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# perspectives de l'aqüicultura a Catalunya

Una mirada  
des de la gestió,  
la investigació  
i la indústria



perspectives  
on aquaculture  
in Catalonia  
Management,  
research,  
and industry

# **ORAL COMMUNICATIONS**

## Importància de l'estudi de biofilms bacterians per a l'aqüicultura

**Sergi Baillés<sup>1</sup>, Marc Tejero<sup>1</sup>, Ignasi Sanahuja<sup>1</sup>, Antoni Ibarz<sup>1</sup>, Carlos Balsalobre<sup>2</sup>, Cristina Madrid<sup>2</sup>, Laura Fernández-Alacid<sup>1</sup>**

- 1- Departament de Biologia Cel·lular, Fisiologia i Inmunologia, Facultat de Biologia, Universidad de Barcelona (08028, Barcelona).
- 2- Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universidad de Barcelona (08028, Barcelona).

L'aqüicultura ha estat responsable del creixement impressionant i continuat del subministrament de peix per al consum humà, continua creixent més ràpidament que la resta de grans sectors agroalimentaris, presentant-se com una alternativa pràctica i rendible a la pesca extractiva per a proporcionar aliments altament nutricionals. Tot i això, l'increment de la producció piscícola també ha afavorit l'ocurrència de malalties infeccioses; la seva propagació epizoòtica i la manifestació de malalties emergents cada cop és més freqüent. A la natura, els bacteris s'adhereixen a les superfícies i/o entre ells produint una matriu extracel·lular de biopolímers que envolta les cèl·lules. Aquestes estructures complexes es coneixen com a biofilms i representen la gran majoria de bacteris en ecosistemes naturals i patogènics.

En aqüicultura, a més de la major dificultat de tractament dels animals infectats, els biofilms preocuperen per la colonització de sistemes, equipament i instal·lacions. D'aquesta manera, la fricció deguda al constant flux d'aigua permet l'alliberació de cèl·lules bacterianes potencialment infeccioses dels biofilms, provocant infeccions recurrents en els sistemes de producció.

Els peixos presenten una continua producció i secreció de mucus epidèrmic que actua com a primera línia de defensa contra les infeccions bacterianes, així com contra una gran varietat de condicions ambientals o estressants. El mucus permet la captura i immobilització de patògens abans que puguin contactar amb les superfícies epiteliais i presenta diversos compostos antimicrobians. Recentment, l'extracció del mucus epidèrmic de manera no invasiva i l'anàlisi de la seva capacitat antimicrobiana *in vitro*, ens permet relacionar diferents factors fisiològics del peix, com ara la seva condició dietètica o els nivells d'estrés, amb la possible susceptibilitat a patir una infecció.

Per a molts patògens, la formació d'un biofilm és un pas necessari per l'establiment d'una infecció. Per aquesta raó, el nostre grup està treballant en l'estandardització de protocols de formació de biofilms *in vitro* de diferents espècies bacterianes patògenes de peixos per poder estudiar l'efecte inhibitori del mucus en diferents condicions de cultiu dels peixos per poder ajudar a la prevenció de possibles infeccions en els sistemes de producció intensius.

## Estudio del efecto de la harina de insecto (*Tenebrio molitor*) en el cultivo de juveniles de trucha arcoíris (*Oncorhynchus mykiss*) utilizando isótopos estables

M. Montblanch<sup>1\*</sup>, I. García-Pérez<sup>1</sup>, E. Sukumaran<sup>1</sup>, A. Sánchez-Moya<sup>1</sup>, J. Gutiérrez<sup>1</sup>, I. Navarro<sup>1</sup>, E. Capilla<sup>1</sup>, S. de Diego<sup>3</sup>, E. Gisbert<sup>2</sup>, J. Blasco<sup>1</sup>

<sup>1</sup>Departament de Biología Cel·lular, Fisiología i Immunología, Facultat de Biología, Universitat de Barcelona, Barcelona; <sup>2</sup>IRTA, Centre de Sant Carles de la Ràpita (IRTA-SCR), La Ràpita, Tarragona;

<sup>3</sup>TEBRIÓ, Doñinos de Salamanca, Salamanca

La harina de insecto ha generado un creciente interés en el sector de la acuicultura como una alternativa a las harinas convencionales, con el objetivo principal de mitigar la alta demanda y los costes ambientales asociados a estas últimas. El objetivo de este estudio fue evaluar los efectos de la inclusión de harina de insecto (*Tenebrio molitor*) en la dieta de juveniles de trucha arcoíris (*Oncorhynchus mykiss*). Las dietas experimentales variaban en el porcentaje de sustitución de harina de pescado por harina de insecto: TM0 (0% de sustitución de harina de insecto, considerada dieta control con un 20% de harina de pescado), TM20 (20% de sustitución), TM30 (30% de sustitución), TM60 (60% de sustitución) y TM100 (100% harina de insecto). Los peces (peso inicial  $29.93 \pm 0.03$  g) se alimentaron durante 60 días con las dietas experimentales mediante comederos automáticos (ración al 3% de la biomasa). Se analizó el crecimiento, la composición proximal y mediante espectrometría de masas de relaciones isotópicas (IRMS), la composición isotópica ( $^{15}\text{N}/^{13}\text{C}$ ) del músculo blanco y de sus reservas energéticas (glucógeno, lípidos y proteínas). Además, se cuantificó el enriquecimiento de los aminoácidos mediante CG-IRMS de tres dietas (TM0, TM60 y TM100). Para analizar las diferencias entre los grupos, se realizó el análisis de la varianza (ANOVA) de una vía y una prueba post-hoc de Tukey.

Al finalizar el experimento, se observó que los peces alimentados con la dieta TM100 mostraron un peso significativamente menor, pero no hubo diferencias en la composición proximal del músculo. La sustitución por harina de insecto quedó reflejada en la firma isotópica, mostrando valores menores de  $\delta^{15}\text{N}$  a mayor inclusión de este ingrediente. El fraccionamiento isotópico en músculo ( $\Delta^{13}\text{C}$  y  $\Delta^{15}\text{N}$ ), mostró valores más altos en TM100, indicando un mayor recambio proteico y una posible síntesis *de novo* de aminoácidos no esenciales en esos peces. Este hecho también se vio reflejado en el  $\Delta^{15}\text{N}$  de los aminoácidos esenciales y no esenciales en la dieta TM100, que sugiere un desequilibrio a nivel proteico. En conclusión, se ha observado que una inclusión de hasta un 12% de harina de insecto (TM60) no perjudica el crecimiento de los juveniles de trucha, pero una sustitución completa provoca imbalances aminoacídicos y pone en riesgo el crecimiento del animal.

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## GONADAL TRANSCRIPTOMES OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) MALES, NEOMALES AND FEMALES

Núria Paisano Cabrera<sup>1,2</sup>, Núria Sánchez-Baizán<sup>1,3</sup>, Francesc Piferrer<sup>1</sup>

<sup>1</sup>Institut de Ciències del Mar (ICM), Barcelona, Spain. <sup>2</sup>Universitat de Barcelona (UB), Barcelona, Spain

<sup>3</sup>Current address: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Palavas-les-Flots, France.

Sex reversal is increasingly being reported in different vertebrate wild populations, like fish and reptiles. This phenomenon is caused by the influence of environmental factors on the sex determining mechanisms of a species, also mediated through epigenetic modifications; the most common factor is a change in temperature. In fish, the persistence of high temperatures usually results in the development of neomales. These are genetic females sex reversed into phenotypic males, which have functional testis and can produce fertile sperm. Nonetheless, studies performed so far have not found a common pattern in the gonadal transcriptomes of neomales when compared to genetic males. For instance, in cases such as that of the tongue sole (*Cynoglossus semilaevis*), which has a chromosomal sex determining system of the ZW/ZZ type, many transcriptomic differences were found in the testes of neomales vs males, while in other species like the domesticated zebrafish (*Danio rerio*), which has no sex chromosomes and has a polygenic sex determining system (PSD), little or no differences were found. The European sea bass (*Dicentrarchus labrax*) is one of the most important cultured teleost fish in Europe. It is a gonochoristic fish with PSD, in which gonadal development is determined by a combination of both genetic and environmental factors, such as temperature. Thus, when reared at elevated temperatures, neomales can develop. In this study, we compared the effects of temperature changes on the sexual development of males, females and neomales of the sea bass, via the contrast of gonadal transcriptomes in juvenile fish of 1 year of age. To do so, we used RNA-seq to compare males and neomales, using females as a common reference. Results revealed that basically no significant differences could be found between male and neomale transcriptomes. Further the number of differentially expressed genes between neomales and females was similar than that of males and females. Thus, it is possible that contrasts in the quantity of differentially expressed genes found between males and neomales of diverse species is related to their sex determining system, since both the sea bass and the domesticated zebrafish have PSD. Therefore, our results confirm that, in a PSD species, differences in the gonadal transcriptome of males and neomales are absent or minimal. However, other species should be examined to confirm whether the provided explanation for this differences among species remains valid.

