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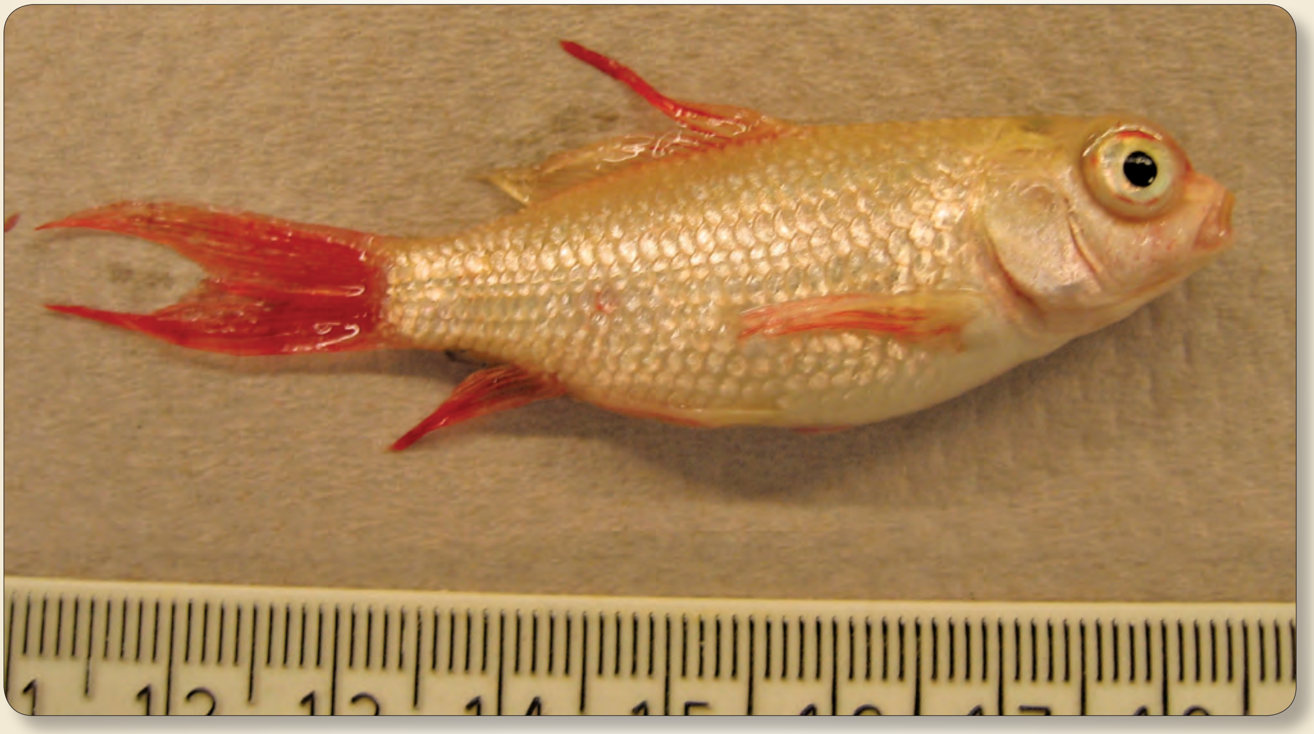
AquaPathogen X: A Template Database for Tracking Field Isolates of Aquatic Pathogens

AquaPathogen X is a template database for recording information on individual isolates of aquatic pathogens and is available for download from the U.S. Geological Survey (USGS) Western Fisheries Research Center (WFRC) website (http://wfrc.usgs.gov). This template database can accommodate the nucleotide sequence data generated in molecular epidemiological studies along with the myriad of abiotic and biotic traits associated with isolates of various pathogens (for example, viruses, parasites, or bacteria) from multiple aquatic animal host species (for example, fish, shellfish, or shrimp). The simultaneous cataloging of isolates from different aquatic pathogens is a unique feature to the AquaPathogen X database, which can be used in surveillance of emerging aquatic animal diseases and clarification of main risk factors associated with pathogen incursions into new water systems. As a template database, the data fields are empty upon download and can be modified to user specifications. For example, an application of the template database that stores the epidemiological profiles of fish virus isolates, called Fish ViroTrak (fig. 1), was also developed (Emmenegger and others, 2011).



Koi (*Cyprinus carpio koi*) infected with an exotic fish virus (spring viremia carp virus) emerging in North America. This fish is exhibiting typical clinical signs of disease, hemorrhaging in all fins and pop-eye.

