

Microflora of *Artemia* nauplii hatched under experimental conditions

○Haruka Kurihara, Hitomi Nimura, Konomi Sekita, Yusuke Tanaka,
Shiro Itoi and Haruo Sugita
(Department of Marine Science and Resources, Nihon University)

Artemia nauplii are widely used as live food for larvae and juveniles of fish and crustacean. However, since the *Artemia* nauplii are often contaminated by fish pathogens such as *Listonella anguillarum*, the nauplii are considered to be one of infection sources in cultured fish. Therefore, this study was performed to investigate the microflora of *Artemia* nauplii hatched under experimental conditions for the better understanding of the microbiological risks in the aquaculture management.

Artemia cysts were chemically decapsulated and incubated in both natural and sterile seawater. Hatched *Artemia* nauplii were homogenized, serially diluted and plated onto 1/20PYBG agar medium. The inoculated 1/20PYBG agar plates were incubated at 25°C for seven days under aerobic conditions. After then, approximately 20 bacteria were isolated from each plate and subjected to the identification to species. Concomitantly, rearing water samples were processed similarly. The 16S rDNA were amplified from each bacterial isolate by the PCR (polymerase chain reaction) method. The 16S rDNA nucleotide sequence was analyzed by a model ABI 3130xl Genetic Analyzer. The similarity in 16S rDNA sequences between a given isolate and other organisms was compared with all sequence data in the DDBJ/EMBL/GenBank databases using the BLAST algorithm and the identification to species was confirmed by phylogenetic analysis using Clustal W software package.

Bacterial populations associated with *Artemia* nauplii were mainly composed of *Planococcus* and *Psychrobacter*, almost of which seemed to be halotolerant. The *Artemia* nauplii hatched in the sterile seawater were mainly colonized with *Psychrobacter* and *Bacillus* while those hatched in the natural seawater were colonized with *Vibrio*. This finding demonstrates that *Artemia* nauplii have high affinity with members of *Vibrio* rather than bacterial species associated with *Artemia* cysts, suggesting that *Artemia* nauplii are easily contaminated with pathogenic vibrios.