Abstract
Live food has been used for feeding and delivery of the compounds to larval stages of aquatic animals. *Artemia* can be used as a possible vector for the delivery of different substances such as nutrients, probiotics and immune-stimulants. Sulfated galactans (SG) from *Gracilaria fisheri* has been demonstrated to stimulate immune activity and against pathogenic infection in shrimp. In vitro study revealed that SG bound with shrimp haemocyte and activated the expression of immune related genes in haemocyte culture. The present study was carried out to investigate the bioencapsulation of *Artemia* with SG for delivery to tissues and activating the expression of immune genes in shrimp. SG conjugated with fluorescein isothiocyanate (FITC-SG) showed no significant toxic in *Artemia*. Bioencapsulation of *Artemia* with FITC-SG (0-100 μg/ml) revealed that FITC-SG was located in *Artemia* gut lumen with a time and dose dependent manners. Tracking of SG in shrimp, shrimp fed with *Artemia* bioencapsulated with FITC-SG demonstrated that FITC-SG presented in shrimp gut until 2 h after feeding. Twenty-four hours after feeding, shrimp tissues were collected and the distribution of FITC-SG was observed in gill, hepatopancreas and haemolymph. FITC-SG was also found to bind with shrimp haemocyte. In addition, shrimp continuously fed with *Artemia* bioencapsulated with SG for 3 days showed activated the expression of immune genes including IMD, IKKβ, dicer and proPO-I, and prolonged high expression levels for a week. These results indicated that *Artemia* bioencapsulation could deliver the SG passed through gut to tissues and bound with haemocyte, and subsequently activated expression of immune genes in shrimp.
Keywords: *Artemia* bioencapsulation; Sulfated galactans; FITC-SG; Shrimp haemocyte; Immunity

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O-005.

Vaccination strategies and IgM responses against PKD in rainbow trout

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Abstract
PKD is one of the most serious diseases affecting trout aquaculture in the UK. Caused by the myxozoan parasite, *Tetracapsuloides bryosalmonae*, PKD is elicited by the temperature-dependent development of parasite spore sacs in colonial bryozoans. Since recovered fish are known to exhibit protective immunity to re-infection, a successful treatment, based on the reduction of kidney pathology and parasite burden, could markedly reduce fish mortalities leading to improved productivity and fish welfare. Our investigations have focused on the selection of putative *T. bryosalmonae* virulence factors to unravel host immune evasion mechanisms exploited by the parasite, whilst shortlisting candidates for vaccine studies.
Here we report the results of three DNA-vaccination field trials using selected antigens administered individually or in combination. Some vaccine groups were found to have a partial protective effect in reducing PKD-associated kidney pathology whilst decreasing parasite load**.** Pathology reduction was improved in successive trials by improving the vaccination strategy.
We have functionally characterized the most promising antigen from our vaccine studies, which represents a novel micro-exon gene (*Tb*-MEG1). MEGs, until now, were thought to be unique to helminth parasites. In schistosomes, they exhibit extensive antigenic variability that is thought to enable greater plasticity in host protein targeting as a mechanism of host immune subversion over the course of infection. Using a validated anti *Tb*-MEG1 MoAb, we have demonstrated the protein to be expressed in and on the surface of parasites and a subset of immune cells within the kidneys of infected fish. We have also demonstrated potent *Tb*-MEG1-specific IgM responses in sera from parasite-infected (farmed) rainbow trout and have successfully induced a specific IgM response after protein vaccination.
DNA vaccines encoding molecules homologous to proteins involved in nutrition acquisition, cell-cell interactions or of unknown function, have shown promise towards the development of a future PKD vaccine, that may also be applicable to the generation of vaccines against other fish parasites. The discovery and characterization of *T. bryosalmonae* antigens has provided valuable insights into host immune evasion by myxozoan parasites, with the present *Tb*-MEG1 studies also having major implications towards understanding the evolution of antigenic variability in metazoan parasites.
Keywords: Vaccination, myxozoan, parasite, antigen, igm response

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O-006.

Demonstration of herd immunity effects in dna vaccinated rainbow trout

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Abstract
While DNA vaccination by intramuscular injection is known to provide highly protective immunity against viral haemorrhagic septicaemia (VHS) in rainbow trout at an individual level, we here aimed at examination of heard immunity effects. Induction of clinical disease following experimental challenge by waterborne exposure of the fish to the pathogens represents a hurdle in vaccine potency testing for many pathogens, but in the case of VHS virus, challenge can be done efficiently by immersion. However, progression and level of mortality depends on the challenge dose. This has some implications for the experimental design and interpretation of the results. Initial experiments indicated that while mortality following a high challenge dose was independent on whether the fish were kept together in one aquarium or in individual aquaria, indirect infection by virus released from primary infected fish seemed to play an important role at low challenge doses. In experimental vaccination trials, it may be assumed that challenge of cohabitant naïve and vaccinated fish represents a harder test of vaccine potency compared to challenging the vaccinated fish separately due to secretion of high amounts of virus from the naïve fish. In challenge trials with high inoculum of virus, this seemed to be true for VHSV challenge of DNA vaccinated rainbow trout as illustrated by increased mortality among vaccinated fish cohabitated with naïve fish. However, at lower inoculum, the effect tended to be opposite in the sense that mortality among the naïve fish was reduced. Implications of the results for experimental vaccine testing will be discussed.
Keywords: Fish rhabdovirus, DNA vaccine, challenge setup, vaccine testing, herd immunity

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O-007.

