1993 Project Abstract
FOR THE PERIOD ENDING June 30, 1995

TITLE: Ecological Impacts of Releasing Genetically Engineered Fishes
PROGRAM MANAGER: Anne R. Kapuscinski
ORGANIZATION: University of Minnesota
LEGAL CITATION: M.L. 93, Ch. 172, Sec. 14, Subd. 12(o)
APPROPRIATION AMOUNT: $175,000 (7/1/95 balance is approximately $9,900)

STATEMENT OF OBJECTIVES

A. To test the null hypothesis: there is no difference in performance traits between transgenic (genetically engineered) fish bearing extra growth hormone genes and non-transgenic fish from the same population and generation. **B.** To develop performance standards (i.e., facility and operating guidelines) for environmentally safe research and development with genetically engineered fish.

OVERALL PROJECT RESULTS

A. A DNA test (called PCR) showed that 39 of 157 northern pike inherited a bovine growth hormone (bGH) gene from parents who had been microinjected with the gene. These transgenic pike (PCR^+) were compared to pike who did not inherit the gene (PCR^-) and control pike (progeny of 1 male and 1 female parent not microinjected with any GH genes). PCR^+ pike had 34% higher growth rate and 34% higher feed efficiency than controls but showed no differences from PCR^- pike. Faster growing, PCR^+ pike had similar feed intake as PCR^- and control fish. After an accidental cold-water temperature shock followed by a parasite outbreak, PCR^+ and PCR^- fish showed greater sensitivity to stress (100% mortality over 120 days) than control fish (67% mortality, with surviving control fish still alive). The three groups showed no significant differences in blood levels of bGH and metabolic rates, but two PCR^+ fish had 3- and 7-fold increases in bGH blood levels. In aquaculture systems, the transgenic northern pike should gain more weight per unit weight of eaten feed than non-transgenic pike. If these fish escaped into natural ecosystems and still grew faster than non-transgenic pike, they should not require a higher food intake to grow faster. Comparisons between transgenic fish and a broader genepool of controls are needed to confirm our findings. **B.** Over 200 persons from the aquatic biotechnology industry, research community, government oversight agencies, and environmental groups contributed to the development of voluntary "Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish." The Standards consist of three interrelated documents: flowcharts, providing the decision making pathway for assessing if the project is safe or if there are specific risks and managing any identified risks; supporting text, giving the scientific background for questions and alternative decisions in the flowcharts; and a worksheet, to trace the decision path and give the rationale for any risk management measures.

PROJECT RESULTS USE AND DISSEMINATION

Media Rare plans a segment on the entire project for a cable TV program. **A.** Presentations of results include: Inaugural Symposium (6/95) of the Univ. of MN Food Animal Biotechnology Center, which comprises advisors from private biotechnology companies and faculty from the University; 125th American Fisheries Society Meeting, the largest fisheries conference in the U. S (8/95); and a paper submitted for publication in a professional journal. **B.** The Standards were mailed to 500+ parties, summarized in the USDA newsletter, *Biotechnology Notes* (mailed to 500,000+ addresses, distributed over the Internet, and released to news wire services), and presented to DNR personnel, Sea Grant Extension staff, aquaculture industry leaders, and at several public meetings. Public comment on the final draft was overwhelmingly supportive. The USDA, Agricultural Biotechnology Research Advisory Committee unanimously endorsed the Standards, recommending to the U. S. Secretary of Agriculture that they be voluntary and fully converted into an interactive, computerized expert system (prototype already exists); and that the USDA convene outreach workshops (1 in MN) to foster state-federal cooperation on use of the Standards.

Date of Report: July 1, 1995

LCMR Final Report - Detailed for Peer Review - Research

I. Project Title: Ecological Impacts of Releasing Genetically Engineered Fishes

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A. Legal Citation: M.L. 93 Chpt. 172, Sect. 14, Subd. 12(o)

Total Biennial LCMR Budget: $175,000
Balance: approximately $9,900

Appropriation Language as drafted 7/27/92: Subd. 12(o) This appropriation is from the trust fund to the commissioner of agriculture in cooperation with the commissioner of natural resources for a contract with the University of Minnesota to assess impacts of the release of genetically engineered fish on Minnesota's gamefish and aquatic ecosystems and formulate recommendations to reduce detrimental impacts through measurement of bioenergetic and behavioral traits. $175,000

B. LMIC Compatible Data Language: not applicable
C. Status of Match Requirement: not applicable

II. Project Summary: The overall goal of this program is to advance ecological safety of genetically engineered fishes (GEFs) via two activities. The first focuses on one type of GEF, and the other takes a generalist approach to safety in aquaculture.

Laboratory experiments will be conducted to measure ecologically important bioenergetic and behavioral traits of existing growth-enhanced GEFs and related non-genetically engineered fishes (non-GEFs, i.e. controls). Findings will be incorporated into existing bioenergetic models to predict risks of GEF releases on Minnesota's gamefish and aquatic ecosystems and recommend ways to reduce risks.

An invitational workshop will convene aquatic biologists, aquaculture engineers, industry representatives, ethicists, and regulators to produce two reports which will foster safe uses of GEFs in Minnesota and nationally. These are: generic performance standards for aquaculture systems, designed to ensure ecological safety of GEF *research*; and future directions for development of ecologically safe *applications* of GEFs. Performance standards will be submitted to the U.S. Department of Agriculture (USDA) for adoption by the federal government. They will assist Minnesota regulators having regulatory oversight of ongoing research on GEFs. We will use additional funds from USDA and Sea Grant to: expand the workshop scope; invite additional participants; and begin workshop planning.

III. Statement of Objectives:

A. Test the null hypothesis: there is no difference in ecologically important, bioenergetic and behavioral traits of genetically engineered fishes (GEFs) bearing extra growth hormone genes and non-genetically engineered fishes (non-GEFs) derived from the same population.

B. Develop (1) draft recommendations for performance standards (i.e., facility and operating guidelines) designed to minimize ecological risks of conducting contained or confined research with GEFs, and (2) recommendations for future development of environmentally sound uses of GEFs.

IV. Research Objectives

A. Title of Objective: Assess ecologically important, bioenergetic and behavioral traits of GEFs, compared to related non-GEFs.

A.1. Activity: We will conduct laboratory experiments to compare bioenergetically important physiological traits of GEFs to related non-GEFs. The traits will include growth rate at various ration sizes, maximum food consumption rate, maintenance ration size, food conversion efficiency, and the effect of temperature on these traits. We will determine preferred temperature to learn if GEFs and non-GEFs might occupy a different thermal niche in the natural environment. We will monitor gonadal development to determine if GEFs and non-GEFs mature at different rates.

A.1.a. Context within the project: A higher growth rate, food consumption rate, conversion efficiency, or earlier maturation may give GEFs a competitive advantage over conspecific non-GEFs in the same water body and lead to displacement of the non-GEFs (Kapuscinski and Hallerman 1990, 1991). A higher food consumption rate among GEFs may also impact the

forage base and lead to widespread changes in the biological community (reviewed in Kapuscinski and Hallerman 1990, 1991). Comparative data on these physiological traits, including preferred temperature, can be incorporated into existing bioenergetics models to predict the impact of GEFs on the forage base in natural ecosystems (Hewett and Johnson 1992).

