



Visakhapatnam Regional Centre of ICAR-Central Marine Fisheries Research Institute, Visakhapatnam, Andhra Pradesh, India; \*sekarrajaqua@gmail.com

**V**iral Nervous Necrosis (VNN) has been causing massive mortality among the larvae, juveniles and adult of marine teleost fishes in most parts of the world, especially in the cultured groupers. The present study, confirmed the presence of VNN in orange spotted grouper brood-stocks maintained in Recirculating Aquaculture System (RAS) and sea cages from Visakhapatnam coast. A total of 50 brood-stock fishes of 2-4 kg size were used for the breeding programme, in which 20 fishes were maintained in the RAS and 30 fishes were maintained in 6 m dia floating HDPE sea cage. After one year of rearing in both the systems, a total of 11 fishes (5 from RAS system and 6 from sea cage) exhibited abnormal behaviour during summer months, which included erratic swimming, opaque eyes, bloated belly and upturned swimming on water surface. Tissue samples from brain, optic nerve, gill, fins and muscle were aseptically collected from fishes that displayed the above clinical signs for VNN. Collected samples were tested for VNN using reverse transcriptase polymerase chain reaction (RT-PCR) with OIE listed VNN specific primers. Presence of 430 bp amplicon confirmed the VNN infection in the fishes. The expression of viral load was more in the tissue collected from brain and optic nerves. The amplified gene was sequenced and submitted in NCBI Genbank database. Sequence analysis confirmed the presence of VNN in the selected fishes. Partial sequence analysis of the virus isolated from the fish showed more similarity with the other virus genes that has been isolated from the fishes of tropical countries. VNN infected fishes were removed and only VNN free brood stock was used, which produced healthier larvae free of VNN infection. Broodstocks of orange spotted grouper are

prone to VNN infection and acts as carriers for the virus, hence screening brooders helps to produce healthy fingerlings.

AH PO 09

### **Measurement of antimicrobial activity of crustacean haemolymph against aquatic pathogenic bacteria and serum protein profiling**

B.H. HARIPRIYA, N. RANJIT KUMAR, T. MUTHULAKSHMI, S.S. GREESHMA, M.M. PRASAD\*

ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India; \*prasadmm@hotmail.com

**C**rustacean haemolymph carries several antimicrobial proteins that are needed for battling with the external pathogens. These proteins serve non-specific immune functions such as nodule formation, phagocytosis and encapsulation of bacterial pathogens. The present study is carried out to determine the antimicrobial activity of crustacean haemolymph against commonly occurring aquatic pathogens. The reference bacterial cultures used in this study are: *Pseudomonas aeruginosa* (ATCC 10145) and *Aeromonas hydrophila* (ATCC 35654). Haemolymph was aseptically drawn from *Litopenaeus vannamei* and *Scylla serrata* using 23 gauge needle. Sterile Brain-heart infusion (BHI) broth was prepared and the freshly grown bacterial cultures were inoculated with supplementation of 1% haemolymph. Incubation was carried out at 30°C for 48 h with measuring the bacterial growth using spectrophotometer at 4, 8, 16, 24 and 48 h for determining antibacterial activity. The results obtained from the above study were compared with readings of two controls [BHI broth with haemolymph and BHI broth with bacteria (without haemolymph)]. The crustacean haemolymph had shown significant effect on the growth of

pathogens which is evidenced from the decrease in optical density in haemolymph supplemented broth for crab and shrimp respectively for (*A. hydrophila* - 0.31, 1.06, 1.18, 1.71, 1.78 & 0.32, 1.25, 1.32, 1.98, 2.04; *P. aeruginosa* - 0.12, 0.31, 0.77, 1.53, 1.62 & 0.30, 0.45, 1.05, 1.52, 1.94) compared to control both for (*A. hydrophila* - 1.27, 1.50, 1.72, 2.16, 2.38 & 1.07, 1.31, 1.53, 2.26, 2.31; *P. aeruginosa*-0.82, 1.18, 1.54, 1.74, 1.94 & 0.62, 1.09, 1.36, 1.68, 2.05). The probable reason may be lysis of bacterial cells by the antimicrobial proteins present in haemolymph. Crustacean serum protein profiling was carried out using SDS-PAGE to detect antimicrobial proteins. In SDS-PAGE, the antibacterial proteins found in shrimp haemolymph were 20, 25, 40 and 60 kDa, whereas in crab haemolymph they were 20 and 25 kDa. In conclusion, crustacean haemolymph has the ability to clear the external pathogens from the haemolymph.

#### AH PO 10

### Antimicrobial activity of fish blood against three pathogenic bacteria and detection of serum proteins

K. VIDYA, N. RANJIT KUMAR, G.K. SIVARAMAN, V. MURUGADAS, M.M. PRASAD\*

ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India; \*prasadmm@hotmail.com

Fish blood contains several antibacterial proteins that play very important role in the immune responses especially innate immune system which has garnered lesser attention when compared to higher vertebrates. The present study reports the antimicrobial property of fish blood on commonly occurring fish pathogens. The reference cultures employed in this study included *Pseudomonas aeruginosa* (ATCC

10145), *Edwardsiella tarda* (ATCC 15947) and *Aeromonas hydrophila* (ATCC 35654). Fish blood was aseptically drawn from the caudal vein using 23 gauge sterile needle. The bacteria were inoculated into sterile Brain-heart infusion (BHI) broth and 1% blood was added to the broth. After inoculation, broth was incubated at 30°C for 48 h with observations on the bacterial density using spectrophotometer at 4, 8, 16, 24 and 48 h intervals. For comparison, readings of two sets of control i.e. one with BHI broth & blood and another BHI broth with bacteria (without blood) were taken. The optical density was measured at 4, 8, 16, 24 and 48 h in blood supplemented broth for *A. hydrophila* (0.69, 1.28, 1.50, 1.50, 1.54); *P. aeruginosa* (0.51, 0.73, 0.92, 0.96, 1.03) and *E. tarda* (0.60, 0.90, 1.23, 1.25, 1.32), whereas increase in optical density for *A. hydrophila* (1.27, 1.50, 1.72, 2.16, 2.38); *P. aeruginosa* (0.82, 1.18, 1.54, 1.74, 1.94) and *E. tarda* (1.03, 1.74, 1.97, 2.14, 2.17) in control broth (without blood) was noticed at same time periods. The clear inhibition of growth was noticed for the three pathogens in BHI broth supplemented with fish blood when compared to control broths. The presence of antimicrobial substances in the fish blood is responsible for the inhibition of growth. The antimicrobial substances present in the fish serum was analysed with 10% SDS-PAGE, which revealed proteins of 20, 22, 23, 33, 34, 44, 45, 46 and 65 kDa. In conclusion, growth of fish pathogenic bacteria is inhibited by the presences of antibacterial proteins in fish blood.

#### AH PO 11

### Haematological, biochemical and antibacterial response of andrographolide in *Labeo rohita* fingerlings