# **Germline Dysfunction Following Acute Exposure to Nanoplastics in Zebrafish Provides Insights into Molecular Pathways Impacted by Pollutants**

Gala Pujol<sup>1,2,\*</sup>, Laia Marín-Gual<sup>1,2</sup>, Laura González-Rodelas<sup>1,2</sup>, Lucia Álvarez-González<sup>1,2</sup>, François Chauvigne<sup>2,3</sup>, Joan Cerdà<sup>2,3</sup>, Mariana Teles<sup>1</sup>, Nerea Roher<sup>2</sup>, Aurora Ruiz-Herrera<sup>1,2,\*</sup>

<sup>1</sup>Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain.

<sup>2</sup>Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain.

<sup>3</sup>Institute of Marine Sciences, Spanish National Research Council (CSIC), 08003 Barcelona, Spain

## **Abstract**

Nanoplastics pollution is a raising environmental concern whose impacts on biodiversity and human health are far from being understood. This is particularly relevant in aquatic ecosystems, where the majority of species depend on external fertilization for fertilization. In the present study, the effects of a short-term (96 h) exposure to polystyrene nanoplastics (PS-NPs) were evaluated in a zebrafish (*Danio rerio*) germ line to further explore the potential effect in reproduction. Present results revealed that, in males, PS-NPs induced testicular histological alterations with abnormal sperm clustering and chromatin compaction, resulting in viable spermatozoa but with reduced motility. Moreover, females showed an alteration in oocyte stages frequencies during oogenesis, possibly reflecting alterations in oocyte growth. RNA-sequencing analysis in males links the alterations caused by the exposure to PS-NPs to the expression of genes related to molecular pathways involved in chromatin structure, meiosis and DNA double-strand break formation, and repair progression. Overall, our results demonstrated that an acute exposure of gonads to PS-NPs can compromise zebrafish reproductive fitness, underscoring the environmental and health impacts of NPs pollution.

Efecto de la acidificación oceánica sobre la mineralización ósea de la dorada (*Sparus aurata*):  
enfoques *in vivo* e *in vitro*

Rodríguez I.<sup>1\*</sup>, Sadeghi N.<sup>2</sup>, García-Pérez I.<sup>1</sup>, Martín-Velaz L.<sup>1</sup>, Mahmoudi K.<sup>2</sup>, Montblanch M.<sup>1</sup>,  
Navarro I.<sup>1</sup>, Gutiérrez J.<sup>1</sup>, Capilla E.<sup>1</sup>, Garcia de la serrana D.<sup>1</sup>

<sup>1</sup>Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat de Barcelona, Barcelona, España;

<sup>2</sup>Departamento de Pesquerías, Facultad de Agricultura y Recursos Naturales, Universidad de Teherán, Karaj, Irán

La acidificación oceánica debida a la emisión antropogénica de CO<sub>2</sub> supone un riesgo para la acuicultura, ya que puede afectar negativamente al crecimiento y desarrollo de los organismos acuáticos. La disminución del pH oceánico compromete la supervivencia, el crecimiento y la calcificación en invertebrados marinos. En peces, se ha demostrado que merma el crecimiento y la supervivencia, pero aumenta la calcificación de otolitos en algunas especies; no obstante, su efecto sobre la mineralización ósea no ha sido suficientemente estudiado. En mamíferos, experimentos *in vitro* han demostrado que un pH extracelular más elevado aumenta la actividad y mineralización de los osteoblastos. En el presente estudio, juveniles de dorada fueron expuestos a un pH experimental de 7.3 (inducido por inyección de CO<sub>2</sub> en el agua), o mantenidos a un pH “control” de 7.9 durante 68 días. Posteriormente, se tomaron muestras de sangre y tejidos, y se analizó el hematocrito, la composición electrolítica del plasma, la mineralización con radiografías óseas, y la expresión de genes implicados en la remodelación y mineralización en hueso, y de canales de intercambio iónico en branquias. Por otro lado, se realizaron cultivos primarios de osteoblastos de dorada y se evaluó la actividad celular y la mineralización en respuesta a diferentes condiciones de pH (8.1, 7.5, 7.3). En el experimento *in vivo*, el pH plasmático fue más elevado en el grupo a pH de 7.3, probablemente debido a una respuesta compensatoria del organismo frente a la acidosis, afectando a los niveles de electrolitos como el potasio. En las radiografías óseas, la relación longitud/altura de las vértebras fue significativamente mayor en los animales expuestos a un pH bajo, pero este grupo mostró unos niveles inferiores de la escala de grises, sugiriendo una menor mineralización. Además, en hueso se observó un aumento de la expresión génica de *tmap* y *mmp9* en el grupo expuesto a pH de 7.3, lo que sugiere una mayor tasa de recambio óseo, pero no se encontraron diferencias en la expresión génica en branquias. Los resultados *in vitro* mostraron un descenso de la actividad y mineralización de los osteoblastos al disminuir el pH extracelular. En conclusión, el estudio demuestra que la acidificación oceánica afecta el balance electrolítico del plasma y disminuye la mineralización ósea en dorada.

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## PROMOTING CIRCULAR ECONOMY BY THE ASSESSMENT AND VALIDATION OF MUSHROOM INDUSTRY BY-PRODUCTS AS SUSTAINABLE INGREDIENTS FOR RAINBOW TROUT *Oncorhynchus mykiss* DIETS

Carl John Saromines<sup>1\*</sup>, Silvia Torrecillas<sup>1</sup>, Francisco Javier Moyano<sup>3</sup>, Ignasi Sanahuja<sup>1</sup>, Nuria Tous<sup>1</sup>, David Torrallardona<sup>1</sup>, Joan Tarradas<sup>1</sup>, Gerard Verge Merida<sup>1</sup>, Sonia Ojeda<sup>2</sup>, Maria Luisa Tello Martín<sup>2</sup>, Yanis Cruz-Quintana<sup>3</sup> and Enric Gisbert<sup>1</sup>,

<sup>1</sup>Institut de Recerca i Tecnología Agroalimentaries (IRTA), La Rápita, Spain. <sup>2</sup>Centro Tecnológico de Investigación del Champiñón (CTICH), La Rioja, Spain. <sup>3</sup>Departamento de Biología y Geología, Facultad de Ciencias Experimentales, Universidad de Almería, Almería, Spain. <sup>3</sup>Facultad de Acuicultura y Ciencias del Mar, Universidad Técnica de Manabí, Ecuador

\*[carl.saromines@irta.cat](mailto:carl.saromines@irta.cat)

Utilization of agricultural by-products is a key strategy in addressing the search for alternative and sustainable feed protein ingredients for aquaculture. The European Union mushroom agriculture sector produces more than one million metric tons of mushroom per year, with Spain ranking as the third largest producer country in Europe, contributing approximately 10% of the total production. According to the European Mushroom Growers Group, each metric ton of cultivated mushrooms and sold as fresh, canned, or frozen generates 150 kg of organic residues. The management of the generated waste imposes an important challenge for the mushroom industry. Under this scenario, the potential use of these agriculture wastes (mushroom stems or not marketable mushrooms) as alternative protein and functional ingredient sources in aquafeeds appears a promising strategy for promoting circularity within the agrifood sector. Therefore, the present study assessed the potential of mushroom co-products from *Agaricus bisporus* (Ab), *Lentinula edodes* (Le) and *Pleurotus ostreatus* (Po) as alternative aquafeed ingredients by evaluating their *in vitro* and *in vivo* digestibility in rainbow trout (*Oncorhynchus mykiss*). Results showed that the *in vitro* digestibility of Le and Ab meals was similar to that of fish meal, as quantified by the liberation of total amino acids, whereas Po meal exhibited lower ( $p<0.05$ ) digestibility values. In the *in vivo* trial, rainbow trout fed Ab, Po and Le based diets for 42 days exhibited reduced growth performance ( $p<0.05$ ) compared to the fish fed with the control diet, which was positively correlated with the lower dietary crude protein ( $r=0.909$ ,  $p<0.05$ ) and lipid content ( $r=0.934$ ,  $p<0.05$ ) of mushroom based diets. Despite lower dietary crude protein content, fish fed with diets containing mushroom meals presented comparable protein efficiency ratio (PER) to the control diet, whereas lipid efficiency ratio (LER) was improved in all mushroom fed groups in relation to the group fed with the control diet. Ab and Po based diets increased feed conversion ratio (FCR) compared to fish fed the control diet. Hepatosomatic index (HSI) and viscerosomatic index (VSI) of fish fed with Ab and Po diets were comparable to fish fed with the control but were significantly increased ( $p<0.05$ ) in fish fed with Le. Similar patterns were observed in some hematological parameters such as levels of albumin, alkaline phosphatase and lipase, which were altered in fish fed the Le diet but not in fish fed Ab and Po. Mushroom meals, regardless of the species origin, reduced total bilirubin ( $p<0.05$ ). Results of apparent digestibility coefficients (ADC) and digestive enzymes activities will be correlated with fish key performance indicators. The findings of the present study provide basis for future research, particularly addressed at establishing the optimal inclusion dose of these new and sustainable raw materials in practical diets for rainbow trout.

