LIBRO DE ABSTRACTS

7th PhDay Biología

5 de octubre de 2023

ESCUELA DE
DOCTORADO
UNIVERSIDAD COMPLUTENSE
DE MADRID

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DE LA COMUNIDAD DE
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Facultad de C.C. Biológicas
UCM

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Profesora de la Universidad Europea de Madrid.

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17:00 – 18:00 **5 visiones del doctorado desde la perspectiva del género**

Ponentes:

Maite González Jaén
Catedrática de Universidad

Cristina Sánchez García
Profesora Titular de Universidad

Mirian Domenech Lucas
Profesora Ayudante Doctora

Gemma Palomar García
Profesora Ayudante Doctora

Elisa Pérez Badás
Contratada post-doctoral

Moderador:

Benito Muñoz Araujo
Decano de la Facultad de Biología

18:00 – 18:30 Entrega de premios y clausura

Resúmenes

Comunicaciones Orales

Kinorhyncha: The mud dragons – The importance of standardisation in Science

Alberto González-Casarrubios[1]

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Keywords: Taxonomy; Measurements; Scalidophora; Database; Repository

Summary

Kinorhyncha or mud dragons are a phylum of meiofaunal, benthic animals that inhabit marine environments around the world.

Knowledge about these animals is very scarce, and much of their biodiversity and basic aspects of their biology are unknown.

However, the taxonomy of kinorhynchs has undergone a strong advance in recent times, mainly because it has a fundamental advantage; it follows a standardised structure. However, we realised that morphological measurement methods, essential for the formal description of species, differed significantly between authors and were therefore not comparable.

Thus, in 2022 we managed, in consensus with the rest of the experts in the phylum, to publish a paper in which we proposed a standardised method for taking and representing the measurements, with the aim of obtaining comparable and repeatable morphometric results. In addition, we published an online repository to make the measurements accessible to all researchers in the same format, facilitating future comparisons and studies. Finally, we made a glossary that compiles and defines all the measures that can be included in the Kinorhyncha descriptions.

Ghrelin involvement in the anxious state of food anticipation in fish

Lisbeth Herrera-Castillo[1], Pinging Chen[1], Nuria Saiz[1], María Jesús Delgado[1], De Pedro Nuria[1], Esther Isorna[1].

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Keywords: anxiety, behavioral tests, food anticipatory activity, food intake, ghrelin.

Summary

Food Anticipatory Activity (FAA) is an increase in locomotor activity before the expected mealtime and may indicate a food-seeking state of anxiety mediated by both hunger and food reward. Ghrelin, an orexigenic hormone, has been suggested to trigger this behavior. The objective of this work was to investigate whether FAA is an anxious-like state in fish, and to determine ghrelin's involvement. Open field and black-white behavioral tests were performed to evaluate anxiety in goldfish (*Carassius auratus*): after 24-h fasting (FAA time); at 3-h post-feeding; and after 30-h fasting (6-h after FAA). Goldfish exhibited more scototaxis and thigmotaxis during FAA than after this period, suggesting that FAA is an anxious state, irrespective of the animal's feeding status (postprandial or fasting). To examine the role of ghrelin in this behavior, 24-h fasting goldfish were injected with ghrelin antagonists (D-Lys and JMV2959), which showed anxiolytic effects. Fed goldfish were injected with ghrelin alone or ghrelin with D-Lys. Results showed that ghrelin is anxiogenic, an effect that was reversed by its antagonist. In conclusion, these findings suggest that ghrelin could mediate the anxious state observed during FAA in fish, contributing with the food-seeking behavior that occurs during the hours preceding the arrival of food.

Food Anticipatory Activity (FAA) refers to an upsurge in physical movement that occurs in the hours leading up to an anticipated mealtime. This increase in activity may suggest an anxious or eager state associated with the anticipation of food, influenced by both hunger and the expectation of food as a rewarding experience.

Ethnobiology of edible palm weevil larvae, *Rhynchophorus palmarum,* in Amazonian Ecuador

T. Jaramillo-Vivanco[1,2], R. M. Cámara[3], M. Cámara[3], H. Balslev[4], J. M. Álvarez-Suarez[5]

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Keywords: Edible insects, Amazonian Ecuador, sustainable practices.

Summary

For centuries, indigenous communities have relied on sustainable methods to gather food. In neotropical areas, the larvae of the *Rhynchophorus palmarum*, a palm weevil, have played a vital role in meeting this need. Extensive research has documented the historical usage of *R. palmarum* as a source of nourishment and as a medicinal insect. Various ethnic groups have developed practices of consuming the larvae and establishing cultivation systems, drawing from their shared harvesting techniques centered around palm trees. Additionally, the larvae are employed for treating different ailments. Analysis of our data revealed that these groups demonstrated similar patterns in the utilization, cultivation, and collection of the larvae, albeit lacking a formalized management plan. While grub collection remains a peripheral activity associated with traditional palm use, these larvae present new opportunities for breeding, commercialization, and consumption of edible insects. Embracing these opportunities as part of a sustainable approach could strengthen food security among Amazonian populations. Moreover, the larvae's potential in medicinal applications and their appeal to tourists highlight untapped prospects related to this year-round forest resource. Exploring and capitalizing on these opportunities could contribute to the formulation of a comprehensive strategy that promotes food security and sustainable practices within Amazonian communities.

Impact of a simplified treatment protocol for acute malnutrition with a decentralized treatment approach in emergency settings

Luis Javier Sánchez-Martínez[1], Pilar Charle-Cuéllar[2], Abdoul Aziz Gado[3], Abdias Ogobara Dougnon[4], Atté Sanoussi[5], Nassirou Ousmane[6], Ramatoulaye Hamidou Lazoumar[6], Fanta Toure[4], Antonio Vargas[2], Candela Lucía Hernández[1], Noemí López-Ejeda[1,7].

