Experimental Infection of Southern Flounder *Paralichthys lethostigma* with *Neoheterobothrium hirame* (Monogenea: Diclidophoridae)

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**ABSTRACT**—Southern flounder *Paralichthys lethostigma* distributed in the Atlantic waters of North America and Japanese flounder *P. olivaceus* were challenged with the monogenean *Neoheterobothrium hirame*, known so far as a parasite of Japanese flounder. Mature worms were obtained from the challenged flounders, both Japanese flounder and southern flounder, although the infection level was higher in the Japanese flounder. The result indicates that southern flounder as well as Japanese flounder can serve as the host of *N. hirame*.

**Key words:** *Neoheterobothrium hirame*, *Paralichthys olivaceus*, *Paralichthys lethostigma*, Monogenea, flounder

The monogenean *Neoheterobothrium hirame* suddenly appeared as a new species in 1993 and has since prevailed in wild and cultured Japanese flounder, *Paralichthys olivaceus*, in the waters surrounding Japan (Michine, 1999; Ogawa, 1999; Yoshinaga et al., 2000; Anshary et al., 2001; Mushiake et al., 2001). This parasite is considered to be the causative agent of the anemia that has recently spread in Japanese flounder (Yoshinaga *et al.*, 2000, 2001a, 2001b; Mushiake et al., 2001). The prevalence of the parasite and the occurrence of anemia were found to be roughly 60% and 30%, respectively, in wild Japanese flounder collected from various waters around Japan (Mushiake *et al.*, 2001). It is therefore a growing concern that the parasite may have a serious negative impact on the wild stocks of Japanese flounder. In the waters around Japan, *N. hirame* has been recorded only from Japanese flounder. Monogeneans tend to be highly host-specific, but there is a possibility that *N. hirame* could infect closely related fish species if introduced in their areas of distribution. Thus, it is necessary to know the host range of *N. hirame* to prevent possible damage that may occur if the parasite is introduced into areas outside Japan. In this context, an attempt was made to challenge southern flounder *P. lethostigma* naturally distributed in North America, with *N. hirame* in this study.

**Materials and Methods**

Southern flounder (total length: 22–27 cm) and Japanese flounder (total length: 24–30 cm) that had been reared without a history of infection with *N. hirame* at the Central Laboratory of the Marine Ecology Research Institute (Onjuku, Chiba, Japan) and the National Research Institute of Aquaculture (Nansei, Mie, Japan), respectively, were used for the experiment. A challenge experiment with the parasite was carried out as previously described (Yoshinaga *et al.*, 2001a). Briefly, replicates of 5 southern flounder and 5 Japanese flounder were placed together in each of two tanks (150 L). A mesh bag (mesh opening 108 µm) containing either 1600 (low-level) or 4300 (high-level) worm eggs collected from Japanese flounder artificially infected with *N. hirame* was suspended in each tank. The fish were reared in running sea water (water turn-over rate, 30–50
times/day) at 20°C and fed commercial pellets. One southern flounder died of an unidentified cause in the low-level challenge tank during the experiment. On Day 45 post challenge, one southern flounder in the low-level challenge tank was sacrificed and parasitologically examined. On Day 56 post challenge, all the remaining fish were sacrificed and parasitologically examined. The parasitological examination was conducted as follows: the buccal cavity was macroscopically examined for mature worms and the gills were examined for immature worms using a stereomicroscope. All worms collected from the southern flounder and some of worms collected from the Japanese flounder were flattened, fixed in AFA fixative and stained with Semichon’s acetocarmine for morphological observations. The total worm length and the diameters of the most posterior pair of the clamps were measured, and the ratios of the body proper length and isthmus length to the total body length were determined as the features differentiating N. hirame from the most similar species Neoheterobothrium affine.

Results

In the challenge experiment, gravid worms having eggs in the uterus were obtained from the buccal cavity lining of both the Japanese flounder and the southern flounder (Table 1). While worms were observed on both the upper jaw lining and the lining between the pharyngeal teeth in the Japanese flounder, they were found only on the lining between the pharyngeal teeth in the southern flounder. The infection levels were higher in the Japanese flounder than in the southern flounder. No immature worms were obtained from any of the fish on Day 45 or 56. No marked differences were observed in the body size or in the relative ratios of the body parts between the worms collected from the different fish species and from the different infection sites (Table 2).