A.1.b. Methods: Creation of related GEFs and non-GEFs - Rainbow trout and northern pike transgenic and non-transgenic founders existing in our lab will be mated (primarily before the start of this workprogram) to generate F_1 GEF and non-GEF progeny, respectively. Founder GEF parents carry extra bovine or chinook salmon growth hormone genes in their gametes (see Gross et al. 1992 for description of constructs). We have demonstrated growth enhancement in our northern pike GEF founder population, and, because progeny are not mosaic, expect to see more uniform growth enhancement in their transgenic F_1 progeny (Gross et al. 1992). Although growth evaluations of our rainbow trout transgenic founders have not been completed, their progeny are expected to exhibit growth enhancement because they will bear the same construct as that of the growth-enhanced northern pike. Our founder GEF and non-GEF conspecifics were derived from the same genetic population, are of the same age-class, and have been reared in common tanks since juvenile life stages. In some cases, we have the option of generating F_1 fish from founder GEFs and non-GEFs who are full- or half-sibs. Transgenic status of all F_1 GEFs used in Activities A1 and A2 will be confirmed by dot blot analysis of fin tissue; and integrity of the transgene construct in a sample of each F_1 group will be confirmed by Southern analysis. These DNA analyses will be completed mostly prior to initiation of this workprogram, using other existing, federal grants.

Trait comparisons - We will compare the physiological traits of GEFs and non-GEFs of the F_1 generation of rainbow trout and northern pike at ages of approximately 6 months and 1 year old, separately for each species. Each comparison will consist of an approximately 30-day experiment where the fish will be maintained at four constant temperatures bracketing the optimum temperature for growth. Groups of both GEF and non-GEF fish will receive one of three ration sizes, *ad libitum* (maximum consumption rate), no food, and an intermediate ration (50% of maximum). We will gather data on growth in length and weight and food consumption. We will derive conversion efficiency, maximum ration, maintenance ration, and the influence of temperature from the growth and consumption measurements. The experiments will be designed as a 4 x 3 x 2 factorial with two replications (4 temperature, 3 ration sizes, 2 fish groups, i.e. GEFs and non-GEFs). We will use a similar experimental protocol and equipment as Woiwode and Adelman (1991) except that with northern pike we will modify the apparatus so as to keep each fish in a separate compartment. With rainbow trout, we will hold 4-5 fish in each experimental chamber for a total of 8-10 per replication.

Late in the project period, we will excise gonads from GEFs and non-GEFs of both species and examine them histologically for state of maturity using standard histological techniques (Hinton 1990). We will determine the preferred temperature of young-of-the-year and yearlings of each species of GEFs and non-GEFs by subjecting the fish to either gradient tank or shuttle-box experiments (Noakes and Baylis 1991).

Data analyses to predict ecological impacts - Analysis of variance methods will be used for statistical analysis of data on examined traits of GEFs and non-GEFs. Also, we will incorporate the findings from the laboratory experiments into existing bioenergetics models to predict comparative growth rate, food consumption, and impact on prey populations in natural environments by GEFs and non-GEFs. A parameterized model is available for northern pike (Hewett and Johnson 1992) into which we will substitute the data from our experiments. A rainbow trout model is nearly completed (Don Stewart, State Univ. of NY, Oswego, personal communication) and its authors have agreed to give us a completed version before the start of these experiments. We may also be able to incorporate data on capture efficiencies from the predator-prey tests (Activity A.2) into the bioenergetics models. Outcomes of these statistical and bioenergetic analyses, and of predatory behavior tests (see A.2) will be used to assess risks of the following ecological impacts: displacement of conspecific non-GEFs by GEFs; alteration of the forage base leading to disruption of other economically important aquatic species.

A.1.c. Materials: Temperature controls and tanks will be similar to those used by Woiwode and Adelman (1991) except that the tanks will be further partitioned to accommodate individual chambers for northern pike. We will use either a gradient tank or a shuttle box system to determine preferred temperature (Noakes and Baylis 1990). Bioenergetics models will be run on a DOS operating system personal computer.

A.1.d. Budget: $133,400. 7/1/95 balance is $0.

A.1.e. Timeline:	7/93	1/94	6/94	1/95	6/95
Equipment setup	****	*******			
Rainbow growth test		****	****	*******	
Pike growth test			**********	******	
Rainbow temperature test		**		**	**
Pike temperature test			**	**	**
Gonadal histology					***
Rainbow modeling		***		***	***
Pike modeling		**********			***
Final report					**

A.2. Activity: We will examine the ecological significance of predation behavior of GEFs by comparing differences in capture efficiencies and biomass successfully consumed by GEFs and non-GEFs.

A.2.a. Context within the project: If GEFs consume more prey than non-GEFs, either because they are inherently more efficient predators or because they are larger at a given time, the GEFs may have a competitive advantage over conspecific non-GEFs in the same water body. This could lead to displacement of the non-GEFs and also impact the forage base to cause changes in the biological community (reviewed in Kapuscinski and Hallerman 1990, 1991). The data on capture efficiencies and biomass consumed will be analyzed for ecological relevance by incorporation into the bioenergetics model (see Activity A.1.b).

Predator-prey tests have been used in both toxicological and ecological investigations. In studies examining the effect of low level exposure to contaminants on the ability of fish to avoid predation (Sullivan et al. 1978, Woltering et al. 1978, Hedtke and Norris 1980, and Schneider et al. 1980) the predator-prey trials allowed for an evaluation to be made concerning the "ecological significance" of the exposure along with delineating differences induced by various chemicals. Predator-prey tests have also been used to examine intra-and interspecific (Savino and Henry 1991) interactions among fish. Reaction distance to artificial versus natural prey (Henderson and Northcote 1985), influence of suspended solids on vision (Berg and Northcote 1985), age or predator and size (Mills et al. 1984) and many other factors influencing predation efficiency and dynamics have been examined.

A.2.b. Methods: We will compare prey capture efficiency and biomass consumed of related GEFs and non-GEFs of the F_1 generation of rainbow trout and northern pike at ages of approximately 6 months and 1 year old. Fish used in these experiments will be progeny from the same matings described in the first section of Activity A.1.b. Experimental procedures will be similar to those of Savino and Henry (1991). Twenty individual GEFs and 20 non-GEFs of each species will be tested during each testing period. An individual predator will be acclimated to a 100 gal circular test tank for 14 h. Approximately 100 prey fish will be weighed and then acclimated to test tank conditions for the same period but will be visually and physically isolated from the predator by a solid partition. At the end of the acclimation period, we will remove the partition and will observe and record successful and unsuccessful strikes, captures, and prey escapes. Direct observation will take place for 1 hour and the test will continue for another 23 hours. At the end of the test period, we will recover, count, and weigh the remaining prey. We will conduct exploratory trials to determine suitable prey species and number of prey. We will use nonparametric statistics, most likely the Wilcoxin ranked-sign test, to analyze data of GEFs and non-GEFs.

