

ONE HEALTH PhD FORUM

2ND EDITION

ABSTRACTS BOOK | SHORT TALKS

01 - Mismatch negativity and autistic traits: Investigating neural responses to unexpected auditory and visual stimuli

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Introduction: Predictive Processing suggests that the brain continuously generates and updates sensory predictions, minimizing prediction errors between expectations and actual input. Mismatch negativity (MMN), an EEG-based event-related potential, reflects this process by detecting deviations from expected stimuli. This framework has been explored in Autism (ASD), where increased prediction errors may explain symptoms such as hypersensitivity or preference for routines. **Methods:** Considering autistic traits exist on a continuum, with subclinical populations exhibiting neural patterns similar to, but milder than, those with ASD, this study included a community sample of 122 adults (62 females, aged 18-60). Participants completed auditory and visual oddball tasks with two difficulty levels while undergoing EEG recording. Autistic traits were assessed using the Autism Quotient (AQ), and MMN was calculated as the difference between frequent and infrequent stimuli. **Results:** Results revealed that higher Restricted Interests and Detail Orientation (RIDO) scores were associated with enhanced MMN in the difficult visual task, while higher difficulties in communication (AQ Communication subscale) were linked to reduced auditory MMN in the easy task. Notably, RIDO traits did not correlate with Social or Communication traits, supporting distinct social and non-social dimensions within ASD. Our findings partially support the Predictive Processing framework in ASD, showing that prediction errors to unexpected stimuli (indexed by enhanced MMN amplitudes) vary with task demands and sensory modality. **Conclusions:** This investigation showed that trait-specific dimensions (social vs. non-social) differently impact prediction error, with notable effects even at subclinical levels. These results advance dimensional classification systems, emphasizing symptom-specific neurobiological markers, refining perspectives on ASD beyond traditional diagnostic categories.

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02 - Association between microbiota composition and psychopathy scores in humans

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Introduction: Psychopathy is a personality structure characterized by traits such as lack of empathy and deficient emotional responses. Despite its high prevalence (clinical prevalence of approximately 1% in the general population and 10–30% in the prison population), the biological mechanisms underlying psychopathy remain poorly understood, and the role of the microbiota in connection with it has yet to be explored. This study assessed whether microbiota composition is associated with psychopathy scores. **Methods:** We recruited a total of 200 participants from the general population in Porto, Portugal, who completed the Self-Report Psychopathy Scale Short Form (SRP-SF) alongside additional self-report measures of empathy and anxiety. We collected faecal and saliva samples from which we characterized microbiota composition by sequencing the V4 region of the 16S ribosomal RNA gene. We then identified distinct psychopathy-related microbial profiles via K-means clustering in an untargeted approach. **Results:** While gut and oral alpha and beta-diversity did not differ significantly between psychopathy clusters, beta-diversity analyses revealed significant associations with psychopathy scores. Gut taxa *Allisonella*, *Prevotella*, *Ruminococcaceae* DTU089 and *Cloacibacillus evryensis*, as well as oral taxa *Treponema vincentii* exhibited differences in relative abundance between clusters. *Allisonella*, *Prevotella*, and *C. evryensis* showed significant positive associations with psychopathy, while *T. vincentii* was negatively associated. **Conclusions:** These findings suggest that gut microbiota composition is linked to subclinical psychopathic traits, highlighting potential novel pathways for understanding the biological basis of psychopathy.