## TOWARDS A MORE DIVERSE AND RESPECTFUL OF THE ENVIRONMENT AND ANIMAL WELFARE AQUACULTURE

Paula Simó-Mirabet<sup>1\*</sup>, L. Molina-Roque<sup>1,2</sup>, A. Barany<sup>1,3</sup>, A. Caderno<sup>1</sup>, C. Navarro Guillén<sup>4</sup>, E. Perera<sup>4</sup>, J. Fuentes<sup>4</sup>, F. Fonseca<sup>5</sup>, A. Galafat<sup>6</sup>, F.J. Alarcón<sup>6</sup>, J.M. Mancera<sup>1</sup>, J.A. Martos-Siteha<sup>1</sup>

<sup>1</sup>Dpto. Biología, Facultad de Ciencias del Mar y Ambientales, Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI-MAR), Universidad de Cádiz, Puerto Real, España. [paula.simo@uca.es](mailto:paula.simo@uca.es)

<sup>2</sup>Instituto Universitario de Acuicultura Sostenible y Ecosistemas Marinos de la Universidad de Las Palmas de Gran Canaria, Telde, Las Palmas, España.

<sup>3</sup>Dpto. de Genética, Fisiología y Microbiología, Universidad Complutense de Madrid, Madrid, España.

<sup>4</sup>Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Puerto Real, España.

<sup>5</sup>Centro de Investigação Marinha e Ambiental (CIMA), Universidade do Algarve, Faro, Portugal.

<sup>6</sup>Dpto. Biología y Geología, Universidad de Almería, Almería, España.

Aquaculture is key for providing food to the world's expanding population, as it is the fastest-growing and most diverse animal food production sector in terms of species, environment, and technology used. In addition, aquaculture is a source of healthy and high-quality proteins, contributing to a safe and well-balanced human diet. Despite the opportunities of this food sector, it is necessary to face the entailed challenges, especially those related to sustainability and animal welfare.

Therefore, it is essential to generate new knowledge at both technological and biological levels to improve productivity in the aquaculture industry. Some strategies include improving the cultured conditions of already established species and/or promoting the farming of new species, adding economic, social, and ecological value to the sector. The greater amberjack (*Seriola dumerili*) is a good candidate for marine aquaculture diversification due to its high growth rates, flesh quality, and consumer acceptance. However, it has high protein requirements, mostly from fish meal, a finite marine resource. For this reason, replacing marine ingredients with plant proteins could contribute to achieving truly sustainable aquaculture. Nevertheless, diets high in vegetable raw materials can affect the bioavailability and digestibility of nutrients, causing negative effects on fish growth and health. In this sense, the use of biotechnological pre-treatments of plant ingredients and the inclusion of nutraceutical compounds have demonstrated the improvement in the use of these nutrients, providing high growth rates, as well as good intestinal and general health status in highly carnivorous species such as greater amberjack.

On the other hand, raising awareness of the need for improved animal welfare in livestock production has pressured the industry to reduce the stress of aquaculture practices, which can negatively affect fish growth, welfare, and survival. Transport of live fish, one of the most common activities in the sector, induces a stress response in animals, modifying several physiological processes. Thus, improving transport conditions, by using natural compounds (as essential oils) during transport or feeding animals with functional diets before transport could be key to progress in animal welfare issues. Likewise, there is a need to develop less or non-invasive biomarkers to assess accurately stress in animals without compromising their well-being. All these strategies may improve animal health and welfare, increasing productivity and efficiency of this sector and achieving a more animal-friendly and environmentally sustainable aquaculture.

# Monitorització de l'estat del cultiu intensiu mitjançant biomarcadors del mucus epidèrmic de la truita irisada

Marc Tejero<sup>1\*</sup>, Pedro Lodeiro<sup>1</sup>, Maite Korraletxe<sup>1</sup>, Ignasi Sanahuja, , Diego Mendiola<sup>2</sup>, Antoni Ibarz<sup>1</sup>, Laura Fernández-Alacid<sup>1\*</sup>

- 1- Departament de Biologia Cel·lular, Fisiologia i Inmunologia, Facultat de Biologia, Universidad de Barcelona (08028, Barcelona).  
2- Caviar Pirineo S.L.U

## Resum

En aquicultura, les condicions de producció intensiva impedeixen als peixos respondre lliurement a les periodicitats ambientals anuals. Per aquesta raó, el control periòdic de l'estat dels animals esdevé necessari per a poder introduir correccions a les condicions de cultiu que atenguin als seus processos o ritmes biològics i a diferents situacions fisiològiques puntuals. Habitualment, s'ha realitzat la monitorització dels canvis en variables fisiològiques internes, com la concentració d'hormones circulants, paràmetres hematològics o índexs organosomàtics. No obstant, actualment en la indústria aquícola existeix una creixent voluntat de desenvolupar mètodes d'estudi no invasius per a protegir el benestar dels animals, evitant el sacrifici i, així, minimitzant les pèrdues de producció. En aquest sentit, en el present treball, s'ha realitzat una monitorització mensual durant dotze mesos d'un cultiu intensiu de truita irisada (*Oncorhynchus mykiss*) mitjançant l'anàlisi del mucus epidèrmic, la recol·lecció del qual no implica el sacrifici dels animals. Es van capturar mensualment grups de 15 individus de tres estanques diferents de les instal·lacions d'una piscifactoria local (Vivers del Segre, Peramola, Lleida) i es van anestesiar en oli de clau (1mL/L) per a la recol·lecció del mucus epidèrmic. Es van analitzar els següents bioindicadors: proteïna, glucosa, lactat i cortisol com a marcadors d'estrès, i el poder antioxidant, l'activitat lisozim, l'activitat proteolítica total i l'activitat antibacteriana com a biomarcadors de defensa. L'objectiu principal va consistir en poder establir si aquests bioindicadors segueixen uns patrons estacionals i/o alerten d'alteracions mensuals puntuals, per poder transferir aquests resultats a l'empresa de manera ràpida. Amb aquest estudi s'han pogut obtenir, per primer cop, dades del "rang" d'aquests bioindicadors en condicions de cultiu intensiu, sent observable una estacionalitat pels nivells de cortisol (majors durant la primavera-estiu) i l'activitat proteolítica total (incrementant durant els mesos hivernals i primavertals) i la presència de pics "d'incidència" mensuals per la resta de paràmetres (proteïna, glucosa, lactat, poder antioxidant, activitat lisozim i activitat antibacteriana). Malgrat l'escàs coneixement previ que existeix sobre l'ús del mucus epidèrmic per a la monitorització de l'estat dels animals en un cultiu intensiu, aquests resultats ens han permès conoure que el mucus es presenta com una valuosa eina no invasiva de control i monitorització del benestar de els peixos de cultiu al llarg de l'any, al permetre també un ràpid retorn dels resultats a l'empresa.

