologna, Bologna, Italy.

² Grupo de Investigación en Acuicultura (GIA), Instituto Ecoaqua, Universidad de Las Palmas de Gran Canaria, Spain.

In recent years, *Lactococcus garvieae* has attracted increasing interest due to its impact on the Mediterranean fish farms, causing heavy losses. In particular, in Italy, since the summer of 2023 Lactococcosis has begun to cause significant mortality episodes in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) farms, giving rise to serious impacts economic sustainability of affected farms. *Lactococcus garvieae* is a pathogen capable of persisting in the environment and causing disease in farmed fish when temperatures begin to rise. This study is part of a larger experimental *in vivo* trial conducted at the University of Las Palmas, in which a probiotic-based diet was used as a preventative measure, administered to gilthead sea bream experimentally infected with *L. garvieae*. The aim of this work was therefore to obtain a reactivated pathogen and calculate the LD50 in gilthead sea bream fed a commercial diet and maintained in a controlled environment. The experimental study was designed based on previous studies (Akaylı et al., 2022), a total of 120 gilthead sea bream with an average body weight of 130 ± 10 gr, were obtained from the facilities of the University of Las Palmas. The fish were acclimated for one week and divided into 4 different RAS fed with natural seawater at a temperature of $23^{\circ}\text{C} \pm 1$ before the experiment. *L. garvieae* was isolated from diseased gilthead sea bream from an Italian farm at the DIMEVET laboratory of Fish Pathology Unit during the 2024 summer season. The infection test was conducted at the facilities of the University of Las Palmas. After being thawed and seeded in BHI agar (SRL) supplemented with 1.5% NaCl at 26°C for 24 hours, the CFU/ml counts were carried out using the serial dilution method to arrive at 3 different concentrations: 10^4 CFU/ml, 10^6 CFU/ml and 10^8 CFU/ml. To reach the effective concentration, a fresh colony of *L. garvieae* was used and serial dilutions were made after 2, 5, 8, 24 and 48 hours at 26°C of incubation. Once the timing for obtaining the 3 different desired concentrations had been established, gilthead sea breams were divided into 4 different groups, 1 for control and 3 to be infected. The animals were fasted the day before the inoculations. On the day of inoculation, they were anesthetized with clove oil and injected intraperitoneally with 100 μl of the 3 different doses each and the control group with PBS. After 5 days from inoculation, the fish did not die even at the highest concentration but at doses of 10^6 and 10^8 CFU/ml showed signs of hypermelanosis and lethargy, all groups after infection were inappetent., therefore they were sacrificed and *L. garvieae* was isolated from all the inoculated organs (brain, eye, kidney and spleen) in large quantities from each fish in the group at the highest concentration. From the plates, *L. garvieae* isolated from the eye was brought forward in purity. A fresh colony of the reisolated pathogen was then incubated in TSB supplemented for 8 hours to obtain 10^7 CFU/ml to have with the active pathogen, inoculating 100 μl to obtain an inoculum of 10^6 CFU/ml. After the challenge, the fish stopped eating and began to experience mortality after 48 hours. After 13 days from the experimental infection, a survival rate of 50.4% was achieved and after 30 days, at the end of the observation, the survival rate was 37.60%. The fish stopped feeding immediately, the dead and dying showed externally unilateral and bilateral exophthalmos, hypermelanosis, hemorrhages in the body and lethargy. his trial demonstrates that the pathogen, reactivated under appropriate conditions, regains virulence, reaching the LD50 in 13 days at 23°C . This data is optimal for the development of challenge tests with *L. garvieae* even when maintained in the laboratory years after the initial isolation.

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SWIM BLADDER FLOODING IN KOI CARP (*CYPRINUS CARPIO*): DIAGNOSTIC WORK-UP AND OUTCOME OF A CLINICAL CASE

Bonazza Foselli, M.¹, Leone, V.F.², Mandrioli, L.³

¹ Ambulatorio Veterinario Dalmazia, Firenze

² Centro Oncologico Veterinario, Sasso Marconi (BO)

³ Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum Università di Bologna, Bologna

Swim bladder disorders are among the most frequent clinical presentations in ornamental cyprinids, but their aetiology is still poorly characterised. Swim bladder flooding, i.e. the replacement of gas by fluid, is a common endpoint of mechanical, infectious, neoplastic and developmental conditions and causes loss of buoyancy control. We report the work-up, treatment and outcome of a bilateral case in koi carp.

A two-year-old female koi carp (1.265 kg), imported from Japan, was presented four months after introduction into a garden pond with bilateral swelling of the caudal trunk, lethargy and persistent bottom-sitting; appetite was preserved. Water quality was within range (20 °C; dissolved oxygen 10 mg/L; ammonia, nitrite and nitrate not detectable; pH 7.3; hardness 110 mg/L); the remaining stock was unremarkable. The fish was anaesthetised by immersion in alfaxalone (induction 10 mg/L, maintenance 2 mg/L) with branchial irrigation while out of water.

Radiography (60 kV, 15 mAs, 45° lateral recumbency) showed a ventral radiopaque area in both chambers. Ultrasonography (6.5 MHz micro-convex probe) confirmed non-corpusculated hypoechoic content; computed tomography (64-slice, 120 kVp, 248 mAs, 1.25 mm) with 3D reconstruction defined the extent of the flooding and ruled out coelomic masses, retained eggs and skeletal lesions. About 80 mL of clear citrine-yellow fluid were removed by ultrasound-guided aspiration (18 G). Aerobic culture yielded moderate growth of *Shewanella putrefaciens*, while anaerobic culture was negative. The isolate was susceptible to ceftiofur, aminoglycosides, fluoroquinolones and potentiated sulphonamides, and resistant to penicillins, tetracyclines and macrolides. Plasma biochemistry showed hyperglycaemia consistent with handling and anaesthetic stress, with unremarkable hepatic and renal parameters.

After complete drainage, lavage of the swim bladder and local administration of an antibiogram-guided antimicrobial with an anti-inflammatory drug, clinical signs and swelling resolved. At the last follow-up, approximately five years after treatment, the fish did not show clinical signs, had normal buoyancy and had never relapsed.

The patient was a Platinum Ogon, bought as a nisai the year before for € 500. Koi are traded worldwide as high-value ornamental fish, and their commercial value sustains a growing demand for individual clinical medicine and for advanced diagnostics in this species (Andrian et al., 2024).

Combining imaging with ultrasound-guided fluid sampling allowed the aetiological characterisation of the lesion: flooding is a syndrome rather than a diagnosis, and culture-guided therapy may achieve complete recovery even when both chambers are involved.

