Preliminary Studies of the Immune Response of American Eels (Anguilla rostrata) to Glochidia of the Eastern Elliptio Freshwater Pearly Mussel (Elliptio complanata)

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Abstract
American eels migrate from freshwater rivers and streams in the Eastern US to the Sargasso Sea to spawn and die. Their eggs develop in larvae, which drift toward the east coast of North America. They metamorphose into glass eels and subsequently into adults that migrate to upstream freshwater locations, where they mature to the adult stage. The American eel is the obligate host to the glochidial (larval) stage of Elliptio complanata, the Eastern Elliptio mussel. Most freshwater mussels require a fish host for the glochidial stage of their development. The glochidia attach to the gills of the host fish and are encapsulated. The developing glochidia metamorphose to the juveniles that detach from hosts and complete their maturation on stream and river bottom. Non-host fish species infected by glochidia generate glochidia-specific antibodies that cause glochidia to be sloughed off.

Eel migration to the Upper Susquehanna watershed ceased after the construction of hydropower dams in Maryland and Pennsylvania approximately 40 years ago. While eels can live up to 30 years before migrating to the sea, the lack of migration to the Upper Susquehanna has resulted in concomitant reduction in Eastern Elliptio reproduction. The timing of elver migration from coastal regions to tributaries suggests that immunotolerance to Eastern Elliptio is occurring. Eastern Elliptio release glochidia at approximately the time that elvers arrive. If elvers are infected while immunologically immature, they may develop tolerance to the glochidia.

Knowledge of the immune responses of the American eel is limited. Relevant but still limited studies have been done with the European eel (Anguilla anguilla) and the Japanese eel (Anguilla japonica). I propose to examine the immunological response to experimental exposure of glochidia to eels that are (a) wild caught and have been exposed to Eastern Elliptio glochidia in their natural environment, (b) hatchery raised eels that were captured in the Chesapeake Bay as glass eels and reared at the USGS Northern Appalachian Research Laboratory that have never been exposed to glochidia, and (c) Largemouth Bass as a non-host fish. This study will provide important insights into the American eel immune system development and into the lifecycles of both American eel and Eastern Elliptio mussel. These studies will also help shed light on the general question of whether fish can be tolerated to parasites and the specific question of whether fish species other than the American eel can be tolerized to E. complanata glochidia, thereby providing possible alternative hosts for the glochidia.

I, with assistance from numerous undergraduate students, have undertaken preliminary studies to develop immune reagents specific to American eels. Methods have been developed to purify antibodies from Largemouth Bass serum, which methods will be used to purify American eel antibodies. Further, we have shown that a monoclonal antibody produced against European eel IgM is cross-reactive with American eel, which will facilitate development of American eel-specific antibodies.

Eel and Mussel Life Cycles
• Adult silver eels migrate from fresh water to the Sargasso Sea to spawn.
• Leptocephalus larvae drift with currents toward outlets of rivers.
• The larvae metamorphose to glass eels.
• Glass eels migrate upstream and become elvers.
• Elvers mature into yellow eels and remain in fresh water (some reside in brackish water) for many years.
• At maturity, yellow eels become silver eels and repeat the migration to the Sargasso.
• The eggs of freshwater mussels are fertilized inside the shell of the female and mature to glochidia (larvae) before being released.
• They attach to the gills of suitable host fish and metamorphose to juveniles and release and settle into the stream bottom.
• Many species of mussels have strict fish host specificities.

Timing
• Glass eels and young elvers migrate upstream in the spring at approximately the same time that the Eastern Elliptio is releasing glochidia.
• This suggests that the American Eel is being exposed to Eastern Elliptio glochidia while their immune systems are immature, allowing them to become tolerant of future infections and thereby becoming appropriate hosts.

Complications
• Obstructions in the Lower Susquehanna, like the Conowingo Dam, are preventing elvers from migrating upstream.
• Without suitable hosts, the mussels are unable to reproduce.
• One solution would be to simply transport the eels to the obstructions, however the American eel has become infected with an invasive parasite, the swimbladder nematode, Anguillicola crassus. This parasite can also infect other fish.
• While this parasite is in the Hudson, Delaware and Lower Susquehanna river systems, it is absent from the Upper Susquehanna, along with the eels.
• New York State DEC has not granted permission for transporting elvers into the Upper Susquehanna.

Aims of this Study
• To provide insights into the immunological relationship between Eastern Elliptio glochidia and American eels.
• To shed light on the general question of whether fish can be tolerated to parasites.
• To determine whether fish species other than the American eel can be tolerized to Eastern Elliptio glochidia, thereby providing possible alternative hosts for the glochidia.
• To generate immunologic tools to study the immune system of American eels.

Preliminary Results: Monoclonal antibodies that were raised against European eel IgM can be used to detect American eel IgM antibodies.

Methods
• Most samples were collected from wild-caught eels or anesthetized eels via venipuncture of the caudal vein.
• Control samples were collected from anesthetized eels.
• Samples were stored at 0°C and shipped on dry ice.
• Samples were concentrated for five minutes, 3 x 10 minutes.
• Large Mouth Bass serum was used a negative control.

Experimental Design
• Wild-caught eels were exposed to glochidia for five minutes and were visually inspected weekly.
• Captive-raised eels were exposed to glochidia for five minutes and were visually inspected weekly.

Future Studies
• Further studies will be undertaken to optimize conditions for ELISA and Ouchterlonye assays.
• This antibody will be used to study the relationship between American Eel and Eastern Elliptio mussel (Elliptio complanata) in collaboration with an ongoing project to restore the American Eel in the Susquehanna watershed.
• We will investigate the immune response of wild caught eels and captive raised eels from the USGS Northern Appalachian Research Laboratory in Wellsboro, PA to Eastern Elliptio glochidia.

Experimental Design
• If eels are tolertized to Elliptio glochidia, the wild-caught eels should not produce anti-glochidial antibodies, since they were captured in areas where Elliptio are numerous and are presumed to have been exposed as either glass eels or elvers.
• Captive-raised eels did not have the opportunity to become exposed to Elliptio glochidia while in the glass eel and/or elver stages. They will be expected to produce antibody responses.
• A three year project detailing the immune response of eels exposed to glochidia as glass eels and elvers will follow.