Thursday September 4th – Langevin/Cartier
Parasitology 1
Moderators – Ariadna Sitjà-Bobadilla (Inst. Aquacultura Torre de la Sal) **Sarah Poynton** (John's Hopkins Univ.)

10:45 AM	**Parasitology 1**	Poynton - A Global Review of Parasites in Finfish Aquaculture
11:00 AM		**Furtado - Antiparasitic Potential of the Nano-Emulsioned Oil of the Acicula and Resin of *Pinus taeda* Against the Larval Stages of *Lernaea cyprinacea***
11:15 AM		Sitjà-Bobadilla - Parafishcontrol, a European Funded Project to Mitigate Fish Parasitic Diseases in Aquaculture
11:30 AM		Bradley - An Outbreak of *Banamia exitiosa* in Victorian Native Oysters in 2015 and Examination of Risk Factors for Developing clinical Disease
11:45 AM		**Nguyen - Rapid and Specific PCR Assay for Diagnosis of Apicomplexan- "X" (APX) Associated With the Flat Oysters (*Ostrea chilensis*) in New Zealand**
12:00 PM		**Lunch**

A Global Review of Parasites in Finfish Aquaculture

Sarah L. Poynton [1*] and Barbara F. Nowak [2]

[1] Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, 855 Edward D. Miller Research Building, 733 North Broadway, Baltimore, MD 21205 USA spoynton@jhmi.edu

[2] Institute of Marine and Antarctic Studies, University of Tasmania, Launceston, Newnham Dr, Newnham 7248 Tasmania Australia B.Nowak@utas.edu.au

With the rise in global production of finfish in aquaculture, knowledge of the role and control of parasites on a regional basis becomes essential. Although literature is appropriately focused on taxa of parasites with economic importance, geographic coverage is very uneven. Most geographically focused reports are of host-parasite occurrence and ecology, rather than broader issues such as fish health infrastructure. Furthermore, representation in the literature is often disproportional to the global, regional, or local importance of finfish aquaculture, and the parasites therein. There are key gaps in the scientific literature in English, (the current international language of science), about parasite of finfish aquaculture for China, much of Asia, Russia, Africa, and some of Latin America. These gaps exist despite the key importance of some of these countries as significant producers, (whether determined by tonnage of fish produced, or monetary value).

To address these knowledge gaps, we are currently completing assembly of a multi-author book "Aquaculture Parasitology: Global Impacts and Management in Finfish" to be published by Wiley. The "Regional Review" focus of the book describes the global picture in seven chapters, largely following the organizational scheme adopted by the Food and Agriculture Organization (FAO (UN)): China, Asia (excluding China), Oceania, Europe (including Russia), Africa, North American, and Latin America and the Caribbean. For each region, the authors present an overview of aquaculture in the region, infrastructure for health and disease monitoring and management, current and emerging parasite diseases, current practices, and special topics.

When viewing parasites of farmed fish from a global perspective, many interesting contrasts emerge, for example: (ii) parasites of greatest economic importance range from myxozoan and digenean trematodes in North America (primarily salmonid and catfish production) to monogeneans in China (predominantly carp production), and crustaceans in Latin America (predominantly salmonid production); (ii) infrastructure ranges from substantial, as in parts of Europe and North America, and in China (where there has been significant government investment in research and surveillance), to rather limited, as is the case in Russia and in East Africa; and (iii) prevention and treatment practices are very diverse, including rare implementation in East Africa (where there is a history of subsistence aquaculture), to inclusion of traditional herbal medicines in China. Despite the great geographic diversity across regions, some common themes emerged including the importance of non-native fish species, environmental concerns, and the need for better disease control including availability of anti-parasitics suitable for use in foodfish.

Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)

Antiparasitic Potential of the Nano-emulsioned Oil of the Acicula and Resin of *Pinus* c.f. *taeda* Against the Larval Stages of *Lernaea cyprinacea*

William E Furtado[1*], Natalia C Marchiori[2], Fabiano C Bertoldi[2], Bruno C Silva[2], Haluko Massago[2], Maurício L Martins[1]

[1] AQUOS – Aquatic Organisms Health Laboratory, Aquaculture Department, Federal University of Santa Catarina (UFSC), Brazil. we.furtado@hotmail.com

[2] EPAGRI - Company of Agricultural Research and Rural Extension of Santa Catarina, Brazil.