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03 - Self-esteem and self-stigma among persons with schizophrenia spectrum disorders: a cross-sectional study

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Introduction: The relationship between self-stigma and self-esteem in patients with severe Mental illness (SMI) has received increasing attention in recent years. However, studies on this topic remain scarce in Portugal. This study aimed to examine the association between self-stigma and self-esteem in adults diagnosed with schizophrenia spectrum disorders (SSD). Specifically, we sought to assess the prevalence of self-stigma and investigate its correlation with self-esteem in this population. The findings highlight the relevance of addressing self-stigma to support recovery-oriented care in individuals with SSD **Method:** A total of 51 outpatients diagnosed with SMI were enrolled in this cross-sectional study. Participants were recruited from a community psychiatry unit in Porto, Portugal. All assessments were conducted face-to-face by the attending psychiatrist. After obtaining informed consent, data were collected on sociodemographic characteristics, clinical history, and self-reported outcomes. Self-stigma was measured using the Internalized Stigma of Mental Illness (ISMI) scale, and self-esteem was assessed with the Rosenberg Self-Esteem Scale (RSES). Statistical analyses were conducted using SPSS version 28.0 (IBM Corp., Armonk, NY), with significance set at $p < 0.05$. **Results:** The sample was predominantly male (66.7%) with a mean age of 44.8 years ($SD = 11.0$), and 56.9% were single. While 33.3% lived with their parents, 31.4% resided with a partner or spouse. Most participants (60.8%) had a diagnosis of schizophrenia. Regarding education, 58.8% had completed basic education, and 23.5% had upper secondary education. The majority were retired due to illness (62.7%), while 19.6% were employed. Moderate to high levels of self-stigma were observed in 31.4% of participants. No significant associations were found between self-stigma and age, education, age at diagnosis, illness duration, or number of hospitalizations. A strong negative correlation was identified between self-stigma and self-esteem ($\rho = -0.745$, $p < 0.001$). **Conclusion:** This study provides insight into the prevalence and correlates of self-stigma and self-esteem among individuals with schizophrenia spectrum disorders attending a community psychiatric unit in Northern Portugal. The findings reinforce existing evidence that internalized stigma is strongly associated with lower self-esteem. These results highlight the importance of implementing interventions aimed at reducing self-stigma and enhancing self-esteem, as such efforts may meaningfully contribute to improved clinical and psychosocial outcomes in this population.

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04 - The transcription factor FOXM1 mitigates DNA damage-induced epigenetic erosion in aging

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Introduction: Accumulation of DNA damage gradually causes the erosion of the epigenetic landscape, thus driving the aging process. FOXM1, a transcription factor repressed with age, is recognized as a master regulator of DNA repair. However, its role in mitigating DNA damage and preserving epigenetic integrity in aging remains poorly understood. **Methods:** Fibroblasts from young donors were subjected to FOXM1 knockdown using RNA interference, while FOXM1 overexpression was induced in fibroblasts from aged donors via a lentiviral transduction system. DNA damage and protein levels of epigenetic regulators were measured by immunofluorescence. Gene expression changes were measured using RNA-seq, and changes in binding profiles of histone modifications were examined using CUT&RUN. **Results:** FOXM1 knockdown in young fibroblasts results in increased DNA damage due to downregulation of essential DNA repair pathways. This promotes the proteolytic degradation of the G9a methyltransferase and a subsequent loss of the H3K9me2 epigenetic mark. Consistent with FOXM1 repression during aging, G9a and H3K9me2 levels are reduced in aged fibroblasts. Loss of H3K9me2 at specific loci is associated with increased expression of senescence and inflammatory processes. Remarkably, overexpression of FOXM1 in aged fibroblasts reduces DNA damage, which reinstates both G9a and H3K9me2 levels to repress genes involved in inflammation and senescence. **Conclusions:** These findings establish FOXM1 as an age reversal factor capable of restoring epigenetic integrity by enhancing DNA repair, offering a promising therapeutic avenue to address the fundamental causes of aging.