A.2.c. Materials: Tank configurations will be similar to that used by Savino and Henry (1991) except that the bottom substrate will be modified to simulate conditions commonly experienced in the wild by the species and life-stage of the predator being examined.

A.2.d. Budget: $16,600. 7/1/95 balance is approximately $9,900.

A.2.e. Timeline:	7/93	1/94	6/94	1/95	6/95
Equipment setup	****		*******		
Rainbow test		****	****		****
Pike test			****	****	****
Final report					**

A.3 Status:

- Methods Used. *1. PCR Screening*. We used two sets of PCR primers (National Biosciences, Plymouth, MN) to screen GEFs. One set was test primers designed to amplify in vitro only the bovine growth hormone (bGH) transgene integrated into the genome of northern pike and chinook salmon growth hormone gene (csGH)

integrated into the genome of rainbow trout. Genomic DNA was isolated from blood samples of pike and fin tissue samples of trout. The other set was control primers based on northern pike or rainbow trout growth hormone gene to provide a positive control for confirming amplification of sample DNA. An internal standard was used in PCR analyses for northern pike. The standard was whole blood sample from a founder pike, positive for the transgene construct (pRSV/bGH) in both fin and blood samples based on Southern blot hybridization analysis (Gross et al., 1992). There was no such internal standard for trout PCR because all founder fish had died.

When fish were screened by PCR analysis, if blood cells of individuals (whose parents were microinjected with the pRSV/bGH gene construct) contained a bGH gene that was amplified by PCR, the fish were called PCR^+, i.e., these were confirmed transgenic progeny. If blood cells of such fish did not contain a PCR-amplifiable gene, the fish were called PCR^-. Northern pike control fish were offspring of one cross between 1 male and 1 female who had not been microinjected with any gene construct at the zygote stage.

2. *Growth Experiment.* A randomized complete block design was used, in which 24 Northern pike from each group (PCR^+, PCR^- and control) were randomly assigned into 18 experimental unit tanks (3 x 6). Fish were manually fed TROUT GROWER® 1/8" pellets (Supersweet Feeds), 3 times per day to satiation over an 8-week (56 days) experimental period. Satiation was the point at which a few uneaten pellets remained in the bottom of tanks after feeds had been offered for 15 to 20 min. Each of three rearing tanks had a water exchange rate of once per hour with > 7 mg/L of dissolved oxygen and < 0.6 mg/L of total ammonia. Water temperature was controlled at ~20°C throughout the experiment. Photoperiod was provided with fluorescent lights timed as a 14:10h light-to-dark cycle. Experimental fish were measured for weight and length every four weeks.

Data were analyzed by ANOVA with original fish weight used as a covariate for all responses and tank mean used as the experimental unit. Orthogonal contrasts were used to test for a difference in any pair of means if there was a significant difference ($P < 0.05$) existing among the three fish groups.

The finding of no significant difference in weight gains between PCR^+ and PCR^- fish in the 56-day experiment (reported under results below) raised two questions: (1) Did the feeding schedule of three times per day meet nutrient requirements for faster growth in GEFs and (2) did rearing space restrict the fast growth of GEFs? To answer these questions, we first changed the frequency of daily feedings from three to five times for 14 days beginning on Day 57. Then, we relocated the fish from the initial rearing volume (50 L/unit) to individual circular tanks (115 L/unit) beginning on Day 71.

3. *Test of Stress Tolerance Due to Accidental Temperature Shock.* On Day 84, water temperature dropped suddenly from the experimental 20°C to 12°C and remained at the lower value for three days. This resulted from a failure in the building's hot water supply (we have no authority over this hot water system). Beginning Day 92, fish involved in the growth experiments died of a parasite (*Ichthyophtherius multifilis*), as diagnosed by the Pathology Laboratory of the Minnesota Department of Natural Resources. Mortalities were recorded until all PCR^+ and PCR^- fish had died by Day 120. Mortalities among the three fish groups were examined by probit analysis (Finney, 1971) and resulting three regression lines compared by the method of Weisberg (1985).

4. *Plasma Radioimmunassay of Sampled Fish.* In order to better understand why we found differences in stress-induced mortality but no difference in growth between PCR^+ and PCR^- fish, we applied radioimmunassay (RIA) to test for blood concentrations of bGH (i.e., protein product of the transgene). We tested plasma from all remaining 11 PCR^+ fish, from 8 PCR^- and 2 control fish. None of these assayed fish had been used in the prior growth experiment. Prior to collection of blood samples for RIA, temperature of the fish rearing water was raised from the initial 12°C to 19°C with a 1°C-increase-per-day; fish were then acclimated to 19°C for 14 days. At this optimum temperature, fish with high growth rate were presumed to have a high enough bGH concentration in plasma to be detectable by RIA, which was performed by the Endocrinology Laboratory of the Department of Animal Science, University of Minnesota.

5. *Metabolic Rates.* Because GEFs had a significantly higher mortality than control fish in response to the accident temperature shock, we decided it was more important to measure metabolism in a sample of PCR^+, PCR^-, and control fish than to measure behavioral traits as initially proposed. Metabolic rates were determined as oxygen consumption and ammonia excretion using the methods of Cai and Summerfelt (1992) and Brett and Zala (1975). After feeding for 5 hours at 12°C, each fish was measured three times at intervals of 1.5 hours. The difference in dissolved oxygen and ammonia concentrations between the incoming and outflowing waters was used to calculate metabolic rates with reference to flow rate and fish weight.

- Results and Discussion. *1.* Of the screened 157 yearling, progeny pike, we detected the bGH transgene in 39 fish (PCR^+), and did not detect it in 118 fish (PCR^-). We found no PCR^+ fish in the 263 rainbow trout screened for the csGH

transgene. Therefore, we did not conduct any further experiments with these trout.
2. Growth data with northern pike are summarized in Table 1 (below).

Table 1. Growth and feeding traits for northern pike tested in a 56 day experiment (mean values listed in columns labeled control, PCR^+ and PCR^-). Means in the same row with different superscript letters differed significantly from one another ($P < 0.01$).

Response	Control	PCR^+	PCR^-	SE	P <
Weight gain, g/d	0.88^a	1.11^b	1.23^b	0.05	0.001
Specific growth rate, %/d	0.66^a	0.76^b	0.82^b	0.05	0.24
Feed intake, g/d	5.76	5.96	6.04	0.30	0.080
Feed-to-Gain ratio	1.90^a	1.49^b	1.37^b	0.15	0.013

These results address two important questions about transgenic (PCR^+) pike bearing extra bovine growth hormone genes. (1) Do these transgenic fish grow faster? (2) Do faster-growing transgenic fish eat more food? Average daily weight gain of experimental fish indicated that both PCR^+ and PCR^- had 34% greater gain than the control fish ($P < 0.01$) but there was no difference between PCR^+ and PCR^- fish ($P = 0.09$). The reason for this latter result is unclear. Two possible explanations are that: (1) PCR analysis failed to detect low copy numbers of the transgene in some individuals, leading to incorrect scoring of them as PCR^-; or (2) the limited genepool of the controls (1 male and 1 female parent) biased the comparisons between controls and the other two groups (at least 5 male and 6 female parents for each group). Compared to control fish, PCR^+ and PCR^- fish showed similar feed intake ($P = 0.08$), but 34% higher feed efficiency (feed-to-gain ratio) ($P < 0.01$). However, there was no significant difference in these measured traits between PCR^+ and PCR^- fish ($P = 0.36$).