Lernaea cyprinacea L. (Copepoda), popularly known as 'anchor-worm', is a crustacean ectoparasite with worldwide distribution and extremely common in the culture of commercial fish. It may be associated to great economic liabilities due to high mortality rates and its repugnance aspect to fish. Due to the high and frequently great potential damage in the use of therapeutic drugs in fish farms, the management of diseases should focus on less aggressive methods to sick animals, the environment and the professional involved. Phytotherapeutic drugs have proved to be potentially beneficent compounds in fish culture. The *Pinus* species should be underscored due to their bioactive compounds with associated anti-parasite properties. Current analysis established the Minimum Inhibitory Concentration (MIC) of nano-emulsioned essential oils extracted from fresh acicula and from the crude resin of *Pinus* c.f. *taeda* on parasite larvae (nauplii and copepods). Oil extraction was undertaken by hydrodistillation in a Clevenger apparatus in the laboratory of essential oils of the Agro-Livestock Research Firm and Rural Extension of Santa Catarina (Epagri) in Itajaí SC Brazil. Biological material was retrieved during an outbreak of lernosis from broodstocks of silver catfish (*Rhamdia quelen*) in a pond constructed during the summer of 2018 at the Unit for Genetic Improvement of Fish (Itajaí, Epagri). Thirteen animals parasited by *L. cyprinacea* (mean 192 ± 156 parasites per fish, 40 – 479) were captured, anesthetized with eugenol (75 mg/L) and euthanized by brain commotion. Parasites were removed from the host by hand and transferred to a petri plaque with distilled water. The egg-sacs were ruptured to release the eggs which hatched in up to 24 h after the process. Assessment of antimicrobial activity of essential nano-emulsioned oil from the resin, the acicula and of α-Pinene (major compound in the composition of essential oils: 69,96% of acicula and 45,56% of resin) was performed separately by the MIC methodology for the larvae of the parasite (nauplii and copepods). Further, 100 µL of the agent were added to the first well of the flat-bottom microplate of cell culture (96 wells) and 50 µL of distilled water were added as from the second well. A factor 2 series dilution was performed till the 19th well. Finally, 50µL of distilled water with 5 parasites were added to each well. Microplates were monitored during periods of 60 minutes and 24 h. MIC was determined as the lowest dilution of the agent in which total inhibition of the larvae of the crustacean occurred (total absence of body movements) in all triplicates. All tests included one control group with distilled water and the tensoactive used for the preparation of the oil nano-emulsions (Tween® 80). The nano-emulsioned essential oil of the acicula of *P. taeda* had the best result among the chemical agents tested. It inhibited nauplii and copepods of *L. cyprinacea* in concentrations ranging between 20 and 156 ppm, respectively, according to analysis time. Currently, the most efficacious treatment in Brazil against *L. cyprinacea* is based on different commercial solutions with trichlorphon, an insecticide with proved toxicity for fish and authorized for fish farms. Positive results showed the capacity of the Pinus extract as a prophylactic agent in fish.

Conference Session Designation: **(** Parasitology General)
Presentation Format: **(** Oral)
Student Presentation: **(** Yes)

Parafishcontrol, a European Funded Project to Mitigate Fish Parasitic Diseases in Aquaculture

Ariadna Sitjà-Bobadilla

Instituto de Acuicultura Torre de la Sal, Consejo Superior de Investigaciones Científicas (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain parafishcontrol.coordination@csic.es

Horizon 2020 is the largest EU Research and Innovation funding programme ever, with nearly €80 billion of funding available from 2014 to 2020. It promises more breakthroughs, discoveries and world firsts by taking great ideas from the lab to the market. The goal is to ensure Europe produces excellent science, removes barriers to innovation and makes it easier for the public and private sectors to work together in delivering innovation. Here we introduce the H2020 project ParaFishControl (Advanced Tools and Research Strategies for Parasite Control in European Farmed Fish), granted with an EU contribution of € 7.8 million in the first call of this programme, which started on April 2015. The consortium, coordinated by CSIC, comprises 28 academic and public organisations, SMEs and research and industrial enterprises from 13 countries, which are experts in parasitology, immunology, epidemiology, pathology, genomics, nutrition and feeding, biotechnology, chemotherapy, food security, etc. FAO estimates that aquaculture contribution to human food will reach 62% by 2030. This could not be accomplished without reducing the impact of diseases, which can reach 20% production value. Some authors estimate that parasites can produce up to 10% of the annual weight harvest lost in the world. These economic losses in farmed fish can be due to poor growth performance, impaired welfare, and high mortality rates. Therefore, the goal of the ParaFishControl project is to increase the sustainability and competitiveness of the EU aquaculture industry by improving our understanding of fish-parasite interactions and developing innovative solutions and tools for the prevention, control and mitigation of the most harmful parasitic species affecting the main European farmed fish species (Atlantic salmon, rainbow trout, gilthead sea bream, European sea bass, turbot and common carp). The most threatening ecto- and endo-parasites are being studied, including crustaceans, monogeneans, myxozoans, microsporidians, ciliates, dinoflagellates, amoebas, oomycetes and zoonotic helminths. Activities are carried out over five years into nine cross-cutting work packages which integrate all fish host species and their relevant parasites, including: parasite genomics and transcriptomics, life cycles, transmission and host immune response (WP1); wild-farmed fish parasite transfer (WP2); vaccines and immunostimulatory feeds (WP3); diagnostic tools (WP4); innovative treatments (WP5); risk analysis and surveillance, creation of a biobank of parasites (WP6); fish product safety (WP7); dissemination, technology transfer and take-up (WP8); coordination and management (WP9). For more information visit the web site of the project: ***www.parafishcontrol.eu*.**