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Summary

Acute malnutrition is currently a global public health problem, and its effects are specially pronounced in the most vulnerable populations. The World Health Organization estimates that nowadays 45.4 million children under 5 are affected worldwide by acute malnutrition, 2 million of them dying every year as a consequence. This study aims to evaluate the effectiveness of a simplified treatment protocol with Community Health Workers (CHWs) as treatment providers in emergency settings of Mali and Niger.

The main results revealed an increase in treatment coverage in the intervention groups in both Mali and Niger. An increase in the cure rate of children was also observed when using the simplified protocol, especially in moderate cases, which also recovered in a significantly shorter average of days. The hazard ratios calculated by cox regression showed that the treatment and its providers (CHWs) were the most influential factors in the recovery of the children. Finally, machine learning methods were used to establish which socioeconomic factors could affect the severity of children when starting the treatment. The final random forest model provided a ranking of the variables by their importance and the classification success rate was 68% for Niger and 80% for Mali.

Transformaciones naturales e inducidas de humedales, a largo plazo, en La Mancha Húmeda. Efectos sobre el comportamiento de las aves acuáticas

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(Add more if needed)

Keywords: Wetlands, Mancha Húmeda, evolution, induced changes.

Summary

An important part of European waterfowl uses Spanish wetlands during their migratory and winter periods, in addition to the resident population that includes threatened species.

A large plain has been selected, called La Mancha, located at the center of the peninsula, where a large number of wetlands are located, recognized by the Ramsar Convention.

From 1973 to 2023, its evolution and transformations must have been caused by natural reasons, but most of them have been due to human action: erradicating malaria, "recovering" land for agriculture, drained by canals, ditches and wells, used as deposits for solid waste, wastewater and industrial water, and affected by extraction massive subsoil water from aquifers, destroying about 70% of these wetlands. We must add the use of lakes and lagoons as recreational areas (bathing, windsurfing, walks along the shore) and "conservationist" interventions, not always adequately oriented.

Birds' behavior has been affected to different degrees: from an abysmal decrease in the number of reproducers, and abandonment of the classic wintering areas, to the replacement of species, reaching the point of completely disappearing.

A first approach to the origins of Spanish oceanography. The *HMS Challenger* and its impact on nineteenth century Spain (1872 – 1890)

Esteban Del Pozo Márquez[1]

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Summary

The history of oceanography establishes as one of its major events the expedition undertaken by the British ship *HMS Challenger* between 1872 and 1876. Under the scientific direction of the Royal Society, this vessel circumnavigated the globe and gave a significant impulse to the nascent modern oceanographic discipline, which was in a period of evolution. Despite the international relevance of the expedition at the time, Spanish historiography has not paid much attention to the influence of this voyage in the national context. For this reason, it is interesting to study the repercussion that the *Challenger Expedition* may have had on Spanish science.

In this paper we approach the *Challenger Expedition* through the national press and some of the main scientific publications of the time. The aim is to analyse the repercussions and significance that the British expedition had in 19th century Spain. In this way, we present the beginning of the doctoral work and the first results as part of a broader research that seeks to study the origins and development of oceanography in Spain by investigating the past of Spanish science through the history of its institutions, its scientific community and its relations with society.

Lentiviral gene therapy for factor v deficiency. cellular and animal models. a hope for an ultra-rare disease

Juan Andrés De Pablo Moreno[1], Luis Revuelta[2], Andrea Miguel Batuecas[1], Pablo Bermejo Álvarez[3], José Carlos Segovia[4,5], María García Bravo[4,5], Aída García Torralba[4,5], Luis Javier Serrano[4], Mariano García-Arranz[6], Leopoldo González Brusi[3], Antonio Liras[1]

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Keywords: Factor V deficiency; cellular model; mouse model; CRISPR; lentiviral gene therapy

Summary

Ultra-rare diseases have a very low incidence in the population, and many have no specific treatment, such as factor V deficiency. This congenital coagulopathy is characterized by the absence or dysfunction of coagulation factor V (FV), which causes spontaneous or exacerbated bleeding. The scarcity of appropriate animal models of FV deficiency has limited therapy development.

In this study, a recombinant lentiviral vector with a FV cDNA was designed to establish a gene therapy protocol *in vitro*, in a previously generated cell model, and *in vivo* in a mouse model, both deficient in FV using CRISPR/Cas9 technology. The objective is to use this vector to correct the phenotype resulting from mutations in both models.

Before generating the murine model, FV levels, prothrombin time and activated partial thromboplastin time, were standardized in healthy mice using a viscosity-based coagulometer. This established a reference for the animal model, effectively emulating a mild knock-in human mutation. Encouraging results have been observed in the cellular model, with ongoing *in vivo* experiments.

Currently, lentiviral gene therapy exhibits high therapeutic potential, representing a future treatment for FV deficiency. The mouse model also has great potential for studies of recombinant and advanced therapies for congenital coagulopathies.

Paleo-oncology in Ancient Egypt and the Near East

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Keywords: Paleopathology; Paleo-oncology; Ancient Egypt; Ancient Near East; human remains.

Summary

Paleo-oncology is the study of cancer in ancient populations. These studies allow us to reconstruct the existence of cancer in antiquity, through the bones, literary and artistic record; and expand our understanding of this set of diseases that are so widespread today. In order to trace the history of cancer, we go back to the beginning of the first civilizations with the beginning of writing in Egypt and the Near East, and we follow its trail until the Roman domination.

This research reviews the published cases of malignant neoplasms published in the scientific literature. From this bibliographic compilation, 73 cases of malignant neoplasms from Ancient Egypt and 2 from the Near East were obtained. The presence of cancer in antiquity is confirmed, although with an evident lack of published cases in the Near East. With these first results we can evaluate the difficulty of studying cancer in ancient populations, as well as the importance of approaching cancer in paleopathological studies.

In the future, field work will be carried out in museum institutions and archaeological sites in Egypt and the Near East to complete this research.

A cocoa-carob blend modulates gut microbiota in an animal model of type 2 diabetes

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Keywords: type 2 diabetes, polyphenols, gut microbiota, cocoa, carob.

