Environmental Assessment Tool for Land-based Aquaculture in the Great Lakes Version 1.0

by

Deborah J. Brister [1,3]

and

Anne R. Kapuscinski [1,2,3]

[1]University of Minnesota, Department of Fisheries and Wildlife and Conservation Biology
[2] Minnesota Sea Grant College Program
[3] Institute for Social, Economic and Ecological Sustainability

<u>Part One</u>: Introductory Materials, Supporting Text and Summary Documentation

DISCUSSION DRAFT

Prepared for the Great Lakes Fishery Commission
Council of Lake Committees

Acknowledgements

The Great Lakes Fishery Commission, the Minnesota Sea Grant College Program's Extension Program, and the University of Minnesota Institute for Social, Economic, and Ecological Sustainability provided partial funding for this project. Sincere thanks to the Council of Lakes Committee members and other individuals in the American and Canadian natural resource agencies for their time and effort in helping make this a usable tool. Special thanks to Marg Dochoda for her support and assistance in providing valuable reference material. This work is the result of research sponsored by the Minnesota Sea Grant College Program supported by the NOAA Office of Sea Grant, United States Department of Commerce, under grant number NOAA-NA46-RG0101. The U.S. Government is authorized to reproduce and distribute reprints for government purposes, not withstanding any copyright notation that may appear hereon. This paper is journal reprint Number 471 of the Minnesota Sea Grant College Program. This is Article Number 004410001 of the Minnesota Agricultural Experiment Station.

Table of Contents Part 1

Acknowledgements i

Introduction 1

How to Use the Aquaculture Environmental Assessment Tool 4

Supporting Text

I. Determination of Pathway 5

II. Facility Connection to the Great Lakes 8

III. Genetically Engineered Organisms (GEOs) Assessment 20

IV. Disease Assessment 27

V. Effluent Assessment 31

VI. Habitat Alterations 39

VII. Water Source Assessment 41

Optional Precautionary Plans 44

Monitoring 45

Summary Documentation Worksheet 46

Glossary 50

References 53

Environmental Assessment Tool for Land-based Aquaculture in the Great Lakes

Deborah J. Brister and Anne R. Kapuscinski
University of Minnesota

Introduction

An increasing interest in aquaculture development in the Great Lakes region has inspired the development of an aquaculture environmental assessment tool for aquaculture facilities in the Great Lakes. There are approximately 560 aquaculture facilities (including food fish, baitfish, ornamentals, sport or game fish, crustaceans, molluscs and other aquatic animals, algae and sea vegetables) in the Great Lakes basin (USDA-NASS, 1998; Sippel and Muschett, 1999) all under varying degrees of jurisdictional regulation. Jurisdictions include two countries (Canada and the United States), one province (Ontario), eight states (Minnesota, Wisconsin, Ohio, Illinois, Indiana, Pennsylvania, Michigan, New York) and numerous tribal agencies (Native American and First Nations), all of which have some control over how the Great Lakes basin is managed. Although agreements and plans such as the Great Lakes Water Quality Agreement (1987) and the Joint Strategic Plan for Management of the Great Lakes Fisheries (1997) call for agreement and collaboration between the management agencies with respect to water quality and fisheries management, the actual process for consensus is much more difficult. In addition, some managers and other Great Lakes stakeholders may be unaware of other jurisdictions' issues of concern. Because the Great Lakes are interconnected water bodies, organisms may move freely from one body of water to another, making aquaculture management a possible contentious issue between jurisdictions.

To best address the multitude of issues that need to be considered when determining the suitability and environmental effects of an aquaculture facility at a particular site, we have assembled an environmental assessment tool that methodically takes the user through these issues, identifies potential hazards and, when possible, makes risk management recommendations. *This is a tool and not a regulatory document*. It aims to assist decision-makers and other interested parties by providing a systematic and consistent process for assessing aquaculture facilities. Please note that economic effects and cost/benefit analyses were not in the scope of this project. This tool is comprised of three distinct components, the assessment pathway flowcharts, supporting text and summary documentation.

Each component of this environmental assessment tool serves a specific function. First, the assessment pathway guides the user through assessment of potential environmental effects. The user answers a series of carefully worded questions about the species (including genetic strains) and the accessible aquatic ecosystem, identifying whether or not the aquaculture operation under review poses any specific hazards.

Should any hazards be identified, the user is led to consider risk management measures, including culture methods, facilities design and operations management. This would include whether or not measures capable of reducing the risk of the identified hazard currently exist.

Second, the supporting text provides: scientific background, including citations of relevant documents, for the questions and alternative decisions in the assessment pathway; more detail on risk management recommendations; a glossary of scientific terms; and other relevant appendices.

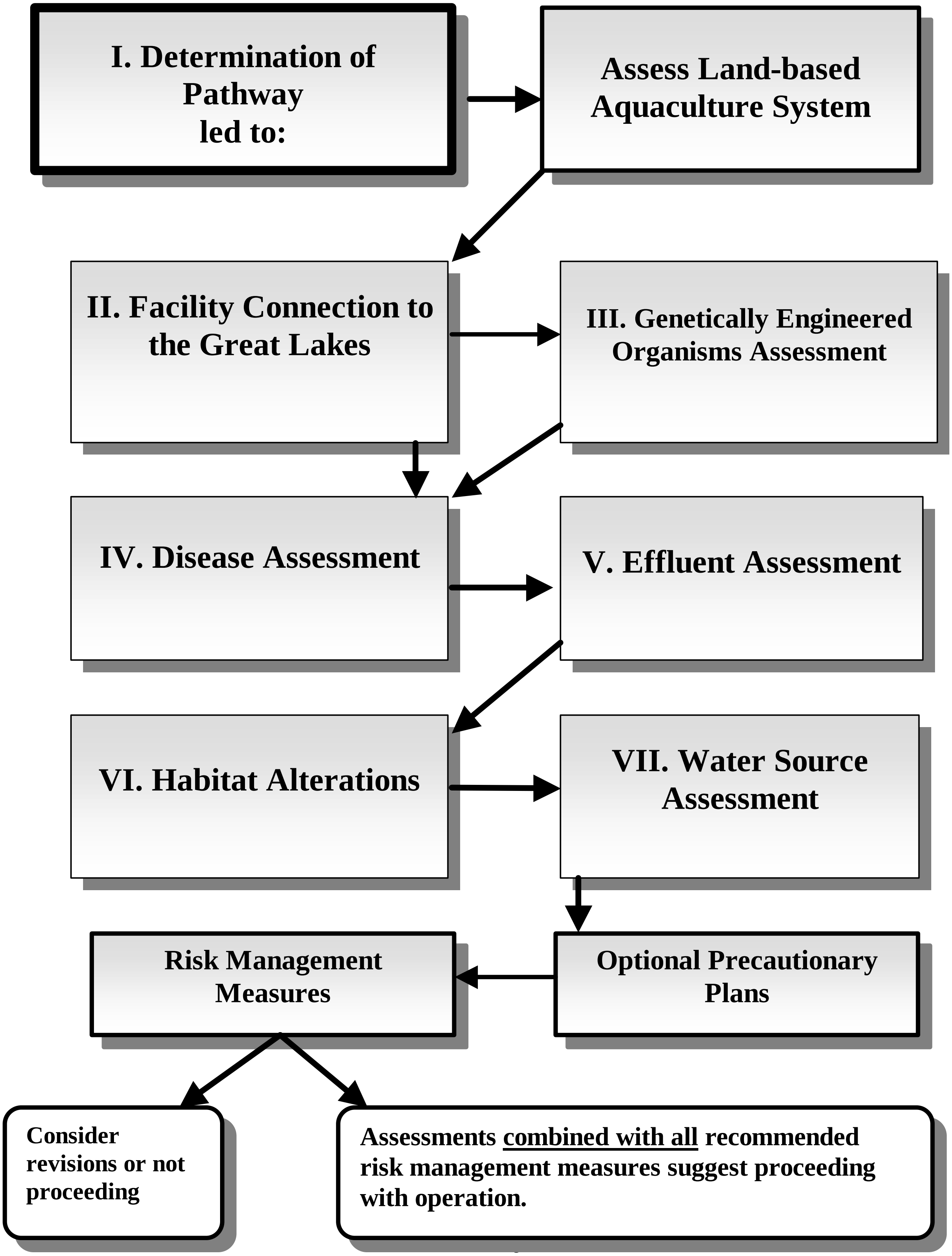
Third, the summary documentation traces the user's path through the assessment pathway and prompts the user to describe the rationale for any selected risk management measures. The summary documentation provides transparent documentation of the systematic assessment process, and will encourage more consistent and systematic use of available scientific and technical information, and regulatory decisions. This will hopefully reduce distrust and some sources of conflict between regulators, aquaculturists and other users.

This assessment tool is dynamic in that it will be periodically updated as new information is available. As stated earlier, this is not a regulatory document. To assist the user, however, links to specific jurisdictional regulations such as those for water quality and approved species lists are included. In addition, the document includes links for specific Great Lakes management plans to assist users in identifying whether or not a proposed aquaculture development conflicts with existing natural resource management plans.

Overview of Land-based Aquaculture Environmental Assessment Tool

The land-based aquaculture assessment tool encompasses aquaculture facilities that are proposed to be located on land within the Great Lakes Basin. The following draft flow-charts parallel the Lake-based assessment tool and include sections on facility connections to the Great Lakes, water source issues, disease management, effluent management and habitat alterations (see Figure 1).

Figure 1. Overview of Sections in Assessment of Land-based Aquaculture Systems

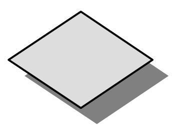


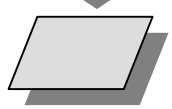
How to Use the Environmental Assessment Tool

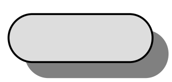
The user is guided through a series of questions relating to a specific section (e.g. Genetic Effects, Disease Effects) of the assessment tool. These questions, usually answered with a yes or no, will assist the user in identifying potential hazards, which then allow the user to determine whether or not to accept the risk associated with each hazard.

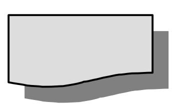
If the answer to a question is unknown, users should refer to the supporting text of the question at issue. The user is assisted in how to find the necessary answer with supporting information, useful links for documents and relevant addresses of people to contact. If the answer is still unknown, the assessment tool directs the user to follow the most precautionary path. This approach is based on the Precautionary Principle as stated in the preamble to the Convention on Biological Diversity (UNEP/CBD/94/1) which suggests that "…where there is a threat of significant reduction or loss of biological diversity, lack of scientific certainty should not be used as a reason for postponing measures to avoid such a threat".

The flowchart symbols consists of:

Questions 

Potential Hazards 

Terminal Points 

Continuation Markers 

This assessment tool uses both the terms hazard and risk. It is appropriate here to distinguish one from the other as they are sometimes used interchangeably in everyday language. For our purposes, *hazard* can be defined as a potentially adverse outcome of an event or activity. *Risk* is the probability of the hazard occurring (Smith, 1992). This assessment tool focuses on the *identification of hazards*. It does not provide guidance on the estimation of specific risks; users may instead consult the extensive literature on risk estimation (see e.g. Burgman et al., 1993 and Stern et al., 1996). The degree of acceptable risk a user is willing to take is left up to the users of this decision support tool. In this way, the assessment tool can be flexible and adaptive to unique circumstances of each case.Throughout the assessment tool, supporting text is provided for almost every question and recommended risk management measures are provided for hazards that have been identified. **The Summary Documentation worksheet should be checked off as the user goes through the flowcharts.** Upon completion of the flowcharts, the user is directed to read supplemental information regarding voluntary *Precautionary Plans*.

Section I.
Determination of Pathway

The Determination of Pathway flowcharts identify which particular series of assessment flowcharts are applicable to the proposed aquaculture operation in question.

Question 1.

The organism at issue is what the aquaculturist proposes to culture. In the event of polyculture, or the rearing of multiple species, the user will need to run through the assessment for each species.

If answer to this question is unknown, consult with operator.

Question 2.

In some instances, broodstock, eggs, fry or other marketable life stages may be collected from the natural environment prior to growout. In other cases, this collection, or harvesting requires no growout except for holding. Examples of this would include (but not limited to) harvesting of baitfish, feeder fish and species collected for aquaria. At issue are the possibilities of inadvertently collecting aquatic nuisance species (ANS) while collecting the desired target organisms, and the possibility of damaging habitat while collecting. Questions 3 and 4 deal with these issues specifically.

If no growout will take place after collection, the user is directed to bypass much of the assessment tool. Conversely, if growout of organisms is part of the operation, the user is directed through the entire assessment.

If organisms/gametes originate from outside the basin, operator should be aware of disease restrictions as outlined in the Protocol to Minimize the risk of Introducing Emergency Disease Agents with Importation of Salmonid Fishes from Enzootic Areas (Horner et al., 1993). To keep abreast of new disease restrictions, users should also contact the Great Lakes Fish Health Committee at: www.glfc.org/staff/health.htm .

If answer to this question is unknown, consult with operator.

Question 3.

Infested waters, or waters that have been found to contain aquatic nuisance species may be unsuitable for collection or harvesting of organisms. Determine first if the responsible government agency considers the water body to be infested. Some states prohibit the collection of organisms from infested waters (e.g. Minnesota State Statute 84D.11 Subd.2a).

Aquatic nuisance species may occur in the waters or substrate from which the target organism is collected or located on the collected target organism (e.g. parasite). These species can be collected and inadvertently sold with the desired baitfish. In a study by of baitfish purchased from 21 North Dakota and Minnesota dealers, 28% of the 21 samples contained a nonbait species (Ludwig et al., 1996). In a Toronto survey by Litvak et al. (1993), 6 species of illegal baitfish were identified in holding tanks of 4 dealerships.

The Great Lakes has many known aquatic nuisance species. Because live bait is harvested from Great Lakes waters, and then shipped to dealers for use in inland regions (Snyder, 1997), caution must be taken to minimize the spread of an accidentally harvested aquatic nuisance species. Indeed, bait bucket transfer and the release of unused bait after fishing is considered to be a major vector for the introduction of non-native species. Litvik et al. (1993) found that 41% of anglers interviewed would release extra, unused bait. Hence, prevention of these unwanted organisms should begin before they reach the anglers' live wells.

Additional information regarding Great Lakes aquatic nuisance species can be found on the Great Lakes Information Network at:
http://www.great-lakes.net/envt/exotic/exotic.html.

If answer to this question is unknown, encourage local natural resource agency to determine if body of water at issue is free from aquatic nuisance species. When the answer is unknown, it is recommended that the user take the precautionary approach and answer yes.

Question 4.

Harvesting wild organisms may have an impact on the ecosystem where collection takes place. Litvak et al. (1993) review possible effects of baitfish harvesting and consider population alteration, trophic alteration and habitat alteration. Population alteration may occur if forage fish are harvested without consideration of sustainable yields. For example, in Wisconsin, Vives (1990) observed that the horneyhead chub, *Nocomis biguttus*, is a keystone species. This is because the chubs' nests are used as spawning substrate by other cyprinids. Trophic alteration may occur if species dependent on the harvested organisms must shift to alternative prey species. Habitat alteration may occur if harvesting uproots vegetation and destroys cover for small or juvenile fish or if waterfowl breeding sites are disturbed.(Litvak et al., 1993).

Habitat damage due to the collection method may occur depending on the vulnerability of the specific environment and the type of equipment used. Factors to consider include but are not limited to: vulnerability of other organisms, type of substrate, vegetation, time period or season of collection and frequency of collection.

If answer to this question is unknown, take the precautionary approach and answer yes.

Question 5.

If growout (i.e., continued rearing) of organisms is included in the operator's plan, the user will be guided through the rest of the assessment. If operator plans to only hold and not feed organisms until they reach market, the user may bypass most of the assessment and go Lake-based Section X., Impacts of Facility and Infrastructure, in order to assess the holding facility.

If answer to this question is unknown, consult with operator.

Question 6.

This question determines whether operations exist within in one of the Great Lakes, any connecting bodies of water, or any tributary of the Great Lakes excluding those that flow out of the Great Lakes, such as the Chicago Sanitary and Ship Canal.

If answer to this question is unknown, consult with operator.

Hazard 1.

Because aquatic nuisance species (ANS) are present in the collecting waters, measures must be taken to reduce the risk of transferring these ANS to other bodies of water that may not contain them. Although this task may be extremely difficult, it is possible, by means of inspecting collected organisms, to reduce this risk. Refer to ANS-HACCP: Aquatic Nuisance Species-Hazard Analysis and Critical Control Point Training Curriculum (National Seafood HACCP Alliance for Seafood Safety Training and Education, 2001).

Hazard 2.

Degrees of impact to the collection area depend on the type of harvesting equipment used. For instance, seining, which involves dragging the net along the bottom may have a greater impact than the use of traps. If the area is particularly vulnerable to the collecting activity, the operator should identify ways to minimize disturbance to the area.

Section II.
Facility Connection to the Great Lakes

Question 7.

An aquaculture facility located on land may still have effluents that reach a Great Lake, a connecting water body of a Great Lake or a tributary of a Great Lake. If this is the case, it is possible that the cultured organisms or pathogens may ultimately reach the Great Lakes. Even if there is no direct link to these water bodies, consideration should be given to levels of different flood events (e.g. 50 year flood events). If there is a high probability that the proposed aquaculture facility will be flooded, users should answer yes to this question.

Land-based aquaculture production allows use of effective mechanical and physical barriers in addition to sterilization of production aquatic animals. The diversity and number of barriers may need to be higher in flow-through systems than in recirculating aquaculture systems. The risk of fish escaping is typically lowest in recirculating systems; this is because no more than 10% of the rearing water is discharged daily and many upstream components of the system (such as solids removal) also act as mechanical barriers to fish escape (Kapuscinski and Brister, 2001).

If answer to this question is unknown, consult with flood level data.

Question 8.

The province of Ontario and each of the eight Great Lakes States has an approved species list. The linked approved lists are valid as of 1999. For a current approved list consult with the managing agencies.
Ontario (http://www.mnr.gov.on.ca/MNR/),
Michigan (http://www.dnr.state.mi.us/),
Wisconsin (http://www.dnr.state.wi.us/),
Minnesota (http://www.dnr.state.mn.us/fish_and_wildlife/fishsec.html),
New York, (http://www.dec.state.ny.us/index.html)
Illinois (http://dnr.state.il.us/),
Indiana (http://www.state.in.us/dnr/index.html),
Pennsylvania (http://www.state.pa.us/PA_Exec/Fish_Boat/mpag1.htm)
Ohio (http://www.dnr.ohio.gov/)
(Note: As of this printing, approved species lists are not known for Native American and First Nations tribal agencies. Consult with the Great Lakes Indian Fish and Wildlife Commission (http://www.glifwc.org/for) and Chippewa/Ottawa Treaty Fishery Management Authority (COTFMA) (http://home.northernway.net/~qitfap/) for more information.

If answer to this question is unknown, consult with agencies listed above.

Question 9.

A new introduction includes any species that does not (to the best of our knowledge) exist currently in the Great Lakes. To best determine this, go to Checklists of the Fish Fauna of the Laurentian Great Lakes and Their Connecting Channels (Cudmore-Vokey and Crossman, 2000). If the proposed cultured species is not listed, the user should answer yes to this question.

