



JOINT MASTERS | AQUACULTURE |
ENVIRONMENT | SOCIETY

Tuesday 6th and Wednesday 7th July 2021

STUDENT CONFERENCE 2019 - 2021

*Presentations from our FIFTH cohort of ACES students
Online (via Webex)*

BOOK OF ABSTRACTS



University of the
Highlands and Islands
Oilthigh na Gàidhe:
agus nan Eilean



UNIVERSITÉ DE NANTES



Tuesday 6th July

09:00 – 09:15 **Welcome and introduction to the Student Conference
2021 - Professor Liz Cottier-Cook, SAMS-UHI**

09:15 – 10:30

Amalia Krupandan: An Integrated Remote Sensing & DEB Model Approach to Assess the Suitability of Sites for Pacific Oyster Mariculture in South Africa

South African Pacific Oyster growth rates are amongst the highest in the world, however, production remains low relative to top oyster-producing countries despite recent governmental promotion of aquaculture. This study aims to determine oyster growth potential within the South African Exclusive Economic Zone and contribute to baseline data for oyster mariculture site selection.

Novel methodology combining the use of satellite-derived environmental data and a Dynamic Energy Budget model to determine oyster growth potential was adapted for the South African environment. MODIS chlorophyll-a and SST products were used to simulate yearly oyster growth (Dry Flesh Mass (DFM) and Total Weight (TW)) for 18 years. Average DFM and TW at the end of the culture period was mapped and compared for prominent sectors. Industry-relevant growth indicators Days to Reach Commercial TW (100g) and the optimal Culture Period Length to reach each of the commercial size classes was also established.

High growth potential occurred in eastern nearshore Agulhas sectors. The highest growth was found in Plettenberg Bay where oysters reached 500g TW within 11 months. Other sectors in the region yielded Large commercial size within 150 days. However, Agulhas growth hotspots were found to be highly variable, and contain low DFM relative to TW. Growth potential was favourable in the northern sectors of the Benguela, where oysters could reach 300g in 11 months, Large commercial size within 200 days, and contained higher DFM relative to TW. Thus, current oyster production sites are unfavourable for growth, and high-growth sites coincide with areas in economic decline or high levels of poverty. However, due to constraints of successfully applying DEB models over large spatial scales, these results should be interpreted with caution and await additional *in situ* verification, as well as a spatial-multi-criteria analysis, before further investment and development.

Herman Prasyad: Topographic Analysis of Oyster-farming areas using Unmanned Aerial Vehicles (uav) very high resolution spatial data.

Oyster culture in France has been established since the 19th century in intertidal areas of the French Atlantic coast. Oysters are traditionally grown in plastic mesh bags set on metal trestles (tables), at a height of *ca.* 1 m off the bottom. This work evaluates the potential of Very High-Resolution (VHR) data obtained from Unmanned Aerial Vehicles (UAVs) and Structure from Motion (SfM) integrated with remote sensing, topographic and spatial analysis techniques to map the characteristics of intertidal oyster farms. Two different UAVs equipped with multispectral cameras were deployed at different altitudes above the ground to produce

various Digital Surface Model (DSM) and orthophotographs spatial resolutions. Firstly, a 10 cm spatial resolution DSM was obtained from flights at *ca.* 110 m altitude to identify the oyster tables' footprints. Topographic indices were tested *i.e.*, openness, geomorphon, and real surface area, on the DSM to identify and map the oyster tables based on the topographic features of oyster concessions.

The results showed that most of the oyster tables could be successfully identified and mapped by using this topographic analysis approach. Accuracy assessment showed a good agreement between observations and models with r^2 value of 0.92, and Root Mean Square Error (RMSE) of 3.12 m². Another flight at *ca.* 50m altitude producing a 7 cm spatial resolution was tested to retrieve oyster tables heights by subtracting a DTM (Digital Terrain Model) data from the DSM. Accuracy assessment showed a good agreement between the modeled and *in situ* measured table heights with r^2 value of 0.92 and RMSE of 0.096 m. Finally, the level of details of the plastic mesh bags containing the oysters was examined for different spatial resolution orthophotographs. This study demonstrates the potential of VHR UAVs data integrated with topographic analysis for a precision mapping of intertidal oyster farms.