Summary

The gut microbiota (GM) is disrupted in type 2 diabetes (T2D) and polyphenols are phytochemicals able to modulate it. In this sense, cocoa and carob are rich in these compounds, and designing a functional food based on them constitutes an attractive approach for recovering a healthy GM.

Zucker diabetic fatty rats were fed on a standard or a cocoa-carob blend (CCB)-rich diet (10%) for 12 weeks. GM composition and phenolic metabolites were evaluated from faeces.

CCB-rich diet modulated the composition of the GM, improving the species richness, diversity and *Firmicutes-Bacteroidetes* relative abundance. At genus level, an increase in beneficial bacteria and a decrease in the pathogens were found. Correlation analysis strengthened the associations between these genera and improved pathological variables in diabetic animals. Moreover, the 2,3-dihydroxybenzoic and 3,4,5-trihydroxybenzoic acids identified in CCB faeces were associated with increased levels of beneficial bacteria.

Our findings support the potential of the CCB supplementation to prevent dysbiosis in T2D, which may delay its progression.

To establish the reference intervals (ris) of the reticulocyte profile in our population

Posada Franco, Yolanda[1], Sanz Casla Mª Teresa[2], Hernández Álvarez Elena[2], Martínez-Novillo González Mercedes[2].

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Keywords: reticulocyte, reticulocyte parameters, reference intervals, blood, laboratory.

Summary

Reticulocytes are immature red blood cells. Reticulocyte count in peripheral blood and reticulocyte parameters reflect the erythropoiesis and provide information for the classification and differential diagnosis of pathologies from the laboratory.

The reference intervals (RIs), which allow discrimination between normal and pathological values, are defined as the range of values covering 95% of a healthy reference population between the 2.5 and 97.5 percentiles.

The aim was to establish the RIs of our reference population of reticulocyte count and reticulocyte research parameters provided by the Beckman Coulter® UniCel® DxH 800 and DxH 900 haematological autoanalysers.

175 blood samples were collected from healthy donors (101 males and 74 females between 18 and 65) which were analyzed at the Clinical Laboratory of Hospital Clinico San Carlos.

The RIs were estimated using a nonparametric method based on the 2.5th and 97.5th percentiles with a 90% confidence interval.

Results: RET%: 0.61-2.28; RET#($\times 10^9$/L): 28.67-110.27; MRV(fL): 95.04-115.2; IRF(%): 0.23-0.49; LHD%: 1.14-8.79; MAF: 10.9-14.83; MSCV(fL): 71.13-88.89; HLR%: 0.17-0.97; HLR# ($\times 10^6$/µL): 7.7-46.7; UGC%: 0-0.03; UGC#($\times 10^6$/µL): 0-1; RSF(fL): 89.5-104.62; RDWR-CV(%): 22.93-30.46; RDWR-SD(fL): 23.1-32.97; WROP($\times 10^3$cells/µL): 4.43-12.04; UWROP($\times 10^3$cells/µL): 4.44-12.05.

It is important that, in the absence of standardisation, each laboratory establishes its own RIs according to the methodology used and its population.

Role of TRIB3 in the development of non-alcoholic steatohepatitis and hepatocellular carcinoma

Óscar Gutiérrez Jiménez[1], Kunzangla Bhutia[1], María del Mar Lorente[2], Guillermo Velasco Díez[2]

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Keywords: Hepatocellular Carcinoma, Cancer, Tribbles family, TRIB3, Fatty liver disease

Summary

Liver cancer is the third most deadly type of cancer which, along with its rising incidence, makes it a global health issue. Hepatocellular carcinoma (HCC) is the most common liver cancer type. Viral hepatitis, chronic alcohol consumption, and the occurrence of metabolic disorders that lead to the development of fatty liver disease (NAFLD) are considered the main risk factors for the development of this disease.
Pseudokinases, and specifically Tribbles pseudokinase 3 (TRIB3) regulate many cellular functions via specific interactions with various protein. Previous results of our group showed that TRIB3 exhibit onco-suppressive properties in liver cancer models via its capacity to inhibit AKT phosphorylation by MTORC2. However, the role of TRIB3 in the progression from NAFLD to HCC remains to be elucidated.

To explore this issue, in this project we performed different experimental approaches including the use of *Trib3* knock-out mice that were subjected to diets that induce NAFLD. We found that Trib3-deficient animals developed NAFLD and non-alcoholic steatohepatitis at a higher extent (and exhibited higher inflammation and steatosis) than their wild type counterparts. We also found that TRIB3 regulates these events at least in part by controlling macrophage functioning as well as hepatocyte lipid accumulation.

Relevance of *Streptococcus pneumoniae* serotype 3 in the context of Invasive Pneumococcal Disease

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Keywords: *Streptococcus pneumoniae,* IPD, serotype 3, PCV, epidemiology

Summary

Streptococcus pneumoniae is a respiratory pathogen with up to 103 serotypes described so far, although, about 23-25 serotypes are responsible for the majority of all invasive pneumococcal disease (IPD) episodes. Serotype 3, is one of the most prevalent causing IPD despite being included in the current pneumococcal vaccines. It has positioned itself as the leading cause of IPD in children and adults in 2022-2023.

Using PCR-MLST we have characterized the circulating genotypes within serotype 3 from 2009 to 2018. ST180 has established itself as the most frequent, replacing others such as ST260. The explanation may be related to differences in the levels of lung adhesion and immune evasion. ST180 strains had a higher ability to adhere the lung epithelium, and showed increase resistance to complement-mediated phagocytosis and higher evasion of complement C3 component.

Serotype 3, despite being one of the major causes of IPD every year, has an irregular evolution. As pneumococcal infection has been associated to influenza virus infection, we have observed that epidemic years with high incidence of serotype 3 are correlated with high circulation of influenza virus. To confirm this hypothesis we are establishing co-infection assays using lung cells, influenza strains and different genotypes of serotype 3.