In recent years, the emergence of aquatic pathogens in North America has escalated, particularly for fish rhabdoviruses (Goodwin, 2002; Elsayed and others, 2006; Garver, and others, 2006). Increased numbers of disease outbreaks have occurred in wild, food-based aquaculture, and ornamental fish stocks. Global fish trade, increased aquaculture production, pathogen evolution, and climate change have been suggested as possible reasons for the rising prevalence of viral aquatic pathogens and ensuing epidemics (Walker and Winton, 2010).

To assess these possible hypotheses, monitoring of the emerging pathogens is required. As the number of aquatic pathogen incidences increases, it is especially important to catalog the epidemiological data associated with each pathogen isolate in order to perform disease risk analyses and discern disease patterns. Proper surveillance and assessment of pathogen incursions also requires consistent data parameters for making valid science-based analyses to support sanitation orders and validate the implementation of disease control policies.

U.S. Department of the Interior
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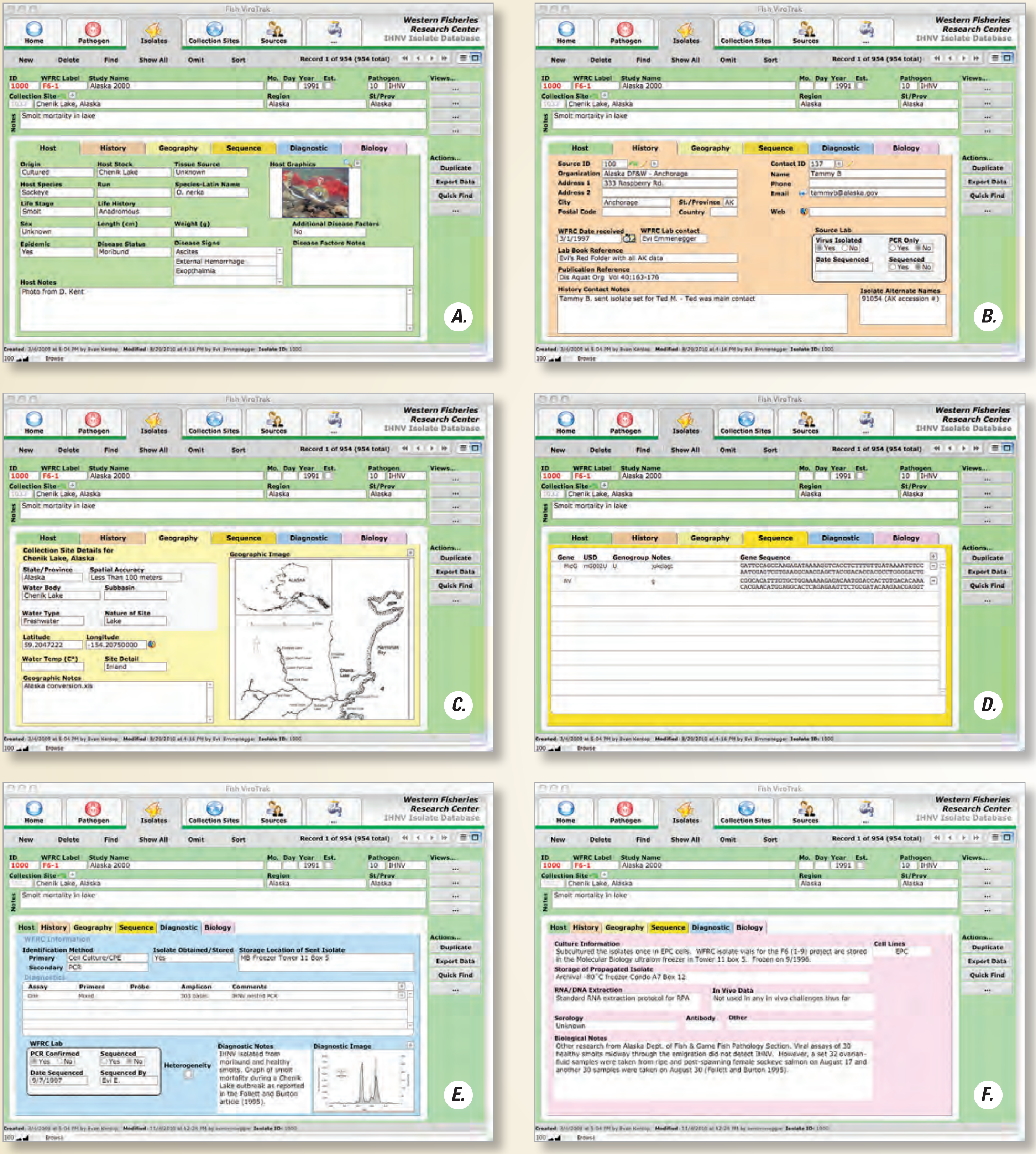