Feed intake was similar among the three fish groups and this was true whether these fish were fed three times or five times per day (Table 2 below).

Table 2. Total daily food consumed by the three groups of experimental fish fed three and five times per day (mean ± SD).

Progeny	Total intake, g/day	
Northern pike	Three times[a]	Five times[b]
Control	6.23 ± 1.20	6.34 ± 0.77
PCR^+	6.76 ± 1.30	6.09 ± 0.82
PCR^-	7.17 ± 1.36	6.46 ± 1.04

[a]Averaged from 28 days' feeding records (8/20/94--9/16/94).
[b]Averaged from 14 days' feeding records (9/17/94--9/3094).

The higher feed efficiency of PCR^+ pike compared to controls suggest that, in aquaculture growout systems, these transgenic pike should gain more weight per unit weight of food than controls. If transgenic pike escaped into natural ecosystems and still grew faster than nontransgenic counterparts, results suggest that they might not require more food to grow faster. However, comparisons between confirmed transgenic fish (PCR^+) and a broader genepool of controls are needed to confirm our results.

3. We were able to address another important question: what is the sensitivity of transgenic pike bearing bGH genes to environmental stress? One component of fitness both in aquaculture systems and in the wild is tolerance of temperature shock. When all experimental fish were subjected to a 3-day cold temperature shock, PCR^+ and PCR^- fish showed significantly higher mortality than controls

(P< 0.05, Figure 1). After this cold temperature shock, the last PCR$^+$ fish died 26 days later and the last PCR$^-$ fish died 33 days later, whereas 9 control fish are still alive. These results are consistent with findings of detrimental effects on the general health of transgenic pigs bearing extra growth hormone genes (Pursel et al., 1989) and certain pathologic syndromes in transgenic mice bearing different foreign transgenes (reviewed by DeTolla 1991).

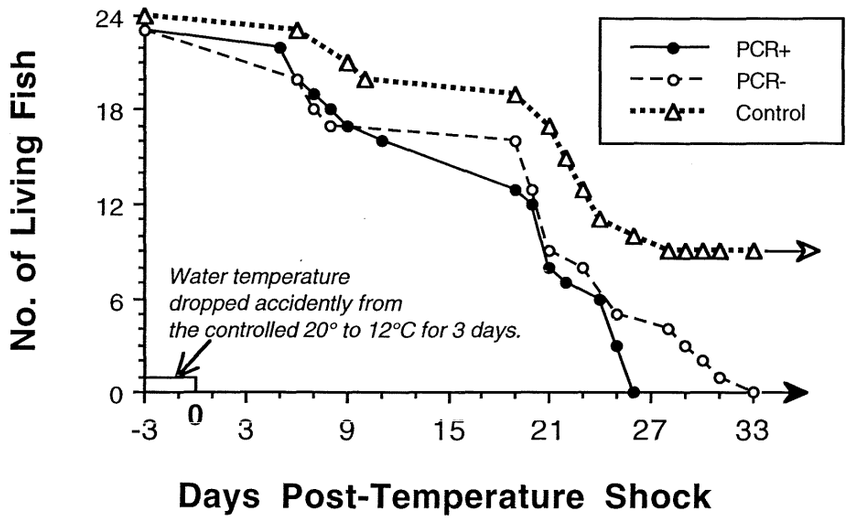


Figure 1. Surviving numbers of experimental fish over experimental days.

4. Two PCR$^+$ fish showed 3- and 7-fold increases in plasma bGH levels compared to the background exhibited by all other assayed fish (Table 3).

Table 3. Plasma bGH concentrations of individual progeny northern pike, detected by radioimmunassay. [a]Fish used for metabolic rate estimates (see Table 4).

Fish ID	Fish type	bGH (ng/mL)
7F7E574D7F[a]	PCR$^+$	17.4
7F7F212545[a]	+	45.1
7F7F213902[a]	+	7.6
7F7F190944[a]	+	6.4
7F7E574166	+	3.5
7F7F142C66[a]	+	4.6
7F7F14311F	+	5.3
7F7F1F0703	+	7.2
7F7E574B2A	+	7.7
7F7F18671B	+	7.3
7F7F212466	+	6.2
7F7F214F5D[a]	PCR$^-$	9.6
7F7E574C0D	-	6.6
7F7F212344	-	5.6
7F7F21362E	-	4.5
7F7F220521[a]	-	6.7
7F7F142C6B	-	6.2
7F7F1F2075	-	6.8
7F7F217D39	-	4.7
7F7F212537[a]	Control	5.3
7F7F190A32	Control	7.3

5. Eight fish sampled from the three groups of radioimmunassayed fish (Table 3, superscript a) had similar rates of oxygen consumption and ammonia excretion when they were held at a constant water temperature (Table 4).

Table 4. Metabolic rates of the sampled progeny northern pike (mean ± SD).

Fish ID	Fish weight (g)	Fish type	bGH level by RIA (ng/mL)	Oxygen cnsmptn (mg/kg.h)	Ammonia excretion (mg/kg.h)
7F7E574D7F	150	PCR+	17.4	27 ± 1	.20 ± .13
7F7F212545	106	+	45.1	44 ± 5	.83 ± .38
7F7F213902	110	+	7.6	39 ± 3	.31 ± .35
7F7F190944	131	+	6.4	25 ± 6	.15 ± .23
7F7F142C66	134	+	4.6	16 ± 3	.14 ± .65
7F7F214F5D	215	-	9.6	13 ± 1	.09 ± .04
7F7F220521	150	-	6.7	19 ± 4	.13 ± .38
7F7F212537	97	Ref.	5.3	36 ± 2	.40 ± .35

- Conclusion. Experimental results suggest that GEFs (confirmed transgenic northern pike, PCR^+) progeny had higher growth rate, similar feed intake, higher feed efficiency, but were more sensitive to stress and disease, than non-GEFs (control) of the same population. Comparisons between transgenic fish and a broader genepool of controls are needed to confirm these results.

Problems: Regarding Activity A.1, the unexpected results of no differences between PCR^+ and PCR^- fish placed a higher priority on testing for actual levels of bovine growth hormone in the PCR^+ fish (i.e., to determine if the hormone was successfully expressed by the inherited bGH genes). Thus, we conducted radioimmunassays instead of bioenergetics modeling. Regarding Activity A.2, we did not complete behavioral experiments because we lost a large number of the required PCR^+ and PCR^- pike when there was an accidental die-off. Fortunately, we were able to take advantage of this situation by obtaining important information about stress tolerance of the GEFs (as summarized in the status section), even though stress testing was not in our initial plans. To take full advantage of the stress tolerance results, we also added testing of metabolic rates.