Transcriptomic Analysis Sheds Light On Enhanced PHBV Production In A *Rhodospirillum rubrum* Mutant With Unpaired Pigmentation Synthesis

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Keywords: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate), Bioplastics, *Rhodospirillum rubrum*, Transcriptomic analysis, Electron sink.

Summary

Photosynthesis (PS) in most Purple Non-Sulphur Bacteria (PNSB) is tightly regulated to minimize unnecessary energy consumption and prevent oxidative stress when oxygen is present. PpsR, a transcriptional repressor encoded in the Photosynthetic Cluster (PSC), plays a key role in the regulation of PS. However, in some cases, the sensing function is not directly performed by PpsR itself but by other partner proteins. Rru_A0625 locus is situated adjacent to the *ppsR* gene, and its deletion abolishes pigment synthesis, as is the case of other PpsR anti-repressors. Due to the previously demonstrated capacity of *Rhodospirillum rubrum* for PHBV production, a promising biodegradable bioplastic with similar properties to oil-derived plastics, we hypothesize that mutating Rru_A0625 could redirect the carbon and energy that are not being used for pigment production towards polymer synthesis.

Given the substantial impact of a mutation in Rru_A0625 on *R. rubrum* metabolism, we delved into the transcriptomic consequences of disrupting this gene. This research highlights the far-reaching effects of mutating a regulatory protein on the overall metabolic dynamics of the cell. Furthermore, it demonstrates the potentiality of deleting Rru_A0625 to redirect carbon and electron flow towards PHBV production.

Development of mutants of *Aspergillus niger* defective in OTA prodution using the CRISPR/Cas9 technology

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Keywords: Gene editing*, Aspergillus niger*, ochratoxin A, CRISPR/Cas9, halogenase

Summary

Aspergillus niger is a fungus that produces ochratoxin A (OTA), a toxic secondary metabolite that represents a threat to food safety and health. Different genes are involved in OTA synthesis which are located in a cluster. One of the key genes of the cluster encodes an halogenase. The development of gene editing techniques using CRISPR/Cas9 allows to improve the knowledge about their function and regulation by reducing the homologous recombination problems that occur in some fungi. This work aims to develop a CRISPR/Cas9 protocol targeting the halogenase-encoding gene to generate non-producing mutants in *Aspergillus niger*.

Target-specific crRNAs were designed, as well as primers to prepare the repair template using the hygromycin cassette. Protoplast processing and transformation were performed as previously described[1]. The mutant selection was based on growth on hygromycin-selective medium and validated by PCR.

Several hygromycin-resistant colonies were observed, resulting in a transformation efficiency of 2%. PCR validation of the transformants confirmed the incorporation of the hygromycin cassette (1650 bp). The mutants obtained produced 97% less OTA.

These results demonstrate the usefulness of the CRISPR/Cas9 technique for editing *A. niger* genes involved in OTA synthesis.

[1]Van Rhijn et al. (2020) Fungal Genetics and Biology 145:103479

The combination of insoluble solids and lignocellulosic-derived inhibitors alters *K. marxianus* fermentation performance.

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Keywords: cell robustness, lignocellulosic raw materials, fermentation, non-conventional yeast, biorefineries.

Summary

Kluyveromyces marxianus is a non-conventional yeast with promising applications in lignocellulosic-based biorefineries. This microorganism can grow and ferment at temperatures between 40-45°C, being capable of assimilating a wide range of carbon sources. In contrast, fermentation of lignocellulosic-derived media is very challenging, and the increase of *K. marxianus*' cell robustness is required to make this yeast a potential candidate for the biotechnology industry. In this work, the tolerance of *K. marxianus* CECT 10875 against a synthetic mix of lignocellulosic-derived inhibitors (0-100% v/v) and insoluble solids (0-60% w/w) was studied. In addition, different temperatures (30°C and 42°C) were used to assess the effect of this parameter under stress conditions. Major results showed cell inhibition when combining 50% (v/v) of the synthetic inhibitor mix and 30% (w/w) of insoluble solids within the first 24 hours. These conditions severely affected glucose consumption and cell concentration, reducing these parameters when compared to control assays from 100% and 6 g/L to 48.5% and 1.6 g/L at 30°C, and from 100% and 4.1 g/L to 13.8% and 1.4 g/L at 42°C. These results set the basis to elucidate the mechanisms involved in cell tolerance, which can be then targeted in the future to genetically engineer *K. marxianus*.

Insight on the cellular signaling mechanisms in *Yarrowia lipolytica* strains

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Keywords: *Yarrowia lipolytica*, *quorum-sensing*, biofilm, oleaginous yeast, signal molecules

Summary

In recent years, the yeast *Yarrowia lipolytica* has generated interest in biotechnology due to its ability to efficiently secrete proteins and organic acids. In addition, it can accumulate high amounts of lipids intracellularly and it is very tolerant to low nutritional requirements and various stresses. Therefore, *Y. lipolytica* is an interesting candidate to produce value-added products in the food industry or for the generation of biofuels or biopolymers. To optimize the use of *Y. lipolytica* in industrial fermentation processes, not only in individual cultures but also in microbial consortia, we decided to explore the potential cell to cell signalling mechanisms in this species, in particular the putative quorum sensing (QS) systems. QS mechanisms have been described in other yeasts, such as *Candida* albicans, regulating yeast-hyphae morphological transition and biofilm formation, involving molecules such as farnesol or tryptophol. In this work, we explored the phenotypic impact of eight signal molecules described in yeasts against 14 different strains of *Y. lipolytica.* The response to these molecules is highly strain-dependent, but we found a conserved response to farnesol, which generally inhibits biofilm formation in *Y. lipolytica.* We also performed induction experiments with spent media from different strains and tested the production of farnesol in the supernatants using metabolomics techniques.

Streptomyces exfoliatus: a microorganism source of enzymes with biotechnological interest.

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Keywords: *in silico,* lipases, hexosaminidases, *Rhodococcus*.