ID 1000 | WFRC Label F6-1 | Study Name Alaska 2000 | Year 1991 | Pathogen 10 IHNV

Collection Site Chenik Lake, Alaska | Region Alaska | St/Prov Alaska

Notes: Smolt mortality in lake

A. Host

Origin: Cultured
Host Stock: Chenik Lake
Tissue Source: Unknown
Host Species: Sockeye
Run:
Species-Latin Name: O. nerka
Life Stage: Smolt
Life History: Anadromous
Sex: Unknown
Length (cm):
Weight (g):
Epidemic: Yes
Disease Status: Moribund
Disease Signs: Ascites; External Hemorrhage; Exopthalmia
Host Graphics
Additional Disease Factors: No
Disease Factors Notes:
Host Notes: Photo from D. Kent

B. History

Source ID: 100
Organization: Alaska DF&W - Anchorage
Address 1: 333 Raspberry Rd.
Address 2:
City: Anchorage
St./Province: AK
Postal Code:
Country:
Contact ID: 137
Name: Tammy B
Phone:
Email: tammyb@alaska.gov
Web:
WFRC Date received: 3/1/1997
WFRC Lab contact: Evi Emmenegger
Lab Book Reference: Evi's Red Folder with all AK data
Publication Reference: Dis Aquat Org Vol 40:163-176
Source Lab: Virus Isolated ● Yes ○ No; PCR Only ○ Yes ● No; Date Sequenced: ; Sequenced ○ Yes ● No
History Contact Notes: Tammy B. sent isolate set for Ted M. - Ted was main contact
Isolate Alternate Names: 91054 (AK accession #)

C. Geography

Collection Site Details for Chenik Lake, Alaska
State/Province: Alaska
Spatial Accuracy: Less Than 100 meters
Water Body: Chenik Lake
Subbasin:
Water Type: Freshwater
Nature of Site: Lake
Latitude: 59.2047222
Longitude: -154.20750000
Water Temp (C°):
Site Detail: Inland
Geographic Notes: Alaska conversion.xls
Geographic Image

D. Sequence

Gene | USD | Genogroup | Notes | Gene Sequence

E. Diagnostic

WFRC Information
Identification Method: Primary Cell Culture/CPE; Secondary PCR
Isolate Obtained/Stored: Yes
Storage Location of Sent Isolate: MB Freezer Tower 11 Box 5

Diagnostics

Assay	Primers	Probe	Amplicon	Comments
One	Mixed		303 bases	IHNV nested PCR

WFRC Lab: PCR Confirmed ● Yes ○ No; Sequenced ○ Yes ● No
Date Sequenced: 9/7/1997
Sequenced By: Evi E.
Heterogeneity ☐
Diagnostic Notes: IHNV isolated from moribund and healthy smolts. Graph of smolt mortality during a Chenik Lake outbreak as reported in the Follett and Burton article (1995).
Diagnostic Image

F. Biology

Culture Information: Subcultured the isolates once in EPC cells. WFRC isolate vials for the F6 (1-9) project are stored in the Molecular Biology ultralow freezer in Tower 11 box 5. Frozen on 9/1996.
Cell Lines: EPC
Storage of Propagated Isolate: Archival -80°C freezer Condo A7 Box 12
RNA/DNA Extraction: Standard RNA extraction protocol for RPA
In Vivo Data: Not used in any in vivo challenges thus far
Serology: Unknown
Antibody:
Other:
Biological Notes: Other research from Alaska Dept. of Fish & Game Fish Pathology Section. Viral assays of 30 healthy smolts midway through the emigration did not detect IHNV. However, a set 32 ovarian-fluid samples were taken from ripe and post-spawning female sockeye salmon on August 17 and another 30 samples were taken on August 30 (Follett and Burton 1995).

Figure 1. Screen captures of data category windows cataloging information for each virus isolate record in the Fish ViroTrak database that include: general pathogen information (continuously displayed at the top of each window); (*A*) host background; (*B*) isolate history; (*C*) geographic/spatial data; (*D*) gene sequences; (*E*) diagnostics; and (*F*) biological factors. The sequence field in panel (*D*) expands upon selection to view any input length of nucleotide sequence. The example epidemiological data displayed in the panels are from an infectious hematopoietic necrosis virus (IHNV) isolate sampled during an epidemic in sockeye salmon at an aquaculture facility in Alaska.