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Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)

An Outbreak of Bonamia Exitiosa in Victorian Native Oysters in 2015 and Examination of Risk Factors for Developing Clinical Disease.

Tracey L Bradley[1]*, Jaimie Hunnam[1], John Mercer[2]

[1] Department of Economic Development, Jobs, Transport and Resources, 475 Mickleham Road, Attwood, Victoria 3049, Australia tracey.bradley@ecodev.vic.gov.au
[2] Victorian Fisheries Authority, 2A Bellarine Hwy, Queenscliff, Victoria 3225, Australia

Bonamia ostreae and *Bonamia exitiosa* are significant pathogens of oysters that cause high mortality rates and substantial economic losses to the oyster farming industry globally. Infection by an unidentified Bonamia sp. was responsible for the devastation of experimental aquaculture of the Native Oyster (*Ostrea angasi*) and adjacent wild beds in Victoria, Australia in the early 1990s.

Small scale aquaculture of the Native Oyster was re-commenced in 2010 in Victoria. Surveillance was undertaken by the government to assess the presence of *Bonamia spp* and the results of that testing and the subsequent outbreak of clinical *Bonamia exitiosa* will be described.

A project investigating the factors that cause the development of clinical disease from previously healthy but sub-clinically infected oysters was undertaken both in indoor tanks and in the field.

The tank trials were conducted to examine putative risk factors for clinical expression of Bonamia infection in Native Oysters held in tanks at the Government Queenscliff facility over 2 years. Risk factors examined include water temperature, starvation, agitation, size and provenance. Comparisons between risk factors were examined with measures including mortality rates, PCR and histopathology.

The 2 field trials were undertaken at the "infected" site where clinical *Bonamia exitiosa* has previously been confirmed and the "uninfected" site where the Bonamia parasite have not previously been detected. Different risk factors were examined over 2 years including stocking density, oyster size, depth held in the water column and cleanliness of cages. A full summary of the results for all these trials will be provided.

Conference Session Designation: (Parasitology General or Aquatic Epidemiology)
Presentation Format: (Oral)

Rapid and Specific PCR Assay for Diagnosis of Apicomplexan- "X" (APX) Associated with The Flat Oysters (*Ostrea chilensis*) in New Zealand

Suong T. Nguyen[1, 2*], Jonathan C. Banks[2], Stephen C. Webb[2], Andrew Jeffs[1], Kevin C. Wakeman[3, 4] and Andrew Fidler[1]

[1] Institute of Marine Science, University of Auckland, Auckland, 1142, New Zealand suongthaonguyenvn@gmail.com, andrew.fidler.nz@gmail.com
[2] Cawthron Institute, Nelson, 7010, New Zealand Steve.Webb@cawthron.org.nz, Jonathan.Banks@cawthron.org.nz
[3] Office of International Affairs, Hokkaido University, Sapporo, 060-0810, Japan Wakeman.kevin@gmail.com
[4] Faculty of Science, Hokkaido University, Sapporo, 060-0810, Japan

A PCR assay designed based on *in situ* hybridisation probes was developed to specifically detect the parasite Apicomplexan-X in the flat oyster (*Ostrea chilensis*), endemic to New Zealand, targeted 723 bp DNA product. The specificity of the assay was proved as it didn't amplify any product of other apicomplexan species DNA including *Toxoplasma gondii*, *Neospora caninum*, *Selenidium* sp., *Cephaloidophorida* sp., *Lecudina* sp., *Platyprotepum* sp., and *Thiriotia* sp. The analytical sensitivity of the test was determined as 1pg of APX DNA using dilution series method. Following analytical validation, diagnostic performance was determined by testing samples from flat oysters infected with APX (n = 75) at different intensities estimated by histology. Of 73 flat oysters infected with APX identified by histology, 69 (95%) tested PCR-positive. Failure to amplify an internal control indicated the presence of PCR inhibitors in the 4 PCR-negative samples. This is the first PCR assay for specific detection of the parasite APX in the flat oyster *O. chilensis*. It should be useful for diagnostic testing and active surveillance programs for managing flat oyster health.

Conference Session Designation: **(** Invertebrate & Shellfish Disease / Parasitology)
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