Summary

Streptomycetes are Gram-positive filamentous bacteria, which produce a wide variety of useful enzymes in biotechnology, including lipases or β-N-acetylhexosaminidases. They have attracted considerable attention on the field of functional foods, cosmetics, pharmaceuticals, and agriculture, providing an alternative approach to chemical processes. The Enzyme Biotechnology Group of UCM, where this doctoral thesis is being carried out, has sequenced the genomes of several microorganisms that produce enzymes, for example *Streptomyces exfoliatus*.

In this work we performed an *in silico* search on the *Streptomyces exfoliatus* genome, finding two putative lipases (*Se*LipA and *Se*LipC) and one β-N-acetylhexosaminidases (*Se*Hex).

Therefore, these genes were amplified by PCR and cloned and expressed in *Rhodococcus sp.* 104, a Gram-positive bacteria able to recognise the *Streptomyces* signal peptides and produce the enzymes extracellularly. The enzymes *Se*LipA, *Se*LipC and *Se*Hex were purified from the free-cell broths and characterized. In addition, *Se*LipC and *Se*Hex were immobilised.

Moreover, biotechnological applications were studied. *Se*LipC is able to hydrolyse biopolymers and synthetize sugar fatty acid esters (SFAEs).

Pósteres

Shifts in species dominance in forests of the Iberian Peninsula: underlying drivers and effect of diversity

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Keywords: disturbance regime, diverse forests, forest dynamic, global change,

Summary

Global change is altering the structure and composition of forest ecosystems through increased disturbance frequency. The speed of these changes depends on the species-specific responses to environmental factors. This study evaluated shifts in dominance of conifers, broad-leaved deciduous and broad-leaved evergreen species during the last 30 years. In addition, we analysed the effect of climate, initial forest structure and disturbance regime (management, wildfires, biotic damage and drought) on dominance shifts and the role of diversity in changes in species composition. For this, we used permanent plots of the Spanish National Forest Inventory. We expect higher dominance of broad-leaved species over conifer species, especially in plots that have suffered disturbances, resulting in composition shifts (i. e., intense drought, outbreaks, fires or management). We also expect forest diversity to smooth out changes in species composition, maintaining forest diversity and more productive forests. The results will have implications in the design of management measures for the adaptation of forest stands in the Iberian peninsula to global change, such as the diversification of stands.

Malagasy herpetological diversity

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Keywords: Madagascar, amphibians, diversity, inventory, species delimitation.

Summary

Madagascar harbors one of the richest and unique anuran diversity worldwide, characterized by high levels of endemism. The documented patterns of diversity for the island and the latest taxonomic research suggest that there may be many species waiting to be discovered and described in both, the wild and historical collections. Escalating threats, especially in anuran-rich rainforests where many undescribed species are known to occur, exacerbate conservation concerns. Limited knowledge of the true diversity of Malagasy amphibians impedes distribution understanding and effective conservation. In this study we aim to unveil the potential hidden diversity of Malagasy anurans by analyzing all available 16S rRNA sequences (N=9650), in all 26 endemic genera geographically covering the entire island of Madagascar. Our results uncover ca. 305 undescribed divergent lineages of frogs suggesting that Madagascar, one of the most threatened tropical countries of the world, could host the richest community of insular amphibians across the globe. The existence of 305 divergent lineages mostly distributed in well-studied regions within Madagascar confirms the importance of exploring other poorly explored regions to get a complete inventory of the amphibians and highlights the urgent need to assess and describe these lineages before they go extinct.

Experimental manipulation of nest temperature and relative humidity reduces ectoparasites and affects body condition of blue tits (*Cyanistes caeruleus*)

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Keywords: hen flea, mites, nestling body condition, nest microclimate, *Protocalliphora*.

Summary

Studies exploring the effect of microclimatic changes on host-parasite relationships are scarce. However, many models predict changes in the distribution and incidence of diseases associated with climate change. In this study, we increased both temperature and humidity in blue tit nest-boxes during the breeding season, trying to discern between the effect of both variables on the abundance of ectoparasites reported in previous studies and, also, on the body condition of the hosts. Temperature and humidity were experimentally increased on average of about 2°C and 15 percentage units respectively. The abundance of some ectoparasites, like blowfly *Protocalliphora azurea* pupae, *Dermanyssus* spp. mites, *Ceratophyllus gallinae* flea larvae and biting midges *Culicoides* spp. was significantly reduced in experimental nests. On the other hand, body condition and mass of nestlings were lower in experimental nests. However, the body condition and mass of adults was not significantly affected by the experiment. In conclusion, an increase in temperature and humidity reduces the abundance of ectoparasites, which suggests that these parasites are sensitive to sudden changes in the microclimate in which they develop. In addition, these fluctuations negatively affect the body condition and mass of blue tit nestlings despite the concurrent decrease of parasites in nests.

Stable isotope analysis is a reliable method for identifying migrants and residents in a partial migratory European hoopoe (*Upupa epops*) population in southern Spain

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Keywords: isotopes, isoscapes, migration, morphology, bayesian

Summary

Avian migration is one of the most investigated topics in behavioural ecology. Partial migration is of particular interest, since it allows studying differences among migrants and residents, and the persistence of these two behaviours within one population. However, most studies have been conducted on populations of passerines in which the migratory individuals migrate short distances, making it difficult to distinguish them from residents. The European hoopoe is a non-passerine, long-distance migrant that is partially migratory in southern Spain. We expected that the marked differences in wintering latitude found in these hoopoe populations would facilitate reliably identifying migrants and residents.

The aim of this study was to establish a method to identify migrants and residents and to investigate migration tendencies within a partially migratory hoopoe population. We used stable-isotope ratios of hydrogen in several types of feathers to determine the migratory strategy of individual hoopoes. The, we compared morphological traits between migrants and residents. We found that migrants were more frequent than residents, without an apparent difference between sexes and age. This work highlights the utility of stable isotope analysis to infer variation in individual migratory behaviour in hoopoes but no relation between it and phenotype.

