Aquaculture Medicine

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Bioencapsulation as a mode of drug delivery in marine larval rearing

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Successful marine larval rearing depends largely on the effective prevention and control of diseases, such as bacterial infections. The standard procedure to treat them is by adding prophylactic or therapeutic quantities of agents to the culture water. Marine larval rearing involves feeding the hatched larvae with suitable live feeds (diatoms, rotifers, copepods, nematodes, *Artemia* nauplii and metanauplii, mysids etc.). Most often live feeds are the primary source of bacterial contamination in rearing systems. By virtue of their size and feeding habits, most live feeds are size specific filter feeders. Therefore, it is possible to incorporate into the live feed particles say an antibiotic or therapeutic drug like Romet-30 or a probiotic organism of the appropriate size. This process called bio-encapsulation is thus an innovative means of delivering drugs and probiotic organisms to the larvae. Indeed, for fish larvae that are active sight feeders, it is the only effective means of drug delivery.

The uptake of drugs by *Artemia* metanauplii has been modeled as a batch adsorption process, and in this manner, one can calculate the dosage required for a given population of nauplii. Experiments have also been designed to determine the loss of drugs in nauplii after the bio-encapsulation period. The pharmacokinetics of trimethoprim (TMP), sulfamethoxazole (SMX) and its metabolite N-acetyl-sulfamethoxazole (N-acetyl-SMX), have been studied in *Artemia* nauplii as a function of the duration and temperature of their storage, following their enrichment with the therapeutics using the bio-encapsulation technique. A marked decrease in the therapeutic content of the nauplii was observed upon storage at 18 and 25°C and it was concluded that medicated nauplii should either be administered fresh to fish larvae or after storage for 8 h at 5°C, at the most.

New HPLC analytical methods have been developed for the quantification of the water soluble antibiotic Oxytetracycline in *Artemia* nauplii and of the lipid soluble antibiotic sarfloxacin in nauplii and fish larvae. Oxytetracycline was incorporated in liposomes, which were then administered to the nauplii. Ascending concentrations of liposomal OTC in the enriching medium resulted in proportional increases of OTC levels in the nauplii, although the use of liposomes appeared to be a rather expensive method for use against *Vibrio* infections of fish.

In another study, post larvae of shrimp *Penaeus monodon* were fed *Artemia* sp. enriched with oxytetracycline (OTC). The amounts of this antibiotic in the *Artemia* sp., the shrimp, and the water of the experimental system were measured by radial

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diffusion bioassay. The results indicated that 11% of the OTC was taken up by the *Artemia* sp. After 8 d of being fed OTC-enriched *Artemia* sp., the shrimp were found to contain 3.12 mg OTC/shrimp, which is twelve times greater than the average minimum inhibitory concentration (MIC) cited in the literature for sensitive strains of *Vibrio* spp. The recommended therapeutic dose for treatment of bacterial infections is four times the MIC.

Studies have also been made on the efficiency of *Artemia* nauplii in bio-encapsulating bacteria and this indicates that it strongly depends on the type of bacteria used, time of exposure, and status (live or dead) of the bacteria.