Luca Hertfelder: Title: Opportunities and challenges of aquaculture as a restoration tool: A case study on European flat oyster (*Ostrea edulis*) restoration

Biogenic, temperate reefs are among the most threatened marine habitats in the world. It is estimated that 85% of global oyster reefs have been lost due to overfishing, habitat loss and more recently the spread of diseases. The European flat oyster (*Ostrea edulis*) used to be abundant along Europe's coasts before stocks drastically declined in the 1900s. The growing recognition of *O. edulis* ecosystem services and functions has led to increased restoration efforts across Europe. This study reviews current restoration efforts and approaches and identifies the opportunities and challenges of aquaculture as a restoration tool. For this, a case study on an *O. edulis* restoration project in Loch Melfort, Scotland was carried out, aiming to identify suitable release sites.

Firstly, a categorical abundance survey of the intertidal area was carried out across 27 sites, identifying areas where *O. edulis* was most common. Secondly, five sites with high oyster abundance were revisited for a transect survey, investigating the vertical distribution and numerical abundance of this species. *O. edulis* were significantly more abundant in sheltered sites with freshwater influence. In addition, *O. edulis* were found in the lower intertidal area with the locations of peak abundance varying between sites. The absence of juvenile oysters indicates that no successful recruitment has taken place in recent years, highlighting the need for active restoration in the area. The potential benefit of hatchery produced, disease resistant oyster seed was identified, at the expense of genetic diversity while large scale pond production can maintain relatively high genetic diversity. A combination of these two approaches is necessary to ensure long term success of restoration projects. The results presented in this thesis provide a baseline to evaluate the success of restoration efforts and provide an approach to data-based small-scale release site selection.

10:45 – 12:00

Clairie Ng: Development of an easy to operate hatchery protocol for *Mytilus edulis* seed production: The impact of rearing system and settlement substrate on hatchery success in Denmark

Mytilus edulis, commonly known as the blue mussel, is the second most economically important mussel cultivated in Europe. Faced with increasing spatial limitations inshore, researchers and industry are looking at distant offshore sites for longline cultivation where recruitment of spat is lower. Presently, low, inconsistent seed availability and suboptimal systems not adapted to exposed conditions present obstacles for successful expansion. Hatchery production of blue mussel seeds could be a solution to ensure reliable supply and introduce opportunities for selective breeding and triploid production. However, due to the low cost of natural spat and final sale value of mussels, it remains underdeveloped. In order to overcome the prohibitively expensive cost of hatchery reared spat, optimization is necessary.

The Danish Shellfish Centre, DTU Aqua located at Nykøbing Mors contains a modern hatchery equipped in producing blue mussel, European flat oyster and other shellfish. Currently, the extant Irish protocol for blue mussel hatchery seed production developed within AquaVitae, the Atlantic research consortium for sustainable aquaculture, is not operational for mussel farmers without a traditional shellfish hatchery. The study aims to adapt this protocol to the local context and propose a cheaper alternative “Danish method”. *Mytilus edulis* larvae were grown under two rearing systems to assess impacts on mortality and growth. Pediveligers at the end of the larval rearing stage were exposed to high or low aeration treatments and five settlement substrates available in Denmark. This study demonstrated the cheaper, low-technological Danish method could be a viable alternative. Even though larvae grew at a slower rate, preliminary results indicate higher settlement success than the Irish protocol. This study did not find any significant impact of aeration on primary settlement but found thick, polypropylene ropes and polypropylene trawl nets to be alternatives to the traditional polypropylene belt spat collectors currently used in wild spat collection.

Evgeniya Yastrebova: Novel feed in the novel market: Understanding the value of certification labels in targeting the US market for a novel insect aquafeed product

Targeting a new market with a novel insect product requires tools and strategies to connect with stakeholders and increase the reach of the company’s activities. The work has been undertaken within InnovaFeed company, which is an innovative insect ingredients producer. The aim is to secure a healthy business development of InnovaFeed in the US market using optimal certification labels. The work is organized as a series of case studies that focus on different stages of market awareness. First, this paper analyzed various certifications and their potential benefits for the insect-producing company as well as other members of the value chain. Secondly, the most effective certification labels were identified and prioritized for further analysis. They were measured on their relevance to the insect producer through a decision matrix and The Wheel of Sustainability.

Finally, this paper considered long-term development strategies in the US market to build consumers’ awareness, increase demand, and change the perception of alternative feed

ingredients in a positive direction. These strategies were examined using the SWOT (Strengths Weaknesses Opportunities Threats) analysis, which will give the company the spectrum of possible outcomes and difficulties to consider. In the end, the paper hopes to decrease the risk and increase the momentum of insect ingredients in the US market by using certification labels as a tool. The insect-producing company (or other organizations) could use these findings as a base for their expansion strategy into any international market or for further development in an already studied business environment.