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05 - Preliminary *in vitro* assessment of Low-density polyethylene digestion in the human gastrointestinal tract

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Microplastics (MP) are widespread pollutants found in ecosystems, food chains, and human tissues. While often considered chemically inert, their interaction with the human gastrointestinal (GI) tract—characterized by variable pH, enzymes, bile salts, and microbiota—may alter their surface and behavior. These changes could impact MP' bioavailability, toxicity, and health risks, emphasizing the need to study their transformations during digestion. Low-density polyethylene (LDPE), a common polymer in food packaging, was subjected to a standardized *in vitro* digestion protocol simulating the human GI tract. Samples were collected before and after digestion phases. FTIR analysis revealed slightly spectral changes between virgin LDPE and LDPE post-digestion, especially after the intestinal phase. These modifications suggest that digestion alters the polymer's surface chemistry, potentially enhancing its capacity to interact with biological systems or carry adsorbed substances. The findings indicate that the digestive process can transform MPs, potentially influencing their toxicity and interaction with human health pathways. This study demonstrates that MPs, specifically LDPE, can undergo changes in the GI tract, impacting their behavior and health implications. Recognizing digestion as a transformative step is crucial in assessing MP risks, aligning with the One Health approach to protect environmental, animal, and human health.

06 - Comparative epidemiological study and geographic distribution of melanocytic tumors in humans, dogs, and cats in Portugal

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Introduction Melanocytic tumors (MT) are some of the most commonly diagnosed cancers in both humans and companion animals. However, regardless of their importance, there has been no prior research comparing their epidemiology and spatial distribution and therefore, this study aims to examine the incidence, risk factors, and geographic distribution of MT in humans, dogs, and cats in Portugal. **Methods** Through a retrospective, cross-sectional study, cases of MT were analyzed consulting data from the Portuguese National Cancer Registry (RON) (2011–2021) and the Portuguese Veterinary Cancer Registry (Vet-OncoNet) (2019–2023). ICD-O-3.2 was used to classify human melanomas and Vet-ICD-O-canine-1 for dogs and cats. Data was analyzed through descriptive statistics, calculation of incidence rate (IR), relative risk (RR) assessments, and spatial clustering analyses, including Moran's Index (Moran's I) and Bivariate Moran Local Index (BLISA). **Results** 18,324 human, 1,199 canine, and 104 feline MT cases were obtained. Melanoma, NOS was the most common MT among all species, while melanocytomas were more common in dogs. The IR was higher in dogs (16.1 per 100,000) compared to humans (8.1 per 100,000) and cats (6.3 per 100,000). Rhodesian Ridgebacks (RR=12.2) and Shar-Peis (RR=9.8) were the most predisposed breeds to MT. Spatial analysis showed notable clustering in urban regions, with human and canine cases displaying significant geographical overlap (BLISA=0.345, $p<0.001$). **Conclusions** This study reveals significant similarities in the epidemiology and geographical distribution of MT across humans and companion animals, reinforcing the importance of translational studies, within the One Health concept.

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07 - Annexin A1 in healthy dogs: Influence of age and sex and implications for One Health

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Introduction: Annexin A1 (AnxA1) is a glucocorticoid-regulated, calcium-dependent protein involved in immune regulation and inflammation resolution. In humans, it is a promising biomarker in inflammatory, autoimmune, cardiovascular, and neoplastic diseases. From a One Health perspective, studying AnxA1 in dogs offers valuable insights into conserved inflammatory mechanisms, supporting comparative and translational research across species.

Methods: A total of 45 healthy dogs were prospectively enrolled. All animals underwent comprehensive clinical evaluation, including physical examination, complete blood count, serum biochemistry, and screening for common vector-borne diseases using the UranoTest QUATTRO® assay. Serum concentrations of AnxA1 were determined using a commercially available canine-specific ELISA kit (MyBiosource, Inc., CA, USA), following the manufacturer's instructions.

Results: The median age was 5 years (25th percentile; 75th percentile: 3; 9), and the median body weight was 18 kg (9.25; 23). Notably, older dogs (>7 years, n = 20) had significantly higher serum AnxA1 levels than younger ones (≤ 3 years, n = 13): 1049 pg/mL (550.3; 2254) vs. 267 pg/mL (200.3; 457.1), $p = 0.004$. Furthermore, a positive correlation was identified between age and serum AnxA1 levels (n=45, $r = 0.44$, $p = 0.002$). Regarding sex differences, intact females (n = 17) tended to present higher serum AnxA1 levels than males (n = 26) (864.5 pg/mL (391.9; 6607) vs. 338 pg/mL (231.3; 1148), $p = 0.0535$).