Viral hemorrhagic septicemia virus (strain IVb), is an invasive fish virus emerging in the Great Lakes region. This yellow perch (*Perca flavescens*) is showing evidence of disease (for example, hemorrhaging in the eye and bleeding in a pectoral fin) after exposure to this virus

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Collating accurate and comprehensive aquatic disease information into a computer-based cataloging system is vital to the development of an aquatic pathogen-monitoring program. General aquatic animal disease databases provide accurate overviews of pathogen features, including host specificity, confirmatory diagnostic tests, countries of occurrence, and bibliographies of the listed aquatic animal pathogens, and can be used to document the initial introduction of a pathogen into a new country. The databases do not catalog subsequent incidences of the pathogen, which is needed to monitor the emergence of a novel pathogen, or track the spread of an established pathogen. Researchers attempting to discern disease patterns, or resource managers making critical sanitation decisions at infected sites, require detailed epidemiological information of the aquatic pathogen isolates, such as a geo-referenced isolation site, genetic type, gene sequences, water systems, or host stocks (table 1), to accomplish these tasks.

Table 1. Primary data categories and associated epidemiological information fields available to catalog each pathogen isolate in the AquaPathogen X database.

Categories	Information fields
General Host	Pathogen, In-house label, Record number, Date collected, Collection site origin, Host stock, Run, Tissue, Host species common name, Species Latin name, Life stage, Sex, Life history, Length, Weight, Epidemic, Disease status, Disease signs, Host graphics
History	Source organization, Source contact name, Date received, In-house laboratory contact, In-house laboratory notebook reference, Source laboratory collection information (such as PCR only or pathogen isolated)
Geography	Collection site, State/Province, Water body, Subbasin, Water type, Nature of site (farm, hatchery, spawning channel, or wild), Latitude, Longitude, Spatial accuracy, Water temperature, Site detail, Site graphic
Sequence	Gene region(s) sequenced, Nucleotide sequences, Genogroups, Universal sequence designator (USD)
Diagnostic	Primary identification method, Secondary identification method, Isolate obtained, Diagnostics (such as assay primers or probes), Storage location of original isolate, In-house confirmation (such as PCR or sequencing), Images
Biology	Cell culture, Cell lines, RNA/DNA extraction, *In vivo* data, storage of cultured isolate, serology, antibody reactivity

Development of Template Database: AquaPathogen X

The AquaPathogen X template database was built using FileMaker® Pro, Inc. (2011) (http://www.filemaker.com), a cross-platform user oriented database application. This platform was selected because it has an easy learning curve for a user to customize the application for specific purposes; support is available in 60 countries and 11 languages; it is scalable from single user to multi-user operation; it is a server-based operation with no modification of the AquaPathogen X database; it supports simultaneous Macintosh® and Windows® users in multi-user environment; and it has a fully relational database with open database connectivity (ODBC) capabilities, if required. The database has standard navigation buttons, or the built-in FileMaker Pro keyboard commands and shortcuts. The FileMaker Pro application has built-in features to allow for a wide range of users to modify and customize the database. The template database is distributed with all user and development passwords, which can be customized by the site manager. The AquaPathogen X database currently runs on FileMaker Pro, version 9.0.

Application of Database Template: Fish ViroTrak

At the WFRC, the AquaPathogen X template was used to develop a catalog of fish viral pathogens called Fish ViroTrak. Currently, Fish ViroTrak catalogs isolate data for three aquatic rhabdovirus species, infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), and spring viremia of carp virus (SVCV). For every isolate record, 77 data items are collected and organized into 7 categories: general pathogen information, host background, isolate history, geographic/spatial data, gene sequences, diagnostics, and biological factors (fig. 1). Each category provides several data fields to record specific features associated with a pathogen isolate (table 1).

Database Usage

Any of the epidemiological factors listed in the database can be queried. A navigation page containing the most common search parameters, such as gene sequences, host species, isolation locations, and year of isolation, also is available for rapid database inquiries. Genetic sequence queries can identify identical matches; therefore, molecular identification of pathogen strain type is easily achieved. New genetic types, that may have phenotypic differences, also are quickly discovered. Database mining can be single or multifactorial key word searches (for example, rainbow trout and VHSV G gene nucleotide sequence and Great Lakes). Search results are displayed in various table or report formats. Additional predefined sort options based on user needs are available, as well as customized search and sort options.

The practical application of the Fish ViroTrak and template AquaPathogen X databases is the organization of copious amounts of data associated with pathogen detections, including gene sequences. Aquatic animal health researchers and resource agencies can customize the template as an in-house database to catalog their own aquatic pathogen isolates. AquaPathogen X template database can become a widely distributed resource for expediting the collation and comparisons of emerging aquatic pathogens.

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A complete list of WFRC publications is available at http://wfrc.usgs.gov/cgi-bin/pub_search.cgi?0&view=all.

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