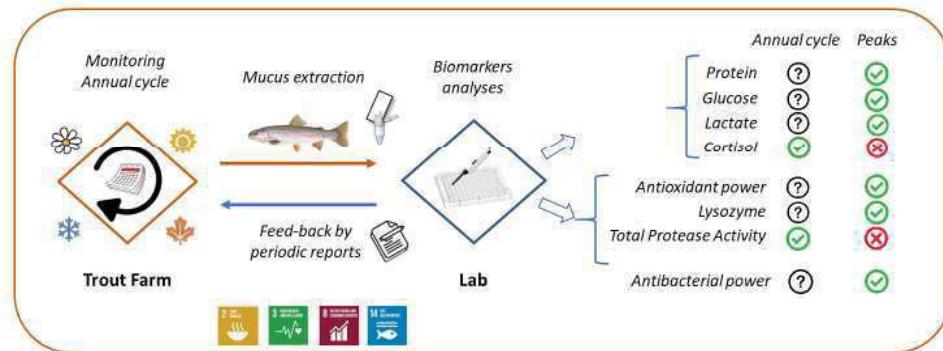


Figura 1. Esquema de l'objectiu del projecte i dels bioindicadors obtinguts mostrant les variacions estacionals ("anual cycle") i/o l'efecte puntual en el cultiu

**POSTERS**

## **Histopathological effects of chronic exposure to high concentrations of polystyrene nanoplastics in Chilean mussels (*Mytilus chilensis*)**

Camila Barría<sup>1,2,3\*</sup>, JoanCarles Balasch<sup>3</sup>, José Luis Iriarte<sup>2</sup>, Doris Oliva<sup>4</sup>, Mariana Teles<sup>3</sup>

<sup>1</sup> Programa de Doctorado en Ciencias de la Acuicultura, Escuela de Graduados Sede Puerto Montt, Universidad Austral de Chile, Puerto Montt 5507210, Chile

<sup>2</sup> Centro de Investigación Dinámica de Ecosistemas Marinos de Altas Latitudes- IDEAL, Instituto de Acuicultura, Universidad Austral de Chile, Punta Arenas, Chile

<sup>3</sup> Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

<sup>4</sup> Instituto de Biología, Centro de Investigación y Gestión de Recursos Naturales (CIGREN), Facultad de Ciencias, Universidad de Valparaíso, Valparaíso 2360102, Chile

### **Abstract**

Several emergent contaminants, including nanoplastics (NPs) in particular, may accumulate in mussels, damaging tissues, altering metabolic and immune performances, and limiting growth and developmental rates. Here we analyse the histopathological alterations associated to long-term exposure (28 days) to high concentrations (5 mg/L) of Polystyrene (PS) NPs in adult *Mytilus chilensis* feed daily with *Isochrysis galbana* (50.000 cells/ml). We found accumulation of PSNPs mainly in the gills and the digestive gland, and to a lesser degree, in the gonadal tissue. Haemocyte infiltrations were observed in branchial tissues, gonads, digestive gland and mantle at the end of exposure time. Morphological changes in the gastrointestinal tract and the gills were also observed, including altered epithelia and inflammation. Taken together, our results suggest a potential impairment of reproductive performance if *M. chilensis* populations remain chronically exposed to elevated concentrations of PSNPs.

# Identification of epigenetic biomarkers of resistance to bacterial infectious diseases in the European sea bass

Silvia Beato<sup>1\*</sup>, Marine Herlin<sup>2</sup>, María López Belluga<sup>3</sup>, Francesc Piferrer<sup>1</sup>

<sup>1</sup>Institut de Ciències del Mar (ICM - CSIC), Passeig Marítim, Barcelona, Spain.

<sup>2</sup>Aqüicultura Balear, S.A.U., Coll D'en Rabassa, Balearic Islands, Spain

<sup>3</sup>Culmarex, S.A.U., Águilas, Murcia, Spain

Infection by *Photobacterium damsela* subsp. *piscicida* is a major threat to Mediterranean aquaculture, including that of European sea bass (*Dicentrarchus labrax*), causing high mortality. Currently, there are no satisfactory preventing or treating strategies. Genomic selection can enhance disease resistance, but it presents economic limitations. Modifications of DNA methylation in host cells, associated with bacterial infection, can trigger immune response or facilitate pathogen survival and replication. Notably, differences in DNA methylation have been observed in various tissues of different species, particularly between resistant and susceptible fish to infectious diseases. These changes were located in promoter, exon, and intron regions of immune-related genes. This indicated the potential for incorporating epigenetic markers into genomic selection for disease resistance and developing diagnostic tools to better manage fish health. This study aimed to analyze methylation differences in sea bass following a *Photobacterium* infection, distinguishing between surviving and non-surviving fish and resistant (RF) and non-resistant (NRF) families. A 19-day challenge was conducted on 593 PIT-tagged and injected fish from 26 families genotyped by microsatellites. A control group consisted of 25 randomly-selected fish from 5 families, which received phosphate-buffered saline (PBS) injections. Spleen samples were obtained from dead fish throughout the challenge, and from both dead and surviving fish at the end of the challenge. Three of the 26 families reached 100% mortality. Additionally, 8 families had a mortality rate of less than 70% (average = 64.4%) and the remaining 15 had more than 70% (average = 78.3%). Based on mortality rates and number of samples available to have enough replicates per family (n = 3), two families with less than 65% mortality and two with more than 80% mortality were selected as RF and NRF, respectively. From the control, two families were selected. Genome-wide DNA methylation was determined by RRBS and bioinformatics analysis completed, averaging 97.9%, 71.4% and 98.9% for trimming, alignment and bisulfite conversion, respectively. Principal Component Analysis revealed a clear clustering of surviving fish, based on their belonging to either RF or NRF, validating our approach. Statistical analysis identified 8,881 Differentially Methylated Cytosines (DMCs) in non-surviving fish from the RF vs NRF, 10,270 in surviving fish from the RF vs NRF, and 8,889 in surviving vs non-surviving fish, from RF. Currently, we are focusing on the 10,270 DMCs, annotating these against the genome to identify affected regions by the infection and performing enrichment analysis to determine the pathways most associated with survival to infection.

**THE IN VITRO LIFE CYCLE AND FECUNDITY OF ANISAKIS PEGREFFII**Harriet Nketiah Birikorang<sup>1,2</sup>, Samantha Moratal<sup>3</sup>, Jerko Hrabar<sup>4</sup>, Ivona Mladineo<sup>1</sup><sup>1</sup>Institute of Parasitology, Biology Centre Czech Academy of Sciences, Ceske Budejovice, Czechia<sup>2</sup>Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Gent, Belgium and Faculty of Veterinary medicine, Universitat Autònoma de Barcelona, Barcelona, Spain<sup>3</sup>Servicio de Análisis Investigación de Animales Silvestres (Saigas), Facultad de Veterinaria, Universidad Cardenal Herrera- CEU, CEU Universities, C/Tirant lo Blanc, Alfara del Patriarca, Valencia, Spain<sup>4</sup>Institute of Oceanography and Fisheries, Split, Croatia**Introduction**

The spotlight on marine nematodes of the genus *Anisakis* has intensified in research circles due to a surge in anisakiasis cases in Europe; a human clinical condition caused by *Anisakis* spp. A recent breakthrough in establishing an *in vitro* life cycle has enabled a more detailed exploration of the biological and physiological characteristics of both *Anisakis pegreffii* and *Anisakis simplex*. This development shows possibility for screening potential drug targets and conducting in-fillet inactivation tests. In their natural habitat, free-swimming second-stage larvae (L2) emerge from eggs released into seawater through the feces of marine mammals, serving as final hosts. These larvae are targeted by crustaceans, primarily euphausiids, and possibly small fish, acting as intermediate hosts, where they moult into third-stage larvae (L3). The intermediate hosts are then consumed by larger fish, acting as paratenic hosts, within which L3 migrate into the visceral cavity and remain in parathesis until final hosts prey upon and digest the infected paratenic host. Within the final hosts, L3 progress through the fourth (L4) and fifth (L5) juvenile stages before maturing into reproductively active adults of distinct sexes. The objective of this study was to assess the egg production and hatchability throughout the lifespan of *Anisakis pegreffii* adults sustained *in vitro*, derived from third-stage larvae (L3) extracted from fish. Examining the reproductive characteristics of this zoonotic nematode, including fertility and lifespan, will aid in future epidemiological modeling and risk assessment estimations.