Binbin Cai: Assessment of the effects of cadmium, samarium and gadolinium on the blue mussel (*Mytilus edulis*): a biochemical and metabolomic approach

The integration of tools relying on physiological responses into environment studies is of great concern to assess environmental risks and to broaden the understanding of the ecotoxicological impact on organisms. Due to the known chemical pressures on the environment, relevant content regulations have been relatively completed for some chemicals, such as cadmium. In contrast, the environmental toxicity of rare earth elements, which are widely used in industry, has only gradually gained attention in recent years. However, are the regulations of the corresponding elements proportional to their environmental toxicity? Here we studied the effects of cadmium, and two rare earth elements, samarium and gadolinium, on a marine mussels (*Mytilus edulis*) under laboratory experiments. We found that after an 8-day exposure, rare earth elements tend to show an impact on 2 biochemical markers of oxidative stress (Glutathione-S-Transferase, Superoxide dismutase) at 500 µg/L. A lipidomic analysis showed that some upregulated lipids are involved in the metallic pressure for the three elements tested. This result showed a possible toxicity of cadmium at two concentrations (50, 500 µg/L), and of samarium and gadolinium (500 µg/L) which were bioaccumulated by the organisms. This study added an improvement to the insight of metal toxicity. Furthermore, the further identification of the nature of the lipids impacted by the metals will be a next step of this work to elucidate their role in the toxic action mechanisms.

12:00 – 13:30 LUNCH

13:30 – 14:45

Despoina Vasileiadi: Engineering beneficial algal microbiomes

The finfish larval hatchery represents a critical production point in aquaculture. Larval mortalities, often caused by opportunistic pathogenic bacteria, can be high especially under intensive rearing conditions. Considering the multiple ways by which opportunistic bacteria are introduced along a hatchery trophic chain, a systemic approach for the application of a probiotic strain antagonistic against fish pathogenic bacteria was examined in this study. The purpose was to investigate the potential of the recently isolated bacterial strain *Phaeobacter piscinae* S26, to antagonize the pathogenic strain *Vibrio crassostreae* DMC-1, which was previously associated with zooplankton and has caused mass mortalities in cod and turbot larvae. Treatment was targeted at the lowest trophic level, that of the algal culture, which is usually fed to live-feed (zooplankton), or directly to the fish larvae. After assessing the extent by which DMC-1 could establish itself in an axenic *Tetraselmis suecica* microalgal culture, thereby rendering it a pathogen vector, the probiotic effect of the *Phaeobacter* strain was examined. *P. piscinae* S26 (initial concentration: 10⁶ CFU/mL), which produces the

antibacterial compound tropodithietic acid (TDA), markedly diminished the numbers of *V. crassostreae* DMC-1 (initial concentration: 10^{3-4} CFU/mL) in a *Tetraselmis suecica* culture (initial concentration: 10^5 CFU/mL), keeping it below detection levels for 5 days. A bioactivity-deficient *P. piscinae* S26 mutant that does not produce TDA, also reduced the DMC-1 numbers in the *T. suecica* culture although to a lesser extent, suggesting that *Phaeobacter piscinae* S26 has an inhibitory effect against *Vibrio crassostreae* DMC-1, with TDA likely being the primary effector compound. Additionally, the application of the wild type and mutant *Phaeobacter* strains did not have any effect on the growth of the algae. These results show that *P. piscinae* S26 is a promising sustainable bio-control agent against *V. crassostreae* DMC-1. However, the research on this new probiotic strain needs to be optimized under more realistic hatchery conditions.

Sandamali Lokuhitige: Investigation of halogenation potential of marine vanadium-dependent haloperoxidases

Halogenated compounds have been described to present numerous biological activities, such as antitumoral and antimicrobial properties. Thanks to different enzymes, many marine organisms produce halogenated compounds. Among them, vanadium-dependent haloperoxidases (vHPOs) present strong interest in green chemistry development. They are robust and tolerate many different substrates and reaction conditions. The literature reviews related to haloperoxidase production by marine fungi are limited. Preliminary studies have shown the potential of several marine fungal strains in the production of vHPOs with the detection of several and isolation of one new enzyme. This study investigates the potential of these marine vHPOs. Three enzymes were taken into consideration: first, two known enzymes; the vanadium-dependent bromoperoxidase, isolated from the brown alga *Ascophyllum nodosum* (AnvBPO) and the vanadium-dependent chloroperoxidase from the fungus *Curvularia inaequalis* (CivCPO). Finally, the newly produced enzyme corresponding to a vanadium-dependent chloroperoxidase isolated from the marine fungus *Hortaea werneckii* (HwvCPO). The reactivity of each enzyme was tested using the MCD assay. AnvBPO showed significantly higher reactivity compared to CivCPO or HwvCPO for bromination.

