

Drop in the ocean: 'ground-truthing' environmental DNA approaches for detecting important aquatic pathogens

Supervisors:

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Hosting institution: Natural History Museum

Project description: Using an environmental sequencing approach, an existing collaboration within the supervisory team has recently demonstrated significant novel diversity within the Haplosporida (Rhizaria, Cercozoa), a previously enigmatic group of protists which includes commercially important parasites of molluscan and crustacean hosts¹. Some of these parasites are listed as notifiable by the World Organisation for Animal Health (OIE) and in EU legislation. Their detection within national water bodies therefore has direct implication for the trading of animal hosts therein to other regions. The proposed study combines customised environmental DNA (eDNA) probing and sequencing methods with advanced phylogenetics, to an important policy setting.

Our previous work has revealed aspects of the lifecycles of haplosporidian lineages and suggests that multi-trophic transfer may occur within the group. The aim of this project is to develop these exciting findings in two main ways: 1) to utilise partner facilities (PCR, histopathology, electron microscopy, and *in situ* hybridisation) to identify host invertebrates of the five recently identified 'groups' of novel haplosporidians in aquatic habitats, for their establishment as novel taxa². 2) To select an invertebrate group in which haplosporidians are appropriately diverse and accessible, but PCR signal (from results of part [1]) suggests infection is patchy at a population level (likely the edible mussel *Mytilus edulis*). The student will a) ascertain which haplosporidian lineages are true parasites of the mussels and in which tissue(s) infection occurs, and b) confirm our previous observation that adjacent mussel individuals in wild populations can differ in presence and/or nature of haplosporidian infection. The next step will be to use next generation sequencing (NGS) to reveal co-infections within the host. This will allow for testing of the hypothesis that infection level (with haplosporidians) is moderated by co-infection by other pathogens. Because the latter could represent a wide phylogenetic diversity (from viruses through bacteria and protists to metazoans), and include highly divergent (and possibly novel) lineages thereof, we will use a metagenomic NGS approach, which is not limited by PCR primer biases and incompatibilities, and also allows for relative quantification of different pathogen lineages within a system. The combined approaches within this proposed project will align the classical taxonomic toolbox with metagenomic approaches to not only identify novel pathogen lineages, but to investigate the role of co-infections in disease outcomes. The project has direct relevance to risk assessment for the sighting of shellfish farming operations as part of proposed future aquaculture strategy in Europe.

¹Hartikainen, H., Ashford, O.S., Berney, C., Okamura, B., Feist, S.W., Baker-Austin, C., Stentiford, G.D., and Bass, D. (2013). Lineage-specific molecular probing reveals novel diversity and ecological partitioning of haplosporidians. *The ISME journal*.

²Stentiford, G.D., Bateman, K.S., Stokes, N.A., and Carnegie, R.B. (2013). *Haplosporidium littoralis* sp. nov.: a crustacean pathogen within the Haplosporida (Cercozoa, Ascomycota). *Dis Aquat Org doi: 10.3354/dao0261*

Training opportunities: The project will develop a diverse skill-base in the student. This will include environmental sequencing and molecular ecology methods (Bass), protistan parasitology and

associated methods (light, fluorescence, and electron microscopy) and histopathology (Stentiford), next generation sequencing (NGS; Illumina Mi/HiSeq) sample preparation techniques, sequencing theory and practice (Bass, Studholme), and bioinformatics analyses (Studholme, Bass). The importance of taxonomic robustness and policy observance in an institution with a remit like that of Cefas will also be covered in detail. Thus the required skills and training will be provided in a fully complementary and balanced way from the three supervisory institutions. As stated below, the combined input from project partners will provide a unique training environment unlikely to be emulated within Europe or globally. The project is truly interdisciplinary and is therefore expected to create a specialist with context. All supervisors are experienced trainers and supervisors from undergraduate through to postdoctoral levels and hold senior positions within their respective institutions.