Getting to Know the Odd-Man-Out: A Model-clade Approach to Investigate the Drivers Shaping Tropical African Plant Diversity

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Keywords: Euphorbiaceae, Odd-Man-Out Pattern, Tropical Africa, Phylogenomy, Biogeography.

Summary

The "Odd-Man-Out" pattern describes the observed lower plant diversity in continental tropical Africa compared to other tropical regions. We examine long-standing hypotheses behind this pattern by adopting a model-clade approach that combines the in-depth knowledge of small-scale clade studies with the power of family-wide metanalysis.

We focus on 31 genera of the plant family Euphorbiaceae, which form 19 clades and include approximately 551 species. Our workflow involves the revision of each genus' alpha-taxonomy and the production of sequences for 431 low-copy nuclear genes per sample using the Euphorbia kit for HybSeq target sequencing. The resulting information is then used to generate genome-wide phylogenies of each group, to examine the specific mechanisms working at the clade-level and the general drivers acting on Afrotropical lineages. We have produced the first extended phylogenomic time-tree including representatives of the three main subfamilies: Acalyphoideae, Crotonoideae and Euphorbioideae. Additionally, we have inferred a species tree for the genus *Croton* including 45 of the 56 continental African species and 18 from Madagascar. Contrasting with previous results, this allowed us to recover highly resolved phylogenetic relationships within this group. Our results support the polyphyly of the continental species in relation to the Malagasy *Croton*.

Cannabinoid receptor 2 in the breast tumor microenvironment: role in tumor progression

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Keywords: breast cancer, endocannabinoid system, cannabinoid receptor 2, tumor microenvironment, immune cells.

Summary

Breast cancer (BC) is a heterogeneous disease in which cancer cells establish dynamic interactions with their surrounding stroma (tumor microenvironment, TME), which determine tumor progression and response to therapy.

In oncology, cannabis is used with therapeutic purposes due to its palliative and potential anti-tumoral actions. The molecular target of the active principles of cannabis [i.e. cannabinoids (CBs)] is the endocannabinoid system (ECS), a complex cell communication system that is deregulated in several pathologies. Specifically in BC, upregulation of cannabinoid receptor 2 (CB_2R) in BC cells is associated with poor patient prognosis, but its activation by CBs triggers antitumor responses. On the other hand, CBs have been linked to immunosuppressive actions due to their action on CB_2R expressed by immune cells, one of the main components of the TME.

Considering the above, we hypothesize that CB_2R is a key mediator in communication in the TME. To validate this hypothesis, we conducted *in vitro* and *in vivo* experiments in genetically engineered mouse models of BC. Our results show that the lack of CB_2R tends to reduce tumor growth and alters the TME composition and function at both cellular and non-cellular levels.

Together, our results suggest that CB_2R expressed by immune cells may play a role in antitumor immunity and thus in BC progression.

Further insight into the effect of polymers in pulmonary surfactant structure and function

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Keywords: pulmonary surfactant, amniotic fluid surfactant, hyaluronic acid.

Summary

Pulmonary surfactant is a lipid/protein complex with the ability to coat the alveolar interface during breathing cycles thanks to its unique mixture of saturated (packable) and unsaturated (unpackable) lipids and the presence of two small and highly hydrophobic proteins, SP-B and SP-C that connect the interface with surfactant reservoirs allowing the incorporation of lipids to it during inspiration (when the surface increases) and helping in the recycling of the unpackable material excluded during expiration.

In order to minimize the surface tension of alveoli at the end of expiration and prevent alveolar collapse, it is crucial to maintain the pulmonary surfactant system working properly. That is the reason why there is a need to restore its functioning when there is a surfactant inhibition. As polymers have been described to have a positive effect over surfactant functionality, here we show thanks to the use of different biophysical technics how surfactant previously exposed to polymers exhibits a different behavior from the unexposed material, being it more similar to the one of a surfactant isolated from human amniotic fluid that preserves the characteristics of a newly synthesized material with higher packing abilities and adsorption capabilities, which can be useful to develop new therapeutical preparations.

A 3-D journey to study the synapses of the Human Entorhinal Cortex

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Keywords: brain, electron microscopy, FIB-SEM, synaptic organization, ultrastructure.

Summary

The Entorhinal Cortex (EC) is a brain region located in the middle temporal lobe. It has been described as the major interface between the hippocampus and the neocortex, playing an essential role in memory functions. In this work, we have used Focused Ion Bean / Scanning Electron Microscopy (FIB/SEM) to investigate, for the first time, the fine synaptic connectivity of all layers of the human EC.

A total of 12974 synapses from 3 human autopsy cases were 3D reconstructed to analyze synaptic density, proportions of asymmetric and symmetric synapses, synaptic size, synaptic shape and postsynaptic elements. Our results showed remarkable uniformity in the synaptic characteristics analyzed, regardless of the cortical layer. All layers had similar synaptic densities; most synapses were excitatory, displayed a random spatial distribution and were similar in size in all layers. However, we also observed some layer-specific features, regarding synaptic shape and postsynaptic targets.

The differences in synaptic characteristics between layers may reveal unique microanatomical synaptic features that may be related to the differential pattern of layer connectivity in human EC. Thus, the data presented here are essential to fully understand one of the most important circuits that shape human brain, both in health and disease.

Biological nanoparticles as platforms for the development of viral detection systems

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Keywords: molecular detection, viral nanoparticles, gold nanoparticles, LAMP, POC diagnosis.

Summary

Early detection of outbreak infections by pathogen viruses is crucial to prevent transmission among the population. Currently, the detection of RNA viruses relies on complex molecular techniques, like the gold standard qRT-PCR, which are not cost-effective tools. However, recent advances in nanotechnology-based sensors are being used for fast throughput detection with high sensitivity values and lower sample consumption.

Viral nanoparticles (VNPs) are emerging as versatile building blocks for several aspects on nanotechnology, while gold nanoparticles (AuNPs), due to their unique optical properties, are especially well-suited for designing colorimetric detection biosensors for Point-Of-Care (POC) diagnosis in resource-limited settings.

