

# Student projects: practical Masters training in action

Over the next three pages we are presenting a selection of abstracts from MSc projects. These abstracts come from papers produced for publication by students following their project period. Students must also produce a press release. This form of reporting of their work provides students with direct experience of publishing their work, both scientifically and in the wider media. You will see that there is a wide range of topics presented here, with students conducting research in many locations including laboratory and field sites, often with commercial or government research partners and sometimes overseas. Often MSc projects provide vital contributions to larger, on-going projects and sometimes the initial research required to identify the questions for future projects. When work is conducted with other partners they are always included in published results, usually as co-authors.

## Investigation into the negative role of ammonia and pH change in marine egg-larval transportation and methods for their control

Iñigo Ruiz de Apodaca Reyes<sup>1</sup>, John Bostock<sup>1</sup>, Philip Bodington<sup>2</sup> and Nikolaos Papanikos<sup>2</sup>

<sup>1</sup>Institute of Aquaculture

<sup>2</sup>Selonda SA, Greece

Egg quality is a factor of high importance for any finfish hatchery, independent of the species cultured. "Egg quality" is a sum of characteristics, some unknown, that directly affect the condition of the future organism: from the hatching probabilities to the swim bladder formation.

Egg transportation has a negative effect on egg quality, which can be minimised using chemicals to control the changes in water quality during transportation. Gilthead sea bream (*Sparus aurata*) eggs, obtained from natural spawning broodstock in a Selonda hatchery, were stored for 8 hours in order to simulate transportation. Changes in pH, ammonia and dissolved oxygen were measured over this period. Before and after the experiments, egg quality was tested using a buoyancy method in order to check the relationship between the simulated transportation, water quality changes and egg buoyancy.

The trials involved using pH buffers (carbon and Trizma) and ammonia control agent (Chloram-X) in order to check the most suitable method to minimize egg quality losses.

**Keywords:** Sea bream, egg transportation, egg quality, water quality, transportation effect

## Development of an enzyme-linked immunosorbent assay to assess serological responses in salmonids to antigens of *Renibacterium salmoninarum*

Daniela Carmen Broekman<sup>1</sup>, Una McCarthy<sup>2</sup>, Kim Thompson<sup>1</sup>

<sup>1</sup>Institute of Aquaculture

<sup>2</sup>FRS Marine Laboratory, Aberdeen

A study was conducted to optimise the use of a direct enzyme-linked immunosorbent assay (ELISA) for use of soluble antigen to detect antibodies against the p57 protein produced by *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD) in sera of salmonids. It was found that protein levels of at least 25 µgml<sup>-1</sup> are required for the assay to yield satisfying optical densities. The use of fish sera required a minimal dilution of 1/8 to avoid inhibition of antigen-antibody binding. To gain knowledge of immune response patterns of diseased fish an ELISA was subsequently used to analyse the profile of antibody titres following an experimental infection challenge in rainbow trout *Oncorhynchus mykiss* (Walbaum). The study was part of a survey to investigate immune responses not only to p57 but also against the whole cell pathogen and the amount of pathogens in the kidney, aiming to provide a reliable tool for epidemiological monitoring. The study demonstrated that specific antibodies against p57 had been produced and could be detected with the ELISA. Fish challenged with BKD demonstrated the highest antibody titre at week 10 post-challenge. However, a specific immune response was apparent in only 6 out of 36 diseased animals. This finding suggests that disease monitoring by serology might be unreliable as the pathogen exhibits immunosuppressive action. However fish

with a specific response can be considered to be resistant against the pathogen.

**Keywords:** Bacterial kidney disease, *Renibacterium salmoninarum*, p57, antibody, ELISA

## Does integrated mariculture present a sustainable solution to mitigating the environmental impacts generated from commercial aquaculture production systems?

Marc S. Towers

Concerns over the environmental impacts arising as a result of producing carnivorous species, often through intensive monoculture practices, poses a potential constraint to future mariculture development. Integrating bioremediatory organisms such as seaweed and shellfish with finfish or shrimp culture has been suggested as a means to reduce the particulate and soluble waste loads within effluents, whilst providing additional farm income. This study examines the current status of integrated mariculture technologies by critically reviewing 51 peer-reviewed studies undertaken in landbased or open-water culture systems. The relevance of the findings with regards to implementing commercial scale integrated mariculture ventures are analysed, and directions for future research identified. It is apparent that basic hurdles in cultivation techniques, logistics, biological processes and experimental design still remain, and much of this is at spatial scales far below those experienced in most commercial mariculture contexts. The failure in detailing economic aspects of production also undermines

the technology. The use of seaweed as a bioremediatory tool is promising; however the efficacy of shellfish as a waste treatment tool is questionable, particularly in an open-water context. A general increase in research efforts and consequent funding will be necessary if commercial integrated mariculture ventures are to become a viable proposition for the industry.