Interestingly, the new HwvCPO, while allowing chlorination, presented much more affinity for bromine than chlorine. Then, assays were carried out on different substrates such as phloroglucinol and thymol and compared the bromination potential of both AnvBPO and HwvCPO. All reactions were analyzed using TLC and HPLC-UV-MS. Both enzymes were able to produce the mono- and di-brominated analogs of the initial substrates. Tri-brominated phloroglucinol could be observed with the use of AnvBPO. The reaction yields were quantified using an internal standard. The second part of the study, a class of rare heptacyclic alkaloids, the communesins, which presented interesting activities in previous studies, were submitted to enzymatic halogenation using the above-mentioned three enzymes to complete structure modulations around this very promising scaffold and further evaluate the potential of marine v-HPOs.

Anne Hobdy: Integrated Multitrophic Aquaculture Preliminary Assay: Nitrogen Metabolism in Urchin-Macroalgae IMTA System

Integrated Multi-Trophic Aquaculture (IMTA) addresses one of the main impacts of the aquaculture industry—excess nutrients from waste—by utilizing it as a food source. The purple urchin *Paracentrotus lividus* is a high-value, low trophic level organism, an ideal

candidate to be incorporated into IMTA systems, yet there remain knowledge gaps that must be filled before *P. lividus*-based IMTA can be implemented on a wider scale. This preliminary study used an experimental IMTA system of *P. lividus* and two macroalgae species to investigate and characterize the kinetics of ammonium production and uptake. In a first phase, ammonium production by urchins of two age groups was followed and characterized linearly. In a second phase, *Ulva spp.* and *Palmaria palmata* were cultured in the effluents of the first, following the absorption of ammonium as well as algae growth, nutrient removal, and biochemical analysis. Maximum ammonium production by older urchins (~2 years) was observed at 170.54 $\mu\text{g NH}_4/\text{L}$ per day, reaching a maximum concentration of 2438.56 $\mu\text{g NH}_4/\text{L}$ in 14 days with no mortalities or health concerns observed.

Urchins in the younger age group (~1 year) produced at a higher rate of 314.26 $\mu\text{g NH}_4/\text{L}$ per day, exhibiting a decline in health around 3000 $\mu\text{g NH}_4/\text{L}$. In the second phase, ammonium consumption by both macroalgae was observed, with complete removal by both species occurring in ≤ 4 days. In each trial, *Ulva* absorbed the ammonium more quickly than *Palmaria*, at a maximum rate of 82.49 $\mu\text{g NH}_4/\text{L}$ per hour. Significant growth of *Ulva* was also observed. This experiment demonstrates the viability of co-culturing *P. lividus* with macroalgae in a system which efficiently utilizes wastes whilst producing food for the urchins. The results will enable the sizing of these systems in the future, helping to optimize organism densities with nutrient availability and water quality.

15:15 – 16:05

Quynh Khanh: Understanding corporate social responsibility initiatives across aquaculture context: a case study of farmed Scottish salmon and Vietnamese shrimp and Pangasius.

Social perceptions have affected positively and negatively the aquaculture industry development. The aquaculture producers have tried to present themselves as responsible businesses through corporate social responsibility (CSR) strategies and practices. However, CSR in the aquaculture industry has not been defined clearly. The absence of the data on the subject might lead to the miss of information to the public, therefore affect their views and support on the development of aquaculture sectors. The dissertation aims to study CSR practices in the aquaculture context; and to identify their key drivers, benefits and challenges, and how aquaculture businesses perceived CSR, especially in the specific context of farmed Scottish salmon and Vietnamese Pangasius and shrimp sectors in relation to stakeholders. An integrated literature review, market report analysis, and interviews were conducted to identify and investigate CSR in aquaculture businesses.

Thematic analysis was employed to identify, analyze and report the themes within the data in the reports of aquaculture businesses. The key components of CSR practices in aquaculture were identified based on 3 main themes: internal social responsibility, external social responsibility, and environmental responsibility. This study indicates the necessity of CSR in aquaculture businesses and their need to meet stakeholders' requirements and expectations for the responsible and sustainable development of the aquaculture industry. There are many drivers governing CSR practices of aquaculture businesses in Scotland and Vietnam. An understanding between the aquaculture producers and their stakeholders can help comprehend their roles and incorporate them to develop aquaculture sectors sustainably through their common needs and goals.

