



## Great Lakes Fishery Commission

ESTABLISHED BY CONVENTION BETWEEN CANADA AND THE UNITED STATES TO IMPROVE AND PERPETUATE FISHERY RESOURCES

### Great Lakes Fish Health Committee/Lake Michigan Technical Committee

#### Minutes

**Great Lakes Wild Fish Health Assessment Workshop  
Ann Arbor, Michigan  
March 14 - 15, 2001**

**By Sue Marcquenski, WDNR**  
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**Greg Wright (CORA)** - provided an overview of the health history of Lake Michigan, with a focus on chinook salmon spring mortalities from 1988 to 1992 and factors that may have contributed to them. Factors included pathogens such as *Renibacterium salmoninarum* (causing Bacterial Kidney Disease) and *Echinorhynchus salmonis* (an acanthocephalan parasite of the intestine), nutritional stress, thermal stress, predator/prey relationships, and bioenergetics. The purpose of this workshop is to develop a suite of parameters that can be measured in individual fish and be incorporated into the Lake Michigan lakewide assessment plan. The goal of this plan is to identify biological aberrations over the long term that can cue biologists that changes are occurring that require management adjustments. If these changes cannot be identified correctly and management adjusted accordingly, spring die-offs, such as those in the late 1980's, may occur again.

*Greg Wright can be contacted at (906) 484-2391 <gwright@sault.com>*

**Steve Kaatari (VIMS)** - provided an overview on immune system function. Factors that can suppress the immune system include exposure to toxins, seasonal changes, infections that cause hypersensitivity, increases in cortisol (resulting from stress), colder water temperatures. Immunocompetency can be determined by measuring the following parameters (most are lab tests, not field tests): lysozyme, complement, total immunoglobulin, leukocrit, antibodies in immunized fish; plasma glucose and cortisol are indicators of stress, not necessarily immune function. How well these parameters correlate with observable effects at the population level would need to be studied as part of a research project.

*Steve Kaatari can be contacted at (804) 684-7362 <kaattari@vims.edu>*

**Darin Simpkins (UWy)**- provided insight to the question: Do condition indices reflect the physiological condition of fish (use of relative weights). In a study where rainbow trout were fasted for 147 days, relative weight did not change between day 23 and 84, suggesting this

measurement is not a good indicator of physiological state. As lipid was utilized, water replaced the lipid, so relative weight did not change. Gross measurement of gut weight (but not as a percentage of total weight) had some predictive value. Because lipid is replaced by water, all weight related indices are not appropriate for assessing change in fish over time. Lipid levels of 1 to 1.5% seem to be the level at which mortality occurs- it is important to look at fatty acid composition of the total lipid to understand why death occurs. It could be useful to measure percent water in the fish- if this percentage exceeds a certain value, this could indicate fish are responding to an environmental stress.

*Darin Simpkin can be reached at (307) 766-2091 <Simpkins@uwyo.edu>*

**Bruce Barton (USD)**- provided clarification regarding the What and So What of Stress Response in Fish. There can be physical stresses, chemical stresses, social stresses, and others. There is a strong behavioral component to stress. Stress can be measured at the molecular level (heat shock proteins), biochemical level, endocrine level (cortisol), physiological level (glucose, osmoregulation, immune system), whole animal level and behavioral level. The stress response can be modified by the environment, genetics, ontogeny, prior experiences, nutrition, temperature and light. There can be interspecific differences in how fish respond to stress, so the assays chosen to measure stress must be evaluated for the species of interest. Fish have cumulative responses to repeated stresses. If there is not time for a recovery period before the next stress, fish will not be able to compensate for the multiple stressors. Ron Pascho shared an example where chinook without BKD and chinook with BKD received three stresses. Cortisol levels in the chinook with BKD did not return to pre-stress levels- they just kept increasing. Fish without BKD were able to bring their cortisol levels back to the baseline.

*Bruce Barton can be reached at (605) 677-6180 <bbarton@usd.edu>*

**Ron Goede (UDWR retired)**- provided a thorough and humorous discussion of his Fish Health Assessment procedure. This procedure was first developed for hatchery fish, but has been modified and applied to wild fish with good success. An important feature of any health assessment is that it is “do-able”. The most useful component to track over time is percent normal. This system is set up to use Excel software. Ron provided copies of two publications which detail the parameters used in the assessment tool: length, weight, eyes, gills, pseudobranch, thymus, fin condition, opercles, mesentary fat, spleen, hind gut, kidney, bile color, blood (hematocrit, leukocrit, plasma protein), deformity index, skin lesion index, and fin deformity index. The citation for the second publication is Goede, R.W. and Bruce Barton. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. American Fisheries Society Symposium 8:93-108.