Discussion

It is clear from the present results that southern flounder can serve as a host of N. hirame, although they were less susceptible to N. hirame infection than Japanese flounder.

Another species of the genus, N. affine, has been recorded from southern flounder (Price, 1943). This parasite is closely related to N. hirame and can be distinguished from N. hirame by the difference in the ratios of the body proper and isthmus length to the total body length; the respective ratios have been found to be 0.62–0.70 and 0.14–0.24 in N. hirame and 0.34–0.51 and 0.38–0.52 in N. affine (Ogawa, 1999). Although we expected that differences of the host species and infection sites may have influenced on the ratios, the ratios of the worms obtained from both the southern flounder and the Japanese flounder were indistinguishable from those of N. hirame described in the original description (Ogawa, 1999). However, in the original description, the discrimination between the two species was based on only 4 specimens of N. affine, including the type specimen, collected from summer flounder Paralichthys dentatus, which is the type host of N. affine. More studies are needed for further conclusions on their discrimination.

Table 1. Experimental infection of southern flounder (SF) and Japanese flounder (JF) with Neoheterobothrium hirame

<table>
<thead>
<tr>
<th>Challenge level</th>
<th>Day post challenge</th>
<th>Host</th>
<th>No. fish infected / No. fish examined</th>
<th>Intensity Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>45</td>
<td>SF</td>
<td>1/1</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>SF</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>JF</td>
<td>4/5</td>
<td>1 (1)</td>
</tr>
<tr>
<td>High</td>
<td>56</td>
<td>SF</td>
<td>2/5</td>
<td>2.5 (2–3)</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>JF</td>
<td>5/5</td>
<td>24.8 (6–43)</td>
</tr>
</tbody>
</table>

Table 2. Measurements of Neoheterobothrium hirame obtained from southern flounder (SF) and Japanese flounder (JF)

<table>
<thead>
<tr>
<th>Host</th>
<th>Habitat</th>
<th>N</th>
<th>TL (mm) Mean (Range)</th>
<th>Clamp (μm)*2 Mean (Range)</th>
<th>BP/TL*3 Mean (Range)</th>
<th>IS/TL*4 Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>Pharynx lining</td>
<td>5</td>
<td>14.2 (12.0–17.1)</td>
<td>351 (321–366)</td>
<td>0.65 (0.60–0.70)</td>
<td>0.23 (0.17–0.32)</td>
</tr>
<tr>
<td>JF</td>
<td>Pharynx lining</td>
<td>14</td>
<td>14.0 (10.9–16.3)</td>
<td>353 (289–407)</td>
<td>0.63 (0.55–0.70)</td>
<td>0.23 (0.15–0.34)</td>
</tr>
<tr>
<td>JF</td>
<td>Upper Jaw lining</td>
<td>8</td>
<td>15.4 (13.1–16.7)</td>
<td>357 (297–420)</td>
<td>0.64 (0.58–0.71)</td>
<td>0.23 (0.16–0.30)</td>
</tr>
</tbody>
</table>

*1: the number of worms examined
*2: the diameter of the most posterior clamps
*3: the ratio of the body proper length to the total length
*4: the ratio of the isthmus length to the total length
The earliest record of *N. hirame* was obtained from Japanese flounder caught in the central part of the Sea of Japan in 1993; *N. hirame* had not been observed in the flounder collected from the same locality from 1989 to 1992 (Anshary et al., 2001). Considering that Japanese flounder is one of the most studied fish species in Japan and the parasite has been found to be widely distributed in areas with different environmental conditions in Japan (Mushiake et al., 2001), it is unlikely that the parasite had existed at low levels and suddenly increased in the flounder population to detectable levels.

On the other hand, all the members of the genus *Neoheterobothrium* other than *N. hirame* have been recorded from pleuronectiformes living in the waters surrounding North and South America (Piasecki et al., 2000), and the genus seems to have evolved in the pleuronectiformes in this area. The sudden appearance of *N. hirame* in the waters around Japan suggests the possibility that the parasite may have been recently introduced into Japan from the waters around the American continents. However, *Neoheterobothrium* species in the American waters have not been investigated enough to discuss the original distribution of *N. hirame*. Further studies are also needed to clarify this point.

Although the presence or absence of *N. hirame* in North America has not been clarified yet, we should be very careful with any oversea transportation of Japanese flounder just in case. The parasite could spread to North America using the southern flounder as a host if introduced there, and could cause ecological and economic damage.

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**References**