James Hedges: A Changing or Deadlocked Debate in Aquaculture.

Salmon farming is an important and rapidly growing industry in Scotland with coverage of the topic increasing in the media. The public is reliant on media for information regarding the industry and its activities. The media also acts as a debate arena in which different groups can voice their views in attempts to garner support, influence policy, and sway public perception. This has created fierce debate and polarization, leading to deadlocks in a number of countries. This thesis aims to identify the main themes and dominant actors involved in the debate. Furthermore, it aims to determine what actors believe the impact of media has on aquaculture policy, and if the debate is deadlocked or moving. Analysis of online articles of four Scottish newspapers show that environmental impacts are the dominant topic of coverage, and that the debate is controlled by a small number of groups, with 25 individuals appearing in over half of all articles.

Through analysis of interview content from these dominant actors, this thesis gives a glimpse into the current state of the aquaculture debate in Scotland. The debate is controlled by two opposing groups that can be divided into the salmon industry and campaigners, with few voices in between. Actors see the debate as polarized, lacking critical journalism, and lacking government intervention. Public understanding of aquaculture is seen to be poor. This thesis also shows themes of perceived change in the debate through the influence of veganism, social media, and increased industry transparency. All actors believe that media effects policy, either directly or indirectly, through lobbying or perceived public perception. The debate is currently seen as deadlocked with the potential to shift with the changing shape of the arena. This thesis aims to improve understanding of the debate within media and how it can influence policy.

16:05 - Closing Remarks from Day 1 from Professor Liz Cottier-Cook

Wednesday 6th July

09:00 – 09:15 **Welcome to Day 2 - Prof Liz Cottier-Cook, SAMS-UHI**

09:15 – 10:30

Joey Arboleda: Behavioural Effects of 11-beta-Hydroxysteroid Dehydrogenase Type-2 (11 β -hsd2) Mutation in both Larvae and Adult Zebrafish Knockout Mutants

Fish respond to stress via activation of the Hypothalamic-Pituitary-Interrenal (HPI) axis and the subsequent release of cortisol which exerts a variety of actions. In this study, we investigated the behavioural effects of 11-beta-Hydroxysteroid Dehydrogenase type-2 (11 β -hsd2) knockout mutation, a key gene in cortisol availability control, in both adult and larvae zebrafish. To this end, the Vibrational Startle Response Assay (VSRA) for larvae and 4 behavioural tests for the adults, namely; Novel Tank Test (NTT), Light/Dark Test (LDT), Open Field Test (OFT) and Social Preference Test (SPT) were carried out. respectively. No significant differences was observed on the distance travelled by the larvae on the first tap, however, 11 β hsd2^{-/-} larvae were observed to significantly increase the habituation as compared to the wildtype group ($p = 0.0268$).

Anxiety like behaviours in adults were observed from all behavioural tests as confirmed by the significant difference ($p = 0.001$) on the preference of both groups in bottom zone than top zone for NTT, dark zone over light zone in DLT, peripheral zone than central zone in OFT, and social zone over neutral and non-social zone for SPT. However, no significant difference was observed between the 11 β hsd2^{-/-} and the control group in terms of their anxiety like behaviour in all four tests. Behavioural tests are considered to be mild and short-term stressors only, the basal and post-test cortisol levels should be measured to confirm if there is a significant increase in the cortisol for the mutation to manifest in the behavioural endpoints.

Sreeradha Mallick: Effect of the temperature on the immunity and microRNAs(miRNA) in the early-life stage development of the European sea bass (*Dicentrarchus labrax*)