Conclusions: Serum AnxA1 levels in dogs appear influenced by age and possibly sex. Higher levels in older dogs may reflect "inflammaging", and the trend in females suggests hormonal modulation. Findings support a One Health approach and highlight AnxA1's potential as a cross-species inflammation biomarker.

08 - Unveiling the faecal microbiota and its association with clinicopathological features in dogs with mast cell tumours

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Introduction Mast cell tumours (MCT) are the most prevalent cutaneous cancer in dogs. Similar to humans, aggressive and indolent presentations have been identified, with limited therapeutic options available, highlighting the need to discover other factors that promote cancer. Evidence has pointed to a significant role of the microbiota in oncological diseases. Hence, the aim of this project was to evaluate if the faecal microbiota could influence the pathogenesis and patient's outcomes in dogs with MCT. **Methods** The faecal microbiota was characterized, through 16S rRNA gene sequencing, in 56 dogs - 28 healthy and 28 diagnosed with MCT. Bioinformatic analyses with Python were performed using a DADA2-based pipeline and models with chi-squared test, T-test and ANOVA that were applied to recognize taxonomic groups related with prognostic and clinicopathological features. **Results** Several bacterial genera uncovered significant differences when stratifying by diseased versus healthy dogs, tumour size, histologic subtype, grade, metastasis, overall survival (OS) and disease-free survival (DFS). The abundance of Proteobacteria was increased in dogs with higher tumour size ($p=0.03$), lower OS ($p<0.001$) and lower DFS ($p<0.001$). Patients with grade III MCT had a higher predominance of Enterococcaceae ($p<0.001$) and *Escherichia-Shigella* was significantly related with a lower DFS ($p<0.001$) and OS ($p=0.004$). Furthermore, *Clostridium sensu stricto_1* was more present in patients with metastases ($p<0.001$) and worse DFS ($p=0.003$). **Conclusions** This research has provided first-hand insights into faecal microbial profiles linked to clinicopathological features of MCT and patient prognosis. In the context of One Health, these studies may contribute to the understanding of tumourigenesis and

emphasize the potential prognostic and therapeutic value of microbiome in oncological diseases.

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09 - Comparison of obesity-linked polymorphisms between human and other mammalian species

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Introduction: Comparative genetics can contribute to the understanding of the molecular basis of genetic diseases. Obesity is a multifactorial disease affecting humans and some other animal species. Because all living organisms share phylogenetic relationships, that can be useful in perceiving risks and susceptibilities to clinical phenotypes, we aimed to compare genetic sequences containing polymorphisms linked to obesity, between human and non-human mammalian species. **Methods:** For this work, we compared orthologous sequences of 10 non-human primate and rodent species, to evaluate the homologous position of 15 polymorphisms linked to obesity. The data was obtained by accessing a public available genome database, Ensembl. **Results:** Multiple sequence alignments showed that, in some cases, the obesity-linked risk allele in humans corresponded to the ancestral allele in other mammals. For example, the risk allele FTO rs9939609-A in humans was found as the ancestral allele in other primates and rodents. Similarly, the obesity-linked allele ESR1 rs712221-T was found as the native allele in primates and in one rodent species. In other cases, the risk allele was only found in a rodent (LYPLAL1 rs4846567-T) or in a primate (5-HT2C rs3813929-T), indicating that they were absent from the most recent common ancestor and the obesity risk allele is a derived allelic state. **Conclusion:** Some polymorphisms, namely alleles linked to obesity in humans, correspond to the ancestral allele in non-human species. This suggests that humans underwent genetic adaptations, and the protective allele is a genetic novelty that emerged during human evolution whereas the ancestral state cannot accommodate the modern human lifestyle conditions.

