

## **Distribution of a fish pathogen *Listonella anguillarum* in the flounder *Paralichthys olivaceus* hatchery**

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### **Introduction**

*Listonella anguillarum* (= *Vibrio anguillarum*) often causes vibriosis for the larvae and juveniles of various marine fish such as red sea bream (*Pagrus major*), tiger puffer (*Takifugu rubripes*) and flounder (*Paralichthys olivaceus*). Effective prevention of the vibriosis needs to understand the ecology of this bacterium in the hatchery. Therefore, the present study was undertaken to investigate the distribution of *L. anguillarum* in a flounder hatchery.

### **Materials and Methods**

Bacterial (2704 isolates) were isolated from the developing fish, live diets and artificial feeds of flounder and their rearing water using marine agar and thiosulfate-citrate-bile salt-sucrose (TCBS) agar. *L. anguillarum* (439 isolates) were identified by a combination of the incubation on TCBS agar at 35°C overnight with the polymerase chain reaction (PCR) detection for the *VAHI* hemolysin gene.

### **Results and Discussion**

*Listonella anguillarum* was detected in all seven rotifer samples, with densities of  $2.5 \times 10^3$  to  $4.6 \times 10^6$  colony forming units (CFU)/g. Both the analyzed samples of a microalga, *Nannochloropsis oculata* contained this bacterium at densities of  $1.6 \times 10^4$  to  $1.4 \times 10^5$  CFU/g. *L. anguillarum* was detected in only one of four samples of *Artemia* nauplii with a density of  $4.8 \times 10^5$  CFU g<sup>-1</sup> (35%) and it was not detected in the two analyzed artificial feed samples. *L. anguillarum* was detected in 11 of 18 specimens of larval and juvenile flounder at densities of  $5.0 \times 10^1$  to  $7.4 \times 10^5$  CFU/g, while it was not detected in the two analyzed egg specimens of flounder. These results indicate that *L. anguillarum* associated with the developing flounder is likely derived from rearing water and live diets such as rotifers. Further, it is strongly suggested that *L. anguillarum* is a transient bacterium of the intestinal microflora for the flounder but is a permanently indigenous one for the flounder hatcheries.