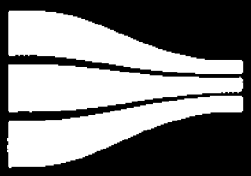

CENTER FOR FISHERIES ENGINEERING RESEARCH

A PILOT HADDOCK HATCHERY FOR MASSACHUSETTS

Clifford A. Goudey and Brandy Moran

MITSG 99-6

MIT Sea Grant College Program

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A Pilot Haddock Hatchery for Massachusetts

by

Clifford A. Goudey

and

Brandy Moran

MIT Sea Grant College Program
Bldg. E38-372, 292 Main Street
Cambridge, MA 02139

Introduction

This project report is in three parts, one covering the design and installation of culture systems in the hatchery facility, a second part covering our efforts in the cryopreservation of haddock eggs and a final brief section of fish tagging.

Part I - Hatchery Design and Operation

The MIT Sea Grant College Program is operating Boston's first marine finfish hatchery at the former Charlestown Navy Yard. The site is within the Boston National Historic Park, only yards from the famed square-rigger USS Constitution. Our goal is to demonstrate pilot-scale techniques for producing new varieties of marine finfish fingerlings. We are also demonstrating that Boston Harbor is ready for an aquaculture industry, something that needed proving, given the harbor's past reputation for pollution.

One might easily ask, "Why promote aquaculture in an urban setting?" The answer is simple: it's the market! When competing for freshness and customer service, distance to market matters, and Boston is the premier seafood market on the east coast. In the short time the hatchery has been in operation, we have met our goal of year-round operations with a variety of species. The development has been made possible through the financial support of the MIT Sea Grant College Program and the Massachusetts Aquaculture Grants Program.

The site is deep within Boston harbor and very near the mouth of the Charles River, the harbors largest source of fresh water. Our approach to intake water processing and system designs was heavily influenced by harbor water quality issues. The ever-present pollution risks in a busy urban harbor determined our water storage capacity.

Water temperatures in Boston Harbor can range from 0° to 22°C. Therefore, indoor, recirculating operation is the only sane approach to year-round productivity. Table 1 provides a general description of our hatchery. Incubator, larval, and fingerling tanks are on the first floor. By adding a mezzanine level, we gained 50% more floor area, which is used for water storage, live-feed production, and lab space.

Floor area	490	sq. ft.
Ambient S.W. storage	1,600	l..
Heated S.W. storage	1,200	l.
Incubator up-wellers	60	l. x 5
Larval rearing tanks	400	l. x 5
Fingerling tanks	2,000	l. x 3
Live feed production	220	l. x 8

Table 1. Hatchery capacities.

Our intake system is diagrammed in Figure 1. The component suppliers are listed in Table 3 with contact information in Table 4. Particle filters and U.V. sterilizers pre-treat the harbor water, which is pumped only during high tide. However, if

necessary, we can go off-line for up to five days to wait out compromised water conditions due to excessive fresh water run-off or industrial pollution. Only once, due to the heavy June rains, have we had this problem.