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O10 - Tracking MDR bacteria in a canine orthopedical surgical site infection: clinical challenges and One Health implications

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Introduction: Surgical site infections (SSIs) pose a major significant challenge in veterinary medicine, often complicating recovery after orthopedic surgery. Microbiological surveillance and antibiotic stewardship are vital for guiding therapy and elucidating the colonization dynamics. This report describes a clinical case of a dog that developed a post-surgical infection after fracture repair, illustrating the emergence, persistence and potential dissemination of multidrug-resistant (MDR) bacteria. Methods: Microbiological samples were collected over three months, including swabs of wound exudate (superficial/deep), surrounding skin of the catheter, axilla, perianal region, as well as the catheter and external fixators. Cultures were performed on selective and non-selective media, including broth enrichment. Bacterial isolates were identified and resistance phenotypes determined, focusing on Methicillin-resistant *S. pseudintermedius* (MRSP) and ESBL-*K. pneumoniae*, initially detected elsewhere. Results: Despite therapy with cefazolin, clinical worsening occurred, prompting further sampling. MRSP and ESBL-producing *K. pneumoniae* have been isolated from multiple locations over time. Enrichment allowed the detection of low-abundance resistant strains. New MDR strains (ESBL-*K. pneumoniae*, ESBL-*E. coli*, MRSP) emerged during follow-up, being re-isolated from the wound, fixators and perianal area, despite clinical improvement. Bacterial load and diversity decreased over time (particularly in wound), yet identical MDR phenotypes were re-isolated in perianal and wound samples at the end of follow-up. Conclusions: This case highlights the complexity of MDR SSIs and the critical role of microbiological monitoring, in order to guide therapy and reduce the risk of spreading MDR strains within veterinary healthcare settings. Fecal carriage of multiple MDR strains heightens the risk dissemination, emphasizing the need of hygiene and patient isolation measures.

O11 - Immunomodulatory fibrin-based hydrogels embedded with Maresin-1-loaded zein nanoparticles for wound healing applications

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Introduction Chronic wounds occur when healing is disrupted, remaining in the inflammatory phase. They significantly affect patients' quality of life and place a heavy burden on healthcare systems. The present research work aims at developing a fibrin-based hydrogel incorporating immunomodulatory zein nanoparticles loaded with maresin-1 to modulate the immune response, resolve inflammation, and create a regenerative microenvironment for effective tissue repair. **Methods** A fibrinogen solution (2 mg/mL) was combined with either maresin-1, empty zein nanoparticles, maresin-1-loaded zein nanoparticles, and maresin-1 plus maresin-1 loaded zein nanoparticles. Thrombin (2 U/mL) was added immediately, and polymerization proceeded for 20 min, at 37°C. Maresin-1 release was quantified via ELISA. Human macrophages isolated from buffy coats of healthy blood donors, by negative selection, were used to assess the M2 polarization potential of the developed hydrogels. **Results** The release assay results exhibited a sustained release of maresin-1 over seven days from fibrin hydrogels containing zein nanoparticles loaded with maresin-1, as well as hydrogels combining maresin-1 and maresin-1-loaded zein nanoparticles. An LDH assay confirmed that these hydrogels were non-cytotoxic to human macrophages. Notably, both fibrin hydrogels incorporating zein nanoparticles loaded with maresin-1, and hydrogels combining maresin-1 and maresin-1-loaded zein nanoparticles, significantly enhanced macrophage polarization towards the M2 phenotype, in comparison with free maresin-1 and empty zein nanoparticles, highlighting their potential for therapeutic applications. **Conclusions** We successfully synthesized fibrin hydrogels incorporating maresin-1 loaded zein nanoparticles. After 7 days of culture with primary human macrophages, the normal cell morphology remained unaffected. Furthermore, these hydrogels nudged macrophages toward an M2 anti-inflammatory and pro-regenerative phenotype.