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CO-CIRCULATION OF CYPRINID HERPESVIRUSES (CYHV-1, CYHV-2) AND CARP EDEMA VIRUS (CEV) IN KOI CARP (*CYPRINUS CARPIO*) FROM A PRIVATE POND IN ITALY

Ciurli G.¹, Coltraro M.¹, D'Alonzo A.¹, Ricci E.¹, Di Blasi L.¹, Cocumelli C.^{1*}, Acri C.^{1*}, Parmigiani R.^{1*}, Bovi E.^{1*}, Bonazza Foselli M.², Susini F.¹

¹ Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Pisa (PI), Italy

^{1*} Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy

² Ambulatorio Veterinario Dalmazia, Firenze (FI), Italy

Cyprinid herpesviruses 1 and 3 (genus *Cyprinivirus*, family *Alloherpesviridae*: CyHV-1, and -3) and Carp Edema Virus (family *Poxviridae*; CEV) are major viral pathogens of common carp and koi (*Cyprinus carpio*); CyHV-2, the causative agent of HVHN and now classified as *Cyprinus carpio* 2 (genus *Cyprinus*, family *Alloherpesviridae*) can also infect common carp and koi, which act as asymptomatic carriers. CEV causes koi sleepy disease (KSD), an emerging infection characterized by lethargy, generalized edema, and severe branchial lesions with gills as the primary target organ for viral replication. Here we describe a mortality event occurred in a private garden pond housing a population of five ornamental koi (*Cyprinus carpio*), consisting of three young-of-the-year (*tosai*, <1 year-old) and two two-year-old (*nisai*) specimens. The introduction history indicated sequential additions to the pond: two fish were introduced approximately two months prior to the outbreak, two fish one month prior, and the final specimen within the same month mortality began. Over a five-day period, the first two fish introduced, died. Clinical signs preceded mortality by six days in the first affected individual and included inappetence, lethargy, respiratory distress characterized by surface piping, and progressive branchial lesions. Post-mortem examination performed on a freshly dead carcass of one of the affected fish revealed hemorrhagic suffusions at the base of the fins, pronounced enophthalmos and severe erosion of the barbels. Free blood within the oral cavity and hemorrhagic gills with multifocal to coalescing necrotic foci were observed; a distended gallbladder and an empty intestinal tract containing minimal mucoid fecal material completed the picture. Gills samples were processed for molecular examination followed by Sanger sequencing, that revealed the presence of Cyprinid herpesvirus 2 (CyHV-2, showing 100% sequence identity) and a positive result for Carp Edema Virus (CEV). Microbiological culture yielded consistent isolation of *Aeromonas* spp., indicating a secondary opportunistic bacterial infection secondary to primary viral gill damage. Histological examination of gills revealed extensive, multifocal to coalescing areas of marked secondary lamellar hyperplasia and epithelial fusion interspersed with necrotic foci. A diffuse, moderate to dense heterophilic infiltrate was present throughout the lamellar stroma. Furthermore, numerous monogeneans morphologically consistent with *Dactylogyrus* sp. were identified in interlamellar spaces, and dense populations of flagellated protozoans consistent with *Ichthyobodo* sp. were observed adhering to the hyperplastic epithelium. Gills from three additional live/co-habiting koi from the same pond - a 1-year-old *tosai* (32 cm), a 2-year-old *nisai* (43 cm), and a second *tosai* (31 cm) - were sampled for molecular analysis because all three fish had also begun to show clinical signs, although none of them died.

The 31 cm *tosai* tested positive for Cyprinid herpesvirus 1 (CyHV-1, exhibiting 90% sequence identity) while the 43 cm *nisai* tested positive for CEV. This outbreak documents a complex, multi-etiological infection in an ornamental koi population underlying the crucial necessity of integrating gross pathology, histopathology, molecular diagnostics, parasitology and bacteriology when investigating mortality events in ornamental cyprinids, as single-pathogen diagnoses may miss complex co-infections.

INTRAHEPATIC INFILTRATIVE LIPOMA IN A GIANT RED-TAILED GOURAMI (*OSPHRONEMUS LATICLAIVUS*): A NOVEL SITE FOR TELEOST ADIPOCYTIC NEOPLASIA

Coltraro, M.¹, Cocumelli, C.^{1*}, Nicolosi, P.², D'Alonzo, A.¹, Ricci, E.¹, Ciurli, G.¹, Di Blasi L.¹, Acri, C.^{1*}, Parmigiani, R.^{1*}, Bovi, E.^{1*}, Susini, F.¹.

¹ Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Pisa (PI), Italy

^{1*} Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy

² Acquario del Museo di Storia Naturale Università Pisa, Calci (PI), Italy

Adipocytic neoplasms are rarely reported in teleost fish, and most documented cases consist of solitary, encapsulated, or infiltrative lipomas involving subcutaneous or soft tissue. Moreover, hepatic adipocytic tumors are exceptionally uncommon in aquatic animals. Lipomas are benign mesenchymal neoplasms composed of mature adipocytes and are generally classified as non-infiltrative or infiltrative according to their growth pattern. Although lipomas have been reported in several freshwater and marine teleost species, their aetiopathogenesis remains poorly understood. Proposed mechanisms include disturbances of lipid metabolism, endocrine or neurological dysfunctions, and excessive differentiation of mesenchymal cells into adipocytes. The scarcity of reported cases limits the possibility of establishing definitive pathogenetic mechanisms.

This study provides pathological features of an intrahepatic infiltrative lipoma in a giant red-tailed gourami (*Osphronemus laticlavius*), an ornamental freshwater species. A 20-year-old adult specimen, measuring specimen long 47cm and 3 kg, was submitted for diagnostic evaluation with severe abdominal distension and an erosive cutaneous lesion extending from the ventral fins to the anal. At necropsy, the liver was enlarged and swollen, with a brown-to-black discoloration and an extremely soft, gelatinous consistency. Representative tissue samples were fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin and eosin (H&E) for histopathological assessment. Microscopic examination revealed an infiltrative proliferation composed predominantly of sheets of well-differentiated, mature adipocytes with peripheralized, flattened nuclei and large cytoplasmic lipid vacuoles, without a discernible fibrous capsule. The neoplastic cells extensively dissected between hepatocytes and pancreatic parenchyma, separating the hepatic parenchyma into irregular areas, without evident cellular atypia, pleomorphism, or mitotic activity. Numerous melanomacrophages and melanomacrophagic centres were observed scattered in the parenchyma. The structural preservation of well differentiated mature adipocytic architecture, the infiltrative behaviour and dissection through hepatic parenchyma, and the conspicuous absence of hepatocellular degeneration, intracytoplasmic lipid accumulation or inflammation rule out metabolic or reactive lipidosis.

Based on these morphological findings, a diagnosis of intrahepatic infiltrative lipoma was established. To the authors' knowledge, this represents the first reported adipocytic neoplasm in the genus *Osphronemus* and the **first documented case** of an intrahepatic infiltrative lipoma in a freshwater teleost. This case expands the spectrum of mesenchymal neoplasms reported in ornamental fish and highlights the importance of histopathological examination in differentiating neoplastic adipocytic proliferation from hepatic lipidosis.