The European Sea bass (*Dicentrarchus labrax*), a poikilothermal species is highly dependent on fluctuations of the ambient temperature. We have studied the impact of the vacillating temperature on the immunity of teleost fishes. They possess a highly developed complex immune system for defense against invading pathogens. The immune system comprises two major components-innate and adaptive. The study involved early developmental stages in *D. labrax* and aquatic organisms usually depend on innate immunity during these stages. A specific group of receptors known as pattern recognition receptors (PRRs), useful in generating threat signals facilitates the triggering of innate immunity in organisms. Our focus was on one such family of recognized PRRs called the Toll-like receptors (TLRs), highly regulated by molecules with complex mechanisms, and responsible for providing a secured

defense against invading pathogens. Since it is bi-directional communication between the immune system and reproductive hormones, our work also involved investigating genes associated with some major gonadal hormones in the European sea bass. MicroRNAs (miRNAs) are a group of highly conserved small nuclear RNAs playing a significant role in the post-transcriptional modification in the regulation of gene expression in a broad range of vertebrates including teleost fishes. Various miRNAs associated with our investigated genes for both immunity and reproduction were studied and it was observed that they were already identified to be influenced by different temperatures during developmental stages. It can be inferred that temperature is crucial in the gene expression of teleost as fluctuations in temperature during early life stage development of the European sea bass not only had an impact on the differential expression of miRNA but also on the immunity and the sex-ratio of the fish.

Emmanouil Diakos: Development of epigenetic biomarkers for aquaculture

Recently, Deans and Maggert (2015), defined epigenetics as “the study of phenomena and mechanisms that cause chromosome-bound, heritable changes to gene expression that are not dependent on changes to DNA sequence”. Gene regulatory events inherited mitotically at the cellular level, and meiotically from parents to offspring, that may also lead to the emergence of physiological phenotypes. DNA methylation, histone modifications, and noncoding RNAs are the three main epigenetic mechanisms implicated in the regulation of gene expression in animals and plants. Epigenetic modifications have been studied broadly, due to their regulatory roles in gene expression or suppression, and, consequently, in the formation of the phenotype, in response to environmental stimuli, such as temperature, stress, and nutrition, as well as due to their implications in cancer, neurodegenerative, cardiovascular, and other diseases.

DNA methylation is considered to be the most studied epigenetic modification in mammals and fish. This epigenetic mark has been used in the development of biomarkers, as it is characterized by its stability and ease of measurement through a wide range of methods that are constantly developed. Thus, scientific research is focusing on the development of biomarkers based on epigenetic changes that could be useful tools for disease treatment, prediction of traits of economic importance in aquaculture, but also in the fields of ecology and evolution. Recent epigenetic changes observed in fish, can pave the way for their incorporation in the development of biomarkers valuable for aquaculture, exploring the genotype to phenotype ‘gap’ and contribute to the sustainability of the sector.

10:45 – 12:00

Natalie Panasiak: Beyond the Crystal Balls: iFishIENCi of Feeding Behavior and Stomach Fullness Methods in RAS Cultivated Rainbow Trout (*Oncorhynchus mykiss*)

The European Union recently funded the iFishIENCi Horizon 2020 project in the interest of promoting sustainable aquaculture and highly controlled growth while optimizing aquafeed use and waste reduction. In a novel study under the framework of the iFishIENCi project, ballotini-incorporated feed intake and X-ray methodology was used in conjunction with machine learning and K-means clustering techniques to investigate the relationship between stomach fullness dynamics and feeding behavior in cultivated fish. Forty-nine adult rainbow

trout (*Oncorhynchus mykiss*) were grown in a recirculating aquaculture system (RAS) and combined feed intake/feeding behavior experiments were conducted on fish conditioned to three distinct meal regimes. Feeding regimes were intended to investigate fish behavior at two extremes and one midpoint of fish satiety: extreme hunger in fish fed a single meal once per day, moderate hunger in fish fed two meals a day, and limited hunger in fish fed three meals a day. Clustering behavior as determined by Calinski-Harabasz score was higher (568.08 ± 163.97) after feeding in fish fed once per day (42 ± 14 g estimated consumed feed) and was higher (526 ± 86.47) before feeding in fish fed twice per day (17 ± 4 g estimated consumed feed). No significant difference in clustering was observed before (500.40 ± 112.14) and after (502.12 ± 112.31) feeding in fish fed three times per day (14 ± 7 g estimated consumed feed). This suggests that the ballotini method is suitable for feed intake analysis in RAS conditions and that clustering may be a useful indicator of feeding behavior. However, further research is needed to precisely define fish clustering effect as well as the relationship between feeding behavior and feed intake dynamics. These results are promising for the development of machine vision to reduce aquaculture wastes by allowing precise delivery of feed rations at strategic time points.