If answer to this question is unknown, refer to Checklists of the Fish Fauna of the Laurentian Great Lakes and Their Connecting Channels (Cudmore-Vokey and Crossman, 2000).

Question 10

The human capability to genetically modify organisms has expanded greatly with the advent of novel techniques of genetic engineering. A genetically engineered organism (GEO) is one that has been constructed by isolating nucleic acids molecules (molecules that encode genetic information) from one organism, and introducing these molecules into another organism in a manner that makes them part of the permanent genetic make-up of the recipient, i.e., capable of being inherited by offspring (Scientists' Working Group on Biosafety, 1998). This definition also includes those organisms constructed by the transfer of subcellular organelles from one cell to another, followed by the regeneration of an adult organism from the genetically altered cell, so long as the alteration can be transmitted to offspring.

In the case of aquatic organisms, interspecific hybridization and chromosome manipulations are so novel that they also warrant careful biosafety assessment (Agricultural Biotechnology Research Advisory Committee, 1995; Scientists' Working Group on Biosafety, 1998). Furthermore, many interspecific hybrids and chromosomal manipulated finfish, shellfish, or plants are derived from parental populations that are close to the wild-type, so these genetically engineered offspring will be ecologically competent if they escape into the wild (Kapuscinski and Hallerman, 1994).

If answer to this question is unknown, consult with operator.

Question 11.

At issue are *naturally* reproducing populations of the same species as the culture species or a closely related species with which the aquaculture escapees can interbreed. The natural populations of concern may be indigenous to the Great Lakes or naturalized descendants of an introduced species that has become socio-economically important (see example of genetically distinct steelhead trout populations discussed in the supporting text for question 55). Many aquaculture operations raise organisms from non-local broodstock sources. In most of these cases, organisms escaping from the aquaculture operation will be capable of surviving to reproduce and interbreed with natural populations in surrounding waters.

It is important to assess if the aquaculture escapees could cross with any closely related species in the accessible ecosystem. Interspecific hybridization among aquatic species is quite common, particularly among fishes (Hubbs, 1955; Lagler, 1977; Turner, 1984; Collares-Pereira, 1987), often yielding fertile hybrids that can backcross to wild populations of either parental species. Interspecific hybrids and their backcrossed descendants may occur naturally but usually at low frequencies; walleye containing introgressed sauger genes, for example, have been found in waters draining into Georgian Bay of Lake Huron (Billington et al., 1988). Frequent or large-scale escapes of fertile hybrids or either parental species from aquaculture operations can substantially increase these frequencies. This then poses a genetic hazard of losing a taxonomically distinct population of a native species. For instance, walleye x sauger hybrids have become a popular culture organism (Held and Malison, 1996). A wild population of walleye could lose its taxonomic and genetic distinctness if large numbers of walleye x sauger hybrids escaping from an aquaculture operation successfully out-crossed with the wild walleye.

It is in the long-term interest of parties interested in aquaculture or capture fisheries to prevent losses of taxonomically distinct populations in the wild. Taxonomically distinct, wild populations are an irreplaceable reservoir of genes (live gene bank) harboring coadapted gene and chromosomal complexes that aquaculture breeders can tap to improve economically important traits, such as disease resistance. Introgressive hybridization would disrupt these gene complexes as well as dilute rare alleles that could be crucially important for aquacultural performance traits. Furthermore, if one half of a hybrid cross comes from an introgressed rather than a pure parental species, the offspring will not show hybrid vigor for the target performance traits, thus undermining the very purpose of making interspecific hybrids in aquaculture. Indeed, Billington (1996a) found saugeye genes in some aquaculture broodstocks presumed to be pure walleye. The loss of coadapted gene and chromosomal complexes and of rare alleles also threatens the long-term sustainability of capture fisheries for reasons explained in greater detail in the remainder of the supporting text.

<u>Panmictic populations versus genetically distinct populations.</u> In cases where wild relatives belong to one panmictic population (probably a rarity in the Great Lakes), interbreeding with aquaculture escapees poses the genetic hazard of reducing the fitness, thus the productivity, of wild populations due to outbreeding depression. In cases where the wild relatives have a number of genetically distinct populations in the Great Lakes, their interbreeding with aquaculture escapees poses two hazards: (1) outbreeding depression that might reduce the near-term fitness and productivity of the wild fish; and (2) homogenization of the genetic differences between populations that might reduce the long-term sustainability of wild populations. Evidence of adverse effects of interbreeding between fish coming from genetically divergent sources has grown in recent years. For instance, see reviews in Kapuscinski and Jacobson (1987), Krueger and May (1991), Heggberget et al. (1993), Busack and Currens (1995:74-75), Leary et al. (1995), Allendorf and Waples (1996:253-254), Lynch (1996:491-493), National Research Council (1996), Reisenbichler (1997), Gross (1998), Youngson and Verspoor (1998), and Miller and Kapuscinski (2000). Further discussion of these potential problems appears under the three sub-headings below.

Although genetic population structure information is missing for many important species in the Great Lakes, substantial information exists for some species. Genetic data may be Great Lakes basin-wide or only lake-wide. For instance, there are data on population structure of lake trout (Ihssen et al., 1988, Krueger et al., 1989; Krueger and Ihssen, 1995), walleye (Billington and Hebert, 1988; Ward et al., 1989; Todd, 1990; Billington et al., 1992; Stepien, 1995), steelhead trout (Krueger et al. 1994; O'Connell et al., 1997), brook trout (Danzmann et al., 1991; Angers et al., 1995; Danzmann et al., 1998), and Northern pike (Senanan and Kapuscinski, 2000). Because new genetic studies are underway all the time, users need to actively seek out the most current information. This involves searching the scientific literature as well as consulting with practicing fisheries geneticists in the region to find out about unpublished results from the most recent studies (e.g., yellow perch population genetic analysis is currently underway for Lake Michigan).

Answers to this question should be based on appropriate genetic analyses of population structure conducted by a qualified population geneticist. Such analyses should examine genetic variation in at least one type of nuclear genetic marker that is polymorphic for the species in question. For example, protein electrophoresis is inadequate for assessing population structure in Northern pike (*Esox lucius*) because studies have shown virtually no variability in these genetic markers (Healy and Mulcahy, 1980; Seeb et al., 1987). Instead, one should use microsatellite DNA, a nuclear genetic marker that has much higher levels of variation and has been used to delineate distinct populations (Miller and Kapuscinski, 1996; Senanan and Kapuscinski, 2000). Likewise, proteins and mitochondrial DNA markers exhibit low variability in yellow perch and prior genetic studies with such markers found very little population structure across broad geographic regions (Todd and Hatcher, 1993; Billington, 1996). Yet, the existence of distinct breeding populations within single lakes has been proposed based on tagging studies, comparative growth and behavior studies, and patterns of egg mass deposition (Aalto and Newsome, 1990). Studies are presently underway to develop higher resolution nuclear DNA markers to search for genetic population structure in yellow perch (Miller and Kapuscinski, unpublished data). It is desirable to confirm population structure results by looking for concurrence between results from two or more types of genetic markers (nuclear or mitochondrial).

The objective of asking this question is to prevent declines in the near-term fitness and productivity and long-term sustainability of wild populations that could be wrought by interbreeding with aquacultural escapees. Genetic diversity is "part of the fabric of a biological resource" (National Research Council, 1996:146). The productivity of the resource, Great Lakes fish populations in this case, cannot be separated from its genetic basis. Escapees that survive and spread to the breeding grounds of a naturally reproducing population could interbreed with the wild organisms. If this happens on a large enough scale, genetic differences between the aquacultural and wild population are eroded, making all the populations simultaneously more vulnerable to environmental change (e.g., pathogens, contaminants, changes in water quality or temperature regimes). An additional outcome can be reduced fitness of the introgressed wild population resulting from outbreeding depression or maladaptive genes from the partially domesticated aquacultural broodstocks. For a review of the genetic basis for fitness and outbreeding depression in wild fish populations, see Busack and Currens (1995:74-77) and Campton (1995:341-342, 345-346).

Increased vulnerability to environmental change due to loss of genetic differences between populations. Genetic differences between naturally reproducing populations of a species provide an evolutionary "bet-hedging" strategy analogous to the adage: don't put all your eggs in one basket. The "eggs" are the different alleles (total genetic variation) harbored within each species. The "basket" is each distinct population. As initially distinct populations become genetically homogenized, they develop the same vulnerability to stressful environmental conditions. The National Research Council (1996:148) expressed the critical importance of conserving between-population genetic differences as follows:

> Consider the extreme where no differences exist between local populations. In that case, a species consists of many copies of the same genetic population and is extremely vulnerable to environmental change. For example, a new disease might be introduced to which most individuals are genetically susceptible; the disease would jeopardize all populations and therefore the entire species. However, in the usual case, where genetic differences do exist between local populations, it is likely that some populations would have a higher frequency of genetically resistance individuals and thus would be relatively unaffected by the disease.

A graphic example of the extreme case was the widespread crash in yields of genetically uniform corn crops across North America in the 1970s due to rapid spread of corn blight disease. Following the precautionary principle, it is desirable to prevent erosion of any existing between-population genetic differences in naturally reproducing populations of fish and other aquatic species in the Great Lakes.

<u>Decreased production and fitness of wild populations due to outbreeding depression</u>. Outbreeding depression is a loss of fitness in the offspring produced as a result of interbreeding between two groups because the parents are too distantly related (Templeton, 1986). Local adaptation in naturally reproducing populations increases the probability that farmed fish x wild fish matings will yield outbreeding depression in the offspring. Outbreeding depression may result from the loss of local adaptation (i.e., through introduction of maladaptive genes) or a disruption in coadapted gene complexes that evolved through many generations of natural selection (Shields, 1993). Reductions in fitness due to loss of local adaptation may occur as soon as the first generation of outbred progeny (F_1). Reductions in fitness because of a disruption of coadapted gene complexes are more likely to occur in the next generation (F_2). For instance, Gharrett and Smoker (1991) documented severe outbreeding depression in F_2 hybrids between even- and odd-year pink salmon from the same stream in Alaska. The reduction in fitness could not be due to loss of local adaptation because both populations are native to the same stream. Instead, the appearance of outbreeding depression in the F_2, but not the F_1 generation, was likely due to breakdown of coadapted gene or chromosomal complexes (Allendorf and Waples 1996:254).

If a substantial proportion of wild fish secure matings with escaped farmed fish, outbreeding depression could cause declines in the wild population's abundance, posing a variety of ecological and socio-economic concerns. Reznick et al. (1997) found adaptive evolution of guppies to a new wild environment in only 7 generations (a mere 4 years for this species). It is thus reasonable to assume that populations of fish and other aquatic organisms in the Great Lakes have persisted in their local environments over enough generations that they have evolved local adaptation.

For example, two studies suggest that local adaptation is important in walleye, a native and economically important species of the Great Lakes. Fox (1993) compared the embryo hatching success of two populations of walleye from two neighboring rivers in Georgian Bay, Ontario. The rivers were 30 km apart and hatching success of both stocks was compared in both rivers. The native population showed significantly higher hatching rates than the non-native population in both rivers. Jennings et al. (1996) found that walleye recruitment to the spawning grounds had a heritable component. Walleye progeny from a river spawning population and a reef spawning population were stocked into an Iowa reservoir containing both river and reef spawning habitat. Upon reaching sexual maturity, the stocked walleye preferred the spawning habitat of their parental populations.

The effects of interbreeding and introgression between genetically divergent populations on the fitness and performance of fish in the wild have not been extensively studied (Campton, 1995; Leary et al., 1995). The published data show that interbreeding between genetically different populations and introgression seldom improve performance of fish in natural environments (reviewed by Krueger and May, 1991; Leary et al., 1995; Waples, 1991, 1995). In a recent study of genetic impacts of a non-indigenous hatchery stock of brown trout on two indigenous populations, Skaala et al. (1996) found that survival was nearly three times higher in wild trout than in hybrids of wild and introduced trout. McGinnity et al. (1997) compared the performance of wild, farmed, and hybrid Atlantic salmon progeny in a natural spawning stream. The progeny of farmed salmon had significantly lower survival to the smolt stage than wild salmon but they grew fastest and competitively displaced the smaller native fish downstream. A related study showed that progeny of farmed fish in this stream and other sites successfully migrated to the sea, homed to their river of escape, and interbred with wild salmon (Clifford et al., 1998). Such introgression is likely to reduce wild populations' fitness and productivity.

Negus (1999) examined the effects of interbreeding between two genetically distinct populations of *Oncorhynchus mykiss* from Lake Superior, a long-naturalized population of steelhead trout and a hatchery-propagated "kamloops" strain of rainbow trout. Embryo survival to hatching and the fright response behavior of fry were compared across progeny of four crosses: pure steelhead crosses, pure kamloops crosses, and the two reciprocal hybrid crosses (steelhead x kamloops, and kamloops x steelhead). Survival to hatching was greatest in the pure steelhead cross. Pure steelhead fry displayed a greater fright response than pure kamloops fry when startled by movements over their tanks. Survival to hatching and fry fright response of hybrids was intermediate to both pure crosses but more closely resembled the maternal source. These results confirm a genetic basis for traits affecting survival and productivity of fish in the wild. They also suggest that interbreeding between a partly domesticated strain (kamloops) and a naturalized strain (steelhead) could reduce the naturalized strain's near-term fitness in the wild. It is reasonable to expect similar fitness reductions in wild populations if partly domesticated strains of rainbow trout escaped from cage culture operations and hybridized with naturalized steelhead trout in the Great Lakes.

Some of the best evidence for outbreeding depression comes from studies comparing the post-stocking performance and introgression between genetically distinct populations of largemouth bass. Long-term studies documented genetic and physiological differences between Northern largemouth bass, *Micropterus salmoides salmoides*, and Florida largemouth bass, *Micropterus s.*

floridanus. The non-native stocks exhibited poorer fitness and performance traits than the native stock (Philipp, 1991; Philipp and Whitt, 1991). Because these comparisons involved stocks that were very distant geographically, follow-up studies compared two much geographically closer stocks, a northern Illinois and a southern Illinois largemouth bass population (Philipp and Claussen, 1995). The Northern Illinois stock demonstrated better survival, reproductive success and growth than did the Southern Illinois stock in northern Illinois and the reverse was true in southern Illinois. This result strongly supports the existence of local adaptation and, consequently, outbreeding depression if non-native fish interbreed with a locally adapted population.

Outbreeding between genetically distinct populations is most likely to yield hybrids with improved fitness in the wild (outbreeding enhancement) when hybridization alleviates inbreeding depression that existed within one or both populations (Waples, 1995). However, inbreeding depression is unlikely in most naturally reproducing populations of aquatic species in the Great Lakes. Ferguson et al. (1988) did find some evidence for superior fitness of *first-generation hybrids* between two non-inbred populations of cutthroat trout. The *superior fitness of hybrids often disappears in subsequent generations* when the hybrids backcross to a parental population (Gharrett and Smoker, 1991). Non-native populations of organisms escaping from aquaculture operations would therefore pose a genetic risk to the wild population in the second and subsequent generations, even if offspring in the first hybrid generation exhibited superior fitness.

<u>Escapees from domesticated aquacultural stocks increase the hazard of outbreeding depression</u>. Most performance traits of aquacultural organisms are partly controlled by genes and, thus, are partly heritable (reviewed in Tave, 1993). Compared to wild-type ancestors, the aquacultural organisms will genetically adapt to the new natural selection forces in the aquaculture environment even when farmers do not actively practice selective breeding. As the organisms become domesticated by genetic adaptation to the aquaculture environment, their adaptation to natural environments declines. This does not mean, however, that aquaculture escapees will be so maladapted to the wild that natural selection will weed them out before they can cross with wild relatives and possibly trigger outbreeding depression (see further discussion below).

Domestication and the commensurate maladaptation to the wild can happen in a fairly small number of generations. Fleming and Einum (1997) documented differences in numerous morphological, behavioral, and physiological traits between a seventh-generation farm strain and its wild founder population of Atlantic salmon. These changes were adaptive responses to the farm environment but most are maladaptive to the natural environment. Another study confirmed that innate predator avoidance ability can be negatively altered through short-term domestication (Berejikian, 1995). Hatchery steelhead fry, whose parents were between one and seven generations removed from the wild population of the Quinault River, Washington survived predation significantly less than fry raised from fertilized eggs of wild Quinault River steelhead adults.

A growing number of studies reveal large differences in aggressive behavior between domesticated finfish and wild counterparts. Heritable changes in aggression in wild offspring of matings between aquaculture escapees and wild fish could make them less fit through various ecological mechanisms. Depending on the life history of the species and its interactions with other species in the wild, either increased or decreased aggression could reduce fitness in the wild. The precautionary approach to sustaining wild populations of aquatic organisms, therefore, is to avoid human-caused genetic changes in aggression.

Numerous studies have shown increased aggression in offspring of domesticated broodstocks, for example, in brook trout (Vincent, 1960; Moyle, 1969) and Atlantic salmon (Einum and Fleming, 1997). Increased aggression (or increased competitive ability) has also been found in hatchery fish including brown trout (Johnsson et al. 1996) and hatchery coho salmon and cutthroat trout (Swain and Riddell, 1990, 1991; Mesa, 1991; Ruzzante, 1991, 1992, 1994; Holtby and Swain, 1992). The reasons for different aggressiveness between hatchery and wild fish could be unintentional artificial selection (imposed when broodstock are chosen for broodstock) or natural selection to the more domestic hatchery environment (reviewed by Jonsson, 1997). For all these salmonine species, increased aggression in wild offspring of hatchery x wild matings would make them more vulnerable to predators (Johnsson and Abrahams, 1991).

Some analysts have argued that maladaptation of escaped farmed fish ensures that their genes would be quickly purged from wild populations by natural selection. Unfortunately, virtually no aquacultural broodstocks have become so intensively domesticated to assure a high death rate in the wild and, thus, rapid purging of maladaptive genes. Furthermore, the ability of natural selection to purge wild populations of maladaptive traits will be severely hindered whenever there is year-after-year escapes and interbreeding of farmed fish with wild fish. Frequent and relatively large escapes of partially domesticated organisms that successfully interbreed with wild organisms would lead to a chronic reduction (genetic load) in the wild population’s fitness and productivity. The decline in the wild population's well being will be in proportion to the frequency of individuals in the mixed population that carry genes from the domesticated farmed fish. Quantification of this frequency is a key step towards quantifying the possible genetic load; see the discussion starting on page 25 of Part I. of the *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (Agricultural Biotechnology Research Advisory Committee, 1995), available at: www.nbiap.vt.edu/perfstands/psmain.html. Although natural selection is expected to remove maladaptive genes from a population, the number of generations required for the process to be completed can be very large (Hartl 1988).

If answer to this question is unknown, consult with fish population geneticists familiar with information for the Great Lakes. They can be reached through fisheries management agencies (sometimes there is a staff geneticist), the Genetics Section of the American Fisheries Society (www.afs.org) or one of the universities in the region. Additionally, the responsible fisheries management agency should conduct a formal estimation of the risk of increased vulnerability to environmental change and decreased productivity and fitness in wild populations. See question 11, supporting text on estimation of genetic load and supporting text for hazard 33 of the lake-based assessment tool for further guidance on risk estimation.