B. Title of Objective: Develop recommendations for environmentally sound uses of genetically engineered fishes.

B.1. Activity: In collaboration with the Office of Agricultural Biotechnology (OAB) of USDA and Minnesota Sea Grant, an invitational workshop in Minnesota will convene a diversity of scientific and regulatory experts to produce draft performance standards for research involving GEFs, and recommendations for future development of environmentally sound uses of GEFs. The workshop's final draft of performance standards will be submitted to the Director of OAB, who will then initiate internal USDA steps for submitting them for federal adoption, including publication in the Federal Register for public review. This draft will also be duplicated for wide dissemination to interested parties. Workshop recommendations for future development of GEFs will be published in a peer-reviewed scientific journal.

B.1.a. Context within the project: Aquaculture is the fastest growing segment of U. S. agriculture and numerous federal and state agencies fund aquaculture research. For example, USDA, currently spends approximately $20.3 million annually on aquaculture research (Al Young, OAB, personal communication) and, since 1987, LCMR has spent approximately $2 million on aquaculture and GEF research. Research expenditures are likely to increase in the future in response to growing consumer demand for high quality aquacultural products and the need to reduce exploitation of natural fisheries to sustainable levels. Genetic engineering techniques make it possible to improve traits desired in aquaculture, such as increased growth rates, improved feed conversion efficiencies, freeze resistance, and resistance to specific disease agents. The performance of aquacultural organisms expressing some of these modified traits may prove to be ecologically novel, thus raising questions about ecological risks associated with using them in research and eventually in commercial operations (Hallerman and Kapuscinski 1992; Kapuscinski and Hallerman 1991; Kapuscinski and Hallerman 1990 and 1990a; Kapuscinski 1990). In order to assess their commercial viability, aquacultural organisms with modified traits must be evaluated as part of different aquaculture systems. Aquaculture systems are diverse in their makeup of both organisms and physical

components. Physical components may involve: static, recirculating, or flow-through incubators and tanks located indoors or outdoors; static or flow-through outdoor earthen ponds; or cages and net-pens suspended in natural waterbodies or artificial reservoirs.

In the U.S., no federal or state standard currently exists for determining whether a particular aquaculture system constitutes containment, confinement, or environmental release of the cultured organisms. To date, in order to ensure compliance of USDA funded research with the National Environmental Policy Act, the Cooperative State Research Service (CSRS) of USDA has had to conduct a costly environmental analysis of each proposed outdoor experiment. Although the state of Minnesota recently adopted environmental safety regulations for research involving releases of genetically engineered organisms within the state, concrete and practicable standards are missing for deciding whether a particular aquaculture research system falls under containment (no need for state permit) or release (requires application for a state release permit). In light of Minnesota's ongoing research on GEFs and aquaculture, state regulators will soon be faced with the need to make such decisions. As an alternative to the unwieldy federal environmental review process used so far by USDA, and to facilitate environmentally sound oversight by Minnesota regulators, the proposed workshop will develop generic performance standards. The intent is that an aquaculture system meeting these standards will comply with the National Environmental Policy Act's "finding of no significant environmental impact" and, if located in Minnesota, will constitute containment and therefore be exempt from a state release permit.

If genetically engineered aquatic organisms do prove to exhibit improved performance traits, many sectors of the aquaculture industry will eventually become interested in using them in commercial operations. It is imperative that such commercialization advance in an environmentally sound manner. Workshop participants, therefore, will also recommend future directions towards development of ecologically safe uses of genetically engineered aquatic animals.

B.1.b. Methods: Prior to the start of this program and with funding from OAB , a Working Group on Aquaculture Biotechnology and Environmental Safety (chaired by Kapuscinski) will outline the content of draft performance standards (to be refined by workshop participants) and will begin workshop planning. Additional funding from OAB and Sea Grant will be used to expand the scope of the workshop beyond fish to other aquatic mollusks and crustaceans, as these are also of aquacultural and economic importance.

A workshop (duration approximately 2.5 days) will be convened in Minnesota in 1993. To ensure that workshop products are scientifically sound, practicable, and consistent with existing regulations, *invited participants* will represent a diversity of technical expertise relevant to assessment of genetically engineered aquatic animals such as: aquatic/fisheries ecology, population dynamics, physiology and behavior, molecular genetics, population and quantitative genetics, evolution, environmental risk assessment, environmental and bioethicists (e.g., current chair of USDA's Agricultural Biotechnology Research Advisory Committee is a bioethicist), aquaculture engineers, industry representatives, and representatives of government agencies involved in regulation or development of aquaculture biotechnology. Following public announcement of its dates (August 17-19, 1993), location (Univ. of MN. Humphrey Institute, block of hotel rooms at nearby Holiday Inn Metrodome) and agenda, the entire workshop will be open to any *interested observers*.

Invited workshop participants will break into smaller groups and address different sets of questions in order to refine the outline of performance standards and formulate recommendations for future development. Each break-out group will be led by a rapporteur who will draft a summary of the group's responses to its assigned questions. Towards the end of the workshop, rapporteurs will present these summaries in an interactive assembly of all workshop participants. After the workshop, these summaries and notes from discussions of the whole assembly will be integrated into a review draft of performance standards and a separate draft of recommendations for future directions. Following review by workshop participants, a final draft of performance standards will be submitted to OAB and draft recommendations for future directions will be submitted to a peer-reviewed journal.

B.1.c. Materials: Meeting rooms, at the University of Minnesota Humphrey Institute, will be rented for holding the workshop. Prior to the workshop, invited participants will receive background materials tailored to the assignment of their break-out group. (USDA funds will be used to prepare much of these materials prior to the start of this LCMR program.) There will be communication and publication costs for organizing the workshop, preparation and review of final reports, duplication of the draft performance standards submitted to USDA, and submission of an article on future directions to a

scientific journal. This LCMR program will cover costs of transportation, lodging, meals, and registration packets for a major portion of invited participants. For other invited participants, these costs will be covered either by MN Sea Grant, or by their home institution (e.g., Agricultural Experiment Station or state Sea Grant program). Outside observers will pay a registration fee to cover additional costs of registration packets, expanded meeting room space, and refreshments at a workshop reception.

B.1.d. Budget: $25,000. 7/1/95 balance is $0

B.1.e. Timeline:	7/93	1/94	6/94	1/95	6/95
Arrangements	****				
Hold workshop		*****			
Report of performance standards					***
Article on future directions					**