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O12 - Balancing inflammation and neuroprotection through P2X7 receptor activation in neonatal Group B Streptococcal infection

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Introduction: Group B Streptococcus (GBS) is a leading agent of neonatal bacterial infection and disease. P2X7 receptor (P2X7R) plays a crucial role in inflammation/ neuroinflammation during infection. Systemic P2X7R activation prevents GBS-induced neonatal mortality, bacterial dissemination, and neurodevelopmental impairment. To understand the beneficial action of P2X7R activation, we explored its impact on the inflammatory profile, neutrophil/ glial activation, and long-term behavioral outcome. **Methods:** Pups of BALB/c dams intravaginally inoculated with GBS were daily treated from postnatal day (PND) 1 to 4 with the selective P2X7R agonist, Bz-ATP (2.5 mg/kg/day), and/or with the selective P2X7R antagonist, A740003 (30 mg/kg/day). At PND3, organs were harvested for cytokine quantification, Ly6G and GFAP immunoreactivity, and myeloperoxidase (MPO) activity. Behavioral performance was assessed in adult survivors. Statistical analyses were performed by ANOVA with Holm-Sidak's post hoc test. **Results:** Systemic activation of P2X7R with Bz-ATP in GBS-infected pups promoted a shift towards a pro-inflammatory state, particularly at the liver and kidneys, with increased ($p \leq 0.05$) (i) TNF- α levels, (ii) percentage of Ly6G+ cells, and (iii) MPO activity, accompanied by decreased ($p \leq 0.05$) IL-10 levels compared to infected untreated pups. At the brain, Bz-ATP treatment induced a rise ($p \leq 0.05$) in TNF- α levels and percentage of IBA+ cells, while reducing ($p \leq 0.05$) GFAP immunoreactivity. Adult survivors showed improved cognitive function and reduced anxiety-like behavior ($p \leq 0.05$). These effects were prevented by co-administering Bz-ATP with A740003. **Conclusions:** Our findings demonstrate that early systemic P2X7R activation plays a protective role in a mouse model of neonatal GBS disease by inducing a pro-inflammatory shift that leads to neutrophil recruitment to infection sites and attenuates astroglial activation, preventing the progression of neurodevelopmental impairments into adulthood.

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013 - Environmental stress and pharmaceuticals toxicity: How temperature and pH influence antibiotic effects in *Danio rerio*?

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Introduction: Aquatic ecosystems face multiple stressors, including rising temperatures, pH fluctuations, and antibiotic contamination (e.g., sulfamethoxazole-SMX, trimethoprim -TRIM, and mixture-MIX). These factors can interact, altering antibiotic toxicity and disrupting vital biological functions. Despite their potential ecological risks, the combined effects of antibiotics, temperature, and pH remain poorly understood, highlighting the need for further research, particularly in climate change scenarios. **Methods:** Two chronic assays were performed to evaluate the toxicity of environmentally relevant concentrations of SMX (150 µg/L), TRIM (30 µg/L), and MIX (150 µg SMX/L + 30 µg TRIM/L) on *Danio rerio*. One assay examined the effects of these antibiotics across three temperature scenarios (26°C, 28°C, and 32°C), while the second assessed the antibiotic impacts under pH fluctuations (6.5, 7.5, and 9.0). A multi-biomarker approach was used to assess *D. rerio* biological health status. **Results:** Temperature and pH variations affected antibiotic's toxicity in *D. rerio*. SMX and MIX increased toxicity, exhibiting moderate toxicity and causing severe alterations (e.g., neurotoxicity and DNA damage), at 28°C, while TRIM showed slight toxicity (antioxidant defense alterations). At 32 °C, MIX was the most toxic, leading to genotoxicity. SMX had stronger effects at acidic pH, whereas TRIM toxicity increased at neutral and alkaline pH, disrupting antioxidant defenses and cellular integrity. MIX was marginally toxic at acidic and alkaline pH but moderately toxic at neutral pH, triggering oxidative stress, lipid peroxidation, and DNA damage. **Conclusions:** The combined effects of antibiotics, temperature, and pH pose significant risks to *Danio rerio*. Its urgent integrated research on multiple environmental pressures (chemical contamination vs climate change-related abiotic stressors), as neglect to address these threats could lead to irreversible damage to aquatic ecosystems and biodiversity.

