Thursday September 4th – Langeve / Cartier
Parasitology 2
Moderators – Ariadna Sitjà-Bobadilla (Inst. Aquacultura Torre de la Sal) **Sarah Poynton** (John's Hopkins Univ.)

1:15 PM	**Parasitology 2**	**Nishiwaki - Histopathological and Ultrastructural Studies on Intracellular Parasites in the Ovary of Skipjack Tuna (*Katsuwonus pelamis*)**
1:30 PM		**Watanabe - Characterization of Proteases of Trophont, the Parasitic Stage of *Cryptocaryon irritans***
1:45 PM		**McAllister - Examination of Parasite-Induced Anemia and Erythropoietic Regulator Gene Expression in *Carassius* auratus During *Trypanosoma carassii* Infection**
2:00 PM		Warland - Nucleospora Cyclopteri (Microspora): Tissue Tropism, Shedding and Non-Lethal Detection
2:15 AM		**Omovwohwovie - Parasites of *Oreochromis niloticus* Observed in the Fisheries Unit of the Niger Delta University Teaching and Research Farm**
2:30 AM		Urawa - Impact of *Spironucleus salmonis* on the Growth and Mortality of Juvenile Masu Salmon *Oncorhynchus masou*
2:45 AM		**Wang - Trichodinid Ectoparasites (Ciliophora: Trichodinidae) From Freshwater Fishes in China, With Notes on Host–Parasite Relationship**

Histopathological and Ultrastructural Studies on Intracellular Parasites in the Ovary of Skipjack Tuna (*Katsuwonus pelamis*)

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Skipjack tuna (Katsuwonus pelamis) is known as a highly migratory fish and is distributed mainly in tropical and subtropical regions of the world. It is also one of the most important target fish species in fisheries. In a previous study, we reported the presence of intracellular parasitic protozoa on ovary tissue of skipjack tuna caught over a number of years in the western central Pacific Ocean (Ashida et al., 2007). Since the protozoa were stained using fluorochrome Uvitex-2B, which binds to chitin, it was presumed that they comprise microsporidian spores with a chitinous wall (Ashida et al., 2007). On the other hand, microsporidian DNA was not detected by general PCR. Therefore in this study we performed histopathological and ultrastructural observations to confirm the detailed morphological structure of the microsporidian-like organisms in relation to classification and localization in the ovaries.

The protozoa are round to ovoid, and 1-3 μm in diameter. No mitochondria were observed, although mitosome-like organelles were present. No cysts could be detected. The parasites were mainly localized in the cytoplasm of phagocytes in connective tissue of the ovary. Some were also found within oocytes. The morphological characteristics of this microsporidian-like organism shows that the protozoa are a new species which have not previously been reported for skipjack tuna.

Ashida H. et al. (2007) Nippon Suisan Gakkaishi, 73(5), 916-918.

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

Characterization of Proteases of Trophont, the Parasitic Stage of *Cryptocaryon irritans*

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Cryptocaryoniasis of marine teleosts is caused by *Cryptocaryon irritans*, an obligatorily parasitic ciliate. This parasite is a major threat to marine aquaculture in tropical and subtropical waters. Many studies have been carried out, aimed at development of control methods against the disease, such as therapeutic drugs and vaccines. However, treatments efficient enough to control the disease have not yet been developed. Recently, proteases have been suggested to play a crucial role in the infection and development of parasitic protozoa such as *Tritrichomonas foetus*, *Tetrahymena* spp., *Leishmania* spp. and *Miamiensis avidus*. It is also thought that proteases play a key role in the infection and development of trophont, the parasitic stage of *C. irritans*, and are a potential target for chemotherapy and vaccines against the parasite. However, proteases involved in the infection of *C. irritans* have not been understood or characterized. In this study, we conducted transcriptome analysis to identify various proteases of *C. irritans*, and examined the activity of trophont proteases by zymography. In addition, we examined the effect of protease inhibitors on the infection, survival and growth of the parasite in vitro. The results show that the activities of serine proteases and cysteine proteases were strong in trophonts. When inhibitors against the proteases were added into the medium for in vitro culture of the parasite, the survival rate declined and growth was delayed. These results suggest that serine and cysteine proteases are important for the parasitic stage of *C. irritans*. This knowledge will assist in the development of new chemotherapeutic drugs or vaccines for cryptocaryoniasis.