B.3 Status: • Development and Endorsement of Performance Standards. Through a workshop (August 18-20, 1993, Humphrey Institute, U of MN) and numerous subsequent opportunities for written and oral input, over 200 people from all regions of the U.S. and seven other countries contributed to the development of voluntary "Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish." The LCMR office received a copy of the two-volume final draft (April 15, 1995).[1] The workshop was co-sponsored by the Minnesota Legislature as recommended by LCMR, USDA Office of Agricultural Biotechnology and Agricultural Biotechnology Research Advisory Committee (ABRAC), MN Sea Grant, and the Dept. of Fisheries and Wildlife at the U. of MN (see attached copies of appreciation letters from the Office of the Secretary of USDA). Workshop participants and later commenters came from industry, academia, government agencies (state and federal), U. S. Congress, non-governmental organizations and represented a broad diversity of technical expertise. The ABRAC felt that "this collaborative effort can serve as a model, both procedurally and substantively, for future efforts to address the potential safety and ecological effects of other groups of genetically modified organisms." Public comment on the final draft was overwhelmingly supportive and many commenters urged conversion of the Standards to an interactive, computerized expert system format. The most frequent comments from all sectors included that: the Standards were developed by an inclusive, open process; are based on well-documented scientific rationales; are already providing useful guidance on existing projects; and are flexible enough to permit research and development to go forward in an ecologically safe manner. The most common concerns of commenters were: (1) the need to convert the Standards to an interactive, expert-system format to make them more user-friendly and easier to update in light of future scientific knowledge; and (2) uncertainty about how government oversight authorities might use the Standards, with a few commenters fearing that they might be used to obstruct research. Regarding the first concern, the USDA, Office of Agricultural Biotechnology already has a prototype computerized version and is seeking funds to complete the conversion. Regarding the second concern, the ABRAC passed several strong motions to allay it and the first page of the final printed version of the Standards (in press) will clearly state that the Standards are intended to facilitate, not obstruct, research and to be voluntary. The ABRAC, of which A. R. Kapuscinski is vice-chair (since 11/94), unanimously endorsed the Standards and forwarded them to the U. S. Secretary of Agriculture with these recommendations: (1) the Performance Standards should remain voluntary; (2) the USDA should expedite conversion of the Standards to a computerized format; (3) in the next year or so, the USDA should convene outreach workshops (1 in MN) to foster state-federal cooperation in use of the Standards and to obtain user feedback on computerized versions; and (4) the USDA should now strongly encourage research and development in aquatic biotechnology in the U. S.

• Synopsis of the Standards[1]. *Purpose*. The voluntary Performance Standards are intended to aid researchers and institutions in assessing the ecological and evolutionary safety of research activities involving genetically modified fish, crustaceans, or molluscs (except for non-applicable organisms as summarized below). Where the need is identified, they are also intended to aid researchers in developing appropriate risk management measures so that the research can be conducted without adverse effects on natural aquatic ecosystems.

Facilitation of environmentally safe research, through use of these Performance Standards, is particularly important because of three features of fish, molluscs, and crustaceans. First, these research organisms are wild-type or nearly so; to date, the domestication of populations or genetic strains of aquacultural species is insufficient to prevent escaped individuals from surviving under natural environmental conditions. Second, the United States is the origin of diversity of numerous fish and shellfish species that are of interest in research and development involving genetic modification. Protection of this natural diversity at genetic, population, and species levels is important because aquatic biodiversity in the United States has suffered dramatic declines. Third, many natural populations of fish, molluscs, and crustaceans are themselves of tremendous economic importance, either because of

commercial fishing, sportfishing, or other recreational activity.

The Standards are designed to apply to research and development with conducted in the public and private sectors. Although information in the Standards may provide a useful starting point for evaluating the environmental safety of intentional environmental introductions in commercial aquaculture or in fisheries management programs, these activities will require additional considerations beyond those addressed in these Standards.

The term "Performance Standards" conveys attributes of the intended guidance. Performance standards define endpoints or goals to be achieved, and they provide guidance and criteria for achieving those goals. They differ from a design standard in that they are not rigid and prescriptive. A performance standard provides flexibility to choose the best and most appropriate method of achieving the goals and meeting the criteria. To ensure this flexibility, performance standards are structured to accommodate a dynamic, rapidly changing state-of-the-art.

For a number of research or development projects involving genetically modified fish or shellfish, contemporary knowledge is insufficient to clearly determine if the project is environmentally safe. The Performance Standards are designed to identify such cases and provide recommendations on how to conduct appropriately confined laboratory experiments or outdoor experiments. Application of the Performance Standards should encourage the conduct of safe research to address important information gaps about environmental effects of particular genetically modified fish and shellfish and facilitate safe development of these modified organisms.

Components. The Standards consist of three interrelated documents: the FLOWCHARTS, which provide the decision making pathway for assessing if research projects have specific reasons for safety (allowing exit from the Standards) or pose specific risks and provide guidance for managing any identified risks; the SUPPORTING TEXT, which gives the scientific background for the questions and alternative decisions in the flowcharts, provides detailed information on options for risk management measures, and gives a glossary and various appendices; and the WORKSHEET, which is completed by the researcher and, where appropriate, describes the rationale for the project's risk management measures. In a forthcoming computerized, interactive version of all three components, explanatory text, literature citations, and a glossary will be accessible from any point in the decision making path and the user's path through the flowcharts will be automatically recorded onto the WORKSHEET. Then, the user will be able to transfer the worksheet to standard word-processing software in order to more easily type in rationales for key decisions and descriptions of risk management measures, if any are needed.

Applicable and Non-Applicable Organisms. Researchers begin by using the first Flowchart to quickly determine whether or not the Performance Standards apply to the research organisms in question. If the conclusion is that they do not apply, then the researcher has completed voluntary compliance with the Standards and exits at this point.

Except for non-applicable cases listed below, the Standards apply to freshwater and marine finfish, crustaceans and molluscs whose genomic structure has been deliberately modified by human intervention. The flowcharts address three categories of deliberately induced changes in genomic structure: (1) deliberate gene changes - including changes in genes, transposable elements, non-coding DNA (including regulatory sequences), synthetic DNA sequences, and mitochondrial DNA; (2) deliberate chromosomal manipulations - including manipulations of chromosome numbers and chromosome fragments; and (3) deliberate interspecific hybridization (except for non-applicable cases discussed below) - referring to human-induced hybridization between taxonomically distinct species.

Research projects involving genetically modified organisms which meet the applicability criteria will not necessarily require precautions beyond those normally practiced in research. Some projects, depending upon combined characteristics of the organism and accessible ecosystems, may be found early in the assessment to have a safety attribute allowing exit from the standards; i.e., further use of the standards for the proposed research is not necessary.

The standards do not apply to organisms whose genomic structure has been modified by humans solely by: (a) intraspecific selective breeding by natural reproductive processes or intraspecific captive breeding, including use of artificial insemination, embryo splitting or cloning; and (b) interspecific hybridization provided that the hybrid is widespread because it occurs naturally or has been extensively introduced (e.g., through stocking) in the environments accessible to organisms escaping from the research site, and there are no indications of adverse ecological effects associated with the specific hybrid in question.

Overview of Flowcharts. (See Figure 2). If the Performance Standards are applicable to the genetically modified organism, the researcher is directed to one of three assessment pathways, depending on the type(s) of genetic modification involved. Each assessment pathway begins with Survival and Reproduction Assessment, involving questions that are easier to answer, in most cases, than the questions that appear later under Ecosystems Effects Assessment Use of Survival and Reproduction Assessment leads to one of four possible conclusions: (1) a specific risk is identified and the researcher is led to guidance for management of that risk; (2) information is insufficient to answer an essential question in the

assessment, so the researcher is directed to risk management guidance; (3) a specific reason for safety of the research is identified and the researcher is directed to EXIT the Standards; or (4) additional information is needed to determine risk or safety and the researcher is directed to proceed to the appropriate section of Ecosystem Effects Assessment.