Our research focuses on the development of an innovative colorimetric molecular system for affordable POC diagnosis. Thus, we funtionalized VNPs of the turnip mosaic virus (TuMV-VNPs) with AuNP-DNA nanoprobes. Preliminar results show that DNA-nanoprobes allows high sensitivity and specificity for viral detection upon LAMP amplification. On the other hand, we ara also handling TuMV-VNPs for antigen detection, using specific antibodies or aptamers. We undergo several experimental designs to develop a diagnostic kit compatible with POC technology using this functionalized VNPs.

Holistic management of epidemics in contexts of limited resources

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Keywords: epidemics, infection, prevention and control.

Summary

In recent years there have been different epidemics of infectious diseases as EVD, especially with the outbreaks in West Africa from 2014 to 2016 and in DRC from 2018 to 2020, the COVID-19 pandemic or the current cholera epidemic in several African countries.

Over the years, the interventions carried out by local authorities have been specialized with the support of both international agencies of the United Nations, as well as Non-Governmental Organizations (international and national), implementing holistic interventions for the control of pathogen dispersion. This includes complementary non-medical activities that require a high technical level, such as water and sanitation, waste management, patient transport, decontamination or safe burials, depending on the pathogen (transmission and survival in the environment) or the environment and the social and economic context.

During the presentation, some of the examples of these interventions carried out by the speaker and that have been included in the doctoral thesis "Holistic management of epidemics in contexts of limited resources" that the authors are carrying out in the Faculty of Biological Sciences of the UCM emphasizing the use of scientific evidence and the experience and lessons learnt for the field.

Time compaction in humans: biological or cultural gender bias?

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Keywords: Human cognition, Spatiotemporal cognition, Dynamic environments, Gender differences.

Summary

Time compaction is a salient cognitive mechanism used by humans to deal with complex dynamic situations, i.e., environments in which spatial relations between subject and elements, and among them, change rapidly over time. This mechanism deletes temporal dimension from moving elements by estimating only the locations of future interactions, i.e., where subject and elements will coincide. The brain then generates an internal map spatially arranging these future interactions, named compact internal representation or CIR. While this cognitive mechanism is salient in men, women use a wider set of cognitive strategies.

This gender bias may result from cognitive sexual dimorphism or from cultural aspects. We focused on sport practice as a paradigm of cultural dimorphism. Thus, we conducted a series of visual discrimination tests on athlete and non-athlete populations, in which the recurrence to CIR's would facilitate a better performance and higher learning speed.

During tests athletes have a lower recurrence to CIR than non-athletes, demonstrating for the first time the effect of environmental factors on time compaction. Furthermore, among athletes, women recur to a greater extent to CIR than men. We hypothesize that this gender bias could be due to various cultural and social traits related to female sport.

Divergent hippocampal representations underlie reference and working memory processes

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Keywords: Hippocampus, memory, neural representation

Summary

The hippocampus is involved in working memory and reference memory tasks. However, reference memory requires the permanent storage of information about a fixed situation, while working memory contents are short-lived and constantly updated. Our understanding of how the hippocampus is engaged in both processes is limited.

We evaluated immediate early gene (IEG) activation using Egr1-CreERT2xR26RCE mice (n=13) running working and reference memory-guided task. Next, using an automatized Arduino-based maze coupled to miniscope imaging, we evaluated spatial neuronal representations from the dorsal hippocampus (n=4, Thy1-GCaMP7 mice).

We first looked for differential IEG activation across the hippocampal formation after working and reference memory-guided tasks. IEG activation exhibited striking proximodistal differences along CA3 and CA1 in the working memory versus reference memory condition. To investigate the underlying representational correlates, we next tested mice in working/reference memory-guided tasks sequentially using 1-photon miniscope imaging. Analysis from CA1 pyramidal cells revealed that, although place field number and size remain similar across tasks, their spatial distribution tends to overrepresent the choice point of the maze in the working memory task, and the rewarded arm in the reference task.

Together, our findings suggest that hippocampal representations may play divergent roles in working memory and reference memory tasks.

A preclinical therapy with cannabinoids, temozolomide and bevacizumab in glioblastoma models

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Keywords: Glioblastoma, cannabinoids, antiangiogenic cancer therapies, bevacizumab, glioma initiating cells.

Summary

Glioblastoma (GB) is one of the most aggressive forms of cancer. It has been proposed that the presence within these tumors of a population of cells with stem-like characteristics, termed glioma-initiating cells (GICs), is responsible for the relapses in GB patients. Another factor that contributes to the aggressive behavior of GB is the tumor microenvironment. Specifically, GB are highly angiogenic and vascularized tumors. Accordingly different antiangiogenic therapies in GB patients, Bevacizumab (BVZ), is a humanized monoclonal antibody that although it has showed promising results, it has not been able to significantly modify the survival of patients with GB. In this context, previous observations by our group and others had shown that cannabinoids are able to inhibit tumor angiogenesis, among other antitumoral properties.

Thus, looking for novel therapeutic approach, in this study we propose the combination of BVZ, cannabinoids and temozolamide (TMZ, the reference agent for the management of GB). Our results show that this combination: (i) decreased the proliferation and self-renewal capacity of GICs; (ii) interfered with the vessel-forming capacity of endothelial cells; (iii) reduced the growth and enhanced the survival of intracranial GICs-derived xenografts. Our findings support the idea that this combination could be explored as a potential therapeutic strategy for the management of GB.

Genetic and phenotypic diversity in the yeasts *Lachancea thermotolerans* and its implications in wine industry

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Keywords: *Lachancea thermotolerans,* diversidad genética, diversidad fenotípica, acidez, fermentación vínica

Summary

Wine fermentation is a complex process involving numerous yeast species. Throughout fermentation, the sugars in grape must are metabolized. However, due to climate change, sugar levels increase while organic acids decrease. These acids are essential in maintaining wine acidity. Altered conditions can lead to various microbiological and technological issues during the fermentation process. *L. thermotolerans* stands out as one of the yeasts employed in winemaking, capable of producing organic acids during fermentation. Its production of lactic acid, combined with its lower fermentation rate, presents an alternative to counter climate change effects on the wine industry.