Conference Session Designation: (Parasitology General)
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Student Presentation: (Yes)

Examination Of Parasite-Induced Anemia And Erythropoietic Regulator Gene Expression In *Carassius Auratus* During *Trypanosoma Carassii* Infection

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Trypanosoma carassii is a flagellated bloodstream parasite of cyprinid fish. Pathogenesis of *T. carassii* manifests primarily as anemia in experimentally infected fish. This anemia is characterized by decreases in the number of circulating red blood cells (RBCs) during peak parasitemia. We examined changes in the key blood metrics and expression of genes known to be important in the regulation of erythropoiesis. Increasing parasitemia was strongly correlated with an overall decrease in the total number of circulating RBCs. Gene expression of critical erythropoiesis regulators was measured in contrast to fish made anemic through injections with phenylhydrazine; a chemical which causes RBC hemolysis leading to severe anemia. Significant upregulation of pro-erythropoietic genes was observed in chemically induced anemia, but not during peak parasitic infection. Mammalian trypanomastids have also been shown to alter erythropoiesis leading to increased morbidity and delayed recovery from infection by the hosts. To examine whether the modulation of key erythropoietic factors was responsible for the observed anemia, we generated recombinant goldfish EPO (rgEPO) and demonstrated that it promoted erythroid colony formation *in vitro*. The administration of rgEPO *in vivo* reduced anemia severity, but was unable to restore erythrocyte numbers in infected fish. The mechanism(s) by which *T. carassii* induce anemia during infection remain unclear. We know that proinflammatory cytokines (IFNγ, TNFα, IL-1β) are upregulated during *T. carassii* infection and that this upregulation has been shown to downregulate EPO levels and consequently, erythropoiesis. It is also possible that the parasites secrete molecule(s) that directly affect EPO production and we are currently testing the excretory/secretory products of *T. carassii* for this activity.

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Presentation Format: **(** Oral)
Student Presentation: (Yes)

Nucleospora cyclopteri (Microspora): Tissue Tropism, Shedding and Non-Lethal Detection

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Farmed lumpfish are now the most important cleaner-fish used in Norwegian aquaculture for salmon lice (*Lepeophtheirus salmonis*) control.

The loss of lumpsucker juveniles is high during a production cycle of Atlantic salmon, mainly due to bacterial diseases. A virus belonging to the Flaviviridae and certain parasites may also be important. *Nucleospora cyclopteri* is a microsporidian parasite mainly affecting lymphocyte-like leukocytes in lumpfish, and has previously been associated with disease and mortality in farmed populations. Renomegaly, sometimes extreme, has been associated with the infection.

A concern is that this parasite could be both vertically transmitted and immunosuppressive.

Among 85 wild caught lumpfish, renomegaly due to *N. cyclopteri* was not observed. Three fish exhibited pale patches on the kidney, particularly affecting the anterior part. A RT-qPCR study of 41 of these fish; included 10 tissues, 6 swab-sites, bile and urine samples. Whole blood and leucocyte fractions were also analysed.

All sample types were positive, but parasite densities were highest in anterior kidney, followed by mid-kidney, posterior kidney, spleen, heart and gills. Prevalence was 59%. Whole blood was positive in only 25% of the infected individuals, leucocyte fractions in 42%. Some bile and urine samples were positive for the parasite, and parasite load in urine correlated with density in the other tissues, suggesting parasite shedding via this route. The parasite could be detected in gill, vent and 4 skin swabs from infected fish, but these samples were also positive in uninfected fish from the same tanks. Control RNA from tank biofilm samples was also positive, so the presence of infected individuals likely contaminated the tank water. Urine and faeces (bile) from infected fish could be a source of this contamination.

Swabs and gill biopsies may be used to examine a lumpfish population, such as potential broodstock, for *N. cyclopteri* presence. However, due to contamination, individual carriers may best be revealed through RT-qPCR analyses on leukocyte fractions from blood samples. However, repeated sampling may be necessary to reveal all infected individuals.