FIRST REPORT OF PROLIFERATIVE KIDNEY DISEASE IN NORTHWESTERN ITALY: OCCURRENCE AND SEASONAL TRENDS

Cotugno, A.¹, Colussi, S.¹, Bondavalli, F.¹, Gini, M.¹, Milanese¹, G., Zicarelli, G.¹, Glorio Patrucco, S.¹, Esposito, G.¹, Mossotto, C.¹, Gabetti, A.¹, Stilo, G.¹, Fariano, L.², Pastorino, P.¹, Prearo, M.¹

¹ Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy

² Azienda Agricola Canali Cavour, Centallo (CN), Italy

Proliferative kidney disease (PKD), caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*, is an important disease of salmonids and a growing concern for trout aquaculture, particularly under increasing environmental temperatures. Despite its occurrence in several Italian regions, the epidemiological distribution and seasonal dynamics of PKD in Italy remain poorly documented. This study aimed to investigate the occurrence and seasonal dynamics of PKD in a rainbow trout (*Oncorhynchus mykiss*) farm located in southwestern Piedmont, an area where the disease had not previously been documented. Between May and October 2024, 787 farmed rainbow trout were examined across three sampling periods: May-June (n = 270), July-August (n = 363), and September-October (n = 154). Water temperature was monitored daily, while fish underwent external and internal examination, parasitological and bacteriological analyses, and molecular detection of *T. bryosalmonae* by endpoint PCR targeting a 435-bp fragment of the SSU-rDNA gene. PKD prevalence showed marked seasonal variation, increasing from 23.0% in May-June to 73.0% in July-August and subsequently declining to 3.9% in September-October ($p < 0.001$), in association with a pronounced seasonal pattern in water temperature, which ranged from 12.4 to 20.3 °C. Interestingly, the highest molecular prevalence was observed during the summer period, when gross pathological findings were comparatively less frequent. In contrast, renal enlargement and splenomegaly were particularly prevalent during the first sampling period, reaching 78.9% and 42.2%, respectively. Multivariable logistic regression identified sampling period as the main predictor of PKD positivity, with significantly higher odds of infection during July-August (OR $\approx$ 8.9) and significantly lower odds during September-October (OR $\approx$ 0.14) compared with May-June. Moreover, PKD-positive fish showed a significantly lower probability of presenting macroscopic lesions (adjusted OR = 0.52, 95% CI: 0.36-0.75, $p < 0.001$). The observed temporal mismatch between molecular detection and gross pathological alterations highlights the complexity of PKD seasonal dynamics and suggests that these diagnostic approaches may reflect different aspects of the host-parasite interaction. Although the absence of histopathological examination and quantitative assessment of parasite burden prevents definitive interpretation of disease progression, the present findings provide the first confirmed evidence of PKD in southwestern Piedmont and extend the documented distribution of *T. bryosalmonae* in Italy. These results underline the importance of continued epidemiological surveillance and integrated diagnostic approaches for PKD in Italian trout farming, particularly in the context of changing environmental conditions and increasing water temperatures.

This study was funded by the Italian Ministry of Agriculture, Food Sovereignty and Forests through the supply chain programme “RESILTROUT-Acquacoltura resiliente ai cambiamenti globali: la ricerca a supporto della filiera trotica italiana” (IZSPV 23E03; CUP: J18H23000800007).

PHILOMETRA FILIFORMIS IN PAGELLUS ERYTHRINUS: EPIDEMIOLOGICAL, MORPHOLOGICAL UPDATES AND MOLECULAR CHARACTERIZATION

De Benedetto G.¹, Riolo K.², Perdichizzi A.³, Armeli Minicante E.³, Veccari G.³, Sanchez Tinjaca Y.V.¹, Giannetto A.², Gaglio G.¹

¹Department of Veterinary Sciences, University of Messina, 98168 Messina, Italy.

²Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, 98166 Messina, Italy.

³Institute for Marine Biological Resources and Biotechnology, National Research Council, 98122 Messina, Italy.

Pagellus erythrinus (Linnaeus, 1758) is a common demersal protogynous teleost in the Mediterranean Sea. Juveniles primarily feed on polychaetes, whereas adults shift their diet towards crustaceans and fish, highlighting a marked ecological flexibility. Nematodes of the genus *Philometra* are known to induce pathological damage, including severe gonadal lesions, also impairing both host reproduction and behaviour. *Philometra filiformis* (Philometridae) is a species-specific nematode infecting the gonads of *P. erythrinus*. It was described for the first time in the Tyrrhenian Sea in 2008 and the only epidemiological survey reported a prevalence of approximately 12%. Given the impact of *Ph. filiformis* on *P. erythrinus* gonads, the present study aims to update epidemiological data, expanding the study area and providing the first molecular characterization of *Ph. filiformis* in *P. erythrinus*.

Between 2024 and 2025, *P. erythrinus* specimens were collected through a collaboration between the University of Messina and the Institute for Marine Biological Resources and Biotechnology (CNR-IRBIM), covering an area from the mouth of the Garigliano river (Lazio, Italy), to Capo S. Vito (Sicily, Italy). All fish were weighed, measured, and dissected to determine sex, maturity stage and the presence of gonadal parasites. Recovered nematodes were fixed in 70% ethanol and diaphanized in glycerine for 24 h for morphological examination or stored at -80 °C for molecular analysis. Genomic DNA was extracted from eight parasite specimens. Two markers, the small *18S ribosomal RNA* (*18S rRNA*) and the mitochondrial *cytochrome c oxidase 1* (*cox1*), were amplified by polymerase chain reaction (PCR).

The *18S rRNA* and *cox1* sequences obtained from the isolates were aligned with the nucleotide sequences of marine philometrid species for phylogenetic analysis. The study area was divided into three sub-areas: Centre (Lazio and Campania), South-Centre (Basilicata and Calabria), and Islands (Sicily). Generalized linear model (GLM) was fitted as a function of gonadal stage and Fulton's condition factor (Kc) as explanatory variables. Descriptive data of prevalence (%), mean abundance (MA) and mean intensity (MI) were calculated.

Of the 717 *P. erythrinus* examined, 494 were females, 184 were males, and 39 were undifferentiated. All male and undifferentiated *P. erythrinus* specimens were negative for nematodes and were therefore excluded from the statistical analysis. Overall, 76 fish were infected with gonadal nematodes (10.6%). Morphological examination allowed to identify the nematodes as gravid and subgravid females of *Philometra filiformis* (P=15.4 %; MA= 0.44; MI= 2.87). Of these, seven were gravid females (mean length, ML = 31.6 ± 11.8 cm; mean width, MW = 828 ± 71.06 µm) and 210 subgravid females (ML = 5.42 ± 0.96 cm; MW = 257.4 ± 20.78 µm). Moreover, ten *Ph. filiformis* larvae were extracted from a gravid female (ML = 527.51 ± 18.54 µm, MW = 16.4 ± 1.9 µm). All isolates showed positive amplification of both *18S* and *cox1* genes. Partial *18S rRNA* (818 bp) and *cox1* (377bp) sequences were identical among the isolates, and representative sequences were deposited in GenBank. Phylogenetic analysis clearly distinguished our isolates from other marine philometrids. This study updates the distribution of *Ph. filiformis* in *P. erythrinus* ten years after the previous epidemiological survey, reporting for the first time the morphometric data of gravid females, and provided the first molecular characterization of *Ph. filiformis*, thereby enhancing current knowledge of its phylogenetic position.