This study sequenced the complete genomes of 150 strains of this yeasts species, allowing the reconstruction of its evolutionary history. Additionally, the phenotypic diversity was studied through a high-throughput screening under 45 different conditions. Fermentative capacity analysis was determined as well for all isolates. The genetic diversity is extremely high and correlates with distinct phenotypic responses, linked to genetic clusters corresponding with geographic patterns. In most cases, these patterns are linked to the species' evolutionary trajectory.

Aspergillus flavus, an emerging risk of aflatoxin contamination in grapes?

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Keywords: *A. flavus*, aflatoxin B_1, grapes, vineyards

Summary

Vineyards are frequently contaminated by mycotoxins such as ochratoxin A, which is the only mycotoxin regulated in grapes and derivatives. As a result of the climate change scenario, new mycotoxin-producing species are emerging. That is the case of *Aspergillus flavus*, an aflatoxin producing species. Our aim was to stablish the risk posed by this species on grapes and grape-derived products.

The incidence of *A. flavus* was determined in 61 grapes samples from Spanish vineyards using a specific PCR assay. 72% of samples were contaminated with *A. flavus*. Due to this high incidence, the growth and aflatoxin B_1 (AFB_1) production ability of three *A. flavus* isolates from grapes were tested in grape-based medium. In general, changes in environmental factors (water activity and temperature) slightly affected the growth rate of the three isolates and only in some conditions (37 °C and a_w 0.9) their growth was severely affected. However, none of the conditions tested were permissive for AFB_1 production.

These results suggest that, despite the frequent contamination of grapes by *A. flavus*, the risk posed to food safety might be low, since the isolates would not be able to produce the AFB_1 in this matrix.

Self-defence mechanism's activation on *Vitis Vinifera* L.

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Keywords: Vine, pathogen, *Fusarium equiseti*, *PGPR,* protein.

Summary

Today's one of the most studied viticulture problems are the Grapevine Trunk Diseases (GTDs.). Versatile pathogens, able to develop under any environmental condition, with a very high growing rate and tough crop protection management; brings a capital importance when to control them. These pathogens expansion from nurseries will suppose a GTD issuing focus to the market. Current study has identified and characterized a phytopathogen vine (*Vitis vinifera* L.) fungus strain *Fusarium equiseti*. Furthermore, Koch's Postulates were demonstrated, evidencing that the strain was pathogen. Some known pathogen strains were compared versus the main one aiming to contrast its virulence (space and nutrient competence and phosphate solubilizing ability, among other tests). Due to the thorough work that implies the graft manufacturing, the high budget necessary when establishing new vineyards, and the scarce benefit margins that today's grape prices return; makes ostensible to minimize factors concurring to farmer's economic loses, besides keeping the ecosystem's ecology as much safe as possible.

The possibility of promoting the activation of the plant's self-defence mechanisms using BCA opens a secondary path much more tolerant ecologically. The reduce of chemical's use is one of the researching community's targets. Provide cost-effective and safe food to the market, a must.

Degradation of ochratoxin A by a bifunctional aminoacylase/carboxypeptidase in *Alcaligenes faecalis* subsp. *phenolicus* DSM 16503[T]

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Keywords: mycotoxin, ochratoxin A, amidohydrolase, biodetoxification, carboxypeptidase

Summary

Ochratoxin A (OTA) is among the most relevant mycotoxins considering its prevalence and toxicity. To guarantee food safety, OTA presence needs to be decreased to values as low as technologically possible. Generally, biological OTA detoxification occurs via the hydrolysis of its amide bond, rendering ochratoxin α and L-β-phenylalanine, both regarded as non-toxic. The potential to detoxify OTA was investigated in *Alcaligenes* strains cultures. An amidohydrolase from *Alcaligenes faecalis* able to hydrolyse OTA (*Af*OTase) has been described. Genome sequence analysis revealed the presence of the *Af*OTase encoding gene in all the *Alcaligenes* strains that showed OTA degradation ability. The *Af*OTase from *A. faecalis* subsp. *phenolicus* DSM 16503[T] was recombinantly hyperproduced and enzymatically characterized. *Af*OTase is a metalloenzyme that possesses all the features and conserved residues identified in M20D family peptidases. *Af*OTase lacks endopeptidase activity but possesses hydrolytic activity on N-acetyl-L- and carbobenzyloxy-L-amino acids. *Af*OTase preferentially hydrolyses amide bonds involving Phe, Tyr, Met, and Leu residues. The amidohydrolase activity of *Af*OTase on C-terminal Phe residues determines its activity on the detoxification of ochratoxins.

Modeling heat transfer during cooking of fish

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Keywords: *Anisakis* larvae, COMSOL Multiphysics, Modeling, Fish cooking, Hake

Summary

Anisakiasis, caused by eating raw or undercooked fish infected with *Anisakis* larvae in the third stage, is considered a significant threat to public health. In Spain, it is estimated an incidence of 12.7–27.5/100,000. The European Union Regulation recommends cooking the fish to a core temperature ≥ 60 °C for, at least, 1 min. However, several studies suggest that cooking at lower temperatures could be equally safe and less detrimental for fish quality, provided that certain minimum cooking time is met.
We recently published a model able to predict *Anisakis* larvae viability after isothermal treatments. Now, it is time to predict viability at non-isothermal conditions as those occurring during fish cooking. The first step to do that is, obviously, to know thermal evolution throughout the sample during cooking. In this work, we used COMSOL Multiphysics to simulate heat transfer throughout a fish cylinder during cooking in a water bath. Experimental and simulated time-temperature evolution at the sample center agreed well. Therefore, next efforts will be focused on coupling this heat transfer model with that performed for larvae viability prediction. The final objective is to obtain a tool able to identify safe cooking conditions for consumers.

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