*Ron Goede can be reached at (435) 752-9650 <rgoede@sisna.com>*

**Michael Arts (NWRI)**- provided information on the significance of looking at the fatty acid composition of lipids and their physiological importance. In the first half of the talk, lipid and fatty acid data from invertebrates crucial to fish and their fry were examined. It was shown, through an analysis of several taxa and groups (e.g. Cladocera, copepods, and amphipods), that there is much variability among species, inferring that fish must have access to a diverse diet in order to obtain the proper mix of essential fatty acids. A different picture can be obtained if lipid classes (using an Iatroscan TLC-FID) are measured compared to only total

lipids. In the second half of his presentation he cautioned that lipid concentrations and fatty acids composition also vary substantially depending on the fish tissue sampled (muscle, brain, retina, liver, mesenteries, eggs). Some fatty acids cannot be effectively synthesized by fish (and by man). These have been termed essential fatty acids. Essential fatty acids are crucial to our health, to the health of fish populations and to ecosystems in general. Such fatty acids are produced primarily by algae and are incorporated into fish (and man) through the diet. In terms of fish health and condition, the most critical fatty acids to measure include: eicosapentaenoic acid (C20:5Ω3), docosapentaenoic acid (C22:5Ω3) and docosahexaenoic acid (C22:6Ω3) commonly abbreviated as EPA, DPA and DHA, respectively. In addition to monitoring individual essential fatty acids, it is of considerable interest to calculate specific ratios of some of the EFA. For example, the ARA/EPA ratio [where ARA = arachidonic acid (C20:4Ω6)] is important because these two fatty acids are the precursors of prostaglandin's G2 and G3. These prostaglandins are important in a whole range of physiological competencies including, for example, smoltification.

*Michael Arts can be reached at (905) 336-6460 <Michael.Arts@ec.gc.ca>*

**Marshall Adams (ORNL)**- provided a stimulating discussion of Multiple and Integrated Measures of Fish Health. It is important to use multiple endpoints that measure fish health – don't rely on single parameters. Points to consider when choosing what to measure include short term vs. long term needs and low vs. high ecological significance. Once parameters are chosen, results from all assays can be plotted on one graph if you plot them as a percentage of the reference site value. Canonical variants can be used to visualize the sum of your test results. Discriminate analysis can then be done to identify the most significant assays, the ones to keep monitoring over time. Assays can be set up at the organism level, suborganism level, or community level – changes are more quickly observed when measuring something at the suborganism level, compared to the community level- so decide how fast you need to detect changes when choosing what to measure. If a measured change is 15% away from the natural variation, the population is probably in trouble. There is a web site for bioindicators: [www.esd.ornl.gov/programs/bioindicators](http://www.esd.ornl.gov/programs/bioindicators). The overall goal is to understand the mechanistic basis of the observed changes in assay results (why and how did the change occur).

*Marshall Adams can be reached at (865) 574-7316 <sma@ornl.gov>*

**Jory Jonas (MDNR)**- provided case history information on monitoring the prevalence of *Renibacterium salmoninarum* using the field ELISA method in Lake Michigan salmonids during summer months. High prevalence in summer 1997 did not result in spring die-offs in 1998.

*Jory Jonas can be reached at (231) 547-2914 ext. 229 <JONASJ@state.mi.us>*

**Rick Nelson (USFWS)**- provided information on accessing data in the U.S. Fish and Wildlife Service's wild fish survey database. These data are now available at the following web site: [www.wildfishsurvey.fws.gov](http://www.wildfishsurvey.fws.gov)

*Rick Nelson can be reached at (608) 783-8441 <rick\_nelson@fws.gov>*

**Roz Stevenson (UG)**- provided a very clear description and graphic illustration of the various methods used to detect *Renibacterium salmoninarum* using fluorescent microscopy (QFAT,

IFAT and DFAT) and their relative sensitivities (IFAT more sensitive than DFAT because there is an additional step that intensifies the fluorescence.). As with any immunological tests, fluorescent antibody microscopy can only be as good as the antiserum reagent used. Whether it's monoclonal or polyclonal, antiserum quality is fundamental to the ELISA-based methods.

*Roz Stevenson can be reached at (519) 824-4120 ext. 3577 <rstevens@uoguelph.ca>*

**John Reddington (DiagXotics, Inc)-** provided insight on using ELISA diagnostic methods, contrasting monoclonal vs. polyclonal techniques. It is important to decide whether you are using the test to detect changes at the individual level vs. population level. To detect changes at the individual level requires a very sensitive test; at the population level, a less sensitive test may be appropriate because your sample size is larger. Decide how specific the test needs to be (purpose of the program, cost/benefit, sample source, sample preparation, interpretation of results). Points to consider about monoclonal ELISA methods: easy to produce consistently; not as likely to cross react; may not be as sensitive; is based on the P57 protein of *R.s.*; reagents must be at room temperature; washing between steps is critical. Points to consider about polyclonal ELISA methods: difficult to produce consistently over time; could cross react, giving false positives.

