Symposium 4 Protists as model organisms to face environmental problems

Organizers: Cristina Miceli (University of Camerino , Italy) and Wei Miao (Chinese Academy of Sciences, China)

Synopsis: Despite general progress in environmental research, the impact of environmental changes on living organisms and human health remains deeply worrying Monitoring of water contamination, air pollution, exposure to metals and global climate change can be faced with the contribution of modern omics techniques applied to key model organisms/systems. Genomics and transcriptomics are used to identify marker genes involved in environmental responses, to analvze differential gene expression under environmental stress, to study the relationship between genotype and



phenotype, including possible epigenetic control Response to stresses and environmental changes is a relevant issue to which protists can provide a strong contribution, due to their wide distribution in many different environmental conditions The proposed workshop is planned with the ambition to gain insight into practical environmental problems using protists model organisms This means that the focus will include applications of genetics/genomics/transcriptomics to contribute to deliver solutions to relevant environmental issues We expect to open a discussion about applications to identify new marker genes, to better understand the adaptation to environmental changes, and to use quantitative genetics and RNAseq to measure cell response to different toxicants and environmental contaminants The progress in technologies is producing new environmental contaminants for which the effect is so far underestimated Just to provide a simple example, the effect of metals has been largely investigated However, the effect on biological processes produced by aggregation of metals in nanoparticles appears a more relevant issue to be unraveled Therefore, it is useful to discuss new practical approaches in a large audience, where the knowledge and the best practices will be shared.

S4-1

Genomic biomonitoring of protistan communities to assess environmental impacts of coastal marine aquaculture

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Traditionally, the backbone of environmental biomonitoring in finfish aquaculture is the collection and identification of benthic macrofauna, which react predominantly to organic enrichment and oxygen (depletion). However, this monitoring strategy with the microscopic identification of macroinvertebrates as biological indicators is very tedious and expensive. Therefore, concerted efforts are to develop alternative monitoring tools. One very promising approach is environmental DNA metabarcoding, which relies on the identification of short DNA fragments amplified from bulk sediment samples. We have used this molecular approach to evaluated the indicator qualities of protists along organic enrichment transects in the vicinity of salmon farms in Scotland and Norway. Significant changes in protistan community compositions, above all in ciliates and diatoms, were typical along these transects. These changes correlated significantly with organic enrichment and corroborated well with benchmark reference data obtained from traditional

macrofauna monitoring of the same sampling sites. We then used supervised machine learning (SML) to train an algorithm, which successfully predicts ecological quality in marine sediments of aquaculture sites based on DNA metabarcodes of protistan communities as features and macrofauna-derived biotic indices as reference data. In conclusion, the genomic biomonitoring of benthic protistan communities as a source of new biosensors in combination with supervised machine learning is an extremely powerful tool for environmental biomonitoring in marine aquaculture. The costs of this technology are notably lower than for macrofauna-based monitoring and results are available within days rather than within months (as for macrofauna monitoring). Hence, we propose the integration of protistan metabarcoding in future compliance biomonitoring regulations in aquaculture.

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S4-2

Microbial community in inland waters in the Anthropocene

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Microeukaryotic plankton and bacterioplankton are critical components of aquatic microbial food webs and play essential roles in the structure and function of aquatic ecosystems. Understanding the processes and mechanisms that community dynamics and assembly of these microorganisms is one of major goals in both pure and applied microbial community ecology. Plankton communities normally consist of few abundant and many rare species, yet little is known about the ecological pattern and role of rare planktonic species. We found the rare bacterioplankton subcommunity had a distinct biogeographical pattern in inland waters that was reasonably similar to the abundant bacteria. However, local processes and factors play the most important role in structuring rare bacterial subcommunity, with regional factors explaining more variation in abundant bacteria. Both deterministic and stochastic processes significantly influenced eukaryotic plankton community assembly, and the stochastic pattern was particularly pronounced for rare taxa. Stochastic processes are sufficient in shaping substantial variation in rare plankton metacommunity in a river-reservoir system across different hydrographic regimes. Co-occurrence network analysis revealed that keystone taxa mainly belonged to rare species, which may play fundamental roles in network persistence following a cyanobacterial bloom event. Both warming and decline in water level can boost cyanobacterial dominance in subtropical reservoirs. The long-term observations revealed that the cyanobacterial biomass cycle created distinct niches between persistent bloom, non-bloom, decrease and increase of cyanobacteria, and therefore associated with distinct eukaryotic plankton patterns. These findings provide a new perspective for the ecological significance of rare plankton in changing aquatic ecosystems, clarifying the contribution of microbial interactions in plankton food web theory.