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AQUACULTURE OPERATORS' AWARENESS OF ANIMAL HEALTH THREATS AND PREVENTION IN ITALY, UNDER REGULATION (EU) 2016/429: DESIGN AND IMPLEMENTATION OF AN E-LEARNING TRAINING COURSE

Donini S.¹, Bissoli F.¹, Ricaldi G.¹, D'Este F.¹, Personeni F.¹, Cassani M.¹, Trevisan S.¹, Nadin A.¹, Lunardi L.¹, Franzago E.¹, Marsella A.¹, Piovan G.¹, Manca G.¹, Toffan A.¹, Dalla Pozza M.¹

¹ Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (Padova), Italy

The Animal Health Law (Regulation (EU) 2016/429) introduced mandatory training requirements for animal keepers, including those operating in the aquaculture sector, with the aim of increasing their awareness and responsibilities in the management of aquaculture facilities in order to prevent the introduction and spread of listed diseases. In Italy these requirements were transposed in Legislative Decree No. 136/2022 and Ministerial Decree of 6 September 2023, as amended by Ministerial Decree of 23 December 2025. The objective is to ensure harmonised knowledge of animal health, biosecurity and animal welfare among operators, thereby strengthening their active role in health surveillance and disease prevention. The Istituto Zooprofilattico Sperimentale delle Venezie (IZSve) was commissioned by some Producers' Associations to design an online training programme for aquaculture operators, taking into account the different types of establishments and aquatic species (fishes, molluscs and crustaceans) farmed in Italy.

According to the legislation, this type of course is mandatory for operators keeping aquaculture animals (fishes, molluscs and crustaceans) registered in the national Identification and Registration (I&R) System. The training programme should be completed by the farm operators by 31 December 2026.

To develop the course, an internal IZSve working group was established, bringing together staff with diverse scientific, technical and IT expertise. The group held numerous meetings starting in autumn 2025. The ministerial decrees were first analysed to identify the main topics to be covered. An organisational framework was then prepared, assigning topics and lecturers to different experts from national, regional and local health veterinary authorities and from IZSve, the national reference laboratory of aquaculture animal pathology, according to their respective areas of expertise. Each lecturer prepared a detailed script, which was subsequently used to record the video lessons, focusing primarily on topics of direct relevance to operators.

The course was developed entirely as an e-learning programme and is hosted on the "learning.izsvenezie.it" platform, with enrolment open from August 2026. The educational design is organised into modules, each of which can be completed according to the participants individual schedule. Each module includes video lessons, interactive exercises and additional PDF materials, for a total duration of 18 hours. Participants can access technical support from the IZSve Training Office.

The four thematic modules cover animal health, identification and registration (I&R), biosecurity and management practices, and animal welfare. Learning outcomes are assessed through multiple-choice tests for each module. Upon completion, participants will receive a certificate valid for five years, after which a six-hour refresher course will be required.

The online training programme for aquaculture operators represents an example of how a regulatory requirement can be translated into an educational tool for farmers. The wide range of topics covered and the flexibility of the training format address the needs of a production sector characterised by farms that vary considerably in size and organisational structure.

We would like to thank all the lecturers who contributed to the development of the course by sharing their expertise and ensuring the high scientific quality of the course content and educational materials: Andrea Maroni Ponti (Ministry of Health), Laura Favero (Veneto Region), Fabio Curto (Veneto Region), Giuseppe Arcangeli (IZSve), Guido Di Martino (IZSve), Luana Cortinovis (IZSve), Alessia Vetri (IZSve), Tobia Pretto (IZSve), Amedeo Manfrin (IZSve), Luigino Bortolotti (ASUIT Trento), Daniela Florio (ULSS 9 Scaligera), Piergiorgio Fumelli (ULSS 5 Polesana), Antonia Paladino (Azienda USL Toscana Sud-Est), Dario Pandolfo (ULSS 3 Serenissima).

EFFECTS OF SINGLE AND COMBINED POLYSTYRENE NANOPLASTICS AND GLYPHOSATE EXPOSURE ON ZEBRAFISH (*DANIO RERIO*) EMBRYOS

Falletti R.¹, Capparucci F.¹, Vallone D.², Foulkes N.S.², Abbate J.M.¹, Lanteri G.¹, Iaria C.¹

¹*Institute for Comparative, Experimental, Forensic and Aquatic Pathology “Slavko Bambir” Università degli studi di Messina, (ME);* ²*Institute of Biological and Chemical Systems (IBCS-BIP), Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany.*

Environmental pollution from plastics and pesticides poses a serious threat to ecosystems. Over time, plastics degrade into nanosized particles that persist in the environment, interact with aquatic and terrestrial organisms and may act as carriers of other contaminants through a “Trojan horse” mechanism. Glyphosate is an environmental herbicide contaminant whose carcinogenic effects have been reported in a long-term rat study, including at doses considered safe by regulatory authorities (Panzacchi et al., 2025). Although the toxicological effects of polystyrene nanoplastics (PS-NPs) and glyphosate (GLY) have been studied individually, the pathological consequences of their co-exposure remain insufficiently characterized. This study aims to investigate the effects of single and combined exposure to 50 nm PS-NPs and GLY using an *in vivo* zebrafish (*Danio rerio*) embryo model, a recognized alternative to adult testing (Directive 2010/63/EU). Experiments were conducted at the “Slavko Bambir” Institute and the Karlsruhe Institute of Technology (KIT). The Zebrafish Embryo Toxicity (ZFET) test was performed in triplicate, following OECD Test Guideline No. 236. Embryos were exposed to eight conditions: four single exposures (PS-NPs at 0.1 and 1 mg/L; GLY at 0.01 and 0.1 mg/L) and four co-exposures (α : GLY 0.01 mg/L + PS-NPs 0.1 mg/L; β : GLY 0.01 mg/L + PS-NPs 1 mg/L; γ : GLY 0.1 mg/L + PS-NPs 0.1 mg/L; δ : GLY 0.1 mg/L + PS-NPs 1 mg/L), while the control groups were maintained in embryo medium alone. Observations were recorded every 24 h to determine lethality based on four endpoints: egg coagulation, lack of somite formation, failure of the tail to detach and absence of a heartbeat. Other morphological alterations were also recorded. In addition, to assess potential motor dysfunction in apparently morphologically normal larvae, a vibration assay was conducted at 120 hours post-fertilisation (hpf) (Hayot G., et al 2024). Mortality rates reached 48% in the group exposed to GLY 0.01 mg/L, 54% in group β and 56% in group γ . Sublethal effects, including skeletal deformities and pericardial oedema, were also assessed. The vibration assay revealed a reduced ability of the larvae to respond adequately to the sensory stimulus. This impairment was particularly evident in groups co-exposed to PS-NPs and glyphosate: 63.3% of the larvae in group β (GLY 0.01 mg/L + PS-NPs 1 mg/L) and 60% of those in group γ (GLY 0.1 mg/L + PS-NPs 0.1 mg/L) exhibited an altered escape response (Kruskal - Wallis, $H=29.98$, $p<0.001$). Indeed, fluorescence microscopy observations also revealed an increased fluorescence signal in the neuromast regions of exposed groups. Further investigation is needed to establish whether this signal reflects contaminant accumulation within these mechanosensory structures. This study integrates morphological and behavioural assessments to investigate potential alterations in mechanosensory structures, such as neuromasts. Characterising these neurobehavioral deficits will guide targeted gene expression analyses to investigate the molecular pathways potentially underlying the observed sensory and pathological effects. These analyses will focus on biomarkers of oxidative stress, inflammation, endocrine-related growth alterations and mechanosensory development. These results indicate adverse effects following combined exposure to PS-NPs and GLY and warrant further investigation of potential synergistic interactions.