*John Reddington can be reached at (203) 762-0279 <john@diagxotics.com>*

**John Wood (Pisces-Molecular, LLC)-** provided an understanding of the polymerase chain reaction (PCR) technique. This is a nucleic acid based test, which can use DNA or RNA and relies on amplifying a target nucleic acid sequence and observing its presence as a band on a gel. The sensitivity of the test is very high, but due to the very high level of signal amplification accurate quantitation of the starting amount of target sequence in a sample is difficult. The procedure takes one day. Several new technologies address limitations of current PCR procedures: Real time PCR combines PCR and a fluorescent detection technology. This allows detection of a target sequence without opening a PCR reaction tube containing large amount of amplified target DNA, greatly reducing the chance of contaminating other samples. Another advantage of real-time PCR is that it can provide accurate quantitation (amount of target sequence) in a sample. Another new technology uses a single tube to isolate nucleic acid from a sample. This “one-tube” test could be used in the field; a major advantage over other PCR procedures that are all laboratory based, however, this technology is not quantitative.

*John Wood can be reached at (303) 546-9300 <jwood@pisces-molecular.com>*

**Ron Pascho (USGS)-** provided a critical comparison of several diagnostic methods used to screen chinook salmon broodstock from Lake Michigan for *R.s.* Immunological assays include ELISA, DFAT and MFAT (membrane filtration of ovarian fluids using DFAT); molecular techniques include PCR; clinical signs are gross visual observations of the fish. ELISA methods are based on the P57 protein and antibodies made to that protein; they rely on color changes read by a spectrophotometer to identify positive samples. The P57 protein confers virulence to the *R.s.* bacterium, circulates throughout the fish, but optical density (O.D.) values are difficult to correlate with actual intensity of infection. ELISA methods work OK on tissue, but poorly on ovarian fluids (limit of detection in ovarian fluids is 10,000 cells/ml). QELISA is only semi-quantitative. Vaccinated fish may take up the P57 protein in the vaccine and thus generate false positive results. Cost is 0.50 to \$3.00 per sample.

PCR can detect live bacteria, 10-1000 cells/mg of tissue. Not fully quantitative yet. Cost is about \$5.00 per sample excluding labor. There is good correlation between PCR, polyclonal ELISA and MFAT at high and medium infection levels. ELISA and PCR can be used on blood.

*Ron Pascho can be reached at (206) 526-6588 <ron\_pascho@usgs.gov>*

Lively discussion followed, lead by Marshall Adams. The bottom line comments are as follows:

The Field ELISA (FELISA) would be an acceptable tool to monitor *R.s.* prevalence as long as steps were taken to reduce variability among tests. These steps include collecting chunks of kidney (rather than just a swab in the field), using clean tools for each fish to minimize cross contamination, running the test under lab conditions, having the same person run the tests to minimize between person variability. QELISA is probably a better choice, but FELISA would be OK under the above conditions.

Ron Goede's assessment technique is applicable to monitoring efforts for salmonids in Lake Michigan, with some fine-tuning. It is do-able and over time can provide a quantitative way to detect deviations from normal.

Additional specific categories to consider including in a suite of assays are those that measure nutrition/bioenergetics, reproductive competence, pathogens, organ dysfunction, metabolic status, performance and immune system competence.

The variability of individual tests should be determined. This could be done by running a pilot study on a large number of fish. The pilot could include a full suite of assays collected over the first year. Then the list of assays could be reviewed and refined based on practicality, cost, variability, etc.

In all of the above, don't lose sight of the original question you want to answer.

Marshall developed an outline of suggested tests for the categories mentioned above:

- I. Nutritional/Bioenergetics
  - consumption
  - blood triglycerides
  - fatty acids (which ones?)
  - body triglycerides (survival, reproductive competence)
- II. Organ Dysfunction
  - electrolyte status (osmoregulation)
  - creatinine (kidney damage)
  - ATPase- gill function
- III. Reproductive Competence
  - Gondal Somatic Index (GSI)
  - fecundity

- atretic oocytes
- vitellogenin- egg diameter

IV. Metabolic Status

- liver glycogen
- LSI
- glucose/lactate?

V. Performance

- disease challenge

VI. Pathogen (R.s.?)

- presence/absence

VII. Immune System Status

- lysozyme
- functional complement
- antibody

VIII. Genetics

- as a research tool