or the age of the fish could have altered the pathological picture.

Summary:
Moderate mortality occurred in March 1993 among ca. 9 cm. long, farmed turbot fry suffering from a severe general oedema with anecia and in many cases rostral protusions. By electron microscopy the outbreak could be associated with herpesvirus-like particles, contained in giant cells in gills and epithelium, and similar to herpesvirus scophthalmi.

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References

SUSCEPTIBILITY OF SOME FRESHWATER SPECIES OF FISH TO INFECTION BY CYTOPHAGA JOHNSONAE

BY M. SOLTANI* B. MUNDAY** & J. CARSON***

Introduction
Although Cytophaga johnsonae has been isolated from a variety of diseased freshwater fish (Christensen, 1977; Lehmann et al., 1991; Riminakil and Bernardet, 1993) its role as a pathogen is uncertain. More direct evidence of pathogenicity has been provided by the isolate of C. johnsonae from juvenile bream (Lates calcarifer) with severe cutaneous erosion (Carson et al., 1993). Of the various reports where C. johnsonae has been isolated from diseased fish, attempts were not made to reproduce infection experimentally. This communication reports experimental induction of the disease in juvenile bream using C. johnsonae from infected bream; in addition, the inability to produce lesions in a number of other species of fish using this strain is described.

Material and Methods
Cultures:
Strain 91/0262 of C. johnsonae (Carson et al., 1993) was used for all experiments. The bacterium was passaged in goldfish (Carassius auratus) and bream 3 times to ensure there was no loss of virulence. For passaging, fish were bated in bacteria at a concentration of 10⁵ cells/ml for 1 hour or injected intraperitoneally with 10⁵ cells/fish. The cultures used for challenge were grown in cytophaga medium (Anacker and Oslad, 1959), with shaking, at 15 or 25°C (Table 1) for up to 72 hours before use.

Figure 1. (A) Dorsal, ventral and anal fin-rot in bream infected by Cytophaga johnsonae. (B) Control fish.
<table>
<thead>
<tr>
<th>No</th>
<th>Method of challenge</th>
<th>Cell no. as cfu</th>
<th>Water temp. (°C)</th>
<th>Fish species</th>
<th>No. fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bath</td>
<td>7.9x10³/ml</td>
<td>25</td>
<td>Goldfish</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Scarification</td>
<td>5x10⁵/ml</td>
<td>30</td>
<td>Goldfish, Guppy</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Scarifying and swabbing</td>
<td>-</td>
<td>15</td>
<td>Goldfish, Guppy, Rainbow trout</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>I.M. injection</td>
<td>4.85x10⁷/ml</td>
<td>25</td>
<td>Goldfish</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>I.P. injection</td>
<td>10²/ml</td>
<td>21</td>
<td>Goldfish</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Immunosuppression and bath</td>
<td>10⁵/ml</td>
<td>25</td>
<td>Guppy</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>Bath</td>
<td>10⁷/ml</td>
<td>25</td>
<td>Barramundi</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>Bath</td>
<td>9.4x10⁶/ml</td>
<td>20</td>
<td>Barramundi</td>
<td>20</td>
</tr>
</tbody>
</table>

- An unchallenged control group was used for each method.
- Challenge time for bath method was 60 minutes except for methods 2 and 8 where fish were exposed to bacteria for 90 minutes.

1) Following anaesthetization, a few scales were removed and the skin lightly scraped with a sterile scalpel blade.
2) Scarifications 10-20x5-10mm were made on the sides of the fish.
3) Scarifications were directly swabbed with plate cultures.
4) Injections were given into the dorsal muscles.
5) Doses used were 10⁷, 10⁶, 10⁵ and 10⁴ cfu/fish.
6) A week prior to challenge, fish were intraperitoneally injected by a single dose (000µg/g B.W.) of trichinose Lactis (TA), and an unchallenged control group treated with TA.
7) Fish were taken from 28°C, immediately challenged at 20°C and then held at this temperature.

Fish stocks: Barramundi, goldfish and guppies (Poecilia reticulata) were maintained in aquaria and rainbow trout (Oncorhynchus mykiss) in a recirculating tank with biofiltration. Water was exchanged 5-10%/day; pH ranged between 7.8-7.5. Juvenile barramundi, 2-5g body weight were maintained at 28°C; goldfish 5-30g body weight and guppies 2-5g body weight were maintained at 15-20°C. Rainbow trout 100-300g body weight were maintained at 12-15°C.

Challenge: Challenge methods are given in Table 1. The fish were observed twice daily for 21 days and any diseased animals removed. All fish with skin or fin lesions were cultured on cytophaga agar; the presence of C. johnsonae was confirmed by the appearance of a characteristic cellular and colloidal morphology and by slide agglutination with rabbit raised polyvalent antisera to C. johnsonae.

Results and Discussion:

No significant lesions were detected in any fish than barramundi and then only in the fish challenged after being changed from water at 28°C to water at 20°C. The lesions induced were not as severe as those described by Carson et al., (1993) and consisted of fin-rot involving especially dorsal and caudal fins (Figure 1). Clinically 45% of fish showed some lesions and exhibited a peculiar swimming behaviour with flexion of the body and rapid rippling of the fins with little actual movement of the fish. Mortality to 21 days after exposure was 25%.

Many factors are involved in predisposing fish to infection with Cytophaga-like bacteria (CLB) and, particularly, to the production of morbidity and mortality associated with these organisms. These factors include fluctuation in environmental temperature, overcrowding, immunity of previously exposed fish, species differences, age, size and condition of fish, differences in strain virulence of the pathogen and interaction with other fish diseases. The outbreak of natural disease in barramundi described by Carson et al. (1993) was associated with a dramatic drop in water temperature from 35°C to 27°C, a situation not dissimilar to that reported for temperature-mediated immunosuppression in channel catfish (Byl and Clem, 1991). It is probably pertinent that disease could only be experimentally produced in barramundi when they were subjected to a sudden drop in water temperature. The reason why barramundi are more susceptible than other species is not entirely clear although preliminary studies suggest that the skin mucus of this species is less inhibitory to C. johnsonae than that of other fish species used (Soltani, unpublished data).

We believe that these studies confirm C. johnsonae as an opportunistic pathogen of barramundi. Also, they illustrate the difficulty of reproducing diseases caused by CLB, and the need to use freshly isolated cultures on fish exposed to environmental conditions similar to those encountered by fish in the wild or held under commercial aquaculture conditions (Ferguson et al., 1991).