Question 12.

Land-based aquaculture production allows use of effective mechanical and physical barriers in addition to sterilization of production aquatic animals. The diversity and number of barriers may need to be higher in flow-through systems than in recirculating aquaculture systems. The risk of fish escaping is typically lowest in recirculating systems; this is because no more than 10% of the rearing water is discharged daily and many upstream components of the system (such as solids removal) also act as mechanical barriers to fish escape (Kapuscinski and Brister, 2001).

Design of Barriers

This subsection discusses factors that should be considered in the design of different barriers used to confine cultured organisms within the operation. For each possible escape path in the water system, the minimum expectation for each project requiring risk management is to have sufficient numbers of barriers in series to achieve either "no / negligible escapes" or the "acceptable number of accidental escapees." Possible aquatic escape paths are discussed in a subsection below. Protection against escape paths beyond the water system is also necessary (see subsection below on this issue).

The entire set of barriers for the water system should prevent escape of the hardest to retain life stage that will occur during the course of production; usually this is the smallest life stage. Because no barrier type is 100% effective at all times, the overall reliability of confinement measures will depend heavily on the number of independent barriers present in series. Operators are expected to determine the appropriate combination of types and total number of barriers needed to achieve the accepted number of accidental escapees. The number of independent barriers is site- and project-specific but will generally range from three to five. Where the surrounding environment (accessible ecosystems) is lethal to all life stages of the cultured organisms (e.g., discharge from a freshwater project into seawater or discharge from a marine project into a hypersaline environment), no barriers beyond the standard types of aquaculture rearing units and effluent screening may be required.

At least four types of possible barriers to aquatic escape paths are available to the researcher:

Physical or chemical barriers

These are manipulations of physical (e.g., water) or chemical (e.g., pH) attributes of rearing water to induce 100% mortality in one or more specified life stages of the cultured organisms before such life stage(s) can reach the accessible ecosystem(s). For example, water temperature or pH can be maintained at lethal values for effluents from incubators or for the final effluent coming from all rearing units. Another example is chemical sterilization of project effluent via addition of a chemical (e.g., chlorine, bromine, ozone) at lethal concentrations followed by appropriate removal of the lethal chemical prior to discharge of effluent water from the project site. Exact dose and contact time with the chemical will depend on species and life stage. Treatment with 10-15 mg/L of chlorine for 15-30 minutes is effective for killing fish in freshwater.

Mechanical barriers

This category includes mechanical structures (either stationary or moving) that physically hold back one or more specified life stages of the cultured organisms from escaping the project site. Mechanical barriers might be placed in series at one or more locations along the water system of the project. For instance, barriers might be located at each point where effluent from a number of rearing units comes together and at the point where effluents of all rearing units form one final effluent stream. Examples of possible mechanical structures include stationary or moving screens (e.g., floor drain screens, standpipe screens), filters made up of one or more types and sizes of media (e.g., gravel traps), grinders with moving parts, and tank covers.

Biological barriers
Biological features or alterations of all or a specific portion of the operation's cultured organisms can serve as barriers if they either (1) prevent any possibility of reproduction at the facility site, thus avoiding risks of escape of small gametes, embryos, or larval stages or (2) greatly reduce the possibility of reproduction or survival of cultured organisms if they accidentally escaped into the accessible ecosystem. A facility's entire set of barriers in series cannot consist solely of biological barriers because inter-individual variability in efficacy of the biological barrier is expected. The operation, therefore, should have at least one other type of barrier in its total number of barriers. Examples of biological barriers are the following: (1) the facility protocol involves killing or removal before they reach a reproductive life stage; (2) only one sex of a solely dioecious cultured organisms is raised in the facility; or (3) all cultured organisms are made permanently sterile before they reach reproductive maturity in captivity.

Barriers for all possible escape paths of the water system
The accidental escape of cultured organisms might occur through any of the following components of the water system: influent water and makeup water (applicable in water reuse systems); effluent and drawdown water; waste slurries collected when filters are backwashed, screens scrubbed, or rearing units cleaned by siphoning; and aerosols from larval hatcheries of some shellfish. Therefore, each water system component should have a sufficient combination and number of mechanical or physical/chemical barriers to prevent escape.

Influent/makeup water. Surface waters require an appropriate set of barriers. Well water, other fully enclosed water sources, and municipal sources do not need barriers.

Effluent and drawdown water. All other factors being equal, the risk of accidental escape increases as the frequency of water discharge increases. Static and closed water systems generally have no discharge except when draining the system. Water reuse systems and ponds may have a minor amount of discharge depending on operations and weather conditions. A flow-through system will have a continuous discharge. Although a sanitary sewer can serve as one barrier, discharge into sanitary sewers alone does not provide an adequate barrier to accidental escape in most cases because (1) many sewers bypass water to storm sewers or surface waters during high-runoff events, or (2) some aquatic animals can survive transit through the sewer and treatment plants. Prior to discharge to a sanitary sewer, effluent and drawdown water should pass through a sufficient set of barriers on the project site to achieve the acceptable number of accidental escapees. For all types of water systems, the effluent drain capacity should be at least two times greater than the normal inflow capacity in order to handle simultaneous draining of a number of rearing units.

For water systems which do not have continuous flow-through, an alternative approach to preventing escapes via effluent and drawdown water is to locate the entire operation in an indoor facility with no floor drains and the capacity to retain water from a specified number of experimental units. For instance, the facility could be designed to retain all the water if there was breakage of 5-20% of the experimental units. Another option is to treat any effluent from such an indoor facility as waste slurry (see below).

Waste slurries. These may hide small or dormant life stages of viable cultured organisms at in the mixture of uneaten food, feces, possibly shells from hatched eggs, and other particulate matter. Batch chemical or temperature treatment known to be lethal to smaller life stages of the cultured organisms is recommended to kill any viable cultured organisms that might be present in waste slurries. For some species, on-site drying of waste slurries might be adequate. Final disposal of treated waste slurries should comply with all applicable environmental regulations; researchers are expected to obtain guidelines and regulations from their institution and, when applicable, from appropriate government units. It is generally illegal to discharge such slurries into an aquatic ecosystem. Examples of appropriate disposal of treated waste slurries might be: discharge to a sanitary sewer; discharge into a septic system, delivery to an institutional hazardous waste facility; or deposit in an approved land site.

Prevent escape via non-aquatic paths
Escape of aquatic cultured organisms might occur through paths other than the facility's water system. Users should determine if their operation poses one or more of the escape paths described below and consider measures to protect against them.

Secure disposal of cultured animals. Certain life stages of some species can survive long periods of time outside of water. For instance, adult bivalves might survive three or more days outside of water as long as temperatures remain relatively cool and surroundings are slightly moist (e.g., a large number of adults packed closely together in a closed container). Therefore, users should consider anticipating and avoiding situations where animals might survive after disposal and get into the hands of persons unaware of the need to prevent their introduction into natural water bodies. The best way to avoid such problems is to: initially place animals destined for disposal in secure, labeled disposal containers on-site; and then deliver the containers to a designated, secure disposal facility, such as a hazardous waste facility or land disposal site.

Equipment cleaning and storage. Certain life stages of certain aquatic cultured organisms could survive for some time if they are accidentally trapped in damp nets, small puddles in fish egg sorting machines, standing water in buckets, gloves or boots of workers attending to the cultured organisms, or other equipment. Therefore, all equipment that comes in contact with live cultured organisms should be properly cleaned and drained after each use. To ensure against accidental transport of live cultured organisms to another insecure site, such equipment should be either: used and stored solely on the project site; or disinfected using treatments lethal to all cultured organisms life stages and thoroughly drained prior to transport off-site. An inventory of equipment is recommended.

If answer to this question is unknown, consult with operator.

Terminal Point 1.

Species must be approved for culture by managing agency.

Section III.
Genetically Engineered Organisms (GEOs) Assessment

Question 13.

The *Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms* is appropriate for assessing commercial-scale aquaculture of genetically engineered animals or plants (Scientists' Working Group on Biosafety, 1998, available at www.edmonds-institute.org/manual.html). It is an expanded version of the USDA's *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (Agricultural Biotechnology Research Advisory Committee, 1995), available at: www.nbiap.vt.edu/perfstands/psmain.html. The manual leads the user through a set of flowcharts, with each user following a case-specific pathway. The manual offers procedures for identifying potential hazards associated with the release of GEOs created from aquatic plants, finfish and shellfish. Where a specific hazard is identified, recommendations are made for minimizing the perceived risk (that is, minimizing the likelihood that a potential hazard will actually occur).

The scientific community has barely begun to conduct the appropriate studies to test for ecological risks of aquatic GEOs. Risk assessment tests need to address two broad issues. What is the ability and probability of a transgene to spread from escaped GEOs into a natural population through outbreeding of the GEO? What is the potential for ecological disruptions, for instance, excessive predation on a prey species or competitive displacement of a wild population, due to altered traits of organisms bearing the transgenes? In addressing both issues, one needs to search for altered traits of the GEO that could affect the outcome. For instance, large size at sexual maturity is known to give a mating advantage to males or females in many fish species. If growth-enhanced transgenic fish are larger than non-transgenics at sexual maturity, they would have a mating advantage that could increase the spread of transgenes into a wild population (discussed in further detail below).

The *Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms* (Scientists' Working Group on Biosafety) directs the user to first assess the potential for transgene spread and, depending on the outcome, then proceed to assess the potential for ecological disruptions. The user assesses the risk of transgene spread by taking a case-specific pathway through portions of flowcharts I through IV.B. In certain cases, the user goes on to assess the potential for ecological disruptions by taking a case-specific pathway through portions of flowcharts V through V.E. This priority order makes sense because conclusions about the potential spread of the transgene into wild populations will affect the range of situations for which one needs to assess ecological disruptions.

One should go on to assess the risk of ecological disruptions when any of three scenarios might apply:

(1) the escaped GEOs could survive and interbreed with wild or feral relatives in the accessible ecosystems and the transgene could spread through the naturally reproducing population;

(2) the escaped GEOs could survive and reproduce among themselves and establish a new population in an accessible ecosystem that lacks wild relatives; and

(3) the escaped GEOs cannot reproduce in the wild (e.g., rendered sterile via triploidy induction in fish) but could survive long enough in the wild to prey on, compete with, or otherwise displace wild organisms in the ecosystem.

The first and second scenarios are of concern for frequent leakage of relatively small numbers of escapees (e.g., holes in mechanical screens of effluent pipes or canals) as well as infrequent but potentially very large numbers of escapees (e.g., floods that temporarily connect constructed aquaculture ponds to natural waters). The third scenario is primarily of concern for infrequent, potentially large numbers of aquaculture escapees, particularly if these recur often enough so that a new wave of escapees tends to replace the earlier wave as it dies off.

The few existing scientific publications that might aid in ecological risk assessment of transgenic fish, although welcome in light of scanty support for such studies (Kapuscinski and Hallerman, 1994), have important shortcomings. They have not estimated the probability of the transgene spreading in wild populations (except for the studies by Muir and Howard discussed below). Devlin et al. (1999) found that dramatically faster growing transgenic coho salmon (*Oncorhynchus kisutch*) had extraordinarily high plasma growth hormone (GH) levels and consumed 2.9 times more feed pellets than the non-transgenic controls in tanks. The elevated GH levels apparently increased feeding motivation or appetite, raising the possibility that escaped GH transgenic fish could compete successfully with wild fish for food. This study confirmed that genetic engineering usually changes non-target traits (feeding motivation, appetite) in addition to changing the target trait (growth rate), thus supporting the need to search for unintended trait changes when assessing the risk/safety of a GEO. This study was not designed to determine if changes in other behavioral traits, such as increased predation exposure due to increased foraging for natural prey, could counteract the higher feeding motivation of the transgenic fish. A second study examining critical swimming speed in tanks suggested that this same transgenic strain might have an inferior swimming ability (Farell et al., 1997). We are left, however, not knowing if swimming ability would offset any feeding-related competitive advantage were these transgenic salmon to escape into natural ecosystems. Also unclear is whether swimming ability and food competition are the most crucial traits to measure in order to assess the ecological impacts of these fish.

Stevens et al. (1998) found that a line of growth-enhanced transgenic Atlantic salmon had higher oxygen uptake (indicating higher metabolic rate) but similar critical swimming speed to similarly sized non-transgenic controls. These transgenic fish also have better food conversion than controls and produce growth-hormone in their tissues year-round (Fletcher et al, 1999; Cook et al., 2000). These isolated bits of information, while potentially useful for demonstrating the desirability of these fish for aquaculture, do not provide the data needed to estimate the probabilities of transgenes spreading from escapees into wild populations and of ecological disruption.

We need a more effective and systematic means of testing aquatic GEOs for possible ecological risk or safety. A step in this direction is the methodology of Muir and Howard (2001,2001a, 2001b) for assessing the risk of transgene spread to wild or feral relatives (scenario 1 discussed above). Their approach focuses on estimating the overall fitness of a GEO by collecting data at critical "check points" in its life history (Muir and Howard, 2000; Prout, 1971a, 1971b). The first step is to conduct controlled experiments to test the transgenic organisms for changes in six fitness components: juvenile viability (survival to sexual maturity), adult viability, age at sexual maturation, fecundity (clutch or spawn size), male fertility, and mating success of both females and males. Then, one integrates the fitness component data to predict gene flow from escapees to wild relatives. Integration of the fitness component data requires the use of simulation models (or multiple generation experiments in simplified, confined ecosystems) to estimate the *joint* effects of all altered fitness components on transgene spread and population size in the wild population. This methodology allows identifying which of the following gene flow scenarios is most likely (detailed in Pew Initiative on Food and Biotechnology, in press):

Purging Scenario—when the net fitness of a transgenic fish is lower than that of its wild relatives, natural selection quickly purges any transgenes inherited by wild relatives. This is the safest scenario in that it does not pose any adverse environmental consequence. It is realistic to expect that some but not all lines of transgenic fish will fit this scenario.

Spread Scenario—Gene flow would lead to spread and persistence of the modified trait in the wild or feral population if the transgenic fish have equal or higher net fitness than their wild relatives. It is important to understand that transgenic fish with greatly reduced viability could still spread their transgenes if the transgenes cause a large enough improvement in other fitness traits. The order of importance of other fitness traits in determining whether the spread scenario applies is age at sexual maturity, followed by juvenile viability, mating advantage, female fecundity and male fertility (Muir and Howard, 2001; Rodriguez-Clark and Rodriguez, 2001).

Trojan Gene Scenario—Gene flow might trigger a steep decline in the wild or feral population under certain conditions of a tradeoff between increase in one fitness trait and decrease in another fitness trait. Recent research has identified two ways this could happen. The first case involves transgenic fish exhibiting a large mating advantage (e.g., fish engineered with growth enhancement genes that are larger at sexual maturity) that overwhelms a simultaneous moderate viability disadvantage. The mating advantage rapidly spreads the transgene in the wild population but the lower survival of each consecutive generation carrying the transgene eats away at the population size (Muir and Howard, 1999, 2001, 2001b). The second case is if transgenic fish show increased juvenile viability (e.g., fish engineered to contain a new disease resistance gene) and reduced fertility (Muir and Howard, 2001b). Increased viability increases the chances of surviving to reproduce successfully, thus spreading the transgene, but reduced fertility in each consecutive generation eats away at the number of progeny born and thus the population size. Unless the decline in either case is stemmed by human intervention or by strong, counteracting natural selection, the decline could drive the wild or feral population to extinction.

If the GEO at issue fits either the spread or Trojan gene scenario, assessment should then proceed to determine the likelihood and severity of undesired consequences (Scientists' Working Group on Biosafety, 1998; Pew Initiative on Food and Biotechnology in press). Undesired consequences might include such issues as loss of genetic resources harbored in the wild population (especially those that are centers of origin for the species); and enhanced predation or competition of the GEO causing harm to threatened or endangered species, sport fish, unique components of aquatic biodiversity, or species that play a key role in maintaining fish community resilience.

If the Trojan gene scenario held true in a real situation, particularly whenever the wild population was already depleted, the local extinction of a wild population could have cascading negative effects on the biological community. It is possible that researchers will eventually identify biological factors that prevent the Trojan gene scenario from happening in nature (and researchers are presently designing experiments to test the Trojan gene effect on fish populations in confined ecosystems). Meanwhile, taking a precautionary approach to any proposed aquaculture of a GEO would involve first requiring laboratory testing for changes in its six fitness components compared to wild-type relatives. In the absence of such key information, the *Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms* (Scientists' Working Group on Biosafety) recommends to "consider disallowing the release" or to implement multiple types of barriers to escape of culture organisms. For land-based aquaculture operations, the latter will likely require changing to a closed, recirculating water system or to a secure facility from which GEOs could not reach a Great Lake, Great Lake connecting body, or Great Lake tributary (see supporting text for question 14 and hazard 5).

If answer to this question is unknown, consult with operator.

Question 14.

If one or more hazards are identified, then the user needs to determine the feasibility of implementing risk reduction measures. A guiding principle is to apply a mix of different types of confinement measures, where each type has a fundamentally different vulnerability to failure (see flowchart VI.C and supporting text in Scientists' Working Group on Biotechnology, 1998). By mixing confinement measures with different vulnerabilities, one increases the chances that failure of one barrier will not breach all the barriers to escape of GEOs from the aquaculture operation. *Physical barriers* induce 100% mortality through such physical alterations as imposing lethal water temperatures or pH to water flowing out of fish tanks or ponds before the effluent is discharged to the environment. *Mechanical barriers* are devices, such as screens, that hold back any life stage of the GEO from leaving the aquaculture facility. *Biological barriers,* such as induced sterilization, are those that prevent any possibility of the GEO reproducing or surviving in the natural environment.

The exclusive farming of monosex, triploid fish that are functionally sterile is a feasible biological barrier for some transgenic fish species, such as salmon and trout (Solar and Donaldson, 1991; Donaldson et al., 1996; Cotter et al., 2000). But sole reliance on biological barriers would violate the risk management principle of applying multiple barrier types. Furthermore, biological barriers to reproduction are unknown for some freshwater aquaculture species, such as crayfish and aquatic plants. For example, there is no feasible way to make a freshwater alga sterile to prevent either sexual or asexual reproduction if some plants were to release propagules into aquaculture effluents or escape the culture facility. Sterilization of farmed genetically engineered algae, therefore, is not an option for helping to reduce establishment of a self-propagating population or to reduce gene flow to locally present wild relatives.

If answer to this question is unknown, seek assistance of the government agencies responsible for management of fisheries and environmental quality in the project area in reviewing the completed biosafety assessment.

Hazard 4.

Lack of a systematic biosafety assessment of the genetically engineered organisms proposed for aquaculture poses a hazard to aquatic biological communities. Although few empirical risk assessments have been conducted on genetically engineered aquatic organisms, a number of studies indicate possible ecological risks (see supporting text for Question 13). Modern evolution and ecology further point to the complex ways in which genetically engineered organisms could harm aquatic communities (Kapuscinski and Hallerman, 1991; Kapuscinski et al., 1999; Johnsson et al., 1999; Johnsson and Björnsson, 2001). Users should conduct a biosafety assessment using the *Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms* (Scientists Working Group on Biosafety, 1998) available at www.edmonds-institute.org/manual.html.

