

persistence capability of these isolates and, suggest that, although all three isolates were capable of reducing *T. leucotreta* infestation in comparison to the control block, *B. bassiana* performed best with an 81.33% reduction. It is thus suggested that future trials focus on the performance of this isolate.

Contributed paper. Tuesday, 11:45. **97-STU**

Wireworm control with entomopathogenic fungi and plant extracts

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Wireworms, the soil-dwelling larvae of click beetles, cause severe damage in arable crops and vegetable production. Currently, no registered and effective insecticides are available. The development of alternative control approaches including botanicals or insect pathogenic organisms are demanded and favoured by EU legislation (Directive 2009/128/EC). Limited efficacy of already tested entomopathogenic fungi (EPF) could be improved by synergistically acting botanicals. In the present study repellency of botanicals towards the wireworm species *Agriotes lineatus* and potential interactions of the most effective repellent with a wireworm-infecting fungus strain (*Metarhizium brunneum*) was investigated. Behaviour and mortality of wireworms were assessed in two-dimensional terraria (40cm x 50cm x 0.6cm) with a peat-sand substrate in a choice test for up to three weeks. Wireworm location was recorded and locomotion trails were manually traced, photographed and trail length determined on the treated and untreated half of the terrarium. We found that the garlic extract R3 repelled wireworms at rates of 1.2 g/L substrate, while this concentration hardly reduced efficacy of the EPF strain. Thyme oil was comparably repellent, but also strongly antifungal. The EPF strain was not repellent. Potential synergies between EPF and efficacy enhancing botanicals will be discussed for a biological control strategy.

Contributed paper. Tuesday, 12:00. **98-STU**

Long-term persistence of *Beauveria brongniartii* BIPESCO 2 used for cockchafer control in the Euroregion Tyrol

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The fungus *Beauveria brongniartii* (Sacc.) Petch has been used to control the European cockchafer *Melolontha melolontha* L. for more than two decades. The goal of this study was to assess persistence of the applied *B. brongniartii* strain in the soil of 20 cockchafer infested sites in East, North and South Tyrol. The sites have been treated with

Melocont[®] Pilzgerste (BIPESCO 2) at different frequencies and time points during the last 20 years. At all sites the density of *M. melolontha* larvae decreased from high levels at the start of treatments to levels below the damage threshold at the time point of sampling in 2012. A selective medium was used to determine *B. brongniartii* density and recover *B. brongniartii* isolates from soil samples. Collected isolates were subjected to genetic analyses to discriminate the applied strain from naturally occurring strains. Highest densities of *Beauveria* spp. (up to 6.8 x 10⁵ CFU g⁻¹ soil dry weight) were detected in soils which have been treated with Melocont[®] Pilzgerste at least three times during the last three years (3 sites) prior to the sampling date. BIPESCO 2 was detected in 7 sites of which one was treated for the last time 15 years prior to sampling. *Beauveria* spp. density varied strongly among and within fields and in 71% of the 162 soil samples no *Beauveria* was detected. Results suggest that periodic applications of the *B. brongniartii* biological control agent increase density and persistence of the fungus in soil and support a long-term control of *M. melolontha*.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

DIS. OF BENEFICIAL INVERTEBRATES 1

Contributed paper. Tuesday, 10:30. **99**

The Curious Case of the PaV1 in Adult Caribbean Spiny Lobsters

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The pathogen PaV1 (Panulirus argus Virus 1) exacts a heavy toll from juvenile Caribbean spiny lobsters with an estimated 24% in Florida dying of it before they reach maturity and recruit to the fishery. Prevalence is also high among adult populations, especially in the northern Caribbean (e.g., Puerto Rico – 17%). However, PaV1 manifests differently in adults. They may act as “carriers” because adults rarely develop visible infections and do not seem adversely affected by the pathogen. Infected adults are not avoided by healthy conspecifics, as occurs among juveniles. Moreover, adult females with subclinical PaV1 infections are often captured from the wild with a spermatophore or fertilized eggs, indicating that males are willing to mate with them. Adults with subclinical infections of PaV1 are not infectious to other adults or to the more susceptible juveniles. Although postlarval lobsters infected with PaV1 occur in the nearshore waters of Florida, experiments indicate that vertical transmission of PaV1 from females to embryos is not the mode of transmission. Instead, postlarvae acquire PaV1 shortly after arriving inshore from the oceanic plankton. These recent results suggest that PaV1 may be of little consequence to adult lobsters in contrast to its major effect on juvenile ecology and population dynamics. Just how adult lobsters retain subclinical infections of PaV1 remains a mystery.