**Materials and Methods**

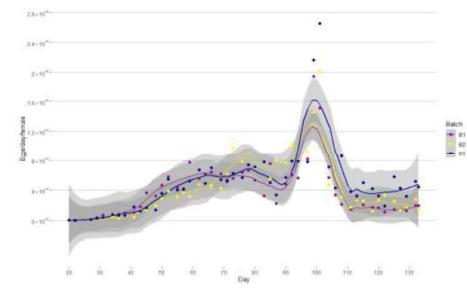
*Anisakis* spp. type I larvae (identified as *A. pegreffii* following genotyping) were isolated from naturally infected blue whiting *Micromesistius poutassou* in the Adriatic Sea (Croatia). Isolated L3 larvae were cultured in Schneider's *Drosophila* media supplemented with 10% chicken serum ( $n=30$  in triplicate; designated as B1, B2, and B3) following a previously published protocol (Mladineo et al., 2023) to attain the adult stage. Progression to adulthood from isolated L3 and the subsequent egg production in the medium were monitored every two days. The collected eggs were then incubated in Sea Salt Solution at 19°C, and the time of hatching was documented. *Anisakis* fecundity was quantified as the daily number of expelled eggs following the method of Moratal et al. (2023). Furthermore, the quantity, gender, and date of adults extracted from the culture were recorded to calculate the sex ratio (expressed as a percentage of females) during each observation. A graph illustrating the daily egg count per reproductive female was generated.

**Results and Discussion**

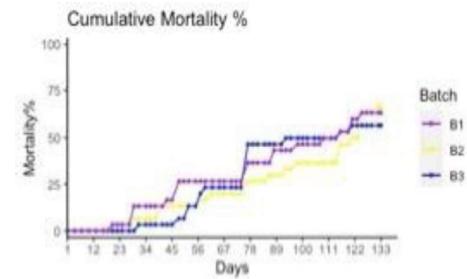
Larvae start transitioning to the fourth stage (L4) by the fourth day post-incubation (dpi), and another shedding of the cuticle, indicating the moult into L5, was observed after 15 days. The onset of the adult stage, characterized by the initial expulsion of eggs, occurred after 17 days. However, the growth rate of the worms was asynchronous, with some individuals developing more rapidly than others. Eggs were detected in the medium from day 17 to day 133 post-incubation. The initial hatching of eggs, indicating the successful fertilization, occurred on day 44 post-incubation. Subsequently, for the next 51 days (until day 95 post-incubation), eggs were fertilized and hatched into L2 larvae. Hatching typically took place 5-7 days after egg incubation. The average fecundity peaked on 100 dpi, being 194,775, 202,038 and 265,763 eggs/day/female for the three replicates. The quantity of eggs harvested from each replicate is shown in the Figure 1. Consequently, a sex ratio of 1:2 was calculated for B1, and 1:3 for both B2 and B3. The number of dead worms was recorded throughout the experiment and the cumulative mortality is shown in the Figure 2.

**Acknowledgement:** Dr. Ivona Mladineo for supervising this project

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**Figure 1:** *Anisakis pegreffii* fecundity (eggs/day/female) *in vitro* culture calculated for each replicate.



**Figure 2:** Cumulative mortality (%) of *Anisakis pegreffii* adults kept in Schneider's *Drosophila* medium enriched with 10% chicken serum.

At the end of the experiment, cumulative mortality rates of 59.99%, 49.99%, and 53.26% were recorded for B1, B2, and B3, respectively. An interesting observation was that deceased worms tended to be smaller, hinting at possible underdevelopment and decreased ability to compete for nutrients. Besides, other biological factors might have also played a role in their mortality.

**Conclusion**

*Anisakis pegreffii* can thrive and reproduce in insect medium for up to four months, but egg fertilization and L2 larvae hatching are viable only within a limited 95-day timeframe post-incubation, consistent with previous research. Despite this, maintaining an adequate number of replicates ensures a satisfactory yield of fertilized eggs for downstream experiments.

# NANOPLASTICS ARE ACCUMULATED IN THE GUT AND BLOOD OF RAINBOW TROUT AND INDUCE A DECREASE IN PLASMA CORTISOL LEVELS

Brandts I.<sup>1,2\*</sup>, García-Meilán I.<sup>3</sup>, Lima J.<sup>4</sup>, Llorca M.<sup>5</sup>, Farré M.<sup>5</sup>, Tvarijonaviciute A.<sup>6</sup>, Tort L.<sup>1</sup>, Teles M.<sup>1,7</sup>

<sup>1</sup> Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

<sup>2</sup> Institute of Marine Sciences (ICM-CSIC), Pg. Marítim de la Barceloneta 37–49, 08003, Barcelona, Spain

<sup>3</sup> Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

<sup>4</sup> Institute of Bioscience, Department of Physiology, Universidade de São Paulo, Brazil

<sup>5</sup> Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain

<sup>6</sup> Interdisciplinary Laboratory of Clinical Analysis INTERLAB-UMU, Regional Campus of International Excellence Mare Nostrum, University of Murcia, Espinardo, Murcia 30100, Spain

<sup>7</sup>Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

\*Email: irene.brandts@uab.cat

Nanoplastics (NPs) are plastic particles of less than 1 µm in size formed in the environment by the degradation of larger plastic waste. These particles can cross biological barriers, such as the gastrointestinal and brain blood barriers, which could make them the most dangerous among plastic debris. The main objective of the present work was to evaluate if polystyrene-NPs (PS-NPs) are recognized as a stressor by the hypothalamus-pituitary-interrenal (HPI) axis of rainbow trout (*Oncorhynchus mykiss*) by measuring plasma cortisol levels. For this purpose, adult rainbow trout (50.2 g mean weight) were orally intubated with PS-NPs (44 nm, 100 µg/L, 1 mL per fish). After 96 h fish were sacrificed, and blood, liver and gut were sampled. Cortisol, and lipid metabolism (glucose, cholesterol, and triglycerides) biomarkers were determined in plasma after PS-NPs exposure. Quantification of PS-NPs levels was carried out in blood, gut and liver of fish. Histology was done in the gut to assess damage. The results showed that PS-NPs were detected and quantified in both blood and gut of exposed fish, but not in liver. Plasma cortisol levels showed a significant decrease in PS-NPs exposed fish when compared to control group. In fish, cortisol is involved in the stress response as well as in many aspects of the endocrine-mediated immune response and therefore the observed decrease could suggest an impairment of the HPI axis. Triglycerides levels in plasma were increased in the exposed individuals, which could point to altered lipid metabolism, potentially affecting the energetic status of rainbow trout. No histological alterations were found.

## **Effects of short-term exposure of nanoplastics on *Dicentrarchus labrax* sperm**

H. Djafar<sup>1</sup>, I. Brandts<sup>1</sup>, E. Okon<sup>1</sup>, J.C. Balasch<sup>1</sup>, D. Sánchez Ruiz<sup>2</sup>, J.M. Mancera<sup>2</sup>, J.E. Rodriguez-Gil<sup>3</sup>, M.M. Rivera del Alamo<sup>3</sup>, M. Teles<sup>1</sup>

<sup>1</sup>Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>2</sup>Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEIMAR), Universidad de Cádiz, Cádiz, Spain

<sup>3</sup>Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

### **Abstract**

In the last decade nanoplastics (NPs) have emerged as a troubling environmental pollutant associated with their impact on the reproductive processes of marine organisms. However, the information on the effects of NPs on fish gametes is still scarce. Here we investigated the NPs uptake and effects of short-term exposure (1h) to environmentally relevant (100 µg/L) and high (5000 µg/L) concentrations of polystyrene (PS) nanoplastics on viability and oxygen consumption in European Seabass (*Dicentrarchus labrax*) sperm cells. We observed an uptake of NPs by sperm cells exposed to both concentrations of NPs, being significantly higher in the group exposed to the high concentration of NPs, suggesting a concentration-dose effect. The percentage of sperm viability was not affected by NPs exposure, but oxygen consumption diminished in samples exposed to both concentrations of NPs, although no difference was observed between environmentally relevant and high concentrations. Previous studies on fish cell lines have demonstrated that NPs can inhibit oxygen uptake by cells, particularly affecting mitochondrial respiratory functioning, which may lead to oxidative stress. This inhibition could be dose-dependent, corresponding with decreased bioenergetic index and ATP-related respiration that may explain the observed decrease in oxygen consumption of *D. labrax* sperm cells exposed to PS-NPs.

# **Plastic pellets can generate micro and nanoplastics and affect the health of marine fish**

A. Martí<sup>1</sup>, I. Brandts<sup>1</sup>, D. Jornet<sup>1</sup>, M.P. Tudela<sup>1</sup>, M. Sugrañes<sup>2</sup>, J. Oliva<sup>2</sup>, M. Teles<sup>1</sup>

<sup>1</sup> Departament de Biologia cel·lular, fisiologia i immunologia, Universitat Autònoma de Barcelona

<sup>2</sup> Good karma projects, goodkarmaprojets.com

Alexxmarti05@gmail.com

With the exponential increase in plastic production and pollution, research has focused on the impact of this material on ecosystems, with special focus on marine ecosystems, since plastic waste tends to end up and accumulate in the seas and oceans by run-off.