**Keywords:** Integrated mariculture, bioremediation, shellfish, seaweed, land-based systems, open-water systems.

### Effects of total substitution of dietary fish oil with a blend of vegetable oils on liver and peripheral blood leucocyte fatty acid composition, plasma prostaglandin E2 and cellular immune parameters in three genetic strains of Atlantic salmon (*Salmo salar*)

Ioannis K. Petropoulos, Kim D. Thompson, Alison Morgan, Alexandra Adams and J. Gordon Bell

Duplicate groups of three genetic strains of Atlantic salmon (*Salmo salar*) smolts were maintained on diets containing either marine fish oil (FO) or a blend of vegetable oils (VO) supplied as 100% of added lipid, and these were assessed for differences in liver and peripheral blood leucocyte fatty acid composition. The impact of different strains and diets on selected innate immune parameters and levels of plasma prostaglandin E2 were also monitored. Two of the chosen strains were supplied by Landcatch and have been selected as being "fat" or "lean" in terms of their muscle fat storage. The third strain was supplied by Marine Harvest. Total replacement of the dietary FO with VO resulted in significantly reduced levels of docosahexaenoic acid (DHA;22:6n-3) and eicosapentanoic (EPA;20:5n-3) in liver lipids while oleic (OA;18:1n-9), linoleic (LA;18:2n-6) and  $\alpha$ -linolenic (ALA;18:3n-3) acids were all significantly increased in VO-fed fish. Fatty acid composition of blood leucocytes showed similar changes. EPA and DHA levels were affected by diet in Marine Harvest and Landcatch "lean" strains. No significant differences were found in plasma concentrations of prostaglandin E2 among the different treatments. Evaluation of non-specific immune function showed that in the "fat" strain the number of circulating leucocytes was significantly lower in fish fed the VO diet. There was also a significant effect of the "lean" strain on serum lysozyme activity. It was also found that in the "lean" strain, feeding fish the VO diet resulted in

reduced haematocrit compared with fish fed with FO diet. In contrast, in the "fat" strain the haematocrit value was significantly higher in VO-fed fish. This study provides some evidence of strain differences in liver-leucocyte fatty acid composition and non-specific immunity in Atlantic salmon fed either of the two diets.

**Key words:** Atlantic salmon, vegetable oil, fatty acid composition, prostaglandin, immune parameters, genetic strain

**An expanded abstract of an MSc project by Saviour Caruana is presented here in the context of on-going research**

### Plant extracts fight fungus

Gil Ha Yoon, Saviour Caruana and Andy Shinn

Fungal fish pathogens arguably rank second, after bacterial infections, in terms of loss to disease in freshwater aquaculture worldwide (Meyer, 1991). Of these, the genus *Saprolegnia* (Order Saprolegniales) is among one of the most important parasitic fungal groups on fish (Hatai *et al.*, 1990; Willoughby, 1994) (see Figure 1), with species being saprophytic and necrotrophic (Bruno & Wood, 1999). Apart from causing disease in captive fish, they have also been implicated in the decline of wild salmonid populations (van West, 2006). The frequent occurrence of *Saprolegnia parasitica* (Coker) in freshwater sources may result in problems for freshwater hatcheries (Rach *et al.*, 2005). The increase in production in the aquaculture sector has put more pressure on hatcheries to supply an ever-increasing amount of fingerlings. As a consequence of this, hatcheries have resorted to increased egg loading densities to meet the required production and such conditions are known to promote fungal infections (Schreier *et al.*, 1996; Brock & Bullis, 2001; Rach *et al.*, 2005). Control of *Saprolegnia* spp. infections during

egg incubation is essential since outbreaks may result in the loss of entire batches of eggs or a reduction in egg survival to hatching. Traditionally *Saprolegnia* spp. infections have been effectively controlled with malachite green, formalin and hydrogen peroxide, however, the ban on the use of malachite green in aquaculture has left farmers with very limited options for control of fungal pathogens.