Hazard 5.

One or more specific ecological hazards of the genetically engineered organisms proposed for aquaculture have been identified. Although few empirical risk assessments have been conducted on genetically engineered aquatic organisms, a number of studies indicate possible ecological risks (see supporting text for Question 13). Modern evolution and ecology further point to the complex ways in which genetically engineered organisms could harm aquatic communities (Kapuscinski and Hallerman, 1991; Kapuscinski et al., 1999; Johnsson et al., 1999; Johnsson and Björnsson, 2001).

Switching to a closed, recirculating aquaculture system and relocation to a more secure site should also involve implementing different types of barriers to escape (as discussed in supporting text for question 14). Land-based farming allows implementing a mix of effective mechanical, physical and biological barriers. The diversity and number of barriers may need to be higher in flow-through systems than in recirculating aquaculture systems. The risk of fish escaping is typically lowest in recirculating systems because no more than 10% of the rearing water is discharged daily and many upstream components of the system (such as solids removal) also act as mechanical barriers to fish escape.

For further information on mechanical and physical barriers, refer to the risk management sections of existing biosafety assessment guides (Agricultural Biotechnology Research Advisory Committee, 1995; Scientists' Working Group on Biosafety, 1998). For biological barriers to reproduction, refer to the supporting text below.

Biological barriers: triploid induction and production of all-female lines.
The exclusive farming of monosex, triploid fish that are functionally sterile is a feasible biological barrier for some transgenic fish species, such as salmon and trout (Solar and Donaldson, 1991; Donaldson et al., 1996; Cotter et al., 2000). But sole reliance on biological barriers would violate the risk management principle of applying multiple barrier types. Furthermore, biological barriers to reproduction are unknown for some freshwater aquaculture species such as crayfish and algae.

Triploidy induction is widely accepted as the most effective method for producing sterile fish for aquaculture (Tave, 1993; Benfey, 1999). Triploidy induction disrupts gonadal development to *some* extent. Typically, gonadal development is more fully disrupted in females than in males. In general, ovarian growth is greatly retarded whereas testes grow to near normal size. Triploid males often produce viable sperm but at greatly reduced numbers and with aneuploid chromosome numbers and other abnormalities. In most though not all species, fertilization of eggs with milt from triploid males produces progeny that die at embryonic or larval stages. Typically, triploid females do not produce mature oocytes, although several studies that went beyond the normal first time of sexual maturation in diploids did report the occasional production of mature oocytes by triploid females. In summary, the production of all-female lines of triploids in fish and shellfish (Benfey, 1999; Thorgaard and Allen, 1992) is the best way to maximize disruption of gonadal development as a biological barrier to reproduction of aquacultural escapees. The commercial culture of all-female lines is now widespread in chinook salmon farming in British Columbia and rainbow trout farming North America, Europe and Japan. Monosex triploid trout are also widely grown and monosex triploid Atlantic salmon are grown commercially in Tasmania and possibly in Scotland (reviewed by Donaldson and Devlin, 1996:980).

Methods of triploidy induction are well described (see reviews in Benfey, 1999 and Thorgaard, 1995). Triploidy has been induced in numerous aquaculture species such as channel catfish, African catfish, various trout species, various salmon species, common carp, grass carp, various tilapia species, yellow perch, red sea bream, and various loach species (Benfey, 1999:51). The methods for production of all-female lines of fish vary depending on whether the species has an XY sex-determining system or a WZ sex-determining system, are also well described and have been used successfully on a broad variety of aquacultural species (reviewed by Tave, 1993:268-277).

Donaldson et al. (1996: figure 5) summarized the production cycle for integrating triploidy induction into a monosex line with additional detail provided by Donaldson and Devlin (1996a) for salmon, trout and other species with an XY sex-determining system. Applying this production cycle to transgenic fish involves initially developing an all-female line of transgenic fish, then fertilizing transgenic eggs with milt from the sex-reversed females and inducing triploidy on the newly fertilized eggs. Triploidy induction must occur every time the all-female transgenic line is bred to produce offspring for growout. Under experienced hands, one can expect rates of successful triploidy in the 90th percentile in large-scale production but this will vary with fish strain, egg quality, age of spawners, and induction conditions.

The critical risk management issue is whether to screen every individual destined for growout for the all-female triploid condition or only a sub-sample of each production lot. Screening for the all-female condition only needs to occur once in the development process. The most common screening method is progeny testing, although male-specific DNA probes provide a faster alternative in chinook salmon and perhaps someday in other species (Devlin 1994, Donaldson et al. 1996, Clifton and Rodriguez, 1997). Screening for triploidy must occur in every generation of production fish.

Individual screening has long been required for large-scale stocking of grass carp in Florida (Wattendorf and Phillippy 1996, Griffin 1991). The most effective screening method involves particle size analysis of fish blood samples with a Coulter Counter and Channelyzer (Wattendorf 1986, Harrell and Van Heukelem 1998). Estimated labor and supply costs in 1986 were $0.08 to $0.20 U.S. per screened fish (Wattendorf 1986). It should be possible to maintain or lower this cost at year 2000 prices through economies of scale and the application of computer automation technology. In any event, the cost of individual screening is a small fraction of the current market price of salmon smolts, trout fingerlings, or other early life stages purchased by grow-out farmers.

It is hypothetically possible to induce sterility in fish through gene transfer that aims to disrupt the production of a key enzyme or hormone involved in gonadal development. Some fish research in this direction is at a very early stage of development (e.g., Alestrom et al. 1992). The feasibility of this approach has not yet been proven. Induction of sterility solely by gene transfer might not be a good option because of vulnerabilities known to be inherent to gene transfer. Expression of the transgene responsible for sterility induction could be turned off at any time through methylation, something that genetic engineers do not know how to prevent. The transgene could also undergo rearrangement in the founders or descendants, thus possibly disrupting the expression needed to induce sterility.

Section IV.
Disease Management

Question 15.

Wild Fish Health Surveys are currently being conducted by the US Fish and Wildlife Service in partnership with individual states. These surveys will investigate all major watersheds throughout the United States and identify existing fish species, pathogens, number of sites, GPS coordinates and season/time of fish and pathogen collections. A database is currently being set up on the Internet that will enable users to download information (http://wildfishsurvey.fws.gov). Comparable Canadian Data should be considered where available.

If answer to this question is unknown, consult Richard Nelson, Director, Lacrosse Fish Health Center,U.S. Fish and Wildlife Service at 608-783-8441 for more information.

Question 16.

No additional supporting text.

If answer to this question is unknown, consult Richard Nelson, Director, Lacrosse Fish Health Center, U.S. Fish and Wildlife Service at 608-783-8441 for more information.

Question 17.

Some stocks from outside the state/province may be prohibited by the state of province.

If answer to this question is unknown, consult with management agency in your jurisdiction.

Question 18.

Some breeding programs have been developed to enhance resistance to disease. For example, Kaastrup et al. (1991) developed viral hemorrhagic septicemia resistance in some strains of rainbow trout (Plumb, 1994).

If answer to this question is unknown, consult operator or broodstock manager otherwise take a precautionary approach and answer no.

Question 19.

It is now possible to vaccinate fish for a number of bacterial diseases, particularly those that affect salmonids (Beveridge, 1996). Prevention prior to an outbreak may be more economical than treatment once an outbreak has occurred. The alternatives include treating fish with antibiotics or destroying all fish and starting over with disease-free stock (Souter, 1983). Because antibiotics are often administered through feed, and sick fish often go off feed, it is possible that excess food could 1) cause a buildup of settleable solids on benthic communities (see Lake-based Tool, Section VII, Settleable Solids for further discussion), 2) allow wild populations of fish and shellfish to consume feed containing antibiotics (Samuelson et al., 1992), or 3) develop bacterial populations that become resistant to antibiotics (Pillay, 1992).

Further information regarding vaccines can be found in the Guide to Drug, Vaccine and Pesticide Use in Aquaculture (Federal Joint Subcommittee on Aquaculture, 1994) and can be found on the Web at:
http://ag.ansc.purdue.edu/aquanic/publicat/govagen/usda/gdvp.htm or contact the Great Lakes Fish Health Committee.

If answer to this question is unknown, answer no.

Question 20.

Consult with operator.

If answer to this question is unknown, answer no.

Question 21.

Optimal conditions are species-specific and should be known before much effort is put into a proposal. Sub-optimal conditions may result in a stress response by the cultured organisms. Stress can be defined as a set of physiological events that result from biotic or abiotic challenges or forces that extend the homeostatic forces of an animal beyond its ability to control normal physiological function (Barton et al., 1991). A stimulus (the stressor) such as sub-optimal temperature or dissolved oxygen can result in a stress response, initially an adaptive response to adjust to the stressor. These primary stress responses include the release of hormones in the circulatory system.

If the stressor is prolonged, the animal exhibits secondary, mal-adaptive stress responses that compromise its biological functions. These include, for example, increases in ion and water fluxes, heart rate and output, respiration rate and glycogen to glucose metabolism in the liver (Barton et al., 1991), all secondary responses that require additional energy input. If the organism cannot move to more optimal environmental conditions, tertiary stress responses occur. These affect the whole body's function and health and include decreased growth and reproductive potential, increased disease susceptibility and, finally, mortality (Alabaster et al., 1980; Pickering, 1981; Anderson, 1990; Schreck, 1990).

If an organism's ability to maintain homeostasis is compromised by conditions such as temperature, dissolved gasses and current speeds that are sub-optimal for the specific species, mal-adaptive stress responses will result as the fish expends energy to compensate for the condition. Long-term or tertiary stress responses include decreased immunocompetence and can result in the organism succumbing to disease. There is an increased risk to cultured organisms if conditions are sub-optimal, and they are reared in waters that have had positively identified diseased fish. For a thorough review of stress responses of cultured fish, see Pickering (1998).

If answer to this question is unknown, refer to fish health specialist.

Question 22.
Disease control in the Great Lakes is essential for both cultured organisms and wild stocks. Due to the nature of culture conditions that are potentially stressful (refer to question 16), the likelihood of a disease epidemic is greater than for wild fish because of a pathogen's ease of transmission in water from fish to fish. For example, Kingsbury (1961) found a correlation between furunculosis outbreaks and specific environmental conditions such as water temperature above 10º C, dissolved oxygen levels below 5.5-6.0 mg/l, handling for size and transportation, and excessive crowding.

Disease transmission is most commonly documented with net-cage operations. For example, in 1985, furunculosis was found in Norwegian cage cultured Atlantic salmon after receiving smolts imported from Scotland. The disease was verified in 16 farms in Central Norway by the end of 1985 and, by 1991, 507 farms had been affected (Heggberget et al., 1993).

The Great Lakes Fish Health Committee of the Great Lakes Fishery Commission developed a Control Policy and Model Program (Hnath, 1993) in order to minimize the degree to which disease agents enter the Great Lakes. This document provides detailed inspection procedures and methods of diagnosis that a certified fish health specialist should follow. Note that this model program is currently under revision.
For information on the revised document, contact John Hnath at Hnathj@state.mi.us .

If answer to this question is unknown, consult with operator.

Question 23.

Removal of sick and dead animals can minimize the spread of pathogenic populations. The disposal of infected fish directly into a Great Lake is hazardous to both wild and cultured fish. Methods for storage and transfer of culled and dead fish should be identified and detailed in a written plan. The nearest land-based disposal site should also be identified here. Any additional construction of structures necessary for waste disposal should also be included in the disposal plan.

Hazard 6.

No additional supporting text.

Hazard 7.

No additional supporting text

Hazard 8.

A hazard to cultured organisms has been identified due to potential exposure to disease agents and suboptimal conditions. In addition, it is either not feasible to vaccinate fish or the operator is unwilling to vaccinate. These combined conditions increase the likelihood of cultured organisms succumbing to disease and possibly enhancing exposure to wild populations.

Hazard 9.

A hazard to the Great Lakes has been identified due to cultured stocks not verified as free of certified pathogens. Recommendations include alternative stocks or the use of adequate barriers (refer to Facility Connection to the Great Lakes section, question 4).

Hazard 10.

No additional supporting text.

Section V.
Effluent Management

Question 24.

If effluent is not released from the production system (for instance a static pond that is never drained), answer no to this question and proceed to the Habitat Alterations section. Fore all other situations should answer yes.

If unknown, consult with operator.

Question 25.

Most states have classifications that are analogous to Outstanding National Resource Waters. A description and discussion of Outstanding National Resource Waters is provided by the United States Environmental Protection Agency (May 18, 2001, personal communication) as follows:

> “The term ONRW arises from Federal Regulations at 40 CFR 131.12. ONRWs represent the third tier of antidegradation. 40 CFR 131.12 (a) (3) states:
>
> > ‘Where high quality waters constitute an outstanding National resource, such as waters of National and State parks and wildlife refuges and water of exceptional recreational or ecological significance, that water quality shall be maintained and protected.’
>
> Lowering of water quality in such waters is thus prohibited. As discussed in the preamble to the Antidegradation Regulation, limited short-term and temporary lowering of water quality is allowed.
>
> There are no specific criteria for designation of ONRWs. States and Tribes may designate as ONRWs any waterbodies they consider to be of exceptional significance. The only water quality criterion that applies to ONRWs is that existing water quality be maintained and protected.
>
> <u>State Classifications Analogous to ONRW</u>
>
> Illinois
> Antidegradation is found in Illinois' water quality standards at Section 302.105 Nondegradation. Illinois has no provisions analogous to Federal ONRWs. Illinois does not have the capability under existing State regulations to afford special protection to waters of exceptional significance.
>
> Indiana
> The antidegradation requirements for the State of Indiana are found at 327 IAC 2-1-2, Section 2. Indiana's state resource waters receive essentially equivalent

protection from reduced water quality that ONRWs do. Specific waters are so designated in Indiana's water quality standards. Any additions to this list would require a change to Indiana's water quality standards.

Indiana State Resource Waters

- The Blue River from river mile 57.0 to river mile 11.5
- Cedar Creek from river mile 13.7 to its confluence with the St. Joseph River
- The North Fork of Wildcat Creek from river mile 43.11 to river mile 4.82
- The South Fork of Wildcat Creek from river mile 10.21 to river mile 0.00
- The Indiana portion of Lake Michigan
- All waters incorporated in the Indiana Dunes National Lakeshore

Michigan

Michigan's antidegradation provisions are found at R 323.1098 of the State's water quality standards (Rule 98). Section 9 of this rule specifies that:

> ‘ ...[w]ild rivers designated under the wild and scenic rivers act,... rivers flowing into, through, or out of national parks or national lakeshores, and wilderness rivers designated under Act No. 231... shall not be lowered in quality.’

The protection given to these rivers is analogous to that given by ONRW status. No lakes receive this level of protection. Rivers could only be added by being included in one of the protected categories. This requires legislation by either the State or Federal governments.

Minnesota

Under 7050.0180, Nondegradation of Outstanding Resource Value Waters, Minnesota recognizes two levels of protection. The more stringent level provides protection equivalent to that of ONRWs. For waterbodies in this class, no new or expanded discharge is permissible. Waters to which this protection is extended include:

- all waters within the Boundary Waters Canoe Wilderness
- all waters within Voyageur's National Park
- all waters within Minnesota-designated scientific and natural areas
- Federal or State designated wild river segments.

Additional waters may be added provided there is an opportunity for a public hearing prior to designation.

Ohio

Ohio's water quality standards at 3745-1-05 are Ohio's antidegradation policy. Paragraph C establishes the category of State resource waters. State resource waters are defined as:

> '...surface waters that lie within national, state and metropolitan park systems, wetlands, and wildlife refuges, areas and preserves, and also include wild, scenic and recreational rivers, publicly owned lakes and reservoirs and waters of exceptional recreational or ecological significance... as determined by the director of (the) Ohio environmental protection agency.'

For such waters, there may be no lowering of water quality for toxic substances or those substances which might interfere with the designated use. Due to Ohio's broad definition of State resource waters, many waterbodies are so designated.

Wisconsin
Wisconsin's antidegradation implementation procedures are found at NR 207. NR 207 recognizes a class of waters identified as "Outstanding Resource Waters". Wisconsin's regulations require that waterbodies be specifically designated as Outstanding Resource Waters. All waters that are either National or State wild and scenic rivers and all Class I Trout streams are designated as Outstanding Resource Waters (See NR 102.10). Wisconsin allows new or increased discharges to Outstanding Resource Waters provided that the concentrations of the pollutants in the discharge are set equal to background concentrations in the receiving water.

Conclusions

All of the States in Region 5 except Illinois have some form of ONRW protection for waterbodies of exceptional significance. However, the extent of that protection varies from state to state. The water quality standards of Indiana and Minnesota appear to most closely approximate the Federal regulations."

New York does not follow ONRW classification and instead regulates waters of significance through a mechanism it deems as "discharge restriction categories." A brief discussion can be found under the following headings: NY State Department of Environmental Conservation Regulations; Chapter X - Division of Water Resources; Subchapter A. General; Article 2. Classes and Standards of Quality and Purity; PART 701 Classifications-Surface Waters and Groundwaters; Discharge Restriction Categories; 701.20 Purpose & 701.21 Criteria**.**
http://www.dec.state.ny.us/website/regs/701.htm#701.20 and
http://www.dec.state.ny.us/website/regs/701.htm#701.21 (Tom Snow, New York Department of Environmental Conservation, June 11, 2001, personal communication).

If answer to this question is unknown, consult state or province water management specialist.

Question 26

As of December, 2001, the United States Environmental Protection Agency is working with the Joint Subcommittee on Aquaculture Effluent Task Force to assess the need for revised effluent regulations. To keep abreast of activity, refer to the Joint Subcommittee on Aquaculture Effluent Task Force webpage at:
http://ag.ansc.purdue.edu/aquanic/jsa/effluents/index.html or contact Gary Jensen, Chair, gjensen@reeusda.gov. (At this time, National Pollutant Discharge Elimination System (NPDES) permits are required in the United States under the Clean Water Act. section 402 (Title 33, Chapter 26, § 1342, USC). For more information about NPDES permits, see the following:
Illinois: http://www.epa.state.il.us/about/org/bureau-of-water.html#dwpc
Indiana: http://www.state.in.us/idem/owm/npdes/guide/index.html
Michigan: http://www.deq.state.mi.us/swq/
Minnesota: http://www.pca.state.mn.us/water/permits.html
Ohio: http://chagrin.epa.ohio.gov/programs/permits.html
Wisconsin: http://www.dnr.state.wi.us/org/water/wm/ww/
New York: http://www.dec.state.ny.us/website/dow/index.html
Pennsylvania: http://www.epa.gov/reg3wapd/npdes/

The permitting process in Ontario is explained below by Mark Muschett, Aquaculture Poicy and Planning Biologist, Fish Culture Section, Fish and Wildlife Branch, Ontario Ministry of Natural Resources (June 20, 2001, personal communication).