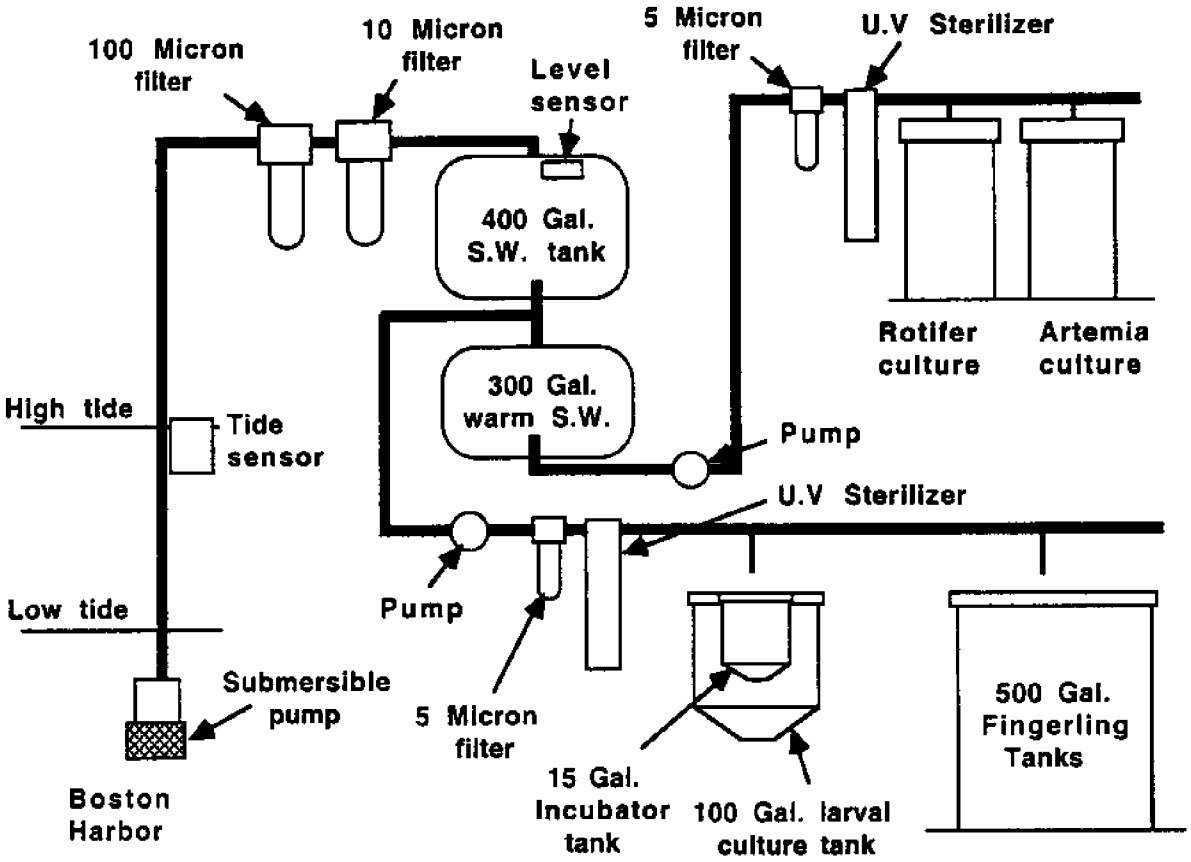

Figure 1. Boston Harbor finfish hatchery sea water intake system.

Our 1/2-hp submersible intake pump is controlled by two float switches, one detecting the tide level and one detecting the tank level. For intake water filtration, we use two filter vessels plumbed in series and fitted with 100- and 10-micron filter bags. Water is stored in a pair of 200-gallon polyethylene (P.E.) tanks at the mezzanine level and can be gravity-fed to the culture systems. These tanks are insulated, keeping the water at ambient harbor temperatures. From these tanks, water is gravity-fed through a plastic float valve to a pair of 150-gallon tanks, each of which is equipped with four immersion heaters to maintain a supply of 24° C. sea water (S.W.) for the live-feed cultures. Before use, both S.W. supplies pass through canister filters fitted with 5-micron filter cartridges and 40-Watt U.V. sterilizers. Supply pumps can be operated if flows in excess of gravity-fed rates are needed.

Because of space limitations, we place our incubator up-wellers inside our five larval culture tanks. This concentric approach provides the temperature stability of the full 2,400 liter system while minimizing early-stage feeding volumes. When growth dictates, the smaller tanks are simply upset within the larger ones, causing little stress to the larvae. Both before and after hatching, we provide water

movement using a flow of bubbles up the center of the tanks, a method developed by Dr. Linda Kling at the University of Maine.

The water-processing system for this larval culture system is shown in Figure 2, and relies on gravity flow from the culture tanks to a weir box where standpipes dictate the level of each tank and where an immersion heater is situated. Water then flows into an open-top bag filter fabricated from 8" PVC pipe. The felt filter bag is 25 micron. Water is pumped from the filter through a chiller unit, then to a protein skimmer also fabricated from 8" PVC pipe. Bubbles for the skimmer are generated by a side stream from the pump into a venturi fitting. A ball valve between the pump and the chiller is used to throttle the flow while generating the pressure needed for the venturi.