Bioflavonoids and essential oils extracted from many plants have been used in traditional oriental medicine for thousands of years but it is only comparatively recently that they have been shown to possess a broad range of activities. These include anti-inflammatory (Middleton & Kandaswami, 1992), anti-viral (Kaul, Middleton & Ogra, 1985), anti-carcinogenic (Hertog, Hollman & Katan, 1992) and anti-oxidant properties. When included within the diet these substances have been suggested to confer an array of health benefits (Hertog & Feskens, 1993; Formica & Regelson, 1995; Nijveldt *et al.*, 2001).

A recent study undertaken at the Institute of Aquaculture set out to establish whether selected natural plant extracts and bioflavonoids could significantly reduce the incidence of fungal pathogens of fish and to assess their suitability as replacements for synthetic chemotherapeutants currently used in the aquaculture industry.

A total of 24 crude plant extracts and 3 isolated bioflavonoids were tested using an *in vitro* agar diffusion method to assess their effect on the growth of *S. parasitica* at 22°C over 72 hours. A 10,000 ppm solution of Compound E was found to wholly prevent germination of *S. parasitica*, while concentrations of 10 ppm of Compounds A, B, E-H and K inhibited growth (Table 1).

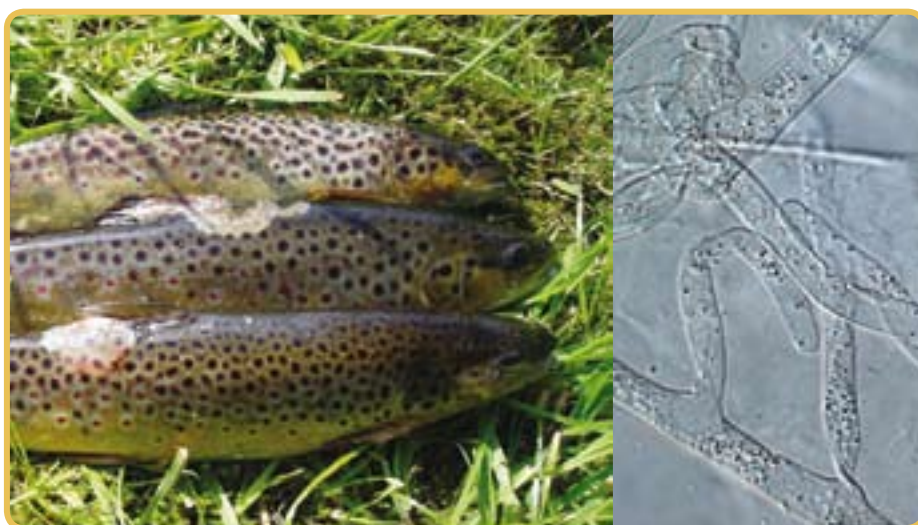
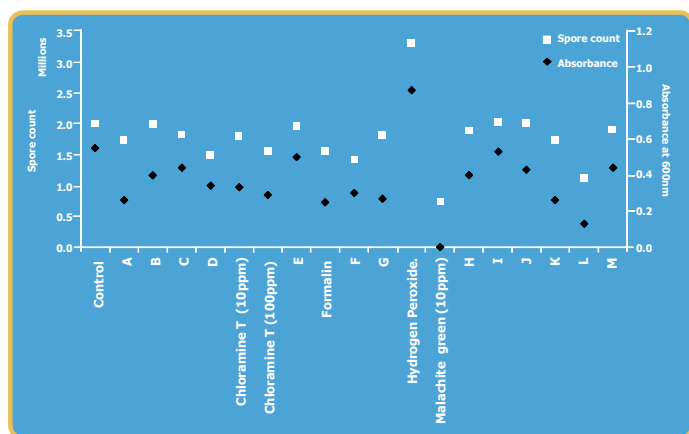


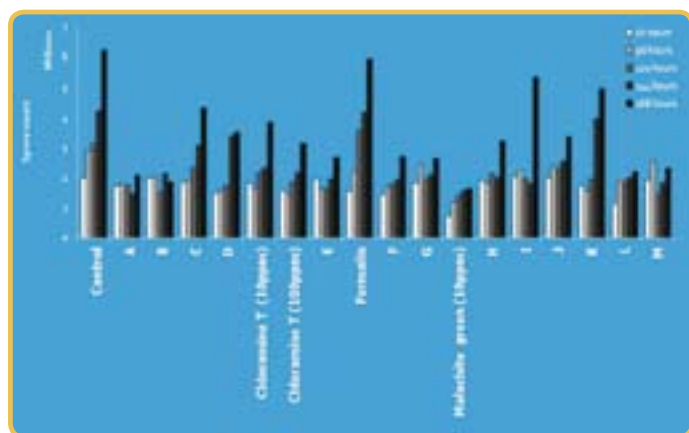
Figure 1. Brown trout, *Salmo trutta*, infected with *Saprolegnia parasitica*.