> "The Ministry of the Environment's legislative authority to manage water comes primarily from two acts, the Ontario Water Resources Act (OWRA) and the Environmental Protection Act (EPA). Links to the relevant MOE legislation at - http://www.e-laws.gov.on.ca/home_E.asp?lang=en
>
> The OWRA gives the MOE extensive powers to regulate water supply, sewage disposal and to control sources of water pollution. The EPA prohibits the discharge of contaminants to the natural environment, including water except where specifically permitted by a Certificate of Approval. In addition, the Environmental Assessment Act (EAA) and the Pesticides Act also apply. The EAA sets out a planning procedure to ensure that potential social, cultural, economic and natural environmental effects and any actions necessary to mitigate/enhance these effects are considered before works are constructed. The Pesticides Act seeks to control pesticides by regulating their sale, use, transportation and disposal and through the licensing of commercial applicators. Aquacultural chemicals may either be classed as pesticides or drugs depending upon whether the chemical has a PCP number or a DIN number. PCP numbered chemicals are pesticides and are regulated under Provincial legislation. DIN numbered chemicals are classed as drugs and are regulated Federally by Health and Welfare Canada.
>
> The Water Management Policies, Guidelines and Provincial Water Quality

Objectives of the Ministry of the Environment, more commonly known as the ’Blue Book’ are a tool through which best management practices to safeguard water quality are established. The policies and guidelines themselves do not have any formal legal status but assist in making decisions related to the mandate and legislation of the Ministry. For example, they give directions that assist in defining site-specific effluent limits, which then may be incorporated into Certificates of Approval or Control Orders. These control documents are issued under the authority of the legislation, and thus become legally binding and constitute the basis for compliance and enforcement actions.

The surface water policies contained in the Blue Book require a minimum acceptable level of water quality to be defined for the waters of the Province. This defined level of water quality provides a baseline for assessing the quality of the waters of the province and acts as a simple, surrogate measure of ecosystem health. The minimum acceptable level of water quality is represented by the Provincial Water Quality Objectives (PWQOs). The PWQOs are scientifically based and are structured for the protection of aquatic life and recreation uses.

Two of the most important MOE policies contained in the Blue Book relate to surface water quality. These policies set out the overall framework for preserving surface water quality in the Province and form the basis for abatement decisions and actions by the MOE's Operations Division. As stated they are:
Policy 1: ‘In areas which have better quality than the PWQO, water quality shall be maintained at or above the Objectives.’
Policy 2: ‘Water quality which presently does not meet the PWQO shall not be degraded further and all practical measures shall be taken to upgrade the water quality to the Objectives.’

The Blue Book does not contain detailed procedures on how to achieve the protection of water resources as defined by the goals and policies. Towards this end, numerous procedures, guidelines and /or regulations have been developed over the years by the various MOE Divisions and Branches whose mandate it is to protect water quality. Implementation of MOE's water policies and guidelines is undertaken by their Operations Division. Where guidelines do not exist, interim operational procedures are usually developed at the regional level through a lead Director responsible for that activity. Internal consultation, consultation with stakeholders and consultation with other Ministries normally occurs before a final guideline, water quality standard or regulation is written or set. More formal public consultation through the Environmental Bill of Rights (EBR registry) must occur for all legal Ministry instruments (i.e. policies, regulations, water quality limits and most MOE guidelines).

> The protection of water quality is the key consideration for the MOE with regard to the development and operation of new and existing aquaculture sites Through the approval process (section 53, OWRA) and the Permit to Take Water ((PTTW) section 34, OWRA), the water quality limits, policies, guidelines and procedures of the Ministry are translated into legal requirements as terms and conditions on Certificates of Approval for sewage works and conditions on permits to take water for water taking facilities. Traditional land based aquaculture operations which have a piped discharge to the environment, require both an approval for sewage works and a permit to take water under MOE legislation."

If answer to this question is unknown, contact state pollution control agencies, the Canadian Ministry of the Environment or Ontario Ministry of the Environment for assistance.

Question 27.

At issue are severely degraded geographic areas in the Great Lakes basin called Areas of Concern (AOCs). These areas have been defined by the Great Lakes Water Quality Agreement as geographic areas that fail to meet the general or specific objectives of the agreement where such failure has caused or is likely to cause impairment of beneficial use of the area's ability to support aquatic life (Annex 2 of the 1987 Protocol, http://www.ijc.org/agree/quality.html#ann2). An impaired beneficial use means a change in the chemical, physical or biological integrity of the Great Lakes system sufficient to cause any of the following:

- restrictions on fish and wildlife consumption
- tainting of fish and wildlife flavor
- degradation of fish wildlife populations
- fish tumors or other deformities
- bird or animal deformities or reproduction problems
- degradation of benthos
- restrictions on dredging activities
- eutrophication or undesirable algae
- restrictions on drinking water consumption, or taste and odor problems
- beach closings
- degradation of aesthetics
- added costs to agriculture or industry
- degradation of phytoplankton and zooplankton populations
- loss of fish and wildlife habitat

43 sites (Figure 2.) have been identified and Remedial Action Plans (RAPs) have been developed for each. Aquaculture facilities within a zone of influence may adversely affect recovery plans for a given Area of Concern.

Figure 2. Areas of Concern (Environment Canada http: www.cciw.ca/glimr/raps/aoc-map-large.html).

General information about AOCs can be found at http://www.great-lakes.net/places/aoc/aoc.html .Details including background, updates and contacts of each AOC can be located at: http://www.cciw.ca/glimr/raps/aoc-map.html

Note: In addition to Areas of Concern, Aquatic Biodiversity Inveestment Areas should also be considered. These are currently defined as “a specific location or area within a larger ecosystem that is especially productive, supports exceptionally high biodiversity and/or endemism and contributes significantly to the integrity of the whole ecosystem” (Koonce, et al., 1999). Presently, 168 sites within the Great Lakes basin have been identified with 49% of those sites identified as supporting 'high biodiversity' and 39 of the sites are located within IJC designated Areas of Concern (Koonce, et al., 1999).

If answer to the question is unknown, refer to map and web sites mentioned above.

Hazard 11.

No additional supporting text.

Hazard 12.

Supporting text in preparation, will cite relevant scientific publications.
.

Habitat Alterations

Question 28.

Wetland habitat for fish and wildlife is at issue. In addition, wetland habitat may be essential for the recovery of species at risk. The United States Endangered Species Act (ESA) of 1973 (http://endangered.fws.gov/esa.html) states that "the purposes of this Act are to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved…" To identify habitat necessary for recovery, the Act requires determination and designation of critical habitat for listed species unless it is determined that 1) identification activities would harm the species (for example, if the identification of critical habitat for an endangered species would increase the risk of a 'taking' for species or the associated human activity would threaten the species); or 2) it is not determinable due to insufficient information. Unfortunately, as of August 1999 only 113 species have designated critical habitat of the 1179 federally listed species (64 Federal Register 31871).

In the United States, federal species at risk in the Great Lakes basin are managed by Region 3 and Region 5 of the U.S. Fish and Wildlife Service (USFWS). Contacts can be found at http://endangered.fws.gov/contacts.html.

Region 3, the Great Lakes-Big Rivers Region, includes: Illinois (25 federally listed species), Indiana (24 federally listed species) , Michigan (21 federally listed species), Minnesota (12 federally listed species), Ohio (22 federally listed species), and Wisconsin (15 federally listed species). http://endangered.fws.gov/statl-r3.html.

Region 5, the Northeast Region includes: New York (15 federally listed species) and Pennsylvania (16 federally listed species). http://endangered.fws.gov/statl-r5.html.

The above include both terrestrial and aquatic species. In addition to federally listed species, each state has its own list of species at risk. Therefore, consultation with both the USFWS and state managing agencies should take place to identify if possible critical habitat may be affected by either the proposed aquaculture facility's infrastructure or by a large accidental release of cultured organisms.

In Canada, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (http://www.cosewic.gc.ca/) evaluates and designates the status of species considered to be at risk (currently 150 species have been listed for Ontario). The Committee on the Status of Species at Risk in Ontario (COSSARO), the provincial committee that evaluates and makes recommendations for at risk species is also a member of COSEWIC. Range maps, legal and management measures, overviews of biological traits and other important information for the endangered or threatened species of Ontario can be accessed by the Species at Risk Module, jointly assembled by the Royal Ontario Museum (ROM) and the Ontario Ministry of Natural Resources at http://www.rom.on.ca/ontario/risk.html.

In the United States, under the Clean Water Act, Section 404, a permit is required to alter wetlands. In Ontario, no one entity has sole oversight of wetlands in the Province. The Ministry of Natural Resources creates the regulations for wetlands (as a general classification) and then municipalities interpret the regulations and decide how to apply these regulations to wetlands on a case by case basis.

If answer to this question is unknown, contact the agencies mentioned above. A formal risk assessment may be necessary.

Hazard 13.

A hazard to fish and wildlife habitat has been identified. Filling or draining of wetlands and clearing of vegetation are alterations that can completely eliminate species and biological communities, cause fragmentation of the ecosystem, increase edge effects, eliminate connectivity and reduce a natural area so that it is too small for a viable population (National Research Council Committee on Restoration of Aquatic Ecosystems-Science, Technology and Public Policy, 1992; Nature Conservancy Great Lakes Program, 1994).

Section VII.
Water Source Issues

Question 29.

The water source will be surface water, ground water or city/municipal water.

If answer to this question is unknown, consult with operator.

Question 30.

Habitat preservation is critical in the recovery of species at risk. The United States Endangered Species Act (ESA) of 1973 (http://endangered.fws.gov/esa.html) states that "the purposes of this Act are to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved…" To identify habitat necessary for recovery, the Act requires determination and designation of critical habitat for listed species unless it is determined that 1) identification activities would harm the species (for example, if the identification of critical habitat for an endangered species would increase the risk of a 'taking' for species or the associated human activity would threaten the species); or 2) it is not determinable due to insufficient information. Unfortunately, as of August 1999 only 113 species have designated critical habitat of the 1179 federally listed species (64 Federal Register 31871).

In the United States, federal species at risk in the Great Lakes basin are managed by Region 3 and Region 5 of the U.S. Fish and Wildlife Service (USFWS). (Contacts can be found at http://endangered.fws.gov/contacts.html.)

Region 3, the Great Lakes-Big Rivers Region, includes: Illinois (25 federally listed species), Indiana (24 federally listed species) , Michigan (21 federally listed species), Minnesota (12 federally listed species), Ohio (22 federally listed species), and Wisconsin (15 federally listed species). http://endangered.fws.gov/statl-r3.html.

Region 5, the Northeast Region includes: New York (15 federally listed species) and Pennsylvania (16 federally listed species). http://endangered.fws.gov/statl-r5.html.

The above include both terrestrial and aquatic species. In addition to federally listed species, each state has its own list of species at risk. Therefore, consultation with both the USFWS and state managing agencies should take place to identify if possible critical habitat may be affected by either the proposed aquaculture facility's infrastructure or by a large accidental release of cultured organisms.

In Canada, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (http://www.cosewic.gc.ca/) evaluates and designates the status of species considered to be at risk (currently 150 species have been listed for Ontario). The Committee on the Status of Species at Risk in Ontario (COSSARO), the provincial committee that evaluates and makes recommendations for at risk species is also a member of COSEWIC. Range maps, legal and management measures, overviews of biological traits and other important information for the endangered or threatened species of Ontario can be accessed by the Species at Risk Module, jointly assembled by the Royal Ontario Museum (ROM) and the Ontario Ministry of Natural Resources at http://www.rom.on.ca/ontario/risk.html.

If answer to this question is unknown, contact the agencies mentioned above. A formal risk assessment may be necessary.

Question 31.

Some states and provinces require water withdrawl permits. For example, Ontario requires a permit for facilities using more than 50,000 liters/day; Wisconsin for more than 70 gallons/minute (Wisconsin DNR, 2001); Indiana for more than 100,000 gallons/day. Some states such as Michigan, Pennsylvania, New York and Illinois do not regulate water withdrawal (Pennsylvania Department of Environmental Protection, 2001, personal communication; Rice, Michigan Department of Environmental Quality, 2001, personal communication, New York Department of Environmental Conservation, 2001, personal communication).The Riparian Rights Doctrine is recommended by most agencies.

If answer to this question is unknown, consult with state or province water management specialist.

Question 32.

So far, no known scientific documentation of adverse effects from aquaculture due to water withdrawal in the Great Lakes Basin exists, presumably due to the small scale of total water use by existing operations. With expected growth in aquaculture production, however, it is important to be proactive in addressing water withdrawal questions so that problems do not arise in the future.

Adverse affects of water withdrawal from aquifers may include..(in preparation, will cite relevant scientific papers). User should consider multiple use effects and planned expansion of the aquaculture operation at issue.

If answer to this question is unknown, consult with state or province water management specialist.

Question 33.

So far, no known scientific documentation of adverse effects from aquaculture due to water withdrawal in the Great Lakes Basin exists, presumably due to the small scale of total water use by existing operations. With expected growth in aquaculture production, however, it is important to be proactive in addressing water withdrawal questions so that problems do not arise in the future

Adverse affects of water withdrawal from surface waters may include..(in preparation, will cite relevant scientific papers). User should consider multiple use effects and planned expansion of the aquaculture operation at issue.

If answer to this question is unknown, consult with state or province water management specialist.

Question 34.

Cumulative multiple users or the eventual expansion of the aquaculture operation at issue may cause adverse effects to the watershed in which the operation is located. Presently, no known adverse watershed effects due to aquaculture operations exist, however given the rapid expansion of the aquaculture industry over the past two decades and the increasing competition between multiple water users, potential cumulative effects may need to be examined and evaluated more closely.

If answer to this question is unknown, consult with state or province water management specialist.

Hazard 14.

No additional supporting text.

Hazard 15.

The aquifer may be at risk resulting in a lower water table that could eventually affect other users of the watershed at issue.

Hazard 16.

Water withdrawal may pose a hazard to the water body at issue by changing instream flow.

Hazard 17.

The aquifer may be at risk if multiple users required increasing amounts of water or the aquaculture operation were to expand and require larger volume of water.

Optional Precautionary Plans

Optional plans include an emergency recovery plan for escaped fish, a fish health contingency plan, a fish disposal plan, and a predator prevention plan. To be truly useful, aquaculture facility managers should have written versions of these plans and train staff to implement the plans.

Emergency Recovery Plan

The purpose of this plan is to define the most common types of emergencies that might occur at a facility and outline measures to prevent loss of the cultured fish.

Responsible party. The facility operator or designated proxy must be available in person or by phone at all times to respond to emergency problems.

Notification of loss of confinement. In the event of loss of confinement, the responsible party must notify responsible local agencies. In most cases, the first local agency to contact is the local office of the state or provincial fisheries management agency.

Mitigation or recovery plan. The emergency response plan should include a plan for mitigation or recovery of escaped cultured fish in cases where the facility site and biological features of the cultured fish allow recovery or mitigation. The state or provincial fisheries management agency should be involved in development of such a plan because it will probably have oversight authority over any recovery or mitigation actions that occur in natural waters. (Agricultural Biotechnology Research Advisory Committee, Working Group on Aquatic Biotechnology and Environmental Safety, 1995: 46-47).

Predator Prevention Plan

The purpose of this plan is to minimize the impact that piscivorous birds and mammals have on cultured fish. Mortalities, infection as a result of injury caused by piscivores, and rearing unit damage from predator actions, (leading to escape of cultured fish), all may result in serious economic loss. Many piscivores exist in the Great Lakes and this plan should identify populations that may prey on fish reared in the proposed facility as well as preventative measures that will be taken to minimize encounters between predators and cultured fish.

Monitoring

In the event that a hazard has been identified and the risk accepted, effective monitoring for the specific hazard and its environmental effects should be part of the plan. Before an aquaculture facility begins operation, baseline measurements of the site's relevant biological, chemical, and physical variables should be taken to allow valid comparison of changes against pre-operation conditions. Threshold limits should be identified and agreed upon before the start of production, thereby reducing the need for emergency measures. The operator should know what specific actions to take if monitoring suggests conditions are approaching threshold limits. For example, if escapes exceed a specified percentage of production stock, additional safeguards such as stronger netting, improved methods of handling, or better predator control may be suitable actions to reduce the risk of reaching a threshold limit.

In another example, if the assessment tool has identified a hazard to benthic organisms, it would be appropriate to take a baseline measurement of sediment chemistry and benthic biota. Monitoring of sediments can assist operators in identifying whether an operational change such as different feeding strategies or if a reduction in production volume will be necessary to adjust to the assimilative capacity of the local environment. Fallowing, although accepted in many marine production areas, should be considered only as an emergency measure, for instance, to break a disease cycle. The preferred solution to excessive organic buildup from aquaculture food and feces is to achieve a production volume that matches the assimilative capacity of the local environment. Because it takes much longer for a benthic environment to recover than it does to load the site with organic wastes, fallowing merely increases the areas impacted by an aquaculture facility (Black, 1998). Thus, it is not appropriate as a routine method of managing overproduction of waste. Operating at an appropriate production volume combined with relevant monitoring can better minimize the hazard to the benthic environment.

Although often costly and logistically difficult to carry out, monitoring that employs feasible data collection methods, with sufficient statistical power to detect change, should be considered for any hazards identified in the assessment tool. Conclusions drawn from statistical analysis of monitoring results might involve one of two types of error. A type I error occurs when the statistical analysis indicates that the aquaculture facility has an adverse effect when in fact no such harm exists. A type II error occurs when the analysis indicates that the aquaculture facility has no adverse effect when in fact it does cause environmental harm. The potential for harm is greater when a type II error occurs than when a type I error occurs. Most environmental harms involve long time lags before recovery and some environmental damage is irreversible (Dayton, 1998). Type I errors, in contrast, are usually limited to short-term economic costs (Dayton, 1998). Monitoring activities should therefore seek to minimize type II errors.

Flowchart Summary Documentation Worksheet

No. Flowchart Section

I. Determination of Assessment of Pathway led to:

___Hazard 1: Harvesting organisms in these infested waters may pose a hazard to the Great Lakes aquatic ecosystem if aquatic nuisance species were accidentally released. Operator must demonstrate acceptable specific points during processing that enable the operator to identify and remove aquatic nuisance species organisms.

___Risk accepted. Demonstration is acceptable.

___Risk not accepted. Demonstration is not acceptable. Harvest in water bodies that do not contain aquatic nuisance species.

___Hazard 2: Collection methods may pose hazard to habitat. Operator must identify methods to minimize impact of collecting or harvesting in this area.

___ Risk accepted. Methods have been identified.

___Risk not accepted. Harvest in areas less vulnerable to collection methods.

___Go to Lake-based Assessment Tool.

___Go to Section II, Facility Connection to the Great Lakes.

___Go to Lake-based Section X, Impacts of Facility and Infrastructure.

II Facility Connection to the Great Lakes led to:

___Hazard 3: Introducing (a) a new species or (b) a genetically novel population of an existing species poses a hazard to the Great Lakes. Refer to the Council of Lake Committee's Procedures for Consultation for Introductions in the Great Lakes Basin (1992).