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CONCURRENT EDWARDSIELLOSIS AND *POSTHODIPLOSTOMUM ANTEROVARII* PARASITISM IN LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*): A CASE STUDY

Giroto, M.¹, Basso, A.¹, Boscarato, M.¹, Pretto, T.¹, Manfrin, A.¹, Cortinovis, L.¹

¹Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD).

Edwardsiella species, namely *E. tarda*, *E. anguillarum*, *E. piscida* and *E. ictaluri*, are important Gram-negative bacterial pathogens of freshwater and marine fish, with a worldwide distribution and a significant impact on aquaculture. Since the first description of the genus in the 1960s, edwardsiellosis has been reported in a wide range of fish species, including eels, salmonids, tilapia, sea bream, sea bass, catfish, turbot and largemouth bass. The disease is generally systemic, with clinical signs including exophthalmia, haemorrhages, skin ulceration and abdominal distension. The anatomopathological findings commonly involve multifocal necrotic and granulomatous lesions in the liver, spleen, kidney and other internal organs. *Posthodiplostomum anterovarium* is a digenean trematode belonging to the family Diplostomidae. Its metacercarial stage is found in freshwater fish, in which the parasite develops within cysts in coelomic organs, such as liver, spleen and kidney, while the adult stage has been reported in piscivorous birds. The simultaneous occurrence of these bacterial and parasitic agents may therefore result in complex multiorgan lesions, representing a relevant but poorly characterized condition in *M. salmoides*. In September 2025, following an outbreak of mortality in a Recirculating Aquaculture System (RAS), six Largemouth bass were submitted for diagnostic examination. At necropsy skin ulcers on the head and marked skin mucous production were observed. Internally, livers displayed an uneven coloration and nodular texture, with additional small (2 mm) multifocal nodules containing trematode metacercariae. Other findings included the presence of multifocal white nodules in spleen, kidney and heart. For bacteriological examination, samples of spleen, kidney, brain, heart and liver were streaked on blood agar (BA) and incubated at 22°C. Portion of tissues were collected for histological examination. Excysted metacercariae were collected for molecular identification. After 24 hours, an abundant growth of Gram-negative colonies was observed in all organs. The isolates were identified as *Edwardsiella* sp. using biochemical assays, MALDI-TOF mass spectrometry (Bruker), and 16S rRNA gene amplification and Sanger sequencing. Antimicrobial susceptibility testing was performed through disk diffusion method and the determination of the Minimum inhibitory Concentration (MIC). The isolates did not display any resistance towards antimicrobials authorized for aquaculture in Italy. Histological examination highlighted the presence of multifocal necrotic foci associated with coccobacilli in the splenic parenchyma and multiple trematode cysts were observed in kidney, spleen, liver and heart. Metacercariae were identified by amplification and Sanger sequencing of the large subunit of ribosomal DNA (LSU rDNA) (900 bp amplicons). BLASTn analysis showed 99.31% nucleotide sequence similarity with *Posthodiplostomum anterovarium* (accession number MZ710943). This case documents the concurrent occurrence of *Edwardsiella* sp. infection and *P. anterovarium* metacercarial parasitism in largemouth bass reared in a RAS. The management of bacterial infections in this type of aquaculture system is particularly challenging, as the application of antimicrobial treatments may negatively affect water quality and the biological filtration system, limiting their use and complicating disease control. This case highlights the importance of an integrated diagnostic approach for the accurate identification of multifactorial diseases in aquaculture. Further studies are needed to clarify the potential interactions between *Edwardsiella* sp. and *P. anterovarium* in largemouth bass.

ISOLATION AND MOLECULAR IDENTIFICATION OF LACTOCOCCUS GARVIEAE ISOLATED FROM RAINBOW TROUT FROM A SICILIAN FRESHWATER AQUACULTURE FARM

Iaria C.¹, Lo Monaco D.², Krezou K.¹, Carrozzo A.², Reale S.², Scibetta S.², Salogni C.³, Di Gaudio F.², Marino F.¹

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy;

²Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy. ³Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna (IZSLER) Via Bianchi, 9. 25039 BRESCIA

An episode of mortality in farmed rainbow trout (*Oncorhynchus mykiss*) at a Sicilian freshwater aquaculture facility supplied with filtered river water was investigated in February 2026, when the water temperature was approximately 11 °C. Moribund and symptomatic fish, together with a limited number of apparently healthy fish, underwent diagnostic examination. Clinical signs included reduced activity, abnormal swimming, exophthalmos, pale or congested gills and fin alterations. Necropsy revealed mild ascites, hepatomegaly, splenomegaly, renal congestion and petechial haemorrhages in several organs.

Bacteriological examinations were performed using samples of coelomic fluid, eyes, gills, liver and kidney. Samples were cultured on blood agar both directly and after enrichment in brain heart infusion broth and 10% NaCl peptone water at 25 °C for 24-48 h. Small, white colonies showing greenish discolouration on blood agar were recovered from the examined organs. The isolates consisted of cocci arranged in chains and tested negative for catalase and oxidase. API 32 Strep yielded a presumptive identification of *Lactococcus garvieae*. Sanger sequencing of the 16S rRNA gene showed 100% sequence identity with both *L. garvieae* and *L. petauri*, precluding differentiation between the two species.

Illumina whole-genome sequencing data from the isolate were subsequently used to investigate the 16S-23S rRNA internal transcribed spacer (ITS), a marker reported to differentiate between *L. garvieae* and *L. petauri*. Read mapping to the ITS reference sequence of *L. garvieae* strain LMG9472 revealed no differences between the consensus and reference sequences across the analysed region, with reported sequencing coverage of 20-150×. This result supported identification of the isolate as *L. garvieae*.

