

(Macherey-Nagel, Bethlehem, USA), DNAzol reagent (Thermo Fisher Waltham, USA) and 4 conventional protocols viz., phenol:chloroform:isoamyl alcohol method, protocol for DNA from human stool, total nucleic acid extraction protocol (Hodgson, 2000) and CTAB/EDTA method of DNA extraction (protocol developed by the University of Exeter, UK) were examined for the extraction of DNA from the stomach, hepatopancreas and intestine of shrimp for NGS analysis. The DNA was assessed for concentration, purity and integrity by spectrophotometry and agarose gel electrophoresis. Except for the CTAB/EDTA method, none of the tested protocols resulted in genomic DNA of desired quality for MiSeq - Illumina sequencing. However, when tested with fish tissues, all the extraction methods except DNAzol yielded sufficient quantity of DNA of desired quality. The inability of DNA extraction kits to yield sufficient DNA from shrimp gut might be due to either enzymatic degradation of DNA or the presence of some inhibitors which compromise the DNA binding ability of silica columns. The CTAB/EDTA DNA extraction method evaluated and standardized in this study is a reliable and cost-effective method of extraction of shrimp DNA for metagenomic studies.

AH PO 37

An emerging pathogen of marine finfish culture *Photobacterium damselae* sub sp. *damselae* from southeast coast of India

M. ROSALIND GEORGE^{1*}, K. RIJI JOHN¹, M.M. MANSOOR¹, S. EZHIL NILAVAN², M. PETCHIMUTHU¹, M.S. KAVITHA¹

¹Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India; ²ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India; *rosalindmg@yahoo.com

Hheavy mortality in cage farmed cobia (*Rachicentron canadum*) in the southeast coast of Tamil Nadu occurred during the summer of 2016 was investigated. About 60% of the fish died following a few days of high temperature and low wind in the coastal region. The fish had an average size of 27.5 cm length and 200 g weight. The affected fish had focal erythematous lesions on the ventral distended abdomen, peritoneal serosanguinous fluid accumulation, splenomegaly and swollen kidney. Bacteriological examination of the peritoneal fluid, kidney and spleen revealed the presence of Gram negative short rods in large numbers. The bacteria grew as green colonies on thiosulphate citrate bile salt sucrose agar (TCBS). The tissues were also screened for the presence of nervous necrosis nodavirus by PCR and cell culture but did not yield positive result. The bacterial strains isolated from the tissues were identified as *Photobacterium damselae* sub sp. *damselae* (*Pdd*) using API 20 E microbial identification kit. The species was confirmed by multiplex PCR for 16S rRNA and urease gene. Sequence analysis of 16S rRNA showed 99% homogeneity with *P. damselae* subsp. *damselae*. One of the isolates was used for the pathogenicity study in sea bass (*Lates calcarifer*) fingerlings (4.1 g average weight and 6.9 cm average length). The experimental infection was done by intraperitoneal administration of 50 µl of two dilutions of the culture at 4.2×10^5 and 4.2×10^3 cfu/fish. The experiment revealed that the bacteria were highly pathogenic to seabass and could induce 100% mortality in 36 h. The same bacteria were re-isolated from kidney sample of moribund fish and were confirmed as *Pdd* with API test kits and 16S rRNA sequencing. The isolates were also screened for the presence of virulence genes.