___For new species: endorsed

___For new species: not endorsed. Revise operation proposal so that cultured organisms and effluent will not reach a tributary that flows into the Great Lakes, a connecting water body of the Great Lakes or one of the Great Lakes.

___For genetically novel population: add more barriers

___For genetically novel population: relocate operation

___For genetically novel population: conduct full risk assessment

___Terminal Point 1: Contact managing agencies for approved species.

___Go to Section III, Genetically Engineered Organisms Assessment.

III ____Genetically Engineered Organisms Assessment led to:

___Hazard 4: Genetically engineered organisms that enter a Great Lake pose hazards to the Great Lakes aquatic communities.

___Conduct assessment using Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms.
___Did not conduct assessment.

___Hazard 5: Biosafety assessment identified specific hazards posed by the genetically engineered organisms.

___Revised project to include switching to closed, recirculating water system.
___Revised project to include relocating to a secure land-based facility where GEOs and effluent cannot reach a Great Lake, G.L. connecting body or G.L. tributary
___Revised project to include switching to culture of non-engineered organisms. .
___Did not revise project.

___ Go to Section IV. Disease Assessment

IV. Disease Assessment led to:

___Hazard 6: Water supply may contain pathogens hazardous to cultured animals. There is an additional risk to cultured organisms if there are livestock or aquaculture facilities upstream.

___Risk accepted.
___Risk not accepted.

___Hazard 7: Stocks from outside the state/province may be prohibited by the state/province. Consult with the managing agency.

___Stocks accepted.
___Stocks not accepted.

___Hazard 8: Culturing fish in sub-optimal conditions and exposing them to disease agents pose a hazard to both the cultured fish and subsequently to wild fish if a disease outbreak occurs.

___Risk accepted.
___Risk not accepted.
    ___Relocate facility to more optimal conditions.

___Hazard 9: Broodstock/Production stock not verified free of certified pathogens may pose hazard to Great Lakes if animals or effluent entered a Great Lake tributary or lake.

___Risk accepted.
___Risk not accepted.

___Hazard 10: Mortalities not properly disposed of and accessible to predators pose a hazard to both cultured and wild aquatic animals by spreading pathogens.

___Risk accepted.
___Risk not accepted.

___ Go to Section V. Effluent Assessment

V. Effluent Assessment led to:

___Hazard 11: Effluent discharge poses a hazard to waters that have an outstanding resource value. Effluent discharge may be prohibited outright or restricted (based on water quality factors) for some water bodies. Consult with relevant federal, state or provincial agency for assistance**.**

___Risk accepted.
___Risk not accepted.

___Hazard 12: Effluent discharge may pose hazard to aquatic organisms in receiving water bodies. Consult with appropriate agencies.

___Risk accepted.
___Risk not accepted.

___Go to Section VI, Habitat Alterations.

VI Habitat Alterations led to:

___Hazard 13: The operation poses a hazard to wild organisms including those that are already "at risk" of decline or extinction. Consult with appropriate management agency.

___Approval granted.
___Disapproved. Relocate to area that will not adversely affect "at risk" organisms.

___Go to Section VII, Water Source Assessment.

VII Water Source Assessment led to:

___Hazard 14: The operation poses a hazard to wild organisms including those that are already "at risk" of decline or extinction. Consult with appropriate management agency.

___Approval granted.

___Disapproved. Relocate to area that will not adversely affect "at risk" organisms.

___Hazard 15: Aquifer may be at risk resulting in lower water table.Consider utilizing partial water recovery by recirculating technology or reducing projected production level.

___Risk accepted..

___Risk not accepted.

- ___Use partial water recovery.
- ___Reduce production volume.

___Hazard 16: Withdrawal may pose hazard to stream users. Consider utilizing partial water recovery by recirculating technology or reducing projected production level. Consider utilizing partial water recovery by recirculating technology or reducing projected production level.

___Risk accepted..

___Risk not accepted.

- ___Use partial water recovery.
- ___Reduce production volume.

___Hazard 17: Aquifer may be at risk resulting in lower water table. Consider utilizing partial water recovery by recirculating technology or reducing projected production level. Consider utilizing partial water recovery by recirculating technology or reducing projected production level.

___Risk accepted..

___Risk not accepted.

- ___Use partial water recovery.
- ___Reduce production volume.

___Go to Optional Precautionary Plans.

Upon completion of Assessment Pathway flowcharts and Summary Documentation Worksheet, proceed to Monitoring.

Glossary

Benthos - bottom-dwelling aquatic plants and animals.

Bioaccumulation - the net accumulation of a substance by an organism as a result of uptake from all environmental sources. As an organism ages, it can accumulate more of these substances, either from its food or directly from the environment. Bioaccumulation of a toxic substance has the potential to cause harm to organisms, particularly to those at the top of the food chain.

Biodiversity - The variety of life and its processes, including the variety of living organisms, the genetic differences among them, and the communities and ecosystems in which they occur.

Critical habitat - Specific geographic areas, whether occupied by listed species or not, that are determined to be essential for the conservation and management of listed species, and that have been formally described in the Federal Register.

As defined by the Endangered Species Act of 1973, critical habitat means i) the specific areas within the geographical area occupied by the species, at the time it is listed in accordance with the provisions of section 4 of this Act, on which are found those physical or biological features (I) essential to the conservation of the species and (II) which may require special management considerations or protection; and (ii) specific areas outside the geographical area occupied by the species at the time it is listed in accordance with the provisions of section 4 of this Act, upon a determination by the Secretary that such areas are essential for the conservation of the species.

Endangered - The classification provided to an animal or plant in danger of extinction within the foreseeable future throughout all or a significant portion of its range.

Exotic – "Exotic", "alien", "introduced", "nonindigenous" and "nonnative" are all synonyms for species that humans intentionally or unintentionally introduced into an area outside of a species' natural range.

Feral - refers to a fish or another aquatic species that is not native to a natural water body, but has established a self-reproducing population in the water body, resulting from successful reproduction of intentionally or accidentally introduced individuals.

Fitness - in population and evolutionary biology, the success in survival and reproduction of an individual organism, a population, or a species, relative to other individuals, populations or species; the number of offspring that survive to reproduce.

Habitat - The location where a particular taxon of plant or animal lives and its surroundings (both living and nonliving) and includes the presence of a group of particular environmental conditions surrounding an organism including air, water, soil, mineral elements, moisture, temperature, and topography.

Harm - An act which actually kills or injures wildlife. Such acts may include significant habitat modification or degradation when it actually kills or injures wildlife by significantly impairing essential behavioral patterns including breeding, feeding, or sheltering

Hazard - an act or phenomenon that has the potential to produce harm or other undesirable consequences to humans or what they value (e.g. a fish species, biodiversity, an entire ecosystem). Hazards may come from physical phenomena (such as floods, fire), chemicals (pesticides, antimicrobial agents), organisms (introduced species, pathogens), commercial products, or human behavior

Infested waters – Waters that have aquatic nuisance species.

Listed species - A species, subspecies, or distinct vertebrate population segment that has (in the United States) been added to the Federal lists of Endangered and Threatened Wildlife and Plants as they appear in sections 17.11 and 17.12 of Title 50 of the Code of Federal Regulations (50 CFR 17.11 and 17.12).

Great Lakes Water Quality Agreement - an agreement signed in 1978 by the United States and Canada and amended in 1987. Its purpose is to restore and maintain the chemical, physical and biological integrity of the waters of the Great Lakes Basin ecosystem.

Growout - Farming of aquatic organisms can be divided into different stages. For example, fish farming can be generally divided into broodstock and egg production, rearing of juveniles and finally growout. Growout is the stage of production that ends with a marketable organism. An aquaculture facility may include all production stages or specialize in one area.

Keystone Species - A species on which the persistence of a large number of other species in the ecosystem depends.

Net cages - Net cages are floating open mesh containment areas for rearing aquatic organisms. These structures can be flexible to rigid. The cages allow for ambient water to freely move into and out of the rearing area. The shape can be square, rectangular, circular, octagonal or hexagonal. These cages vary in size, but can be 10-15 square meters and 10-35 meters in depth. The cages can be moored independently or in an array with 2 cages to a group to 60 or more.

Nuclear marker - information about nuclear genes (in contrast to genes found in animal mitochondria or plant chloroplasts); includes proteins, which are encoded by nuclear genes, chromosomal structures (such as chromosome banding patterns), RNA or DNA.

Outbreeding Depression - a reduction in fitness due to mating of genetically divergent individuals. Like inbreeding depression, outbreeding depression can result from loss of local adaptation, or breakdown of coadapted genes or chromosomes at different loci. Reductions in fitness due to loss of local adaptation may occur in the F_1 generation whereas reductions due to breakdown of coadapted gene complexes are more likely to occur in the F_2 generation because F_1 hybrids retain an entire chromosomal array from each parent (Allendorf and Waples 1996).

Panmictic - refers to a population in which mating is completely random (as opposed to assortative mating between certain adults in the population).

Polymorphic - having two or more forms (alleles) of a gene.

Population - a local (geographically defined) group of conspecific organisms sharing a common gene pool; also called deme.

Propagule - asexual portions of an organism that are capable of dispersal and formation of a new individual.

Recovery -The process by which the decline of an endangered or threatened species is arrested or reversed, or threats to its survival neutralized so that its long-term survival in nature can be ensured.

Resilience - the ability of an interconnected community of living organisms to recover from shocks caused by nature (e.g., storms, floods, land-slides) or humans (e.g., toxic waste spills, ballast water introductions of nuisance species). A resilient fish community has enough of its living and non-living components in healthy enough condition that it can recover from such shocks and settle into a state resembling its pre-shock state, retaining such desirable features as abundant fisheries and clean water. A fish community that has lost resilience responds to such shocks by shifting, often rapidly and with only subtle warning, into an unstable and degraded state, for example, sudden over-dominance of a nuisance species, collapses of fish catches, or degradation of water quality.

Risk- an estimate of the probability or likelihood of occurrence of an identified hazard.

Species at Risk- includes those species or populations classified as endangered or threatened under the United States Endangered Species Act and those classified as endangered, threatened or vulnerable as designated by Committee on the Status of Endangered Wildlife in Canada under the Canadian Species at Risk Act.

Strain - an intraspecific group of organisms possessing only one or a few distinctive traits, usually genetically homozygous (pure-breeding) for those traits and maintained as an artificial breeding group by humans for domestication (e.g., in agriculture or aquaculture) or experimentation.

Threatened - The classification provided to an animal or plant likely to become endangered within the foreseeable future throughout all or a significant portion of its range.

Transgenic - refers to organisms whose genetic composition has been altered to include specific genes from other organisms of the same or different species by methods other than those used in traditional breeding; this is typically accomplished through recombinant DNA or cloning methods.

Vulnerable - A species of special concern because of characteristics that make it particularly sensitive to human activities or natural events.

Some definitions come from or are adapted from:
Bagheera and ESBN (1996); COSEWIC (1998); Environment Canada (2000); US Fish and Wildlife Service Region 3 (1997); King and Stansfield (1990); Stern and Fineberg (1996); Scientists' Working Group on Biotechnology (1998); United States Environmental Protection Agency (2000)

References for Land- and Lake-based Assessment Tools

Agricultural Biotechnology Research Advisory Committee, Working Group on Aquatic Biotechnology and Environmental Safety. 1995. Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish. Parts I & II. United States Department of Agriculture, Office of Agricultural Biotechnology, Documents No. 95-04 and 95-05. Washington, D.C.

Aalto, S.K., and G.E. Newsome. 1990. Additional evidence supporting demic behavior of a yellow perch (*Perca flavescens*) population. Canadian Journal of Fisheries and Aquatic Sciences 47:1959-1962.

Ackefors, H. and M. Enell. 1994. The release of nutrients and organic-matter from aquaculture systems in Nordic countries. Journal of Applied Ichthyology 10:225-241.

Alabaster, J. and R. Lloyd. 1980. Water quality criteria for freshwater fish, 2nd edition. Butterworth, London.

Alestrom, P. G. Kisen, H. Klungland and O. Anderson. 1992. Fish gonadotropin-releasing hormone gene and molecular approaches for control of sexual maturation: development of a transgenic fish model. Molecular Marine Biology and Biotechnology 1:376-379.

Allendorf, F.W. and R. S. Waples. 1996. Conservation and genetics of salmonid fishes. Pages 238-280 *in* J.C. Avise and J.L. Hamrick, eds. Conservation Genetics: Case Histories from Nature. Chapman and Hall, New York.

Alverson, D. and G. Ruggerone. 1997. Escaped farm salmon: environmental and ecological concerns. Discussion Paper Part B. In Salmon Aquaculture Review. Technical Advisory Team Discussion Papers Vol. 3. Environmental Assessment Office, Victoria B.C.

Anderson, D. 1990. Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. American Fisheries Society Symposium 8:38-50.

Anderson, R. 1999. Atlantic salmon escape into Sound from pens. Seattle Times. 15 June 1999.

Animal and Plant Health Inspection Service. 1997. Bird Predation and Its Control at Aquaculture Facilities in the Northeastern United States. APHIS 11-55-009. United States Department of Agriculture.

Angers, B., L. Bernatchez, A. Angers and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology 47(Suppl A):177-185.

Atlantic Salmon Federation. 1999. ASF finds Infectious Salmon Anemia (ISA) in aquaculture escapees and wild Atlantic salmon. Atlantic Salmon Federation Research and Environment Department Research Update (available on the Internet at: http://www.asf.ca/Research/ISAinwild.html).

Bagheera and ESBN. 1996. In the wild spotlight. (available on the Internet at: http://www.bagheera.com/inthewild/spot_spkey.htm).

Bartley, D. M., and G. A. E. Gall. 1990. Genetic structure of chinook and gene flow in chinook salmon populations of California. Transactions of the American Fisheries Society 119:55-71.

Barton, B. and G. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases 1:3-26.

Bastien, Y. 1999. Speech given by Y. Bastien, Canada's Commissioner for Aquaculture

Development to British Columbia Salmon Farmers Association 05-20-1999.

Benfey, T.J. The physiology and behavior of triploid fishes. Reviews in Fisheries Science 7(1):39-67.

Berejikian, B.A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (Oncorhynchus mykiss) to avoid a benthic predator. Canadian Journal of Fisheries and Aquatic Sciences 52:2476-2482.

Berris, C. 1997. Siting of Salmon Farms. *In* Salmon Aquaculture Review. British Columbia Environmental Assessment Office (available on the Internet at: http:/eaoluco-web.eoa.gs.gov.bc.ca/project/AQUACULT/SALMON/siting.htm)

Beveridge, M. 1996. Cage Aquaculture, 2nd edition. Fishing News Books, Oxford .

Billington, N. 1996. Genetic markers and stock identification. Pages 323-330 *in* R.C. Summerfelt, ed. Walleye culture manual. NCRAC Culture Series 101. North Central Regional Aquaculture Center Publications Office, Iowa State University, Ames.

Billington, N., R. J. Barette and R.D. Ward. 1992. Management implications of mitochondrial DNA variation in walleye stocks. North American Journal of Fisheries Management 12:276-284.

Billington, N. and P.D.N. Hebert. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Canadian Journal of Fisheries and Aquatic Sciences 45:643-654.

Billlington, N., P.D. Hebert and R.D.Ward. 1988. Evidence of introgressive hybridization in the genus Stizostedion: interspecific transfer of mitochondrial DNA between sauger and walleye. Canadian Journal of Fisheries and Aquatic Sciences 45: 2035-2041.

Billington, N. 1996. Geographical distribution of mitochondrial DNA (mtDNA) variation in walleye, sauger, and yellow perch. Annales Zoologici Fennici 33:699-706.

Black, K. 1998. The environmental interactions associated with fish culture. Pages 284-326 *in* K. Black and A. Pickering, eds. Biology of Farmed Fish. CRC Press, Boca Raton, Florida.

Blankenship, K. 1994. Experiment with Japanese oysters ends abruptly: oysters thought to be sterile found capable of reproducing. Bay Journal 4(5):1-4. Alliance for Chesapeake Bay, Baltimore, MD.

Bureau, D. and C. Cho. 1999. Nutritional strategies for the management of aquaculture wastes. Pages 28-31 *in* Addressing Concerns for Water Quality Impacts form Large-scale Great Lakes Aquaculture. Great Lakes Water Quality Board Report to the International Joint Commission and Habitat Advisory Board of the Great Lakes Fishery Commission (available on the Internet at: http://www.ijc.org/boards/wqb/aquaculture.)

Burgman, M., S. Ferson and H. Akcakaya. 1993. Risk assessment in conservation biology. Chapman and Hall. New York

Busack, C.A. and K. P. Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. American Fisheries Society Symposium 15:71-80.

Campton, D.E. 1995. Genetic effects of hatchery fish on wild populations of Pacific salmon and steelhead: what do we really know? American Fisheries Society Symposium 15:337-353.

Carr, J. G. Lacroix, J. Anderson, and T. Dilworth. 1997. Movements of non-maturing cultured Atlantic salmon (Salmon salar) into a Canadian river. *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.

Cho, C. J. Hynes, K. Wood, and H. Yoshida. 1994. Development of high-nutrient-dense, low-pollution diets and prediction of aquaculture wastes using biological approaches. Aquaculture 124:293-305.

Cho, C. and D. Bureau. 1998. Development of bioenergetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture. In Proc. Of the 3rd International Symposium on Nutritional Strategies and Management of Aquaculture Waste. Aquatic Living Resources 11:199-210.

Clifford, S.L., P. McGinnity and A. Ferguson. 1998. Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. *Journal of Fish Biology* 52:118-127.

Clifton, D. R. and R. J. Rodriguez. 1997. Characterization and application of a quantitative DNA marker that discriminates sex in chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 54:2647-2652.

Coberly, C. and R. Horrall. 1980. Fish spawning grounds in Wisconsin waters of the Great Lakes. WIS-SG-80-235, University of Wisconsin Sea Grant Institute, Madison, Wisconsin.

Collares-Pereira, M.J. 1987. The evolutionary role of hybridization: The example of natural Iberian fish populations. Pages 83-92 in K. Tiews, ed. Selection, hybridization, and genetic engineering in aquaculture. Verlag, H. Heenemann GmbH, Berlin, Germany.

Committee on the Status of Endangered Wildlife in Canada. 1998. Definitions of Terms and Risk Categories.(http://www.cosewic.gc.ca/COSEWIC/Terms.cfm)

Convention on Biological Diversity. 1994. Text and Annexes. Switzerland. UNEP/CBD/94/1.

Cook, J.T., M.A. McNiven, G.F. Richardson and A.M. Sutterlin. 2000. Growth rate, body composition, and food digestibility/conversion of growth-enhanced Atlantic salmon (Salmo salar). Aquaculture 188 (1-2):15-32.

Cotter, D., V. O'Donovan, N.O. Maoiléidigh, G. Rogan, N. Roche and N.P. Wilkins. 2000. An evaluation of the use of triploid Atlantic salmon (Salmo salar L.) in minimizing the impact of escaped farmed salmon on wild populations. Aquaculture 186(2000):61-75.