Parallelly, a great amount of controversy and social interest has been shown towards plastic pellets found on beaches, especially since the discharge of millions of them in Galicia last December, polluting the northwest coastline of the Iberian Peninsula. Due to the visual impact, because it is visibly mixed with the sand on the beaches, there was a huge coverage by the media. These pellets are the raw material from which all plastic products are produced and moulded.

In the present work, we aimed to study the impact they on marine species, focusing mainly on micro (5 mm-1 µm, MPs) and nanoplastics (1 nm-1 µm, NPs). With this goal in mind, pellet samples were collected from the beach in Costa Daurada (Tarragona) and a process was carried out to obtain microplastics and nanoplastic. Plastic pellets were fused and degraded with the help of a multitool Dremel 3000 with a rotatory diamond burr to avoid metal contamination. Dust was collected and processed through a metal 0.2 mm sieve, digested with TFA, treated with SDS, and cleaned with Mili-Q water and ethanol to obtain the suspended plastics. For the NPs, the sample was left to precipitate for an hour and was collected from the supernatant section. Instead, for the MNPs, no sedimentation was needed and was directly obtained from the suspension. After cleaning and centrifugation, the plastic pellet was resuspended at a desired concentration (10 mg/mL).

After obtaining them from the pellets, a bioassay was carried out with the marine fish Gilthead bream (*Sparus aurata*). Three conditions were studied: control (0 g/L), nanoplastics (30 - 100 nm, 0.001 g/L), and micro and nanoplastics (30 nm - 200 µm, 0.001 g/L) for a 96-hour exposure. Afterwards, blood, and liver was sampled. The blood was immediately used for haematological analysis such as haematological profiling, erythrocytic nuclear abnormalities (ENAs), and cortisol and glucose levels, while the liver was snap frozen and kept at -80°C for posterior gene expression analysis.

## **Col·laboració interuniversitària entre la UPC-BarcelonaTech i la UniSave (Moçambic): Estudi de viabilitat d'explotacions aquícole a la Badia de Inhambane**

I. Masaló<sup>\*1</sup>, M. Grifoll<sup>1</sup>, G. Solana<sup>2</sup>, E. Pavo-Fernàndez<sup>1</sup>, L. Reig<sup>1</sup>

<sup>1</sup> UPC- BarcelonaTech

<sup>2</sup> UniSave (Moçambic)

L'IHD de Moçambic l'any 2021 fou de 0.446 situant-lo en el lloc 185 dels 191 països inclosos en l'informe del 2022 del PNUD; el govern de Moçambic en el seu pla quinquennal (2020-2024) i també l'Organització de les Nacions Unides en l'informe anual sobre Moçambic del 2023 a través del seu programa Mundial d'Aliments han reconegut la seguretat alimentaria i nutricional com a prioritat clau enfatitzant la importància de l'accés als aliments i la millora de les condicions de vida. Els productes aquícoles representen un font de proteïna animal addicional, contribueixen a la seguretat alimentària, i l'aquicultura com a activitat estimula el desenvolupament regional, generant nous llocs de treball i disminuint la pressió sobre les poblacions silvestres. Tot i que Moçambic té un extensa zona costanera (2700 km) i unes condicions climàtiques adients per l'assentament de cultius aquícoles aquests són minoritaris, i concretament a la badia d'Inhambane és una activitat inexistent.

Qualsevol producció aquícola cal que sigui sostenible, d'espècies autòctones (o al·lòctones) i amb les tecnologies adequades. La implementació de qualsevol activitat aquícola sense un estudi previ podria generar problemes tant en termes mediambientals com de conservació de la biodiversitat. Aquest estudi previ ha d'incloure, entre d'altres aspectes, un аналisis de les característiques hidrogràfiques de la zona per tal d'analitzar quines espècies i tecnologies es podrien adaptar millor als diferents condicionants físics (temperatura, salinitat, corrents marins). Fruit de la col·laboració entre la UPC-BarcelonaTech i la Universidade Save (Moçambic) durant el període 2018-2022 s'ha caracteritzat hidrogràficament la badia d'Inhambane; aprofitant el coneixement i les dades obtingudes, la col·laboració s'ha ampliat durant aquest 2024 per tal d'estudiar la viabilitat d'explotacions aquícoles a la badia.

L'objectiu d'aquesta nova col·laboració és avaluar aquelles tecnologies que puguin tenir un impacte neutre o positiu a la zona, com per exemple, la integració de diferents espècies tròfiques (algues-bivalves-peixos) o bé la silvo-aquicultura (cultiu de peixos en manglars). El resultat principal esperat d'aquesta col·laboració és la identificació de les espècies aquícoles idònies per realitzar-ne el cultiu de manera sostenible a la badia d'Inhambane, així com la identificació de les actuacions necessàries per la seva implementació tenint en compte l'administració i la formació de comunitats locals.

Col·laboracions com les presentades aquí, ajuden a enfortir les relacions entre institucions i posen de manifest la importància de la transferència de coneixement des de Catalunya, des d'un punt de vista aquícola en aquest cas, a països amb menys recursos científics i tecnològics.

## The use of seaweed extracts cultured in Catalonia to increase the yield and quality of local crops

HorPTA- Horticultura: Producció, Transformació i Aprofitament  
Grup de recerca consolidat (SGR 00277)

E.J. Gualda, I. Masalo\*, M. Cendra, I. Roig, A. Rivera, S. Campo, A. Palou, J. Casals, I. Codina, A. Gras, I. Achaerandio, E. Hernandez, F.L. Sepulcre, A. Giné, X. Sorribas, L. Serrano

Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya,  
Esteve Terradas 8, 08860 Castelldefels, SPAIN  
e-mail: [horpta.eab@upc.edu](mailto:horpta.eab@upc.edu)

Horticulture is an essential sector of the Catalan economy and society. Although it currently occupies only 1.2% of the agricultural area of Catalonia, its productivity (26.6 t/ha on average) and its economic weight (8.9% of final agricultural production) make it a dynamic sector of great contribution to overall agri-food economy in Catalonia. Although the actual yield of crops is far from what is achieved in other vegetable growing regions, the sector has the capacity to produce up to 50% of the vegetables consumed in Catalonia. Since the end of the last century, the sector has incorporated technical advances and improved plant production standards to increase the efficiency of the production system while reducing harmful effects on the environment and health.

The objectives proposed by the *Pla director de l'horta a Catalunya* proposed by the *Departament d'Acció Climàtica, Alimentació i Agenda Rural de la Generalitat de Catalunya* and those of the *European Commission's New Green Deal* includes “the reduction of the net emissions of greenhouse gases and environmental degradation, allowing and accelerating the transition towards a fair, healthy and environmentally friendly food system”. In this sense, circular food systems play a key role, since they use practices and technology that minimize the contribution of finite resources (like water and nutrients), encourage the use of regenerative ones, prevent the leakage of natural resources from the food system, and stimulate reuse and recycling of resources, adding the maximum possible value to the food system, facilitating food self-sufficiency in order to boost local markets. Under this premise, the HorPTA research group is studying **the effect of biologically based fertilizers using algae on the yield and quality of vegetable crops and on the resistance of plants to biotic and abiotic stress**.

Togherther with this specific objectives HorPTA is evaluating how to obtain improved local plant genotypes adapted to the edaphoclimatic conditions of the growing area. The use of algae extracts to enhance biotic and abiotic stresses in the local plant genotypes will allow designing circular agricultural systems focused on new products and food ingredients development improving the use of by-products.