Contributed paper. Tuesday, 10:45. **100**

Defining lobster-pathogen interactions via high-throughput gene expression studies: The discovery and description of the interplay between the American Lobster (*Homarus americanus*) and the ciliated parasite *Anophryoides haemophila*

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The American lobster (*Homarus americanus*) fishery is the economic engine for hundreds of coastal communities in Atlantic Canada and represents the last remaining significant wild fishery in Canada. Lobsters appear remarkably resistant to microbes in their natural environment however they are susceptible to the opportunistic ciliated pathogen *Anophryoides haemophila*, the causative agent of bumper car disease, during live holding. We have completed numerous controlled experimental infection studies to define the gross, histopathology, biochemical and molecular responses of lobster to this ciliated parasite. Recently completed high throughput oligonucleotide microarray and RNA-Seq transcriptomics studies have revealed a more comprehensive understanding of the molecular pathogenesis of disease in this unique lobster – parasite interaction. One caveat is interpreting the overwhelming wealth of bioinformatic data generated. This issue will be explored in the context of current annotation limitations for both arthropods and protistan parasites.

Contributed paper. Tuesday, 11:00. **101-STU**

Metabolomic investigation of Bitter Crab Disease in snow crabs (*Chionoecetes opilio*)

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Bitter crab disease (BCD) is a fatal disease of crustaceans caused by parasitic dinoflagellates of the genus *Hematodinium*. This emerging disease has been reported in over forty species of crustaceans world-wide including several commercially important crustacean species. In Atlantic Canada BCD occurs in snow crabs (*Chionoecetes opilio*) off the northern coasts of Newfoundland and Nova Scotia. In the late stages of this disease, the dinoflagellate parasites proliferate within the hemolymph and hemal spaces within the crustacean's organs, with no apparent cellular inflammatory response to the infection. The cause of death in cases of BCD is presumed to be metabolic and osmotic dysregulation. In this study, we use a combination of untargeted and targeted metabolomic approaches to characterize some of the metabolic changes associated with BCD.

Contributed paper. Tuesday, 11:15. **102-STU**

Assessment of immunocompetence in the shore crab, *Carcinus maenas*, to natural exposure of pathogens

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UK populations of the shore crab *Carcinus maenas* host various pathogen assemblages. In particular, two geographically close but distinct populations in Weymouth, (Newton's Cove and Harbour), demonstrated entirely different pathogen profiles. Immune biomarkers were used to assess the immunocompetence of individuals in these populations in relation to their pathogen burden. Selected immune genes included *carcinin*, (antimicrobial peptide), *peroxinectin* (cell adhesion molecule and osponin) and the zymogen *prophenoloxidase*, (cleaved to form active *phenoloxidase*, involved in the melanisation of many invading pathogens). Immune gene expression was quantified using real-time PCR. Histopathology revealed greater pathogen incidence in Newton's Cove (95%) compared with Harbour (37%) and a high dissimilarity in the pathogen profile (82.61% SIMPER) between sites. Host immune expression in relation to the presence and absence of pathogens and number of different infections per crab, revealed significant ($p < 0.01$) differences in transcription between populations, suggesting site-specific factors also influenced immune expression. In addition, host RNA quality was compared between pathogen groups ('viruses', 'bacteria', 'macroparasites' and 'no pathogens' groups). Further analysis may reveal whether RNA degradation is a function of pathogen type within the host. This is the first study to compare immunocompetence and histopathology between different *Carcinus maenas* populations in the wild.

Contributed paper. Tuesday, 11:30. **103-STU**

Effects of artificial infection of juvenile edible crabs, *Cancer pagurus* with the parasitic dinoflagellate, *Hematodinium* sp.

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Parasitic dinoflagellates of the genus, *Hematodinium*, are thought to be significant pathogens of a wide range of crustaceans. Much is known of the ecology and effects of this disease on the sustainability of crustacean populations but significantly less is known about the mode of transmission and fate of infected animals. Attempts have been made to transmit the disease under aquarium conditions to several species of crabs resulting in a great deal of variation in mortality levels and the timescale of disease progression. To determine if *Hematodinium* infections are significant drivers of mortality in juvenile edible crabs (*Cancer pagurus*), crabs were injected with either 1×10^5 *Hematodinium* trophonts from an infected animal or sterile saline. Crabs were bled every four weeks to determine the progression of infection and its effects on the numbers of circulating haemocytes. Thirty three percent of the *Hematodinium*-injected crabs became infected and mortality occurred between 93 and 378 days post-challenge. Infected crabs appeared to moult less frequently than their uninfected counterparts but mortality did not appear to be directly caused by *Hematodinium*, as there was no significant difference in the mean time to death between infected and uninfected crabs. Both *Hematodinium*-infected and uninfected crabs exhibited infections by a number of other disease causing agents including haplosporidium-like parasites, fungi and bacteria. These appeared to be key drivers of the mortality observed. These studies, albeit carried out on small cohorts of edible crabs, imply that *Hematodinium* is not a driver of host mortality at least under aquarium conditions.

Contributed paper. Tuesday, 11:45. **104**

A role of polychaetes in transmission of white spot syndrome virus in shrimp ponds?