**Table 1.** The efficacy of each tested compound in inhibiting the growth of *Saprolegnia parasitica* when tested using an *in vitro* agar diffusion method. The inhibitory concentration of commonly used chemotherapeutants is also shown

Inhibitory conc. of chemotherapeutants (ppm)	Plant extracts inhibiting growth at >100ppm	Plant extracts inhibiting growth at >10ppm
Pyceze >200	C	A
DDQ >1000	D	B
Formalin >50	I	E
Hydrogen peroxide >100	J	F
Malachite green >1	L	G
	M	H
		K



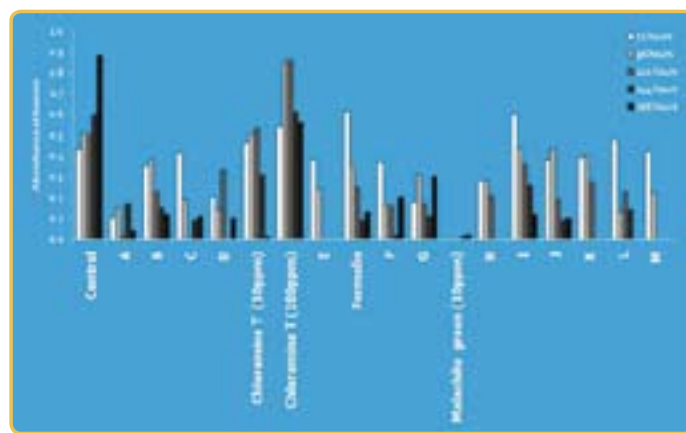
**Figure 2.** The effect of 12 plant extracts (duplicate counts) and 4 different chemotherapeutants on the growth of *Saprolegnia parasitica* 72 hours post-inoculation determined from spore counts and absorbance readings of broth cultures.



**Figure 3.** The effect of 12 plant extracts (duplicate counts) and 3 different chemotherapeutants on the growth of *Saprolegnia parasitica* 72-168 hours post-inoculation determined from absorbance readings of broth cultures.

In a second trial, 12 plant extracts found to inhibit growth at concentrations of 100 ppm or lower in the first trial, were added to cultures of *S. parasitica* in GP broth and the growth monitored spectrophotometrically at 600nm over a 7-day period against three commonly used chemotherapeutants (10ppm chloramine T, 100ppm formalin and 10ppm malachite green). All 12 plant extracts reduced growth of *S. parasitica* (Figures 2 & 3). The spore counts, however, suggested that only Compounds A, B, L and M, were as effective as 10ppm malachite green in slowing the growth of *Saprolegnia*.

In a third trial, an initial dose of plant extract was given at 72 hours post-inoculation, followed by a second dose at 120 hours (Figure 4). Spectrophotometry demonstrated that Compounds C-F and I were the most effective compounds tested. From the *in vitro* study, it is concluded that a range of plant extracts have an impact on the growth dynamics of *S. parasitica* but further research is required to assess their performance *in vivo*.



**Figure 4.** The effect of 12 plant extracts (duplicate counts) and 3 different chemotherapeutants on the growth of *Saprolegnia parasitica*, determined from absorbance readings of the broth cultures, when given as two doses at 72 and 120 hours post-inoculation.



## Commonwealth Fellowship to work on Macrobrachium

Dr Sudha Kappalli of the Sree Narayan College in Kerala was awarded a Commonwealth Fellowship and has been working here for six months from October 07. In Kerala her field of study is on growth and reproduction in crabs so she used the opportunity to extend her research into *Macrobrachium rosenbergii*, a species of increasing importance in Indian aquaculture. She has been working with Janet Brown on looking at variations in ecdysteroid and methyl farnesoate levels in *M. rosenbergii* at different moult stages and different ovarian development stages. She also looked in a preliminary way at the variations found in the different male morphotypes. This work was carried out partly by radio-immunoassay, so we are indebted to the input from Dr John Taylor in this area and for the measurement of methyl farnesoate we were dependant on the HPLC guidance from James Dick.