acts as a potent immunomodulator, acting as an inhibitor of cytokine production and homing, and inducing apoptosis in immune cells. However, less is know about the effect of the stress and cortisol itself on the immune responses after the activation of the immune system or vaccination.
A frequent practice in aquaculture to vaccinate fish against common diseases is the use of formalin-killed bacteria as source of antigens. Hence, in the current study we attempted to determine how stress affects the mucosal and systemic immune and endocrine responses of fish after immersion in *Vibrio anguillarum* bacterin. To this end, four groups of rainbow trout ($n = 8$ fish per group) were used for the experiment: two groups bath treated with PBS and another two groups bath treated with bacterin. 24 hours after immersion, fish were subjected to an acute handling stress (1 minute of anoxia). From the PBS groups, one was used as pure control (no stress) and the other as stressed control. From the bacterin groups, one was used as control of bacterial effect (no stress). Fish were sampled 1h, 6h, 24h and one month after anoxia.
Systemic and mucosal levels of cortisol, prostaglandins, glucose, lactate, lysozyme and complement were measured. To determine gene expression, mRNA levels of IgM and IgT were analyzed by real time RT-PCR. To establish the effect of stress on the B cell response, antibody levels in serum and mucus were measured by western blot and the amount of IgT^+ and IgM^+-B cells in different tissues was determined by FACS.
Results show that cortisol levels increased in plasma and mucus in both stressed groups, with some differences in the vaccinated group. We are currently in the process of measuring changes in IgT and IgM responses. The confirmation of our hypothesis would suggest that after stress episodes, B cell responses are also affected not only after chronic as previously described, but also after acute stress conditions.

* Corresponding author.
E-mail address: david.parra@uab.cat (D. Parra)
§These authors contributed equally to this work.

P-431.

In vitro evaluation of immunomodulatory activity of total extracts of Canelo (*Drimys winteri*) and active compound polygodial in salmon head kidney cells

D. Pereira [1,§], H. Carrasco [2], A. Astuya [1,].*

[1] Cell Culture and Marine Genomics Laboratory, Marine Biotechnology Unit, Faculty of Natural and Oceanographic Sciences, University of Concepcion, Concepcion, Chile;
[2] Departament of Chemical Sciences, Andres Bello University, Viña del Mar, Chile

Abstract
In recent years has intensified the search for natural stimulant of the immune system (immunomodulators), which are considered as a powerful tool for the aquaculture industry, as it strengthens the immunity, disease resistance and stress. This work presents the evaluation of in vitro immunomodulatory activity of total extract of Canelo (*Drimys winteri*) and its isolated active compound polygodial in macrophage-like cell line ASK-1 (Atlantic salmon head kidney cells). We examined the expression of three immune molecular markers: interferon α (IFNα), interleukin 1β (IL-1β) and the major histocompatibility complex class II receptor (MHCII) by QRT-PCR.
Both extracts showed a fast and significant increase in expression of IL-1β similarly to the one observed in the presence of commercial imunomodulator. Moreover, the expression of IFNα and MHCII showed a significant increase versus control when stimulated with polygodial, similarly to the commercial inductor, being less significant increase in expression of these markers when the cells are stimulated with total extract of Canelo.
Additionally, based upon our previous results of the antisaprolegnia activity of these extracts when studied *Saprolegnia parasitica* grown directly on agar plates, we decided to evaluate the effect of these extracts in an acute infection assay using this oomycete in ASK-1 cells. Preincubation with total extract of Canelo or polygodial and subsequent acute infection with Saprolegnia showed a significant increase in expression of IL-1β versus control, without observing a greater difference when *Saprolegnia* is incubated without extracts. Furthermore, during acute infection with *Saprolegnia* a down regulation of IFNα and MHCII expression was observed, suggesting interference of immune function in these macrophage-like cells, which could limit the response of the host. However, the pre-incubation with total extract and especially polygodial produced an increase of these markers expression when ASK-1 cells where acutely exposed to *Saprolegnia*. Our results suggest the transfer of the immunomodulatory properties to this vitro model, which potentially woud allow the recognition and defense against these pathogens.

* Corresponding author.
E-mail address: aastuya@udec.cl (A. Astuya)
§These authors have contributed equally to this work.

P-166.

Syndrome virus using recombinant fortilin expressed in yeast

A. Phongdara [1,], P. Sinthujaroen [1], B. Nupan [1], M. Tonganunt-Srithaworn [2].*

[1] Center for Genomics and Bioinformatics Research, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand;
[2] Department of Microbiology, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

Abstract
Fortilin is a highly conserved protein present in all eukaryotic organisms. Various cellular functions and molecular interactions have been ascribed to this protein, many related to its growth promoting and anti-apoptotic properties. Levels of fortilin are highly regulated in response to various cellular stimuli and stresses including virus infection. In previous work, recombinant fortilin produced from bacteria was used to protect shrimp *P. monodon* from WSSV infection. Here, the advantage of fortilin and yeast cells by produce the recombinant protein in *Saccahromyces cerevisiae* was combining. The possibility to use recombinant fortilin in protection against WSSV infection in *Litopenaeus vannamei* was tested by feeding. The survival rate of 1% and 5% recombinant fortilin fed shrimp (66.7% and 91.7%) was higher than the positive control group (8.3%) or fed with empty vector expressed in yeast (33.4%). At the end of the experiment, the level of WSSV was highly detected by PCR analysis in moribund and fed empty vector treated shrimps, but significantly low in survival shrimps. The stability of this recombinant protein in pH values of 6–9, salinities 15–30 ppt and temperatures of 50–90 °C were investigated. The results showed that this protein was stable in all conditions tested. Recombinant fortilin offers to be a valuable tool in the development of feed additive to control virus infection in shrimp culture. For the functional analysis of fortilin, the mortality of fortilin knockdown shrimp challenged with WSSV significantly increased when compared with control. The result supported the role of fortilin in antiviral activity.

* Corresponding author.
E-mail address: pamornra@yahoo.com (A. Phongdara)

P-236.

Uptake and biological effects of engineered nanoparticles of titanium dioxide (TiO_2) in a fish species (*Dicentrarchus labrax L.*)

S. Picchietti [1,], C. Bernini [1], F. Buonocore [1], A.R. Taddei [2], G. Scapigliati [1].*

[1] Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Viterbo, Italy;
[2] Interdepartmental Centre of Electron Microscopy (CIME), University of Tuscia, Viterbo, Italy

Abstract
Production and use of engineered nanoparticles of TiO_2 (NP-TiO_2) is increasing rapidly with global investment in the range of billions of dollars.