Response: ProjectID 35059

Rapid Detection of White Sturgeon Iridovirus in Spawning Fluids, Eggs and Juvenile Tissues of White Sturgeon

Comment: **"The proposal had poorly stated objectives".**

Response:

This project has three very distinct objectives aimed at satisfying Fish Wildlife Program Priorities for White Sturgeon as set forth in *Future Needs: Priorities for the Mainstem/System-wide Fish Wildlife Program Solicitation*. This project addresses two categories recommended for immediate implementation: (1) "Implement a rapid and sensitive iridovirus screening test to provide appropriate risk assessment capabilities for white sturgeon propagation projects". (2) "Investigate the full geographic range of white sturgeon iridovirus within the basin".

Objective 1 (as described in Section 1 short narrative, Section 5, Section 7, Section 9a, c, and f): Develop a field-ready PCR assay for white sturgeon Iridovirus (WSIV).

At present there is an experimental single-round PCR assay developed by Dr. R.P. Hedrick (UCalDavis) for identification of WSIV. However, the PCR primer set for this assay is not very sensitive and would not be suitable for field use. This project proposes to develop PCR primer sets that will be sensitive enough and specific enough for field use. This project proposes to develop a "nested" PCR assay using two different primer sets for the identification of WSIV. Nested PCRs usually increase the sensitivity of an assay enabling the detection of 10 or less target pathogens in a sample. This should be more than adequate for field detection of infected and/or carrier fish.

Objective 2 (as described in Section 1 short narrative, Section 7, Section 9a, c, and f): Provide early screening and risk assessment capabilities for WSIV in potential wild broodstock.

Because sturgeon broodstock must be sexed well prior to actual spawning, the gametes are available for early PCR analysis to detect presence of WSIV. PCR analysis for WSIV can provide propagation managers with information on infection of broodstock allowing for selection of non-infected fish or, at the least, indicate future virus problem in offspring from infected broodstock.

Objective 3 (as described in Section 1 short narrative, Section 7, Section 9a, c, and f): Distribution of WSIV throughout the Columbia River Basin.

Beginning in 2003 samples will be taken non-lethally from all sturgeon taken during sampling surveys within the basin. These samples will be screened for WSIV in order to determine the distribution of the virus in wild fsturgeon throughout the Basin.

Comment: " neither the proposal nor the presentation provided adequate detail on laboratory or genetic assay methods to provide reviewers confidence that the project's goals are likely to be realized ".

Response:

The polymerase chain reaction is an *in vitro* logarithmic amplification of a specific segment of genomic DNA. It is brought about through the use of a specific enzyme, Taq polymerase, that allows two oligonucleotide primers to bind to the target genome and replicate a small segment of that genome. Since the reaction proceeds logarithmetrically, after 20 to 30 cycles sufficient product is amplified to be detected visually by agarose electrophoresis. While this technique has been around for 15 years or so, it has just recently been adapted and validated for the identification of some fish pathogens.

There are two basic types of PCR; single-round using only one set of primers and nested PCR using two sets of primers in a two-round procedure. The nested PCR is much more sensitive and can detect fewer genomic templates in a sample (in some cases as small as one target organism). The nested assay uses one primer set to amplify a segment of the target genome. This first-round product is then used as the template for PCR by a second set of primers targeting a more precise genomic segment within the first-round product.

Objective 1: develop a nested PCR for the detection of WSIV

Task 1: The genome of WSIV will be analyzed using existing software programs for primer development in order to select a number of candidate primer sets specific for WSIV.

Task 2: Selected oligonucleotide primers will be produce on a DNA synthesizer in Dr. Hedrick's lab at U Cal Davis and will be sent to the Abernathy Fish Technology of preliminary testing and optimization.

Task 3: Primer sets will be screened for their sensitivity in detecting WSIV using known positive and negative samples. The $MgCl_2$ concentration of the PCR will be optimized by running the reaction at 1.5 to 4.0 mM in 0.5 mM increments. The annealing temperature of the assay will be optimized between 35^o and 60^o C as set forth in Taylor and Winton (2002).

Goal: To develop a field-ready assay for the detection of WSIV.

Objective 2: Early screening of sturgeon broodstock and offspring for WSIV.

White sturgeon broodstock collection from the wild is usually conducted in March and April. The fish are sexed immediately by surgically opening the body cavity and removing a biopsy of gonadal material to check for gametogenisis. A small portion of this biopsy can be used for PCR screening for the presence of WSIV.

Task 1: Gonadal samples will be taken by all programs propagating white sturgeon within the Columbia River Basin. At present these samples will be submitted by Project 86-50 (CRIFTC), the Kootanai project, and the Canadian sturgeon project. Samples will be transported on ice for WSIV screening by PCR at the Abernathy Fish Technology Center.

Task 2: Samples will be taken from fry and juveniles from all propagation programs during grow-out and just prior to release. Samples will consist of non-lethal fin biopsies, gill biopsies, or barbell clips. Samples will be iced and sent to the Abernathy Fish Technology Center for screening by PCR for WSIV.

Goal: To detect WSIV infections in broodstock prior to spawning in order to allow managers to make appropriate adjustments in propagation practices and avert disease outbreaks.

Objective 3: To determine the distribution of WSIV throughout the Columbia River Basin.

Task 1: Samples will be taken by all sturgeon survey projects within the Basin during their sampling endeavours and sent to the Abernathy Fish Technology Center for WSIV screening. Samples will consist of fin biopsies, gill biopsies or barbell clips. At present ten agencies will participate in this sampling (ODFW, WDFG, IDFG, USGS, USFWS, Kootenai Tribe, Spokane Tribe, Yakima Tribe, U of Idaho, Canadian Bur. F & O).

Task 2: Create a distribution map of WSIV for the Columbia River Basin

Comment: "…., and how the presence/absence ELIZA-type tests are performed to determine."

Response:

Nonsensical sentence. ELIZA-type tests are serologically based and have no bearing whatsoever on this project. PCR is a much more sensitive and accurate method than ELIZA for identifying target pathogens.