Histopathological examination of ten fish revealed lesions compatible with systemic bacterial infection, including splenic congestion and haemorrhage, multifocal haemorrhages and marked branchial epithelial hyperplasia associated with partial to complete fusion of secondary lamellae. Coccoid bacteria were observed in branchial vessels and mucosal cells.

The findings support the occurrence of an outbreak associated with *L. garvieae* and highlight the limitations of biochemical identification and 16S rRNA gene sequencing for distinguishing closely related lactococci. Analysis of the 16S-23S rRNA ITS region provided additional discriminatory evidence supporting identification of the isolate as *L. garvieae*.

The occurrence of this outbreak in February highlights the importance of considering lactococcosis in the differential diagnosis of systemic bacterial disease in rainbow trout during winter. Further surveillance is warranted to clarify the geographical distribution of *L. garvieae* and the environmental conditions, particularly water temperature, associated with clinical outbreaks.

DESCRIPTION OF WHITE STURGEON, *ACIPENSER TRANSMONTANUS*, MORTALITY POTENTIALLY LINKED TO NOTOCHORD DISPLACEMENT

Marsella A.¹, Pretto T.¹, Buratin A.¹, Biasini L.¹, Cortinovis, L.¹, Maurizio A.², Accini A.², Toffan A.¹

¹ Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy

² Skretting Italia, Mozzecane (VR), Italy

Corresponding author: amarsella@izsvenezie.it

In summer 2025 and January 2026, a commercial sturgeon farm experienced low mortality affecting white sturgeon sub adults. Fish displayed lethargy without any additional clinical signs despite mortality. The mortality occurred in both cases several days after routine farming manipulation. At necropsy, most analysed fish did not display relevant external lesion, while some showed exophthalmos or skin haemorrhages. Internally, splenomegaly with petechial haemorrhages and pale organs was detected in some fish as well as serous liquid in the coelomic cavity or abundant haemorrhagic liquid in the swim bladder. Notably, variable degrees of notochord displacement were observed in almost all specimen. In the most severe cases, the notochord appeared twisted or dislocated in the swim bladder lumen. Bacteriological and virological investigations yielded negative results with no relevant pathogen being detected. Histological analysis on displaced notochords segments highlighted a reduction in diameter and alterations in the central medulla, characterized by the collapse of vacuolated cells. The structural weakness and the reduced attachment of the notochord to surrounding tissues, coupled with physical manipulation during routine operations, likely led to its displacement, triggering potential circulatory disturbances and mortality. Vertebral deformities in fish have been frequently described and, in some cases, linked to different aetiologies, such as temperature, toxic substances, nutritional deficiencies or trauma, especially in larvae or juveniles. Conversely, notochord displacement has been rarely described in fish and little information on its aetiology is available. An underlying genetic predisposition is highly likely to be responsible for the observed case due to the involvement of only one batch of fish, but further investigations are needed to elucidate the causes and pathological mechanisms.

DeformIA: DEVELOPMENT OF AN ARTIFICIAL INTELLIGENCE-BASED PREDICTIVE SYSTEM IN AQUACULTURE FOR THE SELECTION OF THE BEST BREEDING CANDIDATES

Paramatti A.¹, Sciuto S.¹, Tafuro V.¹, Colussi S.¹, Acutis P. L.¹, Pastorino P.¹, Prearo M.¹, Gini M.¹, Milanese G.¹, Vandoni L.², Balzano G.², Revelli M.³, Botto D.³, Milanese M.⁴, Colli L.⁴, Tomasoni M.⁵, Ajmone Marsan P.⁴, Fariano L.⁵

¹ *Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino*

² *Hal Service S.p.A, Borgosesia (VC)*

³ *Informatica System S.r.l., Vicoforte (CN)*

⁴ *Università Cattolica del Sacro Cuore, Piacenza*

⁵ *Azienda Agricola Canali Cavour, Centallo (CN)*

In aquaculture, skeletal deformities represent a major constraint to production efficiency, product quality, and marketability, while also raising important concerns regarding fish welfare and generating substantial economic losses. Although the etiology of skeletal abnormalities is complex, increasing evidence supports a significant genetic contribution to their phenotypic expression. Consequently, the identification of individuals with a favorable genetic background for skeletal robustness represents an important objective in selective breeding programs.

Within this framework, the DeformIA project, started in late 2025, aims to develop an innovative artificial intelligence (AI)-based phenotyping platform integrating deep learning and image-analysis approaches for the identification and ranking of breeding candidates according to their susceptibility to skeletal deformities, specifically targeting the condition known in the literature as "short tail" or Vertebral Column Compression Syndrome (VCCS). The proposed system is designed to exploit standardized photographic images as a non-invasive and scalable source of phenotypic information, with the ultimate objective of providing early, fast, large scale and cost-effective data necessary for the identification of the genetic basis of the defect and the implementation of genomic-based purifying selection strategies. The development of the predictive model is structured into two main phases: the first phase involves the standardized phenotypic and radiographic characterization of individual fish, enabling the definition and annotation of skeletal phenotypes and the generation of a high-quality reference dataset for model training; in the second phase, the deep learning model is optimized by integrating phenotypic information with genomic data generated through GeneTitan-based genotyping. Model performance will subsequently be assessed using an independent validation dataset that is not involved in model training or optimization. Evaluation will include standard classification and prediction metrics, such as accuracy, sensitivity, and specificity, together with an assessment of the concordance between the ranking generated by the AI-based system and that obtained from genomic analyses. This validation framework will provide an objective assessment of the predictive performance, robustness, and potential applicability of the proposed approach within selective breeding programs. Preliminary results have highlighted the presence of a strong genetic component linked to the trait and identified genomic markers associated with deformity under study. At the same time, the penetrance of the trait is strongly influenced by environmental factors such as water temperature. Overall, the DeformIA platform is expected to provide a rapid, non-invasive, and objective tool for supporting genetic selection against skeletal deformities in aquaculture. By reducing the dependence on resource-intensive analytical procedures while enabling the large-scale phenotyping of breeding populations, the approach could contribute to increasing the efficiency and scalability of genetic improvement programs. Furthermore, the underlying methodology may be transferable to other fish species of zootechnical relevance and potentially applicable to additional heritable traits of economic importance, thereby supporting the development of more sustainable, efficient, and competitive aquaculture production systems.