Questions posed under Ecosystem Effects Assessment require more knowledge about evolutionary and ecological issues than the earlier assessment questions. This section addresses the overarching question: if genetically modified organisms did end up in an accessible ecosystem, are adverse effects possible or is there a specific reason to rule out such concern? Use of this section leads to one of the first three conclusions listed above. Thus, certain projects will EXIT the Standards whereas others will proceed to risk management.

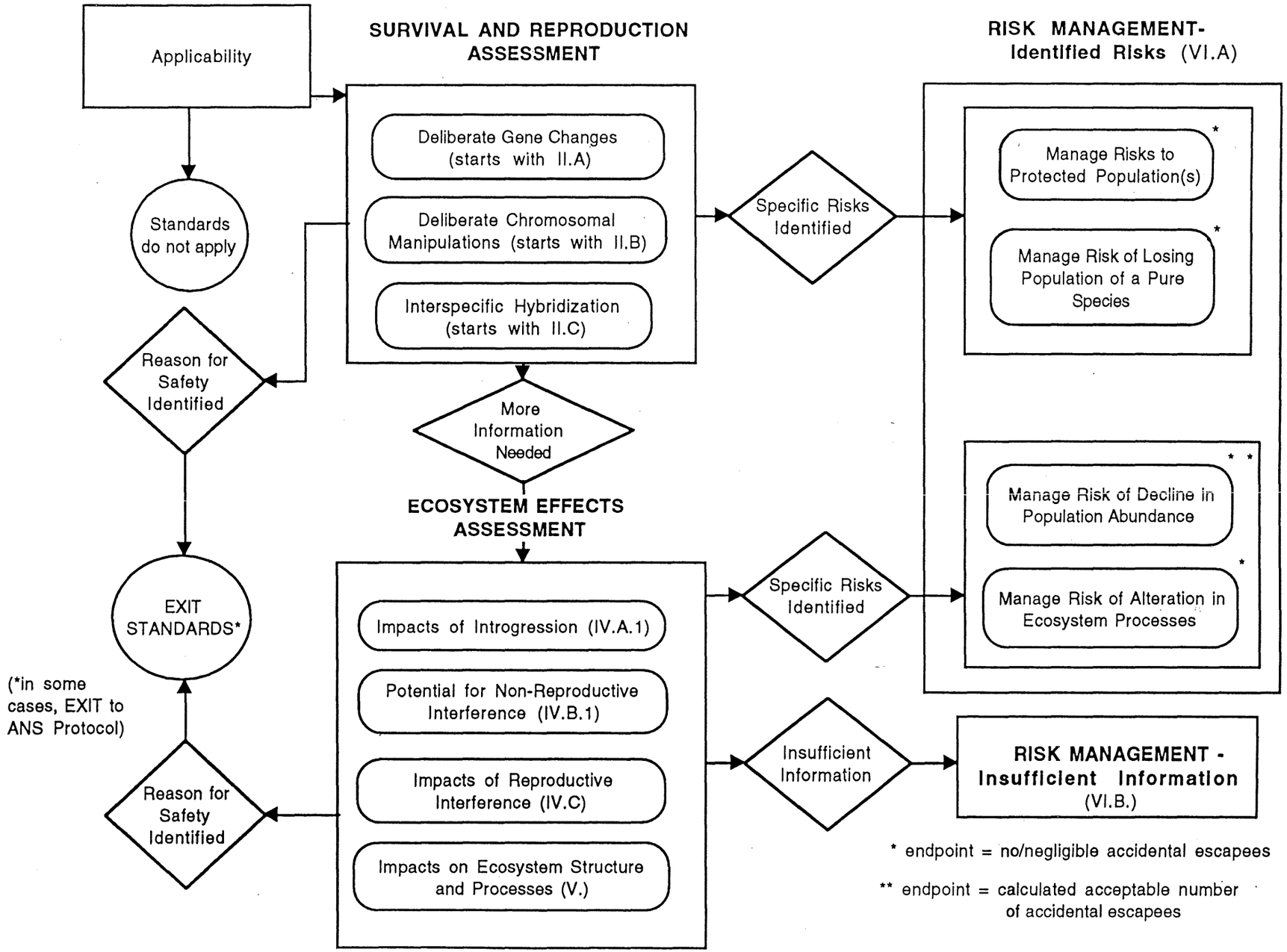
V. Evaluation: Objective A - Incorporation of experimental results into the bioenergetics model will allow us to evaluate ecological safety/risk of growth-enhanced GEFs compared to related non-GEFs, in terms of their potential: to displace conspecific non-GEFs, leading to instability of these populations; and to alter the forage base, leading to disruption of populations of other economically important aquatic species. We will also submit manuscripts reporting our results and safety/risk evaluations to a scientific journal, whose peer review process will yield the best scientific evaluation of the quality of our results and conclusions. Our published results will also provide a case study of one approach to ecological impact assessment for bioenergetic and predatory behavior traits, allowing future evaluation of its general applicability to ecological risk assessments of different types of genetically engineered aquatic animals for different aquatic ecosystems.

Objective B - Extensive evaluation of the generic performance standards will occur as a consequence of their submission to USDA. They will undergo rigorous review by the Agricultural Biotechnology Advisory Committee (ABRAC, a diverse group appointed by the Secretary of Agriculture), then internal review by the agency. Then USDA will submit a revised draft for publication in the Federal Register; public review under stipulations of the National Environmental Policy Act will be solicited. Only after considering all public comments and discussions with other federal agencies will a final version of the performance standards be federally adopted. Minnesota regulators will be: apprised of this review process, encouraged to submit public comments, and encouraged to review the standards for adoption under existing state regulations. The intended outcome of all these reviews is to have standards acceptable both for research and industry (because they are practicable) and for aquatic resource conservation (because they are ecologically sound). Because the workshop recommendations for future directions towards improved safety of GEFs will be submitted to a scientific journal, the peer review process will provide high quality evaluation. Finally, because workshop attendees will represent a broad diversity of perspectives, it may be useful to ask invitees and outside reviewers to critically evaluate these two workshop products.

VI. Context Within Field: Objective A - To date, research on GEFs in Minnesota and elsewhere has focused on actual development of fish which exhibit expression and inheritance of transferred genes. For GEFs bearing growth-promoting genes, few quantitative analyses have been reported on changes in growth rate (Zhang et al. 1990, Gross et al. 1992, Du et al. 1992) and virtually no quantitative information has been reported on ecological risks associated with a given change in growth rate and correlated changes in other ecologically important traits. This current gap of information makes it difficult to reliably assess risks of accidental or intentional environmental releases of growth-enhanced GEFs. Dr. Kapuscinski and colleagues have raised questions about potential ecological impacts of GEFs due to changes in such growth-related traits and in many other traits (reviewed in Kapuscinski and Hallerman 1991), and based on current understanding of factors affecting the health of aquatic ecosystems (e.g., Kapuscinski and Hallerman 1990, 1991). Due to limitations of funding, time, and facilities, proposed experiments will focus on bioenergetics and predatory behavior, thus filling important parts of this information gap, and will build on past research by Drs. Kapuscinski, Hackett and other colleagues aimed at production of growth-enhanced GEFs. These experiments will also complement Dr. Adelman's research program aimed at understanding interactions among various physiological traits of fish and their environment. They will expand Dr. Henry's ongoing application of aquatic environmental impact methodologies from impacts of contaminants to impacts of GEFs.