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014 - Freshwater under pressure: *Daphnia* sp. as sentinels of cyanobacterial stress

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Introduction Water quality is crucial for ecosystem balance, but blooms of cyanobacteria can disrupt it by producing toxins and depleting oxygen. These blooms thrive in eutrophic waters, affecting zooplankton like *Daphnia*, key grazers in aquatic food webs. Understanding these interactions, and how different *Daphnia* species react using *Aphanizomenon flos-aquae* as a food source is essential to assess the ecological impacts of this particular cyanobacterial blooms on aquatic ecosystems. Methods *Daphnia magna*, and two *Daphnia* sp. isolated from Portuguese reservoirs (Rabagão and Aguieira) were used to conduct feeding inhibition assays using *A. flos-aquae* as a food source with different concentrations (cells/mL): 7.5×10^4 (medium risk); 1.5×10^5 (high risk); 3.0×10^5 (high risk); 1.2×10^6 (very high risk). These concentrations were selected based on public health risk values established by the WHO, and their effects on various biomarker responses were also evaluated. Results A significant feeding inhibition for all species was observed when compared with the control. However, an increase in feeding rates was observed with *A. flos-aquae* increasing concentration, although always lower than the control. In all food conditions, *D. magna* showed higher feeding rates, while no differences were shown between natural species. Acetylcholinesterase (AChE) activity increased significantly in all food treatments compared to control group, however a decrease with the concentration increase was recorded. *Daphnia* from Rabagão reservoir showed higher AChE activity suggesting a more active response or greater stress. Conclusions As *A. flos-aquae* concentration rises, *Daphnia* increases food intake to compensate for the poor food quality. *Daphnia* from Rabagão is shown to be more sensitive to the cyanobacteria as a food source. *A. flos-aquae* used as food seems to cause neurophysiological stress, due to elevated AChE activity and reduced feeding rate.

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O15 - A One Health perspective on the risks and management of cyanobacteria and cyanotoxins in Alqueva multipurpose Project (EFMA) reservoirs

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Introduction The One Health recognizes the interdependence of human, animal and environmental health. Harmful cyanobacterial blooms compromise water quality and pose risks to human and animal health through direct or indirect exposure to this hazard. Integrating environmental, public health, and veterinary sciences provides a holistic framework to understand and manage these threats. This study aims to evaluate cyanobacteria and cyanotoxin risks in Alqueva MultiPurpose Project (EFMA) integrating the One Health concept. **Methods** Data on cyanobacteria and cyanotoxins from the Alqueva reservoirs were provided by EDIA (Empresa de Desenvolvimento e Infraestruturas do Alqueva) and were used to analyse their dynamics and assess the potential risks associated with their presence. Additional data from scientific databases, including both occurrence records and reports of poisoning incidents, were also used in this study to evaluate the impacts of cyanobacteria and cyanotoxins in the Alentejo region. **Results** The reservoirs of the Pedrógão subsystem (part of the Alqueva system) serve as sources for drinking water and irrigation. Despite ongoing mitigation efforts, cyanobacterial blooms still occur intermittently. Although the Magra Reservoir maintained *microcystin* and *cylindrospermopsin* levels within safe limits (1 µg/L), recent exceedances were recorded in the S. Pedro and Pedrógão reservoirs, both used for irrigation and animal watering. Not related to the company, a case in Alentejo, where 25 cattle died after drinking from a bloom-affected stream, exemplifies the region's risks and underscores the role of continued monitoring by the company in preventing such outcomes. **Conclusions** Cyanobacterial blooms, often involving toxin-producing species, are becoming more frequent. Effective monitoring programmes and sentinel events have enabled risk assessment and fostered communication across human, animal, and environmental health sectors. Strengthening cross-sector collaboration and recognising these blooms as a multidisciplinary challenge are essential to protect the One Health triad and enhance mitigation strategies.

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