Council of Lake Committees. 1992. Introductions in the Great Lakes Basin Procedures for Consultation. Great Lakes Fishery Commission, Ann Arbor MI. (e-mail: mgaden@glfc.org)

Crozier, W. 1993. Evidence of genetic interaction between escaped farmed salmon and wild Atlantic salmon (*Salmo salar*) in a northern Irish river. Aquaculture 113:19-29.

Cudmore-Vokey, B. and E.J. Crossman. 2000. Checklists of the Fish Fauna of the Laurentian Great Lakes and Their Connecting Channels. Can. MS Rpt. Fish. Aquat. Sci. 2550:v+39p.

Danzmann, R.G., R. P. Morgan II, M.W. Jones, L. Bernatchez and P. Ihssen. 1998. A major sextet of mitochondrial DNA phylogenetic assemblages extant in eastern North American brook trout (*Salvelinus fontinalis*): distribution and postglacial dispersal patterns. Canadian Journal of Zoology 76:1300-1318.

Danzmann, R.G., P. E. Ihssen, and P.D. N. Hebert. 1991. Genetic discrimination of wild and hatchery populations of brook charr, *Salvelinus fontinalis* (Mitchill), in Ontario using mitochondrial DNA analysis. Journal of Fish Biology 39(Suppl. A):69-78.

Dawson, K., R. Eshenroder, M. Holey and C. Ward. Quantification of historic lake trout (*Salvelinus namaycush*) spawning aggregations in Lake Michigan. Canadian Journal of Fisheries and Aquatic Sciences 54:2290-2302.

Dawson, L., A. Pike, D. Houlihan, and A. McVicar. 1997. Comparison of the susceptibility of salmon (Salmo salar L.) and sea trout (*Salmo trutta* L.) to sea lice (*Lepeophtheirus salmonis*) (Kroyer 1837) infections. *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.

Dayton, P.K. 1998. Reversal of the burden of proof in fisheries management. Science 279:821-822.

Department of Fisheries and Oceans. 1995. Federal Aquaculture Development Strategy. Communications Directorate, Department of Fisheries and Oceans/ 5066, Canada.

Devlin, R.H., J.I. Johnsson, D.E. Smailus, C.A. Biagi, E. Jönsson and B. Th. Björnsson. 1999. Increased ability to compete for food by growth hormone-transgenic coho salmon *Oncorhynchus kisutch* (Walbaum). Aquaculture Research 30:479-482.

Devlin, R. H., B. Kelly McNeil, I.I. Solar, and E. M. Donaldson. 1994. A rapid PCR-based test for Y-chromosomal DNA allows simple production of all-female strains of chinook salmon. Aquaculture 128:211-220.

Dodd, Q. 2000. Closed containment promise & pitfalls. Aquaculture and the Environment, [special supplement to Northern Aquaculture] 6:13-14.

Donaldson, E.M., R. H. Devlin, F. Piferrer and I.I. Solar. 1996. Hormones and sex control in fish with particular emphasis on salmon. Asian Fisheries Science 9:1-8.

Donaldson, E.M. and R. H. Devlin. 1996. Uses of biotechnology to enhance production. Pages 969-1020 *in* W. Pennell and B.A. Barton, eds. Principles of Salmonid Culture. Developments in Aquaculture and Fisheries Science No. 29. Elsevier Publishers, Amsterdam.

Draulans, D. 1987. The effectiveness of attempts to reduce predation by fish-eating birds: a review. Biological Conservation 41:219-232.

Edsall, T., G. Kennedy, W. Horns. 1996. Potential spawning habitat for lake trout on Julian's Reef, Lake Michigan. Journal of Great Lakes Research 22:83-88.

Einum, S., and I. Fleming. 1997. Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. Journal of Fish Biology 50:634-651.

Environment Canada. 2000. Lake Erie Lakewide Area Management Plan. (http://www.epa.gov/glnpo/lakeerie/lamp2000/)

Farrell, A.P., W. Bennet and R. H. Devlin. 1997. Growth-enhanced transgenic salmon can be inferior swimmers. Canadian Journal of Zoology 75:335-337.

Fausch, K. 1998. Interspecific competition and juvenile Atlantic salmon (*Salmo salar*): on testing effects and evaluating the evidence across scales. Canadian Journal of Fisheries and Aquatic Science 55(Suppl. 1):218-231.

Fergusen, A., P. McGinnity, C. Stone, J. Taggart, D. Cotter, R. Hynes, and T. Cross. 1997. Will interbreeding between wild and cultured fish have negative consequences? *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.

Ferguson, M.M., R.G. Danzman and F.W. Allendorf. 1988. Developmental success of hybrids between two taxa of salmonid fishes with moderate structural gene divergence. Canadian Journal of Zoology 66:1389-1395.

Fleming, I.A. and S. Einum. 1997. Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. ICES Journal of Marine Science 54:1051-1063.

Fleming I., B. Jonsson, M. Gross, and A. Lamberg. 1996. An experimental study of the reproductive behavior and success of farmed and wild Atlantic salmon (*Salmo salar*). Journal of Applied Ecology 33:893-905.

Fleming, I., K. Hindar, I. Mjølnerød, B. Jonsson. 1997. The simulated escape of farmed salmon in a Norwegian river: breeding success, hybridization and offspring traits. *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.

Fletcher, G.L., M.A. Shears, and S.V. Goddard. 1999. Transgenic fish for sustainable aquaculture. Pages 193-201 in N. Svennig, H. Reinertsen, and M. New, eds. Sustainable Aquaculture. Balkema, Rotterdam.

Fox, M.G. 1993. Comparison of zygote survival of native an non-native walleye stocks in two Georgian Bay rivers. Environmental Biology of Fishes 38:379-383.

Gale, P. 1999. Water quality impacts from aquaculture cage operations in the LaCloche/North Channel of Lake Huron. Pages 51-57 *in* Addressing Concerns for Water Quality Impacts from Large-scale Great Lakes Aquaculture. Great Lakes Water Quality Board Report to the International Joint Commission and Habitat Advisory Board of the Great Lakes Fishery Commission (available on the Internet at: http://www.ijc.org/boards/wqb/aquaculture.)

Garling, D. 1999. Status and expected growth of aquaculture-U.S. experiences. Pages 32-39 *in* Addressing Concerns for Water Quality Impacts from Large-scale Great Lakes Aquaculture. Great Lakes Water Quality Board Report to the International Joint Commission and Habitat Advisory Board of the Great Lakes Fishery Commission (available on the Internet at: http://www.ijc.org/boards/wqb/aquaculture.)

Goodyear, C., T. Edsall, D. Ormsby-Dempsy, G. Moss and P. Polanski. 1982. Atlas of spawning and nursery areas of Great Lakes Fishes. 14 volumes. U.S. fish and wildlife Service, Washington, DC., FWS/OBS-82/52.

Gowen, R., D. Smyth, and W. Silvert. 1994. Modeling the spatial distribution and loading of organic fish farm waste to the seabed. Pages 19-30 *in* B.T. Hargrave, ed. Modeling Benthic Impacts of Organic Enrichment from Marine Aquaculture. Canadian Technical Report of Fisheries and Aquatic Sciences 1949. Department of Fisheries and Oceans, Nova Scotia.

Gowen R.., D.Weston and A. Ervik. 1992. Aquaculture and the benthic environment. *In* Cowey, C. B. and C. Y. Cho, (eds.) Nutritional Strategies in Management of Aquaculture Waste. University of Guelph, Ontario, Canada.

Gharett, A.J. and W.W. Smoker. 1991. Two generations of hybrids between even- and odd-year pink salmon (Oncorhynchus *gorbuscha*): a test for outbreeding depression? *Canadian Journal of Fisheries and Aquatic Sciences* 48:1744-1749.

Great Lakes Fishery Commission. 1997. A joint strategic plan for management of Great Lakes fisheries. Great Lakes Fishery Commission, Ann Arbor MI. (e-mail: mgaden@glfc.org)

Griffin, B.R. 1991. The U.S. Fish and Wildlife Service's Triploid Grass Carp Inspection Program. Aquaculture Magazine Jan/Feb:188-189.

Gross, M. 1998. One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. Canadian Journal of Fisheries and Aquatic Sciences 55(Suppl. 1):131-144.

Gudjonsson, S. 1991. Occurrence of reared salmon in natural salmon rivers in Iceland. Aquaculture 98:133-142.

Hansen, L., J. Jacobsen, and R. Lund. 1993. High numbers of farmed Atlantic salmon, *Salmo salar* L., observed in oceanic waters north of the Faroe Islands. Aquaculture and Fisheries Management 24:777-781.

Hargrave, B. (ed.) 1994. Modeling Benthic Impacts of Organic Enrichment from Marine Aquaculture Canadian Technical Report of Fisheries and Aqautic Sciences 1949 Department of Fisheries and Oceans, Dartmouth. Nova Scotia.

Harrell, R.M. and W. Van Helkelem. 1998. A comparison of triploid induction validation techniques. The Progressive Fish Culturist 60:221-226.

Hartl, D. 1988. A Primer of Population Genetics, Second Edition. Sinauer Associates, Sunderland, MA.

Healy, J.A. and M.F. Mulcahy. 1980. A biochemical genetic analysis of populations of northern pike, *Esox lucius* L. from Europe and North America. Journal of Fish Biology 17:317-324.

Heggberget, T. 1988. Timing of spawning in Norwegian Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Science 45:845-849.

Heggberget, T.G., B. Johnsen, K. Hindar, B. Jonsson, L. Hansen, N. Hvidsten and A. Jensen. 1993. Interactions between wild and cultured Atlantic salmon: a review of the Norwegian experience. Fisheries Research 18:123-146.

Held and Malison. 1996. Culture of walleye to food size. Pages 231-232 *in* R.C. Summerfelt, ed. Walleye culture manual. NCRAC Culture Series 101. North Central Regional Aquaculture Center Publications Office, Iowa State University, Ames.

Hindar K. and B. Jonsson. 1995. Impacts of aquaculture and hatcheries on wild fish. Pages 70-87 *in* D.P. Philipp et al., (eds.) Protection of Aquatic Biodiversity. Proceedings of the World Fisheries Congress, Theme 3. Science Publishers Inc., Lebanon USA.

Hislop, J. and J. Webb. 1992. Escaped farmed Atlantic salmon, *Salmo salar* L., feeding in Scottish coastal waters. Aquaculture and Fisheries Management 23:721-723.

Hnath, J. (ed.) 1993. Great Lakes fish disease control policy and model program (supersedes September 1985 edition). Great Lakes Fishery Commission Spec. Pub. 93-1: 1-38. (available on the Internet at: www.glfc.org/pubs/sp93-1.zip)

Holtby, L.B. and D. P. Swain. 1992. Through a glass, darkly: a response to Ruzzante's reappraisal of mirror image stimulation studies. Canadian Journal of Fisheries and Aquatic Sciences 1968-1969.

Hora, M. 1999. Minnesota's experience with net pen aquaculture in mine pit lakes. Pages 58-62 *in* Addressing Concerns for Water Quality Impacts from Large-scale Great Lakes Aquaculture. Great Lakes Water Quality Board Report to the International Joint Commission and Habitat Advisory Board of the Great Lakes Fishery Commission (available on the Internet at: http://www.ijc.org/boards/wqb/aquaculture.)

Horner, R. and R. Eshenroder (eds.). 1993. Protocol to minimize the risk of introducing emergency disease agents with importation of salmonid fishes form enzootic areas. Great Lakes Fishery Commission Spec. Pub. 93-1: 39-54. (available on the Internet at: www.glfc.org/pubs/sp93-1.zip).

Hubbs, C.L. 1955. Hybridization between fish species in nature. Systematic Zoology 4: 1-20.

Huguenin, J. The design, operations and economics of cage culture systems. Aquacultural Engineering 16:167-203.

Ihssen, P.E., J.M. Casselman, G.W. Martin and R.B. Phillips. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. Canadian Journal of Fisheries and Aquatic Sciences 45: 1018-1029.

International Joint Commission. 1987. Forty-Three Areas of Concern. Environment Canada. (available on the Internet at: Http://www.cciw.ca/glimr/raps/aoc-map.html).

International Joint Commission. 1987. Great Lakes Water Quality Agreement (available on the Internet at:http://www.ijc.org/agree/quality.html).

Jacobsen, J., and E. Gaard. 1997. Open ocean infection by salmon lice (*Lepeophtheirus salmonis*): comparison of wild and escaped farmed Atlantic salmon. *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.

Jennings, M.J., J.E. Claussen, and D.P. Philipp. 1996. Evidence for heritable preferences for spawning habitat between two walleye populations. Transactions of the American Fisheries Society 125:978-982.

Jensen, G. and K. Greenless. 1997. Public health issues in aquaculture. Rev. Sci. Tech. Off. Int. Epiz. 16: 641-651.

Johnsen, B. and A. Jensen. 1994. The spread of furunculosis in salmonids in Norwegian rivers. Journal of Fish Biology *45*:47-55.

Johnsson, J.I. and M.V. Abrahams. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. Canadian Journal of Fisheries and Aquatic Sciences 243-247.

Johnsson, J.I. and B. T. Björnsson. 2001. Growth-enhanced fish can be competitive in the wild. Functional Ecology 15: in press.

Johnsson, J.I., E. Petersson, E. Jönsson, B.T. Björnsson and T. Järvi. 1996. Domestication and growth hormone alter antipredation behavior and growth pattern in juvenile brown trout, *Salmo trutta*. Canadian Journal of Fisheries and Aquatic Sciences 53:1546-1554.

Johnsson, J.I., E. Petersson, E. Jönsson, T. Järvi and B. Th. Björnsson. 1999. Growth hormone-induced effects on mortality, energy status and growth: a field study on brown trout (Salmo trutta). Functional Ecology 13:514-522.

Jonsson, B. 1997. A review of ecological and behavioral interactions between cultured and wild Atlantic salmon. ICES Journal of Marine Science 54:1031-1039.

Jonsson, B., N. Jonsson, and L. Hansen. 1991. Differences in life history and migratory behavior between wild and hatchery-reared Atlantic salmon in nature. Aquaculture 98:69-78.

Joint Subcommittee on Aquaculture. 1994 Guide to drug, vaccine and pesticide use in aquaculture. Texas A&M University, Publication B-5085. College Station, Texas.

Kapuscinski, A. R. and L. D. Jacobson. 1987. Genetic guidelines for fisheries management. Minnesota Sea Grant College Program, St. Paul. 66 pp.

Kapuscinski, A. R. and E. M. Hallerman. 1991. Implications of introduction of transgenic fish into natural ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 48(Suppl. 1): 99-107.

Kapuscinski, A. R. and E. M. Hallerman. 1994. Benefits, Risks, and Policy Implications: Biotechnology in Aquaculture. Contract report for Office of Technology Assessment (U.S. Congress). Aquaculture: Food and Renewable Resources from U.S. Waters. 80 pp.

Kapuscinski, A. R., T. Nega, and E. M. Hallerman. 1999. Adaptive biosafety assessment and management regimes for aquatic genetically modified organisms in the environment. Pages 225-251 *in* Pullin, R.S.V. and D. Bartley (eds.) Towards Policies for Conservation and Sustainable Use of Aquatic Genetic Resources, ICLARM Conf. Proc. International Center for Living Aquatic Resources Management, Makati City, Philippines.

Kelly, L. 1993. Release rates and biological availability of phosphorous released from sediments receiving aquaculture wastes. Hydrobiologia 253:367-372.

King, R. C. and W. D. Stansfield. 1990. A Dictionary of Genetics. Fourth Edition. Oxford University Press, New York.

Kingsbury, O. 1961. A possible control of furunculosis. Progressive Fish-Culturist 23:136-138.

Krueger, C.C. J.E. Marsden, H.L. Kincaid and B. May. 1989. Genetic differentiation among lake trout strains stocked in Lake Ontario. Transactions of the American Fisheries Society 118:317-330.

Krueger, C.C. and B. May. 1991. Ecological and genetic effects of salmonid introductions in North America. Canadian Journal of Fisheries and Aquatic Sciences 48(Suppl. 1):66-77.

Krueger, C.C., D.L. Perkins, R.J. Everett, D.R. Schreiner, and B. May. 1994. Genetic variation in naturalized rainbow trout (*Oncorhynchus mykiss)* from Minnesota tributaries to Lake Superior. Journal of Great Lakes Research 20(1): 299-316.

Krueger, C.C. and P.E. Ihssen. 1995. Review of genetics of lake trout in the Great Lakes: history, molecular genetics, physiology, strain comparisons and restoration management. Journal of Great Lakes Research 21 (Suppl 1): 348-363.

Lagler, K.F., J.E. Bardach, and R.R. Miller. 1977. Ichthyology. John Wiley and Sons, New York, NY.

Laird, L. and T. Needham. 1988. Salmon and trout farming. Fishing News Books, Oxford.

Lawson, T. 1995. Fundamentals of Aquacultural Engineering. Chapman and Hall, New York.

Leach, J., E. Mills, M. Dochoda. In press. Non-indigenous species in the Great Lakes: ecosystem impacts, binational policies and management. *In* Great Lakes Fisheries Policy and Management: a Binational Perspective. Canadian Studies Center, Michigan State University, Michigan.

Leary, R.F., F.W. Allendorf, and G.K. Sage. 1995. Hybridization and introgression between introduced and native fish. American Fisheries Society Symposium 15:91-101.

Levings, C.D., A. Ervik, P. Johannessen and J. Aure. 1995. Ecological criteria used to help site fish farms in fjords. Estuaries 18:81-90.

Littauer, G. 1990. Avian Predators: Frightening Techniques for Reducing Bird Damage at Aquaculture Facilities. SRAC Publication No. 401. Southern Regional Aquaculture Center.

Littauer, G., J. Glahn, D. Reinhold, and M. Brunson. 1997. Control of bird predation at aquaculture facilities :strategies and cost estimates. Southern Regional aquaculture Center SRAC Publication No. 402.

Litvik, M. and N. Mandrak. 1993. Ecology of freshwater baitfish use in Canada and the United States. Fisheries 18:6-12.

Litvik, M. and N. Mandrak. 1999. Baitfish trade as a vector of aquatic introductions.

Pages 163-180 *in* R. Claudi and J. Leach (eds.) Nonindigenous Freshwater Organisms Vetors, Biology and Impacts. CRC Press, Boca Raton, Florida.

Ludwig, H. and J. Leitch. Interbasin transfer of aquatic biota via anglers' bait buckets. Fsheries 21:14-18.

Lund, R., L.Hansen, and F. Økland. 1997. Geographical and temporary distribution of escaped farmed Atlantic salmon, *Salmo salar* L., in relation to farming activity. *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.

Lura, H., and H Sægrov. 1991. Documentation of successful spawning of escaped farmed female Atlantic salmon, *Salmo salar*, in Norwegian rivers. Aquaculture 98: 151-159.

Lynch, M. 1996. A quantitative genetic perspective on conservation issues. Pages 471-501 *in* J.C. Avise and J.L. Hamrick, eds. Conservation Genetics: Case Histories from Nature. Chapman and Hall, New York.

Manninen, C. and R. Gauthier 1999. Living with the lakes: understanding and adapting to Great Lakes water level changes. US Army Corps. of Engineers and Great Lakes Commission (available on the Internet at: http://www.great-lakes.net/envt/water/hydro.html)

Massik, Z. and M. Costello. 1995. Bioavailability of phosphorous in fish farm effluents to freshwater phytoplankton. Aquaculture Research 26:607-616.