Ariel Antinero: The role of starter diets on the development of skeletal abnormalities in zebrafish *Danio rerio* (Hamilton, 1822)

The early use of starter diets in finfish larval rearing has now been practiced by commercial hatcheries to lower production costs and enhance juvenile quality. However, formulation of feed that has a nutritional requirement appropriate for fish larvae is difficult. The imbalanced amount of nutrients in the diets may result to the development of skeletal abnormalities. This study examines the effect of five starter diets (one experimental and four commercial, Ctrl and A –D) on the development of skeletal abnormalities, survival and growth in zebrafish (*Danio rerio*). In addition, using swimming exercise at 8.0 total lengths per second, the efficiency of the three starter diets (A, C and D) was tested for the prevalence of haemal lordosis (a significant skeletal abnormality correlated to excessive swimming performance). At the end of the experiment, fish samples were examined for skeletal abnormalities via whole mount staining for bone and cartilage.

Results showed that starter diets had a significant effect on the development of scoliosis ($p < 0.05$, $19.3 \pm 4.2\%$ - $54.7 \pm 4.2\%$), light gill-cover abnormality ($p < 0.05$, $11.3 \pm 12.7\%$ - $36.7 \pm 8.3\%$) and severe gill-cover abnormality ($p < 0.05$, $1.3 \pm 2.3\%$ - $11.3 \pm 12.9\%$) in different diets, but not of caudal abnormalities, jaw abnormalities, vertebral-arch abnormality, kyphosis, lordosis and of miscellaneous abnormalities ($p > 0.05$). Following the swimming challenge test, starter diets had a significant effect on the incidence of haemal lordosis ($p < 0.05$, $53.3 \pm 17.6\%$ - $80.0 \pm 5.0\%$, in the different diets), but not on the lordosis angle ($p > 0.05$). Survival rate at the end of the trials (18 – 20 dpf) was significantly affected by the nutritional regimes applied ($p < 0.05$, $63.1 \pm 16.0\%$ - $90.3 \pm 14.1\%$ in the different diets), but no significant difference in total length ($p > 0.05$). Overall, these results are beneficial in relation to zebrafish husbandry.

George Chakkalakkal: Identifying and Characterization of piRNAs in Teleosts

Piwi-interacting RNA (piRNA), a group of small non-coding RNAs of length 26-31nt expressed in animal cells, play a major role in the gene silencing of transposons along with some genetic elements during the gonadal development at the levels of transcription and post-transcription. Currently, the data available for piRNA in teleost fishes is restricted to Zebrafish (*Danio rerio*). An in-silico analysis of available data of small non-coding RNA from five selected species: - Zebrafish, Nile tilapia (*Oreochromis niloticus*), Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorhynchus mykiss*), and three-spined stickleback (*Gasterosteus aculeatus*) belonging to Teleostei was performed and the protocol for the identification and characterization of piRNAs was developed. In the initial study using the existing method to identify the piRNA in teleosts, isolation of conserved piRNA sequences in the 5 selected species that matched with already annotated piRNA sequences of zebrafish and humans was conducted. But while investigating the species-specific sequences, it was discovered that the presence of non-conserved piRNA sequences was only in zebrafish with significantly high expression in the gonads. This was, however, not found in other selected test species. It is anticipated that the results would pave the way to the species-specific analysis of piRNA with special emphasis on non-conserved piRNA sequences which made up almost 96.53% of significantly expressed sequences in the gonads of zebrafish. Furthermore, the presence of these unique sequences in the gonads, which could play a major role in germline development and fertility should be further explored at an application-level research focus in Aquaculture.

12:00 – 13:30 LUNCH

13:30 – 14:45

Romarc Moncrieffe: Effects of microplastics containing additives and environmental contaminants, before and after solar light accelerated exposure, on blue mussel (*Mytilus edulis*)

Mytilus edulis is one of the most important commercial species of mussel and is used as an important indicator for the negative impacts of pollution. In aquaculture, *m. edulis* can intake microplastic as a normal part of its feeding cycle, and these microplastics can have a negative impact on the immunological and stress responses of the organism, even at low concentrations. The effects of aged microplastics and aged microplastics contaminated with polyaromatic hydrocarbons (PAH), a ubiquitous environmental pollutant, at environmental levels of 10 mg/L have yet to be fully examined. Current hypotheses indicate that microplastics which have been degraded by ultra-violet (UV) radiation could serve to concentrate PAHs and therefore deliver more contaminants to the organism.