## **Hematological reference values for rainbow trout (*Oncorhynchus mykiss*) using an automated analyser (Sysmex XN-1000V)**

M. Mesalles<sup>1</sup>, I. Brandts<sup>2</sup>, M. Uroz<sup>2</sup>, E. Serrano<sup>3</sup>, R. Cuenca<sup>1</sup>, J. Pastor<sup>1</sup> and M. Teles<sup>1\*</sup>

<sup>1</sup>Servei d'Hematologia Clínica Veterinària (SHCV). Departament de Medicina y Cirurgia Animals. Universitat Autònoma de Barcelona (UAB), Bellaterra, Espanya

<sup>2</sup>Departament de Biología Celular, Fisiología e Inmunología, Universitat Autònoma de Barcelona (UAB), Bellaterra, Espanya

<sup>3</sup>Wildlife Ecology & Health Group (WE&H), Servei d'Ecopatología de Fauna Salvaje (SEFaS), Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona (UAB), Bellaterra, Espanya

\*Corresponding author: [mariana.teles@uab.cat](mailto:mariana.teles@uab.cat)

### **Abstract**

The aquaculture industry has garnered increasing attention worldwide, with rainbow trout (*Oncorhynchus mykiss*) standing out as a significant species. The assessment of blood parameters is an invaluable tool for predicting the well-being status of fish species. Haematological parameters provide critical insights into the health status of fish and their response to changes related to nutrition, water quality, and disease. Previous research on fish haematology has mainly been conducted using manual methods, instead of the automatic methods which has been proved to be faster and more precise than the manual one. Moreover, the establishment of normal blood reference values in fish health is still lacking, as few specific studies address the obtaining of reliable reference values for different fish species, making interpretation of results difficult. The purpose of this study was to establish reference intervals for rainbow trout blood samples using the Sysmex XN-1000V automated haematology analyser. A total of sixty-three blood samples were collected by caudal venipuncture from healthy adult rainbow trout. Fish was anesthetized using MS-222 prior to blood extraction. Blood parameters 'normality' was assessed using the Shapiro-Wilk test ( $p<0.05$ ). Reference intervals were established according to the American Society for Veterinary Clinical Pathology (ASVCP) guidelines. The reference intervals for the blood parameters were as follows: RBC (0.76-1.33  $\times 10^6/\mu\text{L}$ ), Hb (4.33-7.69g/dL), Hct (31.84-60.27%), MCV (331.02-488.02fL), and WBC (10-44.9  $\times 10^3/\mu\text{L}$ ). The results from this study show that values of the reference interval obtained in the present study is similar to those previously obtained by other authors, where the manual method is used. However, a more precise and less variability was observed with the automated method. In summary, in the present study, the reference intervals reported will help to improve the interpretation of haematological results by providing standardized benchmarks for assessing the health status of rainbow trout in aquaculture using a fast and less time-consuming method than the manual method.

**Ekemini Okon**

Department of Cell Biology, Physiology and Immunology

ekemini.okon@ugent.be

## **Effects of emergent contaminants and temperature rise on fish species used for human consumption, in the context of climate change**

**Ekemini Okon<sup>1</sup>, Hayam Djafar<sup>1</sup>, Irene Brandts<sup>1</sup>, Mariana Teles<sup>1</sup>**

<sup>1</sup> Department of Cell Biology, Physiology and Immunology  
Universitat Autònoma de Barcelona,  
Bellaterra, Spain

Plastic waste constitutes a significant threat to the health of aquatic animals. Thus, aquatic organisms, such as fish, are at considerable risk to be exposed to nanoplastics (NPs), with increased risk due to their small size, high surface profile, high mobility and low polarity. This study aims to determine the potential detrimental effects of polystyrene nanoplastics (PS-NPs) and increasing water temperature in the context of climate change, at distinct biological levels in *Sparus aurata* (Gilthead seabream). To this end, we exposed fish for 14 days to PS-NPs (100 µg/L PS-NPs at room water temperature), elevated temperature (0 µg/L PS-NPs at 26 °C), and in co-exposure of the two stressors (100 µg/L PS-NPs + temperature increase - 26 °C). The results show that the biometric indicators (weight, length, Fulton's condition factor and hepatosomatic index) were not affected after the experimental exposures. No significant ( $p < 0.05$ ) effects were observed on white and red blood cell count and platelets count. However, there was a significant decrease in the hematocrit values, and this was significantly lower for the fish exposed to elevated temperature with PS-NPs compared to the control. Haemoglobin concentration reduced significantly in the group of fish exposed to PS-NPs, in the group of fish exposed to an elevated temperature and in the group of fish subjected to high temperature with PS-NPs. There was a highly significant reduction in mean corpuscular cell volume in the groups exposed to elevated temperature, when compared to the control group and in the group of fish exposed to the combination of elevated temperature with PS-NPs ( $123.70 \pm 20.63$ ). A significant decrease in mean corpuscular haemoglobin was observed for the group exposed to PS-NPs ( $20.47 \pm 1.59$ ), elevated temperature ( $17.88 \pm 1.66$ ), and elevated temperature with PS-NPs ( $19.15 \pm 1.84$ ) compared to the control ( $21.37 \pm 1.40$ ). Mean corpuscular haemoglobin concentration was significantly increased in the group co-exposed to elevated temperature and PS-NPs ( $15.68 \pm 1.48$ ) than the control ( $13.87 \pm 1.10$ ). The principal component analysis (PCA) indicated that MCHC increased in the PS-NPs with elevated temperature group, while MCH, MCV, HCT, and HGB concentration decreased. In conclusion, this study showed a significant impact on the haematological parameters of Gilthead seabream after acute exposure to NPs and temperature in the context of climate change.

**Comparació morfolfuncional dels dipòsits de greix en truita irisada  
(*Oncorhynchus mykiss*) i la seva relació amb el metabolisme, la salut i la qualitat  
del peix**

**E. Rosell-Moll, N.T. Kim-My, S. Balbuena-Pecino, M. Montblanch, I. Rodríguez, J. Gutiérrez, D.  
Garcia de la serrana, E. Capilla, I. Navarro.**

**Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de  
Barcelona, Barcelona, Espanya**

**Resum**

El teixit adipós dels peixos teleostis es distribueix en tres dipòsits principals: el visceral (VAT), que envolta els òrgans interns de la cavitat abdominal; el subcutani (SAT), situat sota les capes epitelials, notablement present tant a la part dorsal/superior (SATs) com a la ventral/inferior (SATi) dels salmoníns; i l'intramuscular (IMAT), situat entre els miòtoms. Aquests dipòsits de greix varien regionalment en la seva morfologia i funcionalitat, tenint un impacte significatiu en el creixement, el metabolisme i la salut dels peixos, especialment el VAT; i també modifiquen la qualitat del producte final d'aquicultura, específicament l'IMAT, però també el SAT i VAT, afectant l'adipositat. En aquest treball, s'analitzen les característiques morfològiques i transcriptòmiques dels diferents dipòsits de greix de la truita irisada (*Oncorhynchus mykiss*), una espècie de gran interès aquícola en el nostre país. Els resultats mostren que tant el VAT com el SAT estan constituïts per adipòcits grans i poc nombrosos, suggerint un creixement predominantment per hipertrofia. A més, aquests dipòsits presenten un alt contingut de lípids totals (>85%), provant el fet que el VAT funciona com a principal dipòsit d'energia, mentre que el SAT podria tenir un paper secundari en aquesta funció, a més d'exercir-ne d'altres. Això es pot evidenciar atenent a la composició en àcids grassos del SAT, amb una menor presència d'àcid palmitíic (PA - 16:0) i major d'àcid eicosapentaenoic (EPA - 20:5n-3) comparat amb el VAT i l'IMAT. Els adipòcits de l'IMAT són de menor mida i més nombrosos, independent de la grandària dels animals. A més, presenten una menor activitat metabòlica, en base a l'expressió gènica d'alguns transportadors d'àcids grassos com *fatp1* i *fabp11a*, el transportador de glucosa *glut4* i alguns marcadors de lipòlisi i oxidació d'àcids grassos, més elevada en VAT i SAT en comparació amb l'IMAT. A l'espera dels resultats definitius de seqüènciació massiva, dades preliminars de l'anàlisi bioinformàtic de l'ARNseq indiquen que, tot i detectar-se els mateixos mARNs en els tres dipòsits, hi ha clares diferències en els nivells d'expressió d'aquests entre ells. Això subratlla la importància de comprendre les particularitats de cada dipòsit adipós per optimitzar les estratègies nutricionals o de cultiu que puguin modular el nivells i la distribució d'aquests dipòsits de greix, factors clau en la millora de la salut i el metabolisme dels peixos, així com en la qualitat del producte final.

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Satellite cells isolation and *in vitro* characterization of myogenesis in European sea bass  
(*Dicentrarchus labrax*)

García-Pérez I., Rubio A., Rodríguez I., Díaz-Serrano C., Navarro I., Capilla E., Blasco J., Gutiérrez J.

Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat de Barcelona, Barcelona, España

European sea bass (*Dicentrarchus labrax*) is a species of great importance in the Mediterranean aquaculture. Understanding the cellular and molecular mechanisms underlying muscle development and growth in this species is crucial for enhancing its production efficiency. To this end, we have established and characterized a primary culture of white muscle cells from this species and examined the expression profiles of key genes involved in myogenesis. Primary myocytes were isolated from the dorsal white muscle of fish juveniles (5-10 g) and cultured under standard conditions. Samples for gene expression and immunofluorescence analyses were collected from six independent cultures on days 2, 4, 6, 8, 10, and 12. The transcriptional profiles of myogenic regulatory factors (*myf5*, *myod1*, *myod2*, *mrf4*, *myog*), Gh/Igfs axis molecules (*ghr1*, *ghr2*, *igf1*, *igf2*, *igfbp-5b*, *igfbp-6b*, *igf-1rb*), genes related to Wnt signaling (*wnt1*, *wnt3a*, *wnt4*, *wnt5a*, *wnt5b*), Notch signaling (*notch2*, *notch3*), and other muscle development markers were analyzed. Pena and Myhc expression was examined by immunofluorescence to determine proliferation and differentiation stages, respectively. Gene expression profiles revealed distinct temporal patterns. Early-stage myogenesis markers (*pcna*, *myf5*, *myod2*) peaked at day 4, indicating the initiation of myogenic commitment, while myogenic differentiation markers (*mrf4*, *myog*, *mymk*, *myhc*) increased from the middle stages of the culture until myotube formation. Regarding the Gh/Igfs axis molecules, both *igf2* and *igf-1rb* gradually increased their transcription during the culture, suggesting their involvement in muscle growth and differentiation. In contrast, *igfbp-5b* presented the highest levels at day 2 and then drastically decreased, remaining stable for the rest of the culture. Wnt signaling genes (*wnt4*, *wnt5a*) notably increased during differentiation phases, aligning with their role in promoting myogenic differentiation. Notch signaling genes (*notch2*, *notch3*) displayed relatively constant expression throughout the culture. Immunofluorescence results supported the proper progression of the cell culture, with Pena expression peaking at days 4 and 6 and then decreasing, and Myhc expression increasing as the culture progressed, confirming the proliferation-differentiation transition. In conclusion, this study establishes a primary myocyte culture from European sea bass, providing a valuable tool for studying different signaling pathways and molecular networks involved in muscle development and growth under controlled conditions. These insights are essential for advancing aquaculture practices, improving fish growth and health, and enhancing muscle production efficiency and quality.

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# **REGULATION OF THE FISH STRESS RESPONSE TO A CHRONIC EXPOSURE OF EMERGENT CONTAMINANTS: GEMFIBROZIL AND NANOPLASTICS**

Ruiz N<sup>\*1</sup>, Blonç M<sup>1</sup>, Llorca M, Farré M, Tvarijonaviciute A<sup>4</sup>, Tort L<sup>1</sup>, Teles M<sup>1,2</sup>.

<sup>\*</sup> First and corresponding author

<sup>1</sup> Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

<sup>2</sup> Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

<sup>3</sup> Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEACSC, Barcelona, Spain

<sup>4</sup> Interdisciplinary Laboratory of Clinical Analysis Interlab-UMU, Regional Campus of International Excellence Mare Nostrum, University of Murcia, Espinardo, Murcia 30100, Spain

Affiliation and full address of the authors. Arial 9, justified. <sup>2</sup>Affiliation and full address of the authors. Arial 9, justified.

\*Email: Nuria.Ruiz.Iglesias@uab.cat

Emerging contaminants are environmental wastes of concern, as not only are accumulated in increasing amounts, but they may cause negative effects on the aquatic biota. The present work has focused on whether the stress response is modulated by two of these emergent contaminants, gemfibrozil (a commonly prescribed pharmaceutical lipid regulator) and nanoplastics. Their constant use and discard combined to their environmental persistence and poor removal rates from wastewater makes of these emergent contaminants ubiquitous in aquatic systems. We assessed the effects in hematological, biochemical, oxidative stress and gene expression response of a 28-day waterborne exposure to both an environmentally relevant concentration and a spiked concentration in juvenile fish. Bioaccumulation of these compounds in liver and muscle, as well as possible variations on haematological parameters, blood plasma biochemistry, and gene expression were investigated. The results indicated that both gemfibrozil and nanoplastics accumulated in both liver and muscle. Moreover, significant differences were observed in hematological variables. Regarding plasma cortisol, the response was different depending on the contaminant, since gemfibrozil showed a reduction of plasma cortisol probably associated to its metabolic interaction with the lipid synthesis pathways and therefore, cortisol production. This mechanism was supported by changes observed in the genetic markers of lipid metabolism. Although the cortisol response is different between the two contaminants, gene expression analysis corroborates the biochemical effects of both nanoplastics and gemfibrozil. Overall, the results from the present study first suggest that bioaccumulation of emergent contaminants trigger several reactive responses including the activation of the hypothalamic-pituitary-interrenal axis, and second, that some of them such as gemfibrozil, may interfere with the regulation of the cortisol response.

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# EFFECTS OF DIFFERENT LEVELS OF FISHMEAL REPLACEMENT WITH ALTERNATIVE PLANT PROTEIN SOURCES IN AQUAFEEDS FOR FLATHEAD GREY MULLET (*MUGIL CEPHALUS*) JUVENILES

Sandra Salcedo-Martínez<sup>\*1</sup>, Alicia Estévez<sup>\*1</sup>, Ana Roque<sup>\*1</sup>, Neil Duncan<sup>1</sup>

<sup>1</sup>Institute of Agrifood Research and Technology (IRTA), Sant Carles de la Ràpita, Crta. Del Poble Nou Km 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain

E-mail: [sandra.salcedo@irta.cat](mailto:sandra.salcedo@irta.cat); [alicia.estevez@irta.cat](mailto:alicia.estevez@irta.cat); [ana.roque@irta.cat](mailto:ana.roque@irta.cat)

## Introduction

It is essential to understand the significance of promoting sustainable development in aquaculture, and the need for diversification, using low trophic level species, with nutritional requirements that do not include fish meal (FM) and fish oil (FO) as sources of proteins and lipids. According to APROMAR (2022), feed accounts for about 60% of production costs, so farming species with diets reduced in FM and FO is not only interesting from a sustainability perspective, but also a profitable one. Careful feed management in combination with fish that have good welfare and feeding response will greatly increase sustainability and reduce wastes and costs. Flathead grey mullet (*Mugil cephalus*) is a species that naturally has no nutritional requirements for FM and fish FO, which makes it a good candidate for studying the effects of replacing high levels of FM and FO with alternative protein sources of vegetal origin (Gisbert et al., 2016; Kalla et al., 2003; Wassef et al., 2002). However, there is very little information on how this affects the species' welfare (Fazio et al., 2017). It is necessary to establish criteria that can reflect whether the four principles of animal welfare are met: good feeding, good housing, good health, and appropriate behaviour (Welfare Quality, 2009; Roque et al., 2020). By using parameters established in other species, it will be possible to assess the welfare of mullet fed diets with different levels of FM that was replaced with alternative plant protein (PP) sources. The aim of this study was to assess the welfare and growth of juveniles fed diets with low or no FM.

## Material and Methods

Flathead grey mullet were hatched and reared at IRTA facilities. A total of 495 individuals (initial body mass, ~ 11 g) were distributed randomly in 9 tanks of 500 L coupled to an IRTAmar® water recirculation system, which constituted the 3 experimental groups (in triplicate): i) Control, feed with 20% FM; ii) FM-0, feed with 0% FM; iii) FM-5, feed with 5% FM. After 73 days of *ad-libitum*-feeding trial, a final biometric sampling was performed, and samples of plasma, liver and muscle were collected. Somatic and zootechnical indices were calculated, and samples from each tissue were analysed (biochemical profile in plasma and circulating cortisol levels; proximate composition and fatty acid profile in liver and muscle; and gene expression in liver). Throughout the experiment, swimming and feeding behaviour as well as presence of fin and skin lesions were recorded five days a week in order to make a preliminary evaluation of the mullet welfare.

## Results and Discussion

Results on growth, welfare assessment and plasma parameters will be discussed.

Comité organizador y científico:

Mariana Teles - Universitat Autònoma de Barcelona

Lluís Tort - Universitat Autònoma de Barcelona

Joan Carles Balasch - Universitat Autònoma de Barcelona

Francesc Padròs - Universitat Autònoma de Barcelona

Cristobal Aguilera - IRTA

Jordi Comas - Mørenot

Manuel Blonç - Universitat Autònoma de Barcelona

Nuria Ruiz - Universitat Autònoma de Barcelona

Comité evaluador orales y pósters:

Mariana Teles - Universitat Autònoma de Barcelona

Irene Garcia - Universidad de Barcelona

Ignasi Sanahuja - Universidad de Barcelona