Exposure to antibiotics affects saponin immersion induced immune stimulation and shift in microbial composition in zebrafish larvae

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Abstract
In the last decades, pollution of the environment by large scale use of antibiotics in agriculture and human medicine have led to increased antimicrobial resistance in both the environment and the host animal microbiome. Disturbances in the host microbiome can result in impaired immunity and reduced resilience of aquaculture species. Here, we investigated whether environmentally measured levels of the commonly used antibiotics ciprofloxacin and oxytetracycline influences the host microbiome and susceptibility toward saponin-induced immune stimulation in larval zebrafish. Firstly, neutrophil and macrophage reporter zebrafish larvae were exposed to different concentrations of soy saponin by immersion. A dose-dependent increase in neutrophil presence in the intestinal area was observed together with increased expression of immune genes il1b, tnfa, il22 and mmp9. To investigate the effect of antibiotics, larval zebrafish were immersed in ciprofloxacin or oxytetracycline in the presence or absence of a low dose of saponin. In vivo imaging revealed that antibiotic treatment did not reduce the number of neutrophils that were recruited to the intestinal area upon saponin exposure, although it did tend to lower pro-inflammatory cytokine levels. Microbial sequencing of whole larvae revealed that exposure to a low dose of saponin already shifted the microbial composition. The combination of oxytetracycline and saponin significantly increased α-diversity compared to the controls. In conclusion, the current study provides evidence that the combination of low levels of antibiotics with low levels of anti-nutritional factors (saponin) can induce inflammatory phenotypes and can modify the microbiota, which might lead to altered disease susceptibility.
Keywords: Zebrafish, microbiota, saponin, neutrophils, macrophages, antibiotics

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O-008.

Can passive immunization prevent disease outcome in gilthead sea bream exposed to *Enteromyxum leei*?

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Abstract
Passive immunization is used in humans for treating or preventing some infectious diseases, but it also constitutes an emerging field of interest in aquaculture, particularly with the restrictions for antibiotic use. Intracoelomically-injected antibodies can be detected in fish sera within the first 8 h and their half-life ranges from 7 to 22 days post-injection, depending on the species. The fish models studied so far used fast-acting pathogens such as virus, bacteria or ciliate parasites. The current work aimed to determine if passive immunization could help to prevent enteromyxosis in gilthead sea bream (GSB, *Sparus aurata*). *Enteromyxum leei* is a myxozoan intestinal parasite that invades the paracellular space of the intestinal epithelium, producing a slow-progressing disease, leading to anorexia, cachexia and mortalities. We have previously demonstrated that GSB that survive *E. leei* infection become resistant upon re-exposure, and this resistance is directly related to the presence of high levels of specific serum antibodies.
In the current study, we evaluated whether injection with sera from resistant animals would protect naïve fish when challenged by effluent exposure to the parasite. Serum from a pool of resistant (R) and naïve (N) animals (intact or heat inactivated, 10 μl/g BW) was intracoelomically injected 24 h prior to the *E. leei*-effluent challenge and at 9 days post-challenge (dpc). At 23 dpc, the different groups were allocated in separate tanks and the effluent exposure was terminated. A non-lethal parasite diagnosis was performed at 56 dpc. At the final sampling (100 dpc), blood, serum and tissues were collected for hematology, circulating antibodies, histological and molecular diagnosis and gene expression.
Groups injected with R sera had lower prevalence and intensity of infection than those with N sera, both in the intermediate and final samplings. At 100 dpc, the prevalence of infection in the PBS and N groups was 70%, whereas in R group it only reached 55%. Condition factor (CF) and specific growth rate (SGR), key parameters affected by enteromyxosis, were higher in R group. There was a significant correlation between prevalence of infection and SGR and CF. Immunohistochemistry and gene expression studies will reveal whether this partial protection was due to higher presence of specific antibodies or specific cell populations. These results show that, even with this long term disease, passive immunization can confer some degree of protection. The administration of specific antibodies during exposure, probably provided fish with time to activate the specific defenses before the parasite proliferated.
ACKNOWLEDGEMENTS: This work was funded by EU H2020 program through ParaFishControl Project (634429) and Spanish MINECO project AGL2013-48560. M.C.P. was contracted under CSIC PIE project no. 201740E013 and FPDI-2013-15741 and I.E. under APOSTD/2016/037 grant by the “Generalitat Valenciana”.
Keywords: Passive immunization, Myxozoa, antibodies, gilthead sea bream, parasites.

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O-009.

Bacterial membrane vesicles as vaccines in aquaculture

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Abstract
Infections by two Gram-negative facultative intracellular bacterial pathogens, namely, *Piscirickettsia salmonis* and *Francisella noatunensis,* are causing major problems in aquaculture world-wide. *F. noatunensis* sp is one of the main factors hampering the development of fish farming based on Atlantic cod in Norway and is deleterious to tilapia, a farmed fish that is produced over 3.5 mill tons/year. *P. salmonis* infections have been devastating for salmon aquaculture. As of today no effective treatments are available against the diseases. The immunologically inaccessible intracellular location of *Francisella* and *Piscirickettsia* have until now complicated the development of protective measures. This is in stark contrast to the successful development of efficient vaccines that has been made possible against important extracellular bacterial infections in salmon based on whole inactivated bacteria injected with oil adjuvants. It has been shown that both *P. salmonis* and *F. noatunensis* secrete membrane vesicles (MV). Bacterial MVs has been shown to contain proteins, DNA and RNA and simulate the mother bacteria in a non-replicative form. Bacterial MV has been reported as potential vaccine candidates for a range of host including humans, mice and fish against infection caused by intracellular pathogenic bacteria as they induce both a humoral and cellular immunity. Here the characterization of MV isolated from *P. salmonis* and *F. noatunensis* is described, and their vaccine potential is verified in a zebrafish infection