

in the periphery of the nuclear membrane of the cells. In the second type, granules had a peripheral electron dense area and a vacuolated central portion. Occasionally these vacuolated areas contained fibrous material or crystalloid inclusions. A few mitochondria were also seen in the cytoplasm. The nuclear chromatin appeared condensed on the periphery of the nucleus. Monocytes had abundant cytoplasm with a number of mitochondria and vacuoles. Some of the cells had Golgi apparatus and a few stacks of rough endoplasmic reticulum. Cytoplasm also contained lysosomes. Lymphocytes had very large nucleus and contained very prominent euchromatin and heterochromatin. The cytoplasm contained a few mitochondria and some amount of rough endoplasmic reticulum and abundant polyribosomes. The cytoplasm of thrombocytes contained numerous vacuoles which were interconnected and opened to the exterior through fenestrae. Nucleus was large and consisted of both euchromatin and heterochromatin.

AH PO 01

Effect of organic acids on water microflora used for larval rearing of fishes

N. RANJIT KUMAR¹*, P.K. PATIL², G.M. SIDDAIAH³,
ABHAY KUMAR¹, M.M. PRASAD¹

¹ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India; ²ICAR-Central Institute of Brackishwater Aquaculture, Chennai, Tamil Nadu, India; ³ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha, India;
*nranjeetkumar@gmail.com

Ban on antibiotics usage due to residue problem and development of resistance forces the aquaculturists to find alternative strategies for controlling bacterial infections. In this context, organic acids can be used as one of the alternative for controlling bacteria

mainly the pathogenic *Vibrio* spp. The present study reports the assessment of efficacy of different concentration of organic acids (%) viz., 1, 0.2, 0.1, 0.075, 0.05, 0.025 on total aerobic bacterial count (TPC) and presumptive vibrio count (PVC) from water samples collected from milk fish larval rearing tank, at different time intervals (10, 15, 30 and 60 minutes). The study was conducted separately for all organic acids at six concentrations. Complete inhibition of bacterial flora was observed up to 0.1% for all organic acids at all different time intervals ($p < 0.05$) but for all acids of concentrations below 0.1%, TPC and PVC at different time intervals ranged from 3.19 to 5.75 log cfu mL⁻¹ and from 2.22 to 3.58 log cfu mL⁻¹, respectively. In contrast, TPC and PVC ranged from 6.37 to 6.90 log cfu mL⁻¹ and 4.35 to 4.74 log cfu mL⁻¹ for the untreated water samples (control). Organic acid treated water samples showed a significant decline ($p < 0.05$) in both the TPC and PVC for the concentrations 0.075, 0.05 and 0.025 of all acids compared to control. From the study, it was concluded that the effective concentration of organic acids should be lesser than 0.1% for treating the water used for larval rearing of fishes and the organic acid treated water may be used for larval rearing of fishes at lesser concentrations, since there is decline in pathogenic vibrio count. Further studies are necessitated to evaluate the concentration of organic acids on the survival of the fish larvae.

AH PO 02

Isolation and identification of haemolytic *Vibrio parahaemolyticus* from haemolymph of cultured shrimp *Litopenaeus vannamei*

N. RANJIT KUMAR*, S. EZHIL NILAVAN, V.N.
SREEJITH, T. MUTHULAKSHMI, K. AHAMED BASHA,
M.M. PRASAD

ICAR-Central Institute of Fisheries Technology, Kochi, Kerala,
India; *nranjeetkumar@gmail.com

The pandemic pathogen *Vibrio parahaemolyticus* is normal inhabitant of the marine environment which causes pathogenesis in both the human beings (gastroenteritis) as well as farmed aquatic animals including shrimps (vibriosis and acute hepatopancreatic necrosis disease). In the present study, *V. parahaemolyticus* was isolated from the haemolymph of the widely cultured shrimp, *Litopenaeus vannamei*. The cultured shrimps were brought from local shrimp farm in Ernakulam district to the laboratory and haemolymph was drawn aseptically. The haemolymph was enriched overnight in 3% alkaline peptone water, streaked on TCBS agar and incubated at 30°C for 24 h. Ten well isolated colonies were picked, purified and haemolytic studies were carried out on human blood agar. Among ten isolates, four isolates that produced distinct zone of β -haemolysis on blood agar plate were selected and biochemical identification was carried out using API 20 NE identification system. The results revealed that the four isolates were *V. parahaemolyticus*. PCR detection was carried out using the species specific ToxR gene primers and all the four isolates produced the 368-bp amplicon that is specific to *V. parahaemolyticus*. Thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) are considered to be major virulence factors of *V. parahaemolyticus*. Though the four isolates were haemolytic on human blood, none of the strains were positive for TDH and TRH genes which suggest that some unknown factors might have been responsible for haemolysis. It was concluded that the haemolymph of shrimp harbours pathogenic bacteria such as *V.*

parahaemolyticus which invade when the animal is under stress and produce disease mainly by the production of haemolytic enzymes. Therefore, proper management of the farms has to be carried out regularly to prevent the outbreak of disease.

AH PO 03

Antimicrobial susceptibility of *Lactococcus garvieae* recovered from acute haemorrhagic septicaemia of farmed rainbow trout (*Oncorhynchus mykiss*) in India

S. NEETU*, S.K. MALLIK, S. CHANDRA

ICAR-Directorate of Coldwater Fisheries Research, Bhimtal,
Uttarakhand, India; *shahineetu@rediffmail.com

The aim of this study was to describe bilateral/unilateral haemorrhagic exophthalmia in farmed rainbow trout (*Oncorhynchus mykiss*) of northern Himalayan region of India, and to determine effective antimicrobial treatment. Between March 2013 and October 2016, 38 naturally diseased fish with clinical signs of melanosis, ocular haemorrhage, corneal opacity, exophthalmia, anal prolapsus and haemorrhages in buccal region were obtained from two rainbow trout farms, where the disease had been observed during warmer months, when water temperature elevated over 19 °C. Fifteen *Lactococcus garvieae* strains were isolated from eye, kidney, brain, spleen and lower intestine of diseased fish. All the isolates were found susceptible to penicillin-G, ampicillin, amoxicillin, cefalexin, ofloxacin, tetracycline, vancomycin, ciprofloxacin, chloramphenicol, florfenicol and erythromycin, whereas resistant to oxytetracycline, clindamycin, cloxacillin, oxacillin and amphotericin.