Conference Session Designation: (Cleaner Fish Diseases / Parasitology)
Presentation Format: **(** Oral)

Parasites of *Oreochromis niloticus* observed in the Fisheries Unit of the Niger Delta University Teaching and Research Farm.

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Parasites of Orechromis niloticus was studied at the Teaching and Research Farm of the Niger Delta University. The prevalence, abundance and intensity of infection were determined. The prevalence of Dactylogyrus parasitae in Oreochromis niloticus showed the highest value of 65%, followed by Cestode, ligula 60%, Ergasilus 10% and Protozoa and Nematode 1.66% with the least prevalence. The intensity of Dactylogyrus obtained was 2.79,while Cestode,(ligula)had 1.10,, Ergasilus spp 1.33, Protozoa and Nematode 1.2 respectively.

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

mpact of *Spironucleus salmonis* on the Growth and Mortality of Juvenile Masu Salmon *Oncorhynchus Masou*

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The diplomonad flagellate *Spironucleus salmonis* was detected from the digestive tract of juvenile Masu *Oncorhynchus masou* and Chum Salmon *O. keta* reared at hatcheries in Japan. To elucidate the pathogenicity of *S. salmonis* for juvenile salmon, an infection experiment was conducted at laboratory. Two groups of juvenile Masu Salmon (mean weight 1.5 g; n = 250 fish each) were held separately in 23-l tanks. One group was cohabited with Masu Salmon (n = 50) infected with *S. salmonis* for 2 weeks. The other group served as uninfected controls. Each tank was supplied with running well water, and the water temperature was constant at 10.5ºC. The fish were fed with commercial dry pellet at 2% body weight per day for 10 weeks. Thirty fish were sampled from each group every two weeks, and measured and weighted individually. The contents of stomach, pyloric caeca and intestine removed from each fish were immersed in PBS, and parasite counts were made under the microscope. The intestine tissues were fixed in Bouin's solution or 10% neural buffered formalin, and processed by standard histological technique. Sections were stained with Giemsa's stain or Alcian blue (pH 2.5)/PAS. The parasite was dominantly distributed in the anterior intestine of juvenile Masu Salmon. The abundance of *S. salmonis* increased 2 weeks post infection, peaked at 4,800 parasites at week 4, and declined to less than 100 parasites at weeks 8 and 10. The mortality in the infection group accumulated to 17.9% for 10 weeks, compared with only 1.3% in the control group. The infected fish were significantly smaller than the controls at weeks 6 and 8, and the condition factor of infected fish was also significantly reduced between weeks 4 and 6. Light erosion was observed in the mucosal epithelium of heavily-infected intestine. The present experiment has confirmed that *S. salmonis* infection has a significant impact on the growth and mortality of juvenile salmon. Further laboratory and field studies are required to control the parasite infection at hatcheries.

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Trichodinid Ectoparasites (Ciliophora: Trichodinidae) from Freshwater Fishes in China, with Notes on Host–Parasite Relationship

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Trichodinids, which are morphologically characterized with the presence of a prominent denticular adhesive disc, are probably the most common ciliated protozoan parasites or symbionts of marine and freshwater organisms. Some of them can cause severe disease and mass mortality in their host, which results in considerable economic losses to fishery sector. During a parasitic ciliate survey in China from 2013 to 2018, nine *Trichodina* species and two *Paratrichodina* species were isolated from freshwater fishes. The small subunit ribosomal RNA gene (SSU rDNA) sequences of five *Trichodina* species, that are *T. paranigra*, *T. reticulata*, *T. acuta*, *T. hyperparasitis* and *T. hypsilepis*, were sequenced. Phylogenetic analyses revealed that the five *Trichodina* species investigated in the present study were nested within a clade including several freshwater *Trichodina* species, which indicates that the central granule is a useful taxonomic feature, but it may not be an important phylogenetic characteristic. Our study extended the host range of trichodinids and revealed that invasion of exotic fishe invasion may cause a potential threat to native fishes by carrying or spreading parasitic ciliates. Besides, histopathologic analyses revealed that trichodinids firmly colonized gills, which resulted in discrete hyperplasia and injuries of the gill filaments.

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