In this experiment, we tested the stress response and immunological response of *m. edulis* by recording concentrations of 4 known stress enzymes and one stress biomarker: total proteins (TP), catalase (CAT), superoxide dismutase (SOD), glutathione S-transferases (GST), acetylcholinesterase (AChE), and phosphatase acid (PAP) before after exposure to microplastics (MP), microplastics aged for 1000 days under UV radiation (AMP), microplastics contaminated with PAH (MP PAH), and microplastics aged for 1000 days under UV radiation and contaminated with PAH (AMP PAH). Finally, lipidomics was completed with HP-LC and

ions of potential importance were recorded. We show that under experimental conditions of 10 mg/L, the AChE levels in the mantle were significantly higher for all treatments. This indicates that even at current environmental levels, microplastics can negatively impact the normal feeding behavior of the test organism.

Bernadeth Pananganan: Use of Sediment Geochemistry and Imaging Techniques for Habitat Characterization of *Holothuria poli* (Delle Chiaje, 1823)

Significant exploitation of sea cucumbers for decades has raised global concerns considering their significant trophic role particularly in bioturbation and nutrient recycling in marine benthic ecosystems. This role has led significant researches on sea cucumber inclusion as bioremediators in Integrated Multitrophic Aquaculture (IMTA) systems or polyculture in many countries. In Mediterranean seas, *Holothuria poli* (Delle Chiaje, 1823) is one of the recently exploited and considered candidate extractive species for the IMTA insights of Mediterranean aquaculture. However, little is known and few stock assessments of the species natural populations were done over years. Hence in this study, usage of sediment geochemistry and imaging techniques (Remotely Operated Vehicle–ROV) to determine the environmental variables, population and influence of *H. poli* on sediment geochemical properties and macrofaunal community were investigated. Samplings were done from the month of March–June 2021 in Agios Nikoalos, Crete, Greece at two stations of distinct characteristics (seafront-S1 and enclosed-S2) with objective of establishing a detailed habitat characterization of the species.

Results showed that in S1, where sandy oxic environment with low organic concentration was observed, population density was low ($0.036 \pm 0.01 \text{ m}^{-2}$) but with heavier individuals ($70.42 \pm 17.31 \text{g}$) and higher macrofaunal diversity. Conversely, in silty low redox regime and high organic matter load environment found in S2, population density was higher ($2 \pm 2 \text{ ind. m}^{-2}$) with lighter individuals (43.92 ± 13.49) and low macrofaunal diversity. Also, significant difference of macrofaunal community composition and variances of geochemical properties were observed in sediments taken in the presence/absence of *H. poli*. Seemingly, topography, seasonal variations and sediment geochemical properties influenced the differences on the results of population parameters of *H. poli*. Outputs generated from this study will provide baseline information as a fundamental and imperative aspect for developing plans on conservation, fishery management, restocking and facilitating mariculture activities of *H. poli* inclusion for IMTA system perspectives in Mediterranean aquaculture industry.

Stephanie Pavione: Exploring the economic potential of seaweed land-based aquaculture using industrial scale photobioreactors

Land-based seaweed cultivation can complement at-sea farming by enabling the production of various genera, including high-value but potentially invasive or not suited for mariculture species. Research shows that cultivation in controlled systems is technically feasible and can support both higher production yields and a stable supply of uniform biomass. Despite those advantages, the development of commercial land-based cultivation has been limited by the high capital investment and expenditures required, making economic viability assessments necessary. However only a few studies provide data on productivities in outdoor open systems at commercial scale, and information is even scarcer for closed systems such as

photobioreactors. In the present study, simplified scenarios were employed to estimate the economic viability of *Ulva* and *Porphyra* cultivation with Pandora PBR™ systems from Xanthella LTD, assuming a fully-sold and stable production under the best possible conditions. Within this optimistic framework, productivities of respectively 38 and 26gDW/m²/d for *Ulva* and *Porphyra* could not support a positive profit, and a retail price of 70€/kg of dried *Ulva* was too low to justify its production with Pandora reactors.

Other scenarios considering productivities of 58gDW/m²/d for *Ulva* and 40gDW/m²/d for *Porphyra* and above should not be rejected. Productions became viable for annual productions between 2000-8000kg corresponding to a number of reactors ranging from 13 and 94. Despite the general idea that closed systems are limited to high-value applications, economically reliable scenarios were observed with food price levels. However, this exploratory study presents structural limitations as it relies on non-experimental validated and simplified assumptions, but it could lay the foundation for more advanced estimations.

**14:45 – 15:05 Coffee Break/ Completion of ACES+ Feedback Form/
Collation & confirmation of oral presentation marks (Day 2)
by Programme Leaders**

**15:05 – 15:30 Oral Presentation Award, Student Media Award & Closing
Remarks – Prof Michalis Pavlidis & ACES+ Programme
Leaders**