Objective B - Kapuscinski and colleagues have pointed out that a major gap in federal oversight of biotechnology is environmental impacts of genetically engineered fishes, molluscs, and crustaceans (Kapuscinski and Hallerman 1990a, Hallerman and Kapuscinski 1990). Also, no federal or state generic standards currently exist for determining whether research with a particular aquaculture biotechnology system is environmentally safe or risky. By developing such standards and recommending ways to foster ecologically sound development of aquaculture biotechnology in the future, the proposed work will enhance both effective federal and state oversight and ecologically safe development of aquatic biotechnology. The proposed work is well integrated with Dr. Kapuscinski's current work with USDA as Chair of the ABRAC Working Group on Aquaculture Biotechnology. It also complements her past and ongoing contributions to the state regulatory framework through her appointments to the MN Environmental Quality Board's Genetic Engineering Advisory Committee.

Figure 2 **OVERVIEW of Performance Standards Flowcharts**



VII. Benefits: Objective A - Where no data currently exist on bioenergetic and predatory behavioral functioning of growth-enhanced GEFs, concrete information will be generated for: predicting critical versus negligible impacts of releases on natural gamefish and aquatic ecosystems; use by MN regulators (and in other areas) to assess risks/safety of proposed contained research or proposed intentional releases; and identifying ways to increase safety of GEFs. Objective B - Performance standards will be written in non-technical format and widely disseminated, thus educating interested parties about safe, practicable ways to utilize GEFs. Via their publication in the public domain, recommendations for future directions will encourage: funding agencies interested in aquatic biotechnology to support development of safer genetically engineered aquatic animals; scientists to innovatively build in safety into genetically engineered animals, where such safety is truly needed.

VIII. Dissemination (final status): Media Rare contacted the program manager about producing a short piece on the entire project (Objectives A and B) for a cable TV program, tentatively in fall, 1995. Objective A. Oral presentations of research results include: Inaugural Symposium (6/95) of the University of Minnesota's Food Animal Biotechnology Center, which comprises advisors from private biotechnology companies and faculty from the University; and 125th American Fisheries Society Meeting, the largest fisheries conference in the U. S (8/95, travel at no expense to LCMR). A paper is in preparation for submission to a scientific journal. Objective B. News articles about the Minneapolis workshop appeared in August and December, 1993 issues of *Science*, an internationally distributed and major scientific periodical. Additional news pieces about the Standards or announcements of available review drafts appeared in the Minnesota Sea Grant newsletter, *The Seiche*, two issues of the American Fisheries Society magazine, *Fisheries*, and in a biotechnology industry periodical in France. The Standards were explained and comments sought in oral presentations at two international biotechnology meetings, one fisheries continuing education course in St. Paul (attended by fisheries staff of MN DNR, other state DNR's, and federal agencies), a Great Lakes Sea Grant Extension Network meeting, and five public meetings of the ABRAC or ABRAC Working Group on Aquatic Biotechnology. (Travel to all these meetings was at no expense to the state of Minnesota.) USDA staff informed the Joint Subcommittee on Aquaculture (JSA), a federal interagency coordinating body, about various drafts of the Standards. (The JSA contributed to development of the Standards at several stages.) The Performance Standards (April 1995 draft) were distributed to over 500 parties and summarized in the USDA newsletter, *Biotechnology Notes* (mailed to 500,000+ addresses, distributed over the Internet, and released to news wire services). The final printed copy (expected August 1995) will be distributed similarly and to a growing number of additional interested parties. Three scientific articles about the future directions for environmentally safe research and development were produced (Hallerman and Kapuscinski 1993, 1995, Kapuscinski 1994) and presented orally at three international meetings. Upon invitation by the Office of Technology Assessment, U.S. Congress, A. Kapuscinski prepared a contract report on aquatic biotechnology; OTA will use this report for briefing Congress on reauthorization of the National Aquaculture Act (bill already introduced). This report included a major discussion about the Performance Standards and related issues.

IX. Time: This program is designed to be completed within the two year LCMR funding cycle. As explained in section IV., other funds will be used to conduct preliminary work before the start of the LCMR cycle (before July 1, 1993).

X. Cooperation: Percent time over two yrs.: Dr. Kapuscinski, program manager, - 25%, objectives A & B; Dr. Adelman - 5%, objective A (may also attend Objective B workshop); Dr. Henry - 5%, objective A (may also attend Objective B workshop); Dr. Hackett - 1%, invitee to objective B workshop (also provide fish for objective A)

Dr. Ira Adelman
Professor and Head, Department of Fisheries and Wildlife
University of Minnesota, St. Paul campus

A fish physiologist with extensive research experience on environmental physiology of fishes, Dr. Adelman's primary role will be to design, oversee, and evaluate experiments on physiological traits and to assist with incorporation of experimental findings into bioenergetic models (Activity A.1).

Dr. Mary Henry
Leader, Minnesota Cooperative Fish and Wildlife Research Unit
Department of Fisheries and Wildlife
University of Minnesota, St. Paul campus

An expert on impacts of aquatic contaminants on fishes and aquatic invertebrates, Dr. Henry is also experienced in laboratory measurements of predatory behavior of fishes. Her primary responsibility will be to design, oversee, and evaluate experiments to measure predatory behavioral traits (Activity A.2).

Dr. Perry Hackett
Professor, Department of Genetics and Cell Biology
University of Minnesota, St. Paul campus

DEPARTMENT OF AGRICULTURE
OFFICE OF THE SECRETARY
WASHINGTON, D.C. 20250

DEC 20 1993

Dean Al Sullivan
College of Natural Resources
235 Natural Resources Administration Building
University of Minnesota
St. Paul, Minnesota 55108

Dear Dean Sullivan:

On behalf of the Department of Agriculture (USDA), I want to thank you and the University of Minnesota, College of Natural Resources, for support of the Workshop on Performance Standards for Research with Genetically Modified Fish and Shellfish, held at your institution on August 18-20, 1993.

USDA is committed to a strong national aquaculture program that promotes research and development to enhance seafood quality and production in accordance with environmentally sound practices. As modern biotechnology is incorporated in this important sector of agriculture, we need to seek the best scientific input on issues of safety and environmental protection. Workshops such as the one held at the University of Minnesota provide an important step in achieving those goals. We are pleased that your institution maintains a high interest and support in this area.

We are fortunate in having one of your faculty, Dr. Anne Kapuscinski from the Department of Fisheries and Wildlife, serving on the Agricultural Biotechnology Research Advisory Committee (ABRAC) and chairing the ABRAC Working Group on Aquatic Biotechnology and Environmental Safety. With leadership from scientists such as Dr. Kapuscinski and with support of academic administrators such as you have shown, we can cooperatively advance aquatic biotechnology and address environmental issues of national importance.

Sincerely,

R. D. PLOWMAN
Acting Assistant Secretary
Science and Education

cc: Vice President Anne Petersen
Vice President Eugene Allen
Dr. Ira Adelman