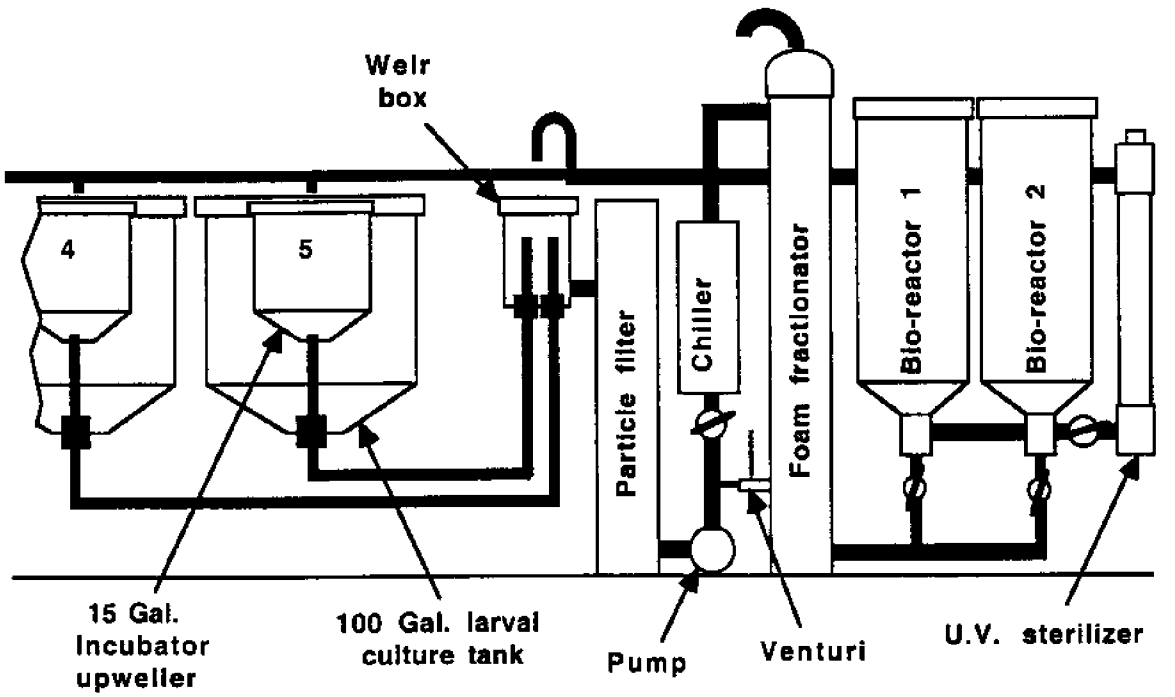

Figure 2. Larval culture recirculating system.

We use a pair of P.E. tanks as bio-reactors. Vigorous aeration keeps high-surface-area P.E. media in constant motion, preventing lazy bacteria. From the bio-reactors, water flows by gravity through a U.V. sterilizer for distribution to the larval tanks. Any unused flow returns to the weir box. This recirculating system has proven very stable and requires servicing of the bag filter only once a day.

Our larger fingerling culture system is very similar in design to the larval system and is diagrammed in Figure 3. We opted for tall tanks fitted with a double drain to isolate solids-laden water taken from the bottom of the tank. We employ two weir boxes, a 1/2-hp circulation pump, larger bio-reactors, and a 40-Watt U.V. sterilizer.

This larger system has worked reasonably well and currently holds haddock from the 1998 winter spawn. Solids removal has provided the most challenge, overwhelming the single filter bag, requiring two or three cleanings per day. We

have also modified the system by putting the foam fractionator and the U.V. on a side stream to allow greater overall flows through the system and greater residence time in the side-stream treatments.