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EURO-PAR-FISH: From Wild Waters to Human Health - A One Health Approach to Zoonotic Helminths in European Freshwater Fish

Vasco Menconi¹, Lisa Guardone², Giuseppe Arcangeli¹, Amedeo Manfrin¹, Federica Tosi¹, Andrea Gustinelli³, Christine Müller-Graf⁴, Mélanie Gay⁵, Monica Caffara³, Marino Prearo⁶, Paolo Pastorino⁶, Gianluca Marucci⁷, Selene Rubiola⁸, Andrea Armani², Maira Mateusa⁹, Sasa Vasilev¹⁰, Aneta Belcik¹¹, Ewa Paździor¹¹, Weronika Korpysa-Dzirba¹¹, Giorgia Angeloni¹, Ewa Bilaska-Zajac¹¹

¹Istituto Zooprofilattico Sperimentale delle Venezie, Italy; ²Department of Veterinary Sciences, University of Pisa, Italy;

³Department of Veterinary Medical Sciences, University of Bologna, Italy; ⁴Federal Institute for Risk Assessment (BfR),

Germany; ⁵French agency for food, environmental and occupational health & safety, France; ⁶Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle D'Aosta, Italy; ⁷Department of Infectious Diseases, Istituto Superiore di Sanità, Italy;

⁸Department of Veterinary Sciences, University of Torino, Italy; ⁹Institute of Food Safety, Animal Health and Environment

BIOR, Latvia; ¹⁰University of Belgrade, Institute for the Application of Nuclear Energy INEP, Serbia; ¹¹National Veterinary Research Institute (PIWet), Poland

Over the last 20 years, consumption of fish products has increased worldwide, including in Europe. Within this context, fish-borne zoonotic helminths (FBZH) represent an emerging yet under-recognised food safety concern. Despite increasing attention to food safety, FBZH have received comparatively limited focus, and robust data to support comprehensive risk assessment remain scarce. In 2026, a consortium led by Poland, with Italy, France, and Germany as partners and Serbia and Latvia as observers, launched the EURO-PAR-FISH project funded by the European Food Safety Authority. Adopting a One Health approach, the project aims to address critical knowledge gaps on zoonotic helminths in wild freshwater fish and strengthen cross-sectoral collaboration across Europe. Project partners will collect freshwater fish samples from selected basins across participating countries and perform parasitological analyses to detect FBZH. Additional activities will include collecting data on human infections from hospital records, complemented by anamnestic investigations focusing on patients' travel history and dietary habits to help identify potential sources of infection. Parasites will be initially identified based on morphological characteristics and subsequently confirmed through molecular approaches, including DNA sequencing. In line with the One Health framework, the project integrates epidemiological investigations, laboratory analyses, and the assessment of consumer habits related to freshwater fish consumption. Epidemiological and molecular data on FBZH across multiple European regions, providing new insights into parasite distribution, host range, and potential exposure pathways to humans. Data on consumer behaviour related to freshwater fish consumption will further contribute to understanding risk patterns. A comprehensive, standardized database will be developed to integrate parasitological, molecular, and epidemiological data. This will support the creation of spatial distribution maps of FBZH and provide a robust foundation for future risk assessment. This project adopts a One Health approach by linking environmental monitoring, animal health, and human exposure to zoonotic parasites. Expected outcomes include the development of standardised operating procedures for parasite detection and molecular characterisation, as well as the establishment of a database integrating epidemiological and genetic data. The project will contribute to evidence-based risk assessment and provide insights into the potential introduction and detection of non-native FBZH associated with the consumption of imported fish products and with international travel, thereby highlighting emerging pathways of exposure. Ultimately, it will support EFSA and public health authorities in improving food safety and protecting consumers from parasitic risks associated with fish consumption.

NADI-ACQUA: DEVELOPMENT OF A DECISION SUPPORT SYSTEM AS AN INNOVATIVE NON-INVASIVE APPROACH FOR EARLY DIAGNOSIS IN AQUACULTURE

Tafuro V.¹, Fariano L.², Paramatti A.¹, Colussi S.¹, Pastorino P.¹, Acutis P. L.¹, Tomasoni M.², Prearo M.¹, Di Nicola M. R.¹, Vandoni L.³, Balzano G.³, Revelli M.⁴, Botto D.⁴, Pascoli F.⁵, Toffan A.⁵, Manfredi M.⁶, Sciuto S.¹

¹ Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino

² Azienda Agricola Canali Cavour, Centallo (CN)

³ Hal Service S.p.A, Borgosesia (VC)

⁴ Informatica System S.r.l., Vicoforte (CN)

⁵ Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD)

⁶ Università del Piemonte Orientale, Novara

Aquaculture represents one of the major productive sectors expected to contribute to global food security; however, the sustainability and competitiveness of aquaculture production are strongly affected by the emergence of infectious diseases. The increasing spread of emerging diseases threatening fish species has significant impacts not only at the economic level but also on public health.

To enable the timely management of infection outbreaks in trout farms, it is necessary to establish an effective fish disease surveillance system through the development of rapid, field-deployable, and non-invasive diagnostic tools. In September 2024, the NADI-ACQUA project was launched, co-funded by the Piedmont Regional ERDF 2021-2027 programme. The project focuses on the development of a technologically advanced decision support system for disease monitoring in aquaculture. The project is currently ongoing and adopts a multidisciplinary approach combining image-based techniques, hydroacoustics, artificial intelligence, and near-infrared spectroscopy (NIR). The diseases investigated are lactococcosis and Infectious hematopoietic necrosis virus (IHNV) in the *in vivo* experimental study, and yersiniosis in the field feasibility study.

Image-based analysis techniques were applied to detect exophthalmia, melanosis and lethargy in fish and showed variation between different time points. NIR analyses showed a separation between samples collected at time points T0 and T2 for both diseases. Proteomic and fatty acids analyses revealed changes in molecular profiles associated with disease status and the temporal progression of infection. Hydroacoustic analysis did not reveal significant differences among the different sampling time points and therefore did not provide discriminatory information useful for characterising the progression of the two diseases.

The feasibility study conducted at a commercial trout farm enabled the applicability of the methodology to be assessed under real-world operational conditions. Spectroscopic analyses performed using the SCiO device showed variations in NIR profiles associated with the temporal progression of infection, in conjunction with molecular biology data relating to the progression of *Yersinia ruckeri* infection and mortality.

Overall, the system developed within the NADI-ACQUA project lays the foundation for implementing intelligent surveillance tools capable of supporting the early diagnosis of infectious diseases, reducing the use of pharmacological treatments, improving animal welfare, and promoting greater economic and environmental sustainability in the aquaculture sector.

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EVIDENCE OF *AEROMONAS EUCRENOPHYLA* INFECTION IN *SALVELINUS FONTINALIS* FROM AN ITALIAN FARM

M. Torricelli¹, E. Radicchi¹, F. Marchetti², R. Di Vincenzo¹, A. Accini², F. Agnetti¹

¹ Istituto Zooprofilattico Sperimentale Umbria e Marche “Togo Rosati”, Perugia-Terni, Italy; ² Skretting Italia S.p.a.