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White spot disease (WSD) is caused by white spot syndrome virus (WSSV) (*Nimaviridae*). WSSV emerged in the early-to-mid 1990s in Southeast Asia and became panzootic since. The disease can be mitigated by introducing rigorous sanitation protocols, proper pond management, use of specific pathogen-free shrimp and by early diagnosis followed by eradication. The virus is transmitted horizontally by healthy individuals preying on diseased ones, via feeding on detritus or by intake of WSSV-contaminated water. WSSV can also be transmitted vertically via broodstock. The virus infects a wide range of crustaceans beyond the penaeids such as crabs and crayfish, and these co-inhabitants of ponds form a reservoir of WSSV for disease transmission to penaeids. Much less knowledge is there on the potential of resident benthic organisms as vectors for WSSV. A literature survey indicates that WSSV is present in a number of non-crustacean invertebrates, which sometimes vector the disease to penaeid shrimp. *Dendronereis* spp. is a most ubiquitous resident annelid in shrimp ponds and used as food source for shrimp. We showed that WSSV replicates in *Dendronereis* spp. and can be transmitted from this polychaete to penaeid shrimp. Furthermore there appears to be a positive correlation between the past incidence of WSD in ponds and the occurrence of WSSV in resident *Dendronereis* spp, whereas there is no correlation with other pond parameters. We hypothesize that *Dendronereis* spp., as a replicative host for WSSV, may serve as a reservoir for WSSV and may be associated with the persistence of this virus in pond systems.

Contributed paper. Tuesday, 12:00. **105**

Novel Pattern Recognition Receptor Protects Shrimp from *Vibrio* Infection by Binding Flagellin and LPS through Different Recognition Modules

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Pattern recognition receptors (PRRs) recognize pathogens through the pattern recognition modules. For example, Toll like receptors recognize the ligands through leucine-rich repeats (LRRs), and C-type lectins bind to glycans on the surface of pathogens by the C-type carbohydrate recognition domain (CRD, also called C-type lectin like domain CTLD). Many PRRs contain more than one kind of modules. In the present study, we identified a novel PRR, named *Leulectin*, which contains several LRRs and a CTLD. Such unique arrangement has not been found in any other organisms. Recombinant *Leulectin* and the modules (LRRs and CTLD) were found to protect shrimp from *Vibrio* infection. An ELISA-based screen was performed to identify the potential ligands the two modules may recognize. Results showed that LRRs could recognize the *Vibrio* flagellins, and CTLD could recognize lipopolysaccharides (LPS). The *Leulectin*-flagellin interaction was determined by the third LRR of *Leulectin* and

the N-terminus of flagellin, and the *Leulectin*-LPS interaction was dependent on the long loop region of CTLD in a calcium-independent manner. The ligand-recognizing activity of LRRs and CTLD was critical for *Leulectin* to bind to bacteria, and the binding was the basis for *Leulectin* to protect shrimp from bacterial infection. This study clearly showed the interesting synergy between distinct modules of a PRR.

Contributed paper. Tuesday, 12:15. **106**

Observations on *Agmasoma penaei* and *Perezia nelsoni* in White shrimp *Litopenaeus setiferus* from the Gulf of Mexico

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In June 2012 a few shrimp from Plaquemines parish LA with the symptoms of microsporidiosis were delivered to the Louisiana Aquatic Diagnostic Laboratory for identification. Light microscopy including examination of Luna-stained paraffin sections, and electron microscopy showed the infection was limited to ovaries and was caused by a microsporidium producing roundish pansporoblasts with 8 spores (3.6 x 2.1µm) and anisofilar (2+6) polar filaments, the features corresponding to the diagnosis of *Agmasoma penaei* (= *Thelohania penaei* Sprague 1950, n.comb Hazrad and Oldacre, 1973). Comparison of the SSUrDNA sequence of the novel isolate to *A.penaei* from Thailand revealed 95% similarity, which suggests these geographical isolates, may be two different species, a conclusion supported by several ultrastructural dissimilarities and different tissue tropism. Phylogenetic analyses places this species as a divergent taxa within the clade IV (microsporidia of terrestrial origin) sensu Vosbrinck, Debruner-Vosbrinck, 2005. In two shrimps infection of ovaries with *A. penaei* was accompanied by heavy infestation of muscles with another microsporidium *Perezia nelsoni*. *P.nelsoni* produces individual spores (2.0 x 1.1µm). Structurally and genetically (SSUrDNA sequence similarity >99%) LA isolate was identical to *Perezia nelsoni* from the Mississippi coast of the Gulf (Canning et al., 2002). Previously reported infection of muscles with *A.penaei* may be due to overlooked double infection with *P.nelsoni*. Supported by Louisiana Department of Wildlife and Fisheries.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

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Contributed paper. Tuesday, 10:30. **107**

Comparison of ecological traits of co-existing *Metarhizium*: What does it take to dominate an agricultural field?

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