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Detection of *Vibrio penaeicida* in Kuruma Prawn after Transport

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In Japan, vibriosis caused by *Vibrio penaeicida*[1] usually occurs in cultured kuruma prawn (*Penaeus japonicus*) during summer and fall[2,3]. The causative bacterium can easily be detected from overtly diseased prawns, but from apparently healthy prawns the detection rate is low. The results of the conventional isolation method of the pathogen does not seem to reflect the true carrier rate because more prawns often come into overt infection after collection-transport-acclimation procedures, although such data have not been published.

In the present study, transport stress was given to apparently healthy prawns to verify the above phenomenon.

Materials and Methods

Experiment 1: Apparently healthy kuruma prawns were collected from a farm in Kumamoto Prefecture and transported to the Kumamoto Prefectural Fisheries Research Center in a container with aerated seawater on a truck. The transport time was approximately 30 min. A group of 30 prawns weighing 4g were stocked immediately in a tank with sand bed supplied with aerated flowthrough seawater and kept at 23–25°C at the research center to monitor mortalities for 3 days. The dead prawns were confirmed to be infected with *V. penaeicida* by bacterial isolation followed by slide agglutination using an anti-*V. penaeicida* (KH-1) rabbit serum.

Experiment 2: Kuruma prawns weighing 7 g were collected from another farm in Kumamoto Prefecture and transported as above. The prawns were divided into 4 groups of 20 prawns each and kept under the previously described condition. In every sampling time at 0 (just after transport), 1, 3, and 5 days post-transport, the lymphoid organs, which were reported as the target organ in this disease,[4] were taken aseptically from all prawns of one group (20 prawns), dead or alive, then homogenized in 1/2 strength seawater. Three 10-fold serial dilutions were made and 0.1 ml from each dilution was plated onto ZoBell's 2216E agar. Plates were incubated at 25°C for 48 h and *V. penaeicida* colonies were confirmed using the antiserum.

Experiment 3: Prawns weighing 8 g were collected from a farm in Hiroshima Prefecture, transported in a box filled with cooled dry sawdust for about 1 h, and stocked in a tank at about 28°C at the Fisheries Laboratory of Hiroshima University. Bacterial isolation was made on site before transport and 0 (just after transport), 1 and 3 days post-transport using 20 prawns each by the same method as above.

Results and Discussion

As shown in Table 1 (Expt. 1), a total of 63% of the prawns died from vibriosis during 3 days post-transport in spite of their healthy appearance at the farm. The detection rate of the pathogen from transported prawns in Experiment 2 was 50% immediately after transport and increased with the lapse of time post-transport (Table 2). In Experiment 3, detection rate of the pathogen was 15% even after 3 days post-transport and no mortality was observed (Table 2).

These results demonstrated that some (Expt. 3) or most (Expt. 2) of apparently healthy prawns harbor *V. penaeicida* and a reliable figure of carrier rate can be obtained by providing prawns with transport stress and appropriate incubation period. The detection rates of the pathogen were much different be-

Table 1. Cumulative mortality among apparently healthy kuruma prawns after transport (Experiment 1)

Days after transport	Cumulative mortality (%)* (n=30)
0	0
1	27
2	60
3	63

* *V. penaeicida* was isolated from all the dead prawns.

Table 2. Detection rate of *V. penaeicida* in kuruma prawns during 3 or 5 days post-transport (Experiments 2 and 3)

Days after transport	Detection rate % Expt. 2 (n=20 × 4)	Expt. 3 (n=20 × 4)
Before	NT*[1]	0
After 0	50 (10+0)*[2]	0
1	75 (15+0)	10 (2+0)
3	65 (11+2)	15 (3+0)
5	100 (16+4)	NT

*[1] NT: not tested, *[2] :(number of *V. penaeicida*-positive prawns, alive + dead, among 20 prawns).

tween the experiments 2 and 3. The lower detection rate in the Experiment 3 may be due to the less stressful method (in a box with sawdust) of transport as well as the intrinsic low carrier rate.

Stress can induce immune suppression in crustaceans, for example, thermal stress reduces the number of hemocytes and levels of phenoloxidase in the crab (*Carcinus maenas*)[5]. It is thought that a similar immune suppression had occurred in transported kuruma prawns in this study. Simulated transport procedure can be used to estimate carrier rate of cultured prawns as with the case in furunculosis of salmonids[6].

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