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Vibrio harveyi and other bacterial pathogens in cultured summer flounder, *Paralichthys dentatus*

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Abstract

A monitoring program for *Vibrio harveyi* and other potential bacterial pathogens of summer flounder was conducted at two facilities in the Northeast United States. Bacterial samples were collected from larval and juvenile fish and live-feed, and identified using API 20E biochemical profiles and 16S rDNA sequencing. Histopathological examinations were conducted in order to relate histological changes with the presence of potential bacterial pathogens. *V. harveyi*, *Vibrio ichthyoenteri*, and *Photobacterium damselae* subsp. *damselae*, three known pathogens of flatfish, were isolated from diseased summer flounder. Although a high proportion of juvenile summer flounder showed microscopic signs of disease, the presence of these potential bacterial pathogens in fish was not associated with large-scale mortalities. An outbreak of flounder infectious necrotizing enteritis with 30% cumulative mortality occurred when juveniles were transported to a new facility. Isolates of *V. harveyi* from the disease outbreak were genetically different from the isolates from the commercial hatchery. *V. harveyi* isolates from both facilities were pathogenic to summer flounder by intraperitoneal injection. *P. damselae* subsp. *damselae*, *V. ichthyoenteri*, and *Vibrio scophthalmi*, also found in fish with gross lesions, were not pathogenic to juvenile summer flounder by intraperitoneal injection. Our research shows that several potential bacterial pathogens are associated with morbidity and mortality in summer flounder larvae and juveniles, especially in situations of stress. Increased knowledge about the environmental conditions that lead to disease, as well as the interactions of *V. harveyi* with other microbial species, could lead to the development of management strategies in summer flounder farms.

Keywords: *Vibrio*; *Paralichthys dentatus*; Intestinal bacteria; Peritonitis; Enteritis; Bacterial identification; Disease management; Bacterial pathogens; Histopathology

1. Introduction

The summer flounder, *Paralichthys dentatus*, is a valuable species of flatfish on the east coast of the United States. A decline in wild stocks resulted in restrictions on the capture fishery (NOAA/NMFA, 1993) and subsequently sparked interest in commercial culture (Bengtson, 1999; Schwarz, 2003). The first commercial summer flounder hatchery opened in New England in 1996, modeled after flatfish culture industries in Europe and Japan and enabled by research performed at the University of Rhode Island (Bengtson, 1999; Bengtson and Nardi, 2000).

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Disease frequently constrains the development of an economically viable industry for the culture of new fish species. In the case of summer flounder, the first epizootic with a well-defined etiology occurred in the summer of 1998 at a grow-out facility in Rhode Island. Shortly after the first batch of juveniles (3–5 g) arrived at the facility, fish began showing signs of disease including distended abdomens, lethargy, and loss of appetite. Mortalities quickly mounted to approximately 35% of the stock with another 15% of the survivors suffering permanent and debilitating intestinal injuries (Soffientino et al., 1999). The causative agent was identified as *Vibrio carchariae* (synonymous with *Vibrio harveyi*, Pedersen et al., 1998; Gauger and Gómez-Chiarri, 2002) and the disease was named Flounder Infectious Necrotizing Enteritis (FINE). The only other published report of a disease epizootic in cultured summer flounder was caused by a *Mycobacterium* species in a re-circulating system in the southeast US (Hughes and Smith, 2002).

Although the outbreak at the Rhode Island farm is the only serious epizootic of FINE reported in cultured summer flounder to date, fish with clinical signs similar to FINE have been found in other summer flounder facilities at enzootic levels (Smolowitz, unpublished). Fish in which the posterior end of the intestine is sealed into a blind end, a lesion observed in survivors from FINE, are routinely found in summer flounder facilities (Specker et al., 2000; Veillette et al., 2006). Unfortunately, it is not known if these conditions were caused by *V. harveyi*, a well-known pathogen of marine finfish and shellfish (Austin and Austin, 1999), or other pathogenic agents. Eddy and Jones (2002) identified *V. carchariae* and *Photobacterium damselae* in a study of the bacterial community of summer flounder fingerlings, but the health status of the fish was not evaluated in their microbiological monitoring program. As the industry grows, it is important to evaluate the threat that *V. harveyi* and other pathogens pose to summer flounder so adequate management and control strategies can be developed. Towards that end, we conducted a monitoring program for *V. harveyi* and other potential bacterial pathogens at GreatBay Aquaculture (GBA), the major summer flounder hatchery in the Northeast US, and the Ann Durbin Marine Aquarium (ADMA), a research hatchery at the University of Rhode Island Graduate School of Oceanography (GSO). Using a combined genetic and biochemical approach, we identified bacteria associated with the intestine and other tissues of larval and juvenile summer flounder, as well as live-feed. We also conducted histopathological examinations in order to correlate histopathologic lesions with changes in the bacterial community associated with summer flounder. Finally, we performed experimental infections with selected bacterial isolates from summer flounder in order to assess their virulence to this species.

2. Materials and methods

2.1. Summer flounder culture and sampling schedule

Summer flounder larvae and juveniles were cultured at GreatBay Aquaculture (GBA) in New Hampshire and the Ann Durbin Marine Aquarium (ADMA) at the University of Rhode Island (URI) following previously described methods (Bengtson and Nardi, 2000; Eddy and Jones, 2002). Incoming seawater for these facilities is pumped directly from Great Bay (New Hampshire) or Narragansett Bay (Rhode Island) and cooled/warmed to a temperature of 18–21 °C (Table 1). A production run of summer flounder was monitored at GBA for the presence of *V. harveyi* and other potential pathogens from July 10, 2002 to November 19, 2002, and on January 10, 2003, two weeks after fish were transported to the Aquatic Pathology Laboratory (APL) at URI. Fish were transported by truck for approximately 2.5 h (170 km) in sealed plastic bags filled with 15 L of water and inflated with oxygen. Early stages of a second production run were sampled at the ADMA on April 4, 2003 and May 2, 2003. The sampling schedule was designed to observe fish in each stage of development and through changes in feed (Table 1).

2.2. Sampling methods

Samples of algal, rotifer, and enriched 72 h artemia-nauplii cultures (Bengtson et al., 1999) were collected in sterile 1.5-ml tubes prior to being used as green water (algae for larval tanks) or feed (rotifers and artemia), centrifuged to remove excess water, and homogenized using sterile plastic pestles. Healthy looking fish were removed from all or a subset of the production tanks (up to 4), euthanized by a lethal dose of tricane methanesulfonate (MS-222, Sigma), and rinsed in 0.1% benzalkonium chloride to remove surface bacteria (Grisez et al., 1997). At early time-points (53 days post hatch (DPH) or fewer), fish were homogenized in microcentrifuge tubes using sterile plastic pestles. Serial dilutions of the homogenate were made and 100 μl were spread on 2–4 trypticase soy agar (TSA), TSA with 5% sheep blood, thiolsulfate citrate bile salt sucrose (TCBS, Kobayashi et al., 1963), and marine minimal medium (3 M, Neidhardt and Bloch, 1974) plates. At the remaining time-points (102–208 DPH), fish were euthanized and disinfected as described above. Samples for microbiological analysis in healthy-looking fish were taken from the intestine as