The *Aeromonas* genus is autochthonous in the aquatic environment, thus detectable both in fresh and salt water. Although accurate epidemiological EUCAST cut-off for antimicrobial resistance (AMR) is still lacking, the role of its genes associated to AMR dissemination in the ecosystems is impactful. In addition, *Aeromonas* spp. are also causing infections in humans and animals, not only in fish (Baron *et al.*, 2017). Globally, *Aeromonas salmonicida* is recognised as one of the major fish pathogens worldwide, causing extensive disease outbreaks and substantial mortality in both cultured and wild fish populations. Among its subspecies, *A. salmonicida* subsp. *salmonicida* is the etiological agent of furunculosis, a highly contagious disease of Salmonids (Wilson *et al.*, 2024). Furthermore, among the existing different bacteria species and sub-species, the broader microbiological studies of freshwater fish, not only restricted to brook trout (*Salvelinus fontinalis*) shown that *Aeromonas eucrenophila* accounts up to approximately 20% of all *Aeromonas* isolates, ranking behind more prevalent species such as *A. veronii*, *A. hydrophila*, *A. sobria*, and *A. caviae* (Silva *et al.*, 2022). Currently, epidemiological studies detecting the specific presence of *Aeromonas eucrenophila* in Italian freshwater farms and ecosystems are very limited, thus an accurate prevalence could not be estimated. On this basis, this work wants to integrate the epi-surveillance data. In an Italian facility with a history of furunculosis outbreaks, where rainbow, brown and brook trout are raised, lesions consistent with *Aeromonas* infection (hyperemia at the base of the fins, rare ophthalmitis, peritoneal petechiae, mild splenomegaly, brain hyperemia) were observed in brook trout during August 2026. The water temperature at the time of sampling was around 22°C. Kidney, liver, spleen and brain samples were collected from 8 specimens and subjected to bacterial culture on blood agar. The isolates were characterized by micromethod biochemical identification system (API 20E/API 20NE) than confirmed by MALDI-TOF MS. Concurrently, antimicrobial susceptibility to amoxicillin, oxytetracycline, sulfamethoxazole/trimethoprim, flumequine, erythromycin and florfenicol was tested by the Kirby-Bauer method and by the determination of the minimum inhibitory concentration (MIC). *A. eucrenophila*, *A. hydrophila*, and *A. sobria* were detected in all examined fish tissues, highlighting widespread and severe septicemia. *A. eucrenophila* susceptibility at the Kirby-Bauer test revealed resistance only to amoxicillin, whereas the isolates were susceptible to all other antimicrobials tested. For *A. eucrenophila* the MIC values were 16 µg/mL for erythromycin, 0.031 µg/mL for flumequine, >32 µg/mL for amoxicillin, 0.25 µg/mL for florfenicol, 0.125 µg/mL for oxytetracycline, and 0.063-1.188 µg/mL sulfamethoxazole + trimethoprim. These findings highlight the importance of maintaining active surveillance of “new possible bacterial pathogens” in aquaculture and the role of the environmental conditions (e.g., too high temperature for brook trout wellness), that can induce stress in fish, potentially leading to the proliferation of secondary pathogens and causing significant clinical conditions. Continuous monitoring is essential to improve farm management and biosecurity strategies, limit the spread of potentially pathogenic and antimicrobial-resistant bacteria and reduce the risk of transmission to other animals and humans, within a One Health framework.

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ESSENTIAL OILS FOR THE CONTROL OF BACTERIAL DISEASES IN AQUACULTURE: A NATURAL ALTERNATIVE

Zicarelli, G.¹, Colussi, S.¹, Glorio Patrucco, S.¹, Cotugno, A.¹, Bondavalli, F.¹, Amadori, M.², Vercelli, C.², Re, G.², Esposito, G.¹, Maganza, A.¹, Merialdi, A.¹, Gasco, L.³, Prearo, M.¹, Pastorino, P.¹

¹Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, Turin, Italy

²Department of Veterinary Science, University of Torino, Grugliasco, Largo P. Braccini, 2, Turin, Italy

³Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, Largo P. Braccini, 2, Turin, Italy

The aquaculture sector is experiencing substantial growth, with production surpassing that of wild fishing in 2024 and becoming the first industry to produce fish animal protein for human consumption, accounting for over 51%. Despite this economic golden age for the sector, the increased production of goods and farmed animals leads to heightened demand for artificial feed supplies, water treatment, environmental degradation, and disease outbreaks. Specifically, ponds can lose over 60% of their annual production due to the spread of diseases, causing high mortality rates and consequential economic loss, which is reported to be globally around 1.05 to 9.58 billion USD/year.

One of the most significant threats to aquaculture facilities and fish is bacteriological disease caused by *Aeromonas* spp., *Streptococcus* spp., *Lactococcus* spp., *Vibrio* spp., *Pseudomonas* spp., and other related genera. These bacteria are commonly treated with synthetic antibiotics, vaccines, and chemotherapies to enhance the immune response. Nevertheless, the over- and uncontrolled use of antibiotics has led to the emergence of multidrug-resistant pathogens and an imbalance in the microbial population, with consequences for the host immune system and the environment.

Consequently, the researcher has sought to identify alternative, more natural solutions to replace antibiotics, either partially or completely, in aquaculture. One valuable and rising option is the inclusion of essential oils (EOs) in the feed used for farmed fish.

Extracted from various parts of medicinal plants (flowers, leaves, seeds, roots, fruits, wood and bark), EOs consist of aromatic and volatile compounds that are insoluble in water. They have been described as regulating quorum-sensing systems, biofilm formation, and the expression of virulence factors. EOs have been shown to possess bioactive properties with phytotherapeutic activity against various bacteria, especially Gram-positive bacteria. This phenomenon can be attributed to the presence of lipoteichoic acids in cell membranes that might facilitate the penetration of substances. Moreover, EOs have demonstrated a variety of favourable functions in farmed fish, such as appetite stimulation, growth promotion, and stress reduction.

In this context, various *in vitro* studies have been conducted to assess the effects of EOs on several bacterial species. The research focuses on groups that mainly affect water and aquaculture facilities, causing death in farmed fish. EOs derived from, for example, *Origanum* spp., *Piper* spp., *Cinnamomum* spp., *Lavandula* spp., and *Eucalyptus* spp. demonstrated growth-inhibitory effects on *Aeromonas* spp. (the most studied bacterial species, such as *A. salmonicida*, *A. veronii*, *A. hydrophila*) in several MIC ranges, from 3 µL/mL to 3628 µL/mL. Similarly, inhibitory effects from diverse EOs were observed for *Vibrio* spp., with MIC ranges from 0.244 µL/mL to 320 µL/mL, *Pseudomonas* spp. (MIC from µL/mL to 640 µL/mL), *Lactococcus* spp. (MIC 4 µL/mL to 1280 µL/mL), *Citrobacter* spp. (MIC > 1600 µL/mL) and others.

To conclude, the *in vitro* tests highlighted positive inhibitory effects of EOs on both Gram-positive and Gram-negative bacteria, suggesting their possible incorporation into aquaculture feed as an ecological and “Green” alternative to antibiotics. Nevertheless, the number of studies conducted on fish affected by these diseases remains limited, underscoring the necessity for further research in this field. Consequently, experimental trials in which farmed fish are fed pellets with different percentages of EOs inclusion would be required in order to enhance comprehension of animals' health status and to verify whether there is an improvement in immunological response.

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