McGinnity, P., C. Stone, J.B. Taggart, D. Cooke, D. Cotter, R. Hynes, C. McCamley, T. Cross and A. Ferguson. 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L. on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES Journal of Marine Science 54:998-1008.

McKinnell, S., A. Thomson, E. Black, C. Guthrie, B. Wing, J. Kierner, and J. Helle. 1997. Atlantic salmon in the North Pacific. Aquaculture 28:145-157.

McVicar, A. 1997. The co-existence of wild and cultured populations—disease and parasite implications. *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.

Mesa, M.G. 1991. Variation in feeding, aggression, and position choice between hatchery and wild cutthroat trout in an artificial stream. Transactions of the American Fisheries Society 120:723-727.

Meyer, F., J. Warren, and T. Carey (eds.) 1983.A guide to integrated fish health management in the Great Lakes basin. Great Lakes Fishery Commission. Ann Arbor, Michigan. Spec. Pub. 83-2. 272pp.

Miller, L. M. and A. R. Kapuscinski. 1996. Microsatellite DNA markers reveal higher levels of genetic variation in northern pike. Transactions of the American Fisheries Society 125(6): 971-977.

Miller, L.M. and A. R. Kapuscinski. 2000. Genetic guidelines for hatcheries used to rebuild fish populations. Pages 00-00 in E.M. Hallerman, ed. Population Genetics of Fishes. American Fisheries Society, Bethesda. *In press.*

Milne, P. 1979. Selection of sites and design of cages, fish pens and enclosures for aquaculture *in* T. Pillay and W. Dill (eds.) Advances in Aquaculture. Fishing News Books, Farnham, Surrey.

Mills, E., J. Leach, J. Carlton, C. Secor. 1993. Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. Journal of Great Lakes Research 19:1-54.

Moyle, P.B. 1969. Comparative behavior of young brook trout of wild and hatchery origin. Progressive Fish Culturist 31:51-56.

Muir, W.M. and R. D. Howard. 1999. Possible ecological risks of transgenic organism release when transgenes affect mating success: Sexual selection and the Trojan gene hypothesis. Proceedings of the National Academy of Sciences 96(24):13853-13856.

Muir. W.M. and R. D. Howard. 2001. Fitness components and ecological risk of transgenic release: a model using Japanes medaka (Oryzias latipes). American Naturalist 158:1-16.

Muir, W.M. and R.D. Howard. 2001a. Methods to Assess Ecological Risks of Transgenic Fish Releases. Pages 355-383 in D.K. Letourneau and B.E. Burrows, Eds. Genetically Engineered Organisms: Assessing Environmental and Human Health Effects. CRC Press.

Muir, W.M. and R.D. Howard. 2001b.Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. Transgenic Research: in press.

National Research Council Committee on Restoration of Aquatic Ecosystems-Science, Technology and Public Policy. 1992. Restoration of Aquatic Ecosystems. National Academy Press. 552 pp.

National Research Council. 1996. Upstream: Salmon and Society in the Pacific Northwest. National Academy Press. 388 pp.

National Seafood HACCP Alliance for Seafood Safety Training and Education. 2001. ANS-HACCP: Aquatic Nuisance Species-Hazard Analysis and Critical Control Point Training Curriculum. Minnesota Sea Grant. 80pp.

Nature Conservancy Great Lakes Program. 1994. The conservation of biological diversity in the Great Lakes Ecosystem: issues and opportunities. University Center, Michigan (available on the Internet at: http://epawww.ciesin.org/glreis/glnpo/docs/bio/divrpt.html)

Negus, M.T. 1999. Survival traits of naturalized hatchery, and hybrid strains of anadromous rainbow trout during egg and fry stages. North American Journal of Fisheries Management 19:930-941.

Nester, R. and T. Poe. 1987. Visual observations of historical lake trout spawning grounds in western Lake Huron. North American Journal of Fisheries Management 7:418-424.

Ohio Sea Grant. 1994. Inland Waters Fact Sheet 058. Ohio State University, Ohio (available on the Internet at: http://www.sg.ohio-state.edu/publications/nuisances/zebras/fs-058.html).

Ohio Sea Grant. 1994. Zebra Mussels in North America Fact Sheet 045. Ohio State University, Ohio (available on the Internet at: http://www.sg.ohio-state.edu/publications/nuisances/zebras/fs-045.html).

O'Connel, M., R.G. Danzmann, J-M. Cornuet, J.M. Wright and M.M. Ferguson 1997. Differentiation of rainbow trout populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. Canadian Journal of Fisheries and Aquatic Sciences 54:1391-1399.

Økland, F., G. Heggberget and B. Jonsson. 1995. Migratory behavior of wild and farmed Atlantic salmon (Salmo salar) during spawning. Journal of Fish Biology 46:1-7.

Pew Initiative on Food and Biotechnology. Genetically Modified Fish in the United States. In press (Expected early 2002 at www.PewAgBiotech.org).

Philipp, D.P. 1991. Genetic implications of introducing Florida largemouth bass, *Micropterus salmoides floridanus*. Canadian Journal of Fisheries and Aquatic Sciences 48(Suppl. 1): 58-65.

Philipp, D.P. and J.E. Claussen. 1995. Fitness and performance differences between two stocks of largemouth bass from different river drainages within Illinois. American Fisheries Society Symposium 17:236-243.

Philipp, D.P., and G.S. Whitt. 1991. Survival and growth of northern, Florida and reciprocal F_1 hybrid largemouth bass in central Illinois. Transactions of the American Fisheries Society 120:56-64.

Pickering, A. 1998. Stress responses of farmed fish. Pages 222-255 *in* K. Black and A. Pickering, eds. Biology of Farmed Fish. CRC Press, Boca Raton, Florida.

Pillay, T. 1992. Aquaculture and the Environment. Halsted Press, New York.

Pickering, A. 1981. Husbandry and stress. Pages 155-169 *in* J. Muir and R. Roberts, eds. Recent Advances in Aquaculture, Volume 4. Blackwell, Oxford.

Plumb, J. 1994. Health maintenance of cultured fishes: principal microbial diseases. CRC Press, Boca Raton.

Prout, T. 1971a. The relation between fitness components and population prediction in Drosophila. I: The estimation of fitness components. Genetics 68:127-149.

Prout, T. 1971b. The relation between fitness components and population prediction in Drosophila. II: Population prediction. Genetics 68:151-167.

Reisenbichler, R.R. 1997. Genetic factors contributing to declines of anadromous salmonids in the Pacific Northwest. Pages 223-244 *in* D.J. Stouder, P.A. Bisson, and R.J. Naiman, eds. Pacific Salmon and Their Ecosystems: Status and Future Options, Chapman and Hall.

Reznick, D.N., F.H. Shaw, F.H. Rodd, and R.G. Shaw. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934-1937.

Ricker, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations. Pages 19-160 *in* R. C. Simon and P. A. Larkin, eds. The stock concept in Pacific salmon, H.R. MacMillan Lectures in Fisheries. University of British Columbia, Vancouver.

Rodriguez-Clark, K.M. and J.P. Rodriguez. 2001. Beware of trojans bearing fish. Trends in Ecology and Evolution 16(8) August 2001:428.

Ruzzante, D.E. 1991. Variation in agonistic behavior between hatchery and wild populations of fish: a comment on Swain and Riddell (1990). Canadian Journal of Fisheries and Aquatic Sciences 1966-1968.

Ruzzante, D.E. 1992. Mirror image stimulation, social hierarchies, and population differences in agonistic behavior—a reappraisal). Canadian Journal of Fisheries and Aquatic Sciences 49:1966-1968.

Ruzzante, D.E. 1994. Domestication effects on aggressive and schooling behavior in fish. Aquaculture 120:1-24.

Samuelsen,O., B. Lunestad, B. Husevag, T. Holleland and A. Ervik. 1992. Residues of oxolinic acid in wild fauna following medication in fish farms. Diseases of Aquatic Organisms 12:111-119.

Schreck, C. 1990. Physiological, behavioral and performance indicators of stress.

American Fisheries Society Symposium 8: 29-37.
Schreiner, D.R., ed. 1992. North Shore steelhead plan. Minnesota Department of Natural Resources, St. Paul.
Schreiner, D.R., ed. 1995. Fisheries management plan for the Minnesota waters of Lake Superior. Section of Fisheries Special Publication No. 149. Minnesota Department of Natural Resources, St. Paul.
Scientists' Working Group on Biosafety. 1998. A Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms. Volumes 1 and 2. Edmonds Institute, 20319-92nd Ave. West, Edmonds, WA 98020, USA. (available on the Internet at: www.edmonds-institute.org)
Seeb, J.E., L.W. Seeb, D.W. Oates, and F.M. Utter. 1987. Genetic variation and postglacial dispersal of populations of northern pike (Esox lucius) in North America. Canadian Journal of Fisheries and Aquatic Sciences 44:556-561.
Sedgewick, S. 1988. Salmon farming handbook. Fishing News Books, Oxford.
Senanan, W. and A.R. Kapuscinski. 2000. Genetic relationships among populations of northern pike (*Esox lucius)*. Canadian Journal of Fisheries and Aquatic Sciences 57:1-14.
Shields, W.M. 1993. The natural and unnatural history of inbreeding and outbreeding. Pages 143-169 *in* N.W. Thornhill, ed. The natural history of inbreeding and outbreeding. University of Chicago Press, Chicago.
Silvert, W. 1994. Modeling benthic deposition and impacts of organic matter loading. Pages 1-18 *in* B.T. Hargrave, ed. Modeling Benthic Impacts of Organic Enrichment from Marine Aquaculture. Canadian Technical Report of Fisheries and Aquatic Sciences 1949. Department of Fisheries and Oceans, Nova Scotia.
Sippel, A. and M. Muschett. 1999. Aquaculture-experiences and lessons: Ontario Ministry of Natural Resources. Pages 48-50 *in* Addressing Concerns for Water Quality Impacts from Large-scale Great Lakes Aquaculture. Great Lakes Water Quality Board Report to the International Joint Commission and Habitat Advisory Board of the Great Lakes Fishery Commission (available on the Internet at: http://www.ijc.org/boards/wqb/aquaculture.)
Skaala, Ø., K.E. Jørstad, and R. Borgstrom. 1996. Genetic impact on two wild brown trout (*Salmo trutta*) populations after release of non-indigenous hatchery spawners. Canadian Journal of Fisheries and Aquatic Sciences. 53:2027-2035.
Skaala, O. and K. Hindar. 1997. Genetic changes in the R. Vosso salmon stock following a collapse in the spawning population and invasion of farmed salmon. *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.
Smith, K. 1992. Environmental Hazards. Chapman and Hall, London, U.K.
Snyder, F. 1997. Commercial fish and baitfish shipments as potential vectors of Ruffe (*Gymnocephalus cernuus*). Page 29 *in* D. Jensen, ed. International Symposium on biology and Management of Ruffe Symposium Abstracts. Minnesota Sea Grant Publications X48.
Solar, I.I. and E. M. Donaldson. 1991. A comparison of the economic aspects of monosex chinook salmon production versus mixed sex stocks for aquaculture. Bulletin of the Aquaculture Association of Canada 91:28-30.
Souter, B. 1983. Immunization with vaccines. In Meyer, F., J. Warren, and T. Carey,

eds. A guide to integrated fish health management in the Great Lakes basin. Great Lakes Fishery Commission. Ann Arbor, Michigan. Spec. Pub. 83-2. 272pp.

Sowles, J.W., L. Churchill, and W. Silvert. 1994. The effect of benthic carbon loading on the degradation of bottom conditions under farm sites. Pages 31-46 *in* B.T. Hargrave, ed. Modeling Benthic Impacts of Organic Enrichment from Marine Aquaculture. Canadian Technical Report of Fisheries and Aquatic Sciences 1949. Department of Fisheries and Oceans, Nova Scotia.

Stepien, C. 1995. Population genetic divergence and geographic patterns from DNA sequences: examples from marine and freshwater fishes. American Fisheries Society Symposium 17:263-287.

Stevens, E.D., A. Sutterlin and T. Cook 1998. Respiratory metabolism and swimming performance in growth hormone transgenic Atlantic salmon. Canadian Journal of Fisheries and Aquatic Sciences 55:2028-2035.

Stern, P. and H. Fineberg (eds.). 1996. Understanding risk: Informing decisions in a democratic society. Committee on Risk Characterization, Commission on Behavioral and Social Sciences and Education, National Research Council. National Academy Press. Washington, D.C.

Stewart, T., R. Lange, S. Orsatti, C. Schneider, A. Mathers and M. Daniels. 1998. Fish Community Objectives for Lake Ontario. Report to the Lake Ontario Committee. Great Lakes Fishery Commission. Spec. Pub. 99-1. 56 pp (e-mail: mgaden@glfc.org)

Stickley, A. 1990. Avian predators on southern aquaculture. Southern Regional aquaculture Center SRAC Publication No. 400.

Stokesbury, M., and G. Lacroix. 1997. High incidence of juvenile Atlantic salmon (*Salmo salar*) escaped from hatcheries in the smolt output of a Canadian river. *In* Final abstracts: ICES/NASCO Symposium. Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. NASCO, Bath, England, U.K.

Swain, D.P. and B.E. Riddell. 1990. Variation in agonistic behavior between newly emerged juveniles from hatchery and wild populations of coho salmon, *Oncorhynchus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences 47:566-577.

Swain, D.P. and B.E. Riddell. 1991. Domestication and agonistic behavior in coho salmon: reply to Ruzzante. Canadian Journal of Fisheries and Aquatic Sciences 48:520-522.

Tave, D. 1993. Genetics for fish hatchery managers, 2nd Edition. Van Nostrand Reinhold, New York. 415 pp.

Templeton, A.R. 1986. Coadaptation and outbreeding depression. Pages 105-116 in M. Soulé, ed. Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, MA.

Tessier, N., L. Bernatchez and J.M. Wright. 1997. Population structure and impact of supportive breeding inferred from mitochondrial and microsatellite DNA analyses in land-locked Atlantic salmon Salmo salar L. Molecular Ecology 1997 (6): 735-750.

Thibodeau, M. and J. Kelso. 1990. An evaluation of putative lake trout (Salvelinus namaycush) spawning sites in the Great Lakes. Canadian Technical Report of Fisheries and Aquatic Sciences. 1739.

Thorgaard, G. H. 1995. Biotechnology approaches to broodstock management. Pages 76-93 *in* N.R. Bromage and R.J. Roberts, eds. Broodstock Management and Egg and Larval Quality. Blackwell Science, Ltd., Oxford.

Todd, T. N. 1990. Genetic differentiation of walleye stocks in Lake St. Clair and western Lake Erie. U.S. Department of the Interior, Fish and Wildlife Service, Fish and Wildlife Technical Report 28:1-19.

Todd, C., A. Walker, K. Wolff, S. Northcott, A. Walker, M. Ritchie, R. Hoskins, R. Abbott, and N. Hazon. 1997. Genetic differentiation of populations of the copepod sea louse *Lepeophtheirus salmonis* (Kroyer) ectoparasitic on wild and farmed salmonids around the coasts of Scotland: evidence form RAPD markers. Journal of Exp. Mar. Biol. Ecol. 210:251-274.

Todd, T.N., and C.O. Hatcher. 1993. Genetic variability and glacial origins of yellow perch (*Perca flavescens*) in North America. Canadian Journal of Fisheries and Aquatic Sciences 50:1828-1834.

Turner, B.J., ed. 1984. Evolutionary Genetics of Fishes. Plenum Press, New York.

USDA-NASS. 1998. 1998 Census of Aquaculture. United States Department of Agriculture, National Agricultural Statistics Service, Washington, DC. (http://www.nass.usda.gov/census/census97/aquaculture/general.htm)

US Environmental Protection Agency. 2000. Lake Erie Lakewide Area Management Plan. (http://www.epa.gov/glnpo/lakeerie/lamp2000/)

US Fish and Wildlife Service Region 3. 1997. (http://www.fws.gov/r3pao/eco_serv/endangrd/index.html)

U.S. Food and Drug Administration. 1999. HACCP: A State-of-the-Art Approach to Food Safety. U. S. Food and Drug Administration, FDA Backgrounder (available on the Internet at: http://vm.cfsan.fda.gov/~lrd/bghaccp.html)

Utter, F. M., G. Miller, G. Stahl, and D. Teel. 1989. Genetic population structure of chinook salmon, *Oncorhynchus tshawytscha*, in the Pacific Northwest. U. S. National Marine Fisheries Service Fishery Bulletin 87:239-264.

Vincent, R.E. 1960. Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchell). Transactions of the American Fisheries Society 89:35-52.

Vives, S. 1990. Nesting ecology and behavior of horneyhead chub *Nocomis biguttus*, a keystone species in Allequash Creek, Wisconsin. American Midland Naturalist 124:46-56.

Waples, R.S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. Canadian Journal of Fisheries and Aquatic Sciences 48(Suppl. 1):124-133.

Waples, R.S. 1995. Genetic effects of stock transfers of fish. Pages 51-69 *in* D.P. Philipp, J.M. Epifanio, J. E. Marsden, J.E. Claussen; R. J. Wolotira, eds. Protection of Aquatic Biodiversity. Proceedings of the World Fisheries Congress, Theme 3. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Ward, R.D., N. Billington and P.D.N. Hebert. 1989. Comparison of allozyme and mitochondrial variation in populations of walleye, *Stizostedion vitreum*. Canadian Journal of Fisheries and Aquatic Sciences 46:2074-2084.

Wattendorf, R. J. 1986. Rapid identification of triploid grass carp with a coulter counter and channelyzer. The Progressive Fish Culturist 48:125-132.

Wattendorf, R. J. and C. Phillippy. 1996. Administration of a state permitting program. Pages 130-176 *in* J. R. Cassani, ed. Managing Aquatic Vegetation with Grass Carp, a Guide for Water Resource Managers. American Fisheries Society, Bethesda, Maryland.

Webb, J., D. Hay, P. Cunningham and A. Youngson. 1991. The spawning behavior of escaped farmed and wild Atlantic salmon (*Salmo salar* L.) in a northern Scottish river. Aquaculture 98:97-110.

Wedemeyer, G. 1997. Effects of rearing conditions on the health and physiological quality of fish in intensive culture. *In* G. Iwama, A. Pickering, J. Sumpter and C. Schreck, (eds.) Fish Stress and Health in Aquauclture.. Society for Experimental Biology Seminar Series 62. Cambridge University Press, Cambridge.

Weseloh, D. and P. Ewins. 1994. Characteristics of a rapidly increasing colony of double-crested cormorants (*Phalacrocorax auritus*) in Lake Ontario: population size, reproductive parameters and band recoveries. Journal of Great Lakes Research 20:443-456.

Weston D.P. 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. Marine Ecology Progress Series 61:233-244.

Williams, T. 1992. Killer fish farms. Audubon 2:14-22.

Youngson, A. and E. Verspoor. 1998. Interactions between wild and introduced Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 55(Suppl. 1):153-160.