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S4-3

The Antarctic ciliate *Euplotes focardii* and its associated bacterial consortium: insights in environmental adaptation and responses to stress from genomics analysis

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Euplotes focardii is an Antarctic ciliate classified as an obligate psychrophilic stenothermal organism. As all ciliates, *Euplotes focardii* is characterized by the presence of cilia on its surface and by nuclear dimorphism: a micronucleus (MIC) that represents the germ line, and a macronucleus (MAC) serving as the somatic line involved in the gene expression during the vegetative stage. We compared *Euplotes focardii* MAC genome with those available from mesophilic Euplotes species to characterize differences that may be consequent to cold adaptation and defense to oxidative stress, the main constraints of the Antarctic marine microorganisms. We focused on the comparison of antioxidant enzymes and heat shock protein (HSP) 70 families, molecules which possess peculiar

characteristics correlated with cold adaptation. We found that SODs and CATs antioxidant enzymes are more numerous in *Euplotes focardii* than in the mesophilic *Euplotes* species. In contrast, there are fewer hsp70 genes in *Euplotes focardii* compared to mesophilic *Euplotes* and these genes respond only to oxidative stress, suggesting a loss of response to heat stress in this Antarctic ciliate adapted to a constant cold water. We also characterized the *Euplotes focardii* associated bacterial consortium. We isolated five members of the consortium and we found that these bacteria can transform chromium, copper, and silver into harmless nanoparticles, that may be a mechanism of heavy metals resistance. Our results suggest that environmental adaptation rely on molecular changes such as peculiar amino acid substitutions and gene duplication. However, a role of associated bacteria cannot be excluded.

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S4-4

Heavy metal bioremediation by sulfide nanoparticle synthesis using *Tetrahymena thermophila* Jiawei Tu¹, Tian Li^{1,2}, Zihan Gao^{1,2}, Wenjun Xiong^{1,2}, Jie Xiong¹, Wei Miao¹ (¹Key Laboratory of Aquatic Biodiversity and Conservation, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China, ²University of Chinese Academy of Sciences, Beijing 100049, China)

Heavy metal pollution is increasingly becoming a problem and has become of great concern due to the adverse effects it is causing around the world. An emerging way to decrease the toxicity of heavy metals is bioremediation using microorganisms. Reasonably, transforming harmful heavy metal ions into stable and less toxic metal sulfide precipitates becomes a possible way to repair the heavy metal pollution in situ. Tetrahymena thermophila, as a eukaryotic model organism, was widely used in toxical studies. It is easy to culture at a large scale, and the genome has been sequenced. Various genetic manipulation methods were established in this organism. These advantages make Tetrahymena thermophila to be a powerful tool for remediation of heavy metal ions polluted water. Herein, intracellular synthesis of HgS and CdS was achieved using Tetrahymena thermophila by adjusting the activity and concentration of metal precursors, the type of sulfur source and the reaction time. Furthermore, the distribution and morphology of heavy metal elements and nanoparticles in Tetrahymena thermophila were analyzed at different time points of the growth process of HgS and CdS nanoparticles to investigate the possible mechanism of this intracellular metal sulfide synthesis. These studies lay a foundation for further identifying the key factors involving in heavy metal ions metabolism, and provided a basis for bioremediation of heavy metals pollution using Synthetic biology approaches in *Tetrahymena thermophila*.

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