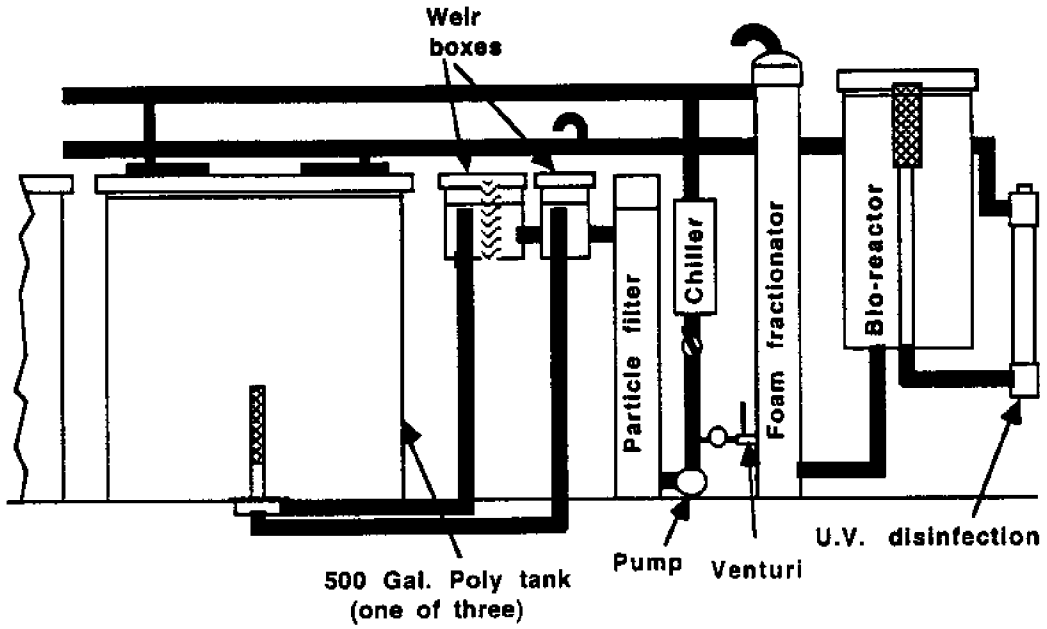

Figure 3. Fingerling grow-out system

To date, these systems have been used for the culture of haddock and tautog. Since operations began in the spring of 1998, culture system water quality has been carefully monitored with variations logically tracking changes in biomass and feeding strategies (see Table 2). We will soon be expanding our facilities to allow for research and demonstrations on grow-out technologies and to develop high-school and professional-development courses in aquaculture.

Parameter	Average (ppm)	Std. Dev.
Ammonia	0.80	1.0
Nitrite	0.06	0.05
Nitrate	0.26	0.22
Dissolved Oxygen	7.6	0.48
pH	7.5	0.14
Alkalinity	114	8.7
CO_2	6.4	1.2
Salinity	26.4	1.2

Table 2. Water chemistry parameters, April 1 - Nov. 1, 1998

Table 3 - System components, part numbers and vendors

Component	Supplier	Stock #
Pro-Drainer submersible intake pump	Aquatic Eco-Sys.	PU7
Mercury float switches (tide)	Aquatic Eco-Sys.	PL1D
Mercury float switches (tank)	Aquatic Eco-Sys.	PL1U
Intake filter vessel	Aquatic Eco-Sys.	FV1
Intake filter bag	Aquatic Eco-Sys.	VB100
Cold S.W. Poly. storage tanks	U.S. Plastics	9319
Warm S.W. Poly. storage tanks	U.S. Plastics	9318
Plastic float valve	Aquatic Eco-Sys.	R401
300 W submersible heaters	Aquatic Eco-Sys.	VT300
SW supply canister filters	Aquatic Eco-Sys.	FXC
SW supply filter cartridges	Aquatic Eco-Sys.	FX5
SW supply U.V. sterilizer	Aquatic Eco-Sys.	UV32
SW supply pump	Aquatic Eco-Sys.	MD5
SW supply canister filter	Aquatic Eco-Sys.	FXC
SW supply 5 micron filter cartridge	Aquatic Eco-Sys.	FX5
SW supply 40 Watt U.V. sterilizer	Aquatic Eco-Sys.	UV32
15 Gal. black Poly. incubator tank	Peabody Eng.	62002-0015B
100 Gal. black Poly. larval tank	Peabody Eng.	62002-0100B
7 Gal. rectangular weir box	U.S. Plastics	6149
Larval system 25 micron bag	Aquatic Eco-Sys.	FB25
Larval system circulation pump	Aquatic Eco-Sys.	PM6
Larval system 1 hp chiller unit	Marine Biotech	TCU-15
Foam Frac. 1/2" venturi fitting	Aquatic Eco-Sys.	4733
30 Gal. Poly. bio-reactor tank	Peabody Eng.	62002-0030C
Larval system PE bio-reactor media	Water Mgmt. Tech.	KMT/Purac
Larval system 25 Watt U.V. sterilizer	Aquacenter	UV25
500 Gal. black Poly. fingerling tank	U.S. Plastics	4306
Fingerling tank double drain	Aquatic Eco-Sys.	D2
11 Gal. midwater weir box	U.S. Plastics	6304
15 Gal. solids water weir box	U.S. Plastics	6308
Fingerling system circulation pump	Aquatic Eco-Sys.	P120
55 Gal. Poly. bio-reactor tanks	U.S. Plastics	3060
Fingerling Sys. 40 Watt U.V. sterilizer	Aquacenter	UV40
Rotifer/artemia production tanks	U.S. Plastics	3060

Table 4 - Component vendor contact information

Aquacenter, Inc. 166 Seven Oaks Road Leland, MS 38756 USA	Tel: 800-748-8921	Fax: 601-378-2862
Aquatic Eco-Systems, Inc. 1767 Benbow Court Apopka, FL 32703-7730 USA	Tel: 800-422-3939	Fax: 407-886-6787
Marine Biotech, Inc. 54 W. Dane Street Beverly, MA 01915 USA	Tel: 978-927-8720	Fax: 987-921-0231
Peabody Engineering 13618-T Alma Ave. Gardena, CA 90249 USA	Tel: 800-348-4408	Fax: 310-324-7247
Unites States Plastic Corp. 1390 Neubrecht Road Lima, OH 45801 USA	Tel: 800-537-9724	Fax: 419-228-5034
Water Management Technologies P.O. Box 66125 Baton Rouge, LA 90249 USA	Tel: 504-627-3930	Fax: 504-627-6918

Part II - Cryopreservation of Haddock Eggs

Cryobiology is the study of the effects of very low temperatures on living organisms (the American Heritage Dictionary, 1991). "A major breakthrough in low temperature biology occurred in 1949 when it was discovered that glycerol provided protection to avian spermatozoa during freezing to - 79° C (Whitengham, 1980). Cryoprotectants are chemicals that can penetrate a membrane, fill up the cell and protect the cellular material so it does not freeze when subjected to sub-zero temperatures. These chemicals have lead to successful cryopreservation of several types of cells and tissues such as embryos for cows, mice, sheep and humans, blood and fish sperm. Successful cryopreservation of fish embryos has been an ongoing research topic.

There are many troublesome aspects in attempting to cryopreserve a fish embryo. First, fish eggs are very large (millimeters) compared to smaller cells that have been preserved (microns). Second, fish eggs are composed of approximately 80% water. This water will develop into ice crystals within the egg upon freezing. The crystals are sharp and can rupture the developing cells inside the egg. And lastly, developing fish embryos have a high yolk content which is has very low permeability.

There are several steps involved with cryopreserving any organism. "The intrinsic biophysical properties of the cells or embryos" (Hagedorn et al. 1997) must be determined. To identify the optimal freezing rate, four factors must be known: "cell volume, membrane permeability (to water and cryoprotectant), chilling sensitivity, and water concentration within the cell. Therefore, cryopreservation procedures for each type of embryo and cell must be tailored individually, bases upon a thorough understanding of cell permeability" (Hagedorn et al. 1997). Answers to these particular steps in the process of cryopreservation will determine what further research needs are and address the actual attempt to cryopreserve marine fish eggs.

To date, there has not been a successful standard cryopreservation technique that proves efficient for a marine finfish egg. At the beginning of the study at MIT Marine Finfish Hatchery, an in-depth literature search of cryopreservation/ cryobiology was conducted as well as attending the World Aquaculture Society conference technical sessions on cryopreservation. While identifying the types and techniques of cryopreservation, several meetings with top researchers in the field of cryobiology were conducted. Ernest Cravelo, a professor specializing in cryobiology at MIT, Mary Hagedorn, a cryobiologist of the Smithsonian Institute, and Mike Russo of Shriners Research Laboratory all participated in the development of this project. A conclusion was made that due to our laboratory capabilities and our inability to gain access to hi-tech laser equipment, a basic toxicity test should be run on the haddock eggs to determine their heartiness. This is baseline information that must to be collected before any sophisticated research on the manipulation of the egg can be conducted. Therefore, preliminary studies to determine the tolerance limits of haddock eggs to 3 commonly used cryoprotectants (DMSO, glycerol, and methanol) were conducted at the MIT Sea Grant Marine Finfish Hatchery in April and May of 1998.

There were two main objectives for this study:

1) To identify the exposure tolerance of haddock eggs in different cyroprotectant solutions
2) To determine at what concentration do cryoprotectants prove to be toxic to the haddock eggs

Materials and Methods

Haddock eggs were supplied by National Marine Fisheries Service Narragansett Laboratory. The egg stage used for this experiment was 24 hours after fertilization. For each experiment, a test tube rack was filled with test tubes each containing 20 eggs and 5 ml of the desired cryoprotectant solution. The cryoprotectants used in this study were DMSO, methanol and glycerol. The eggs and solution were held in a constant temperature of 9=9A C in a water bath.

For the exposure experiment, the eggs were exposed to 1M concentration of the three cryoprotectants for 10, 20, 40 and 60 minute increments. At the expired time, the tubes were removed from the water bath and rinsed with clean sea water until the egg was free of the cryoprotectant. The eggs were then examined under the microscope and survivorship was determined by counting the number of live, healthy egg and the number of dead, opaque eggs.

In the concentration experiment, the three concentrations of cryoprotectants were: 1M, 2M and 3M. The eggs were exposed to the various concentrations for 10 minutes. After 10 minutes, the eggs were rinsed with clean sea water to remove any cryoprotectant and observed under the microscope. Dead and live eggs were counted to determine survivability as the previous experiment.

Within each test, each parameter had 4 replicates. Due to the limited quality and quantity of eggs at the end of the spawning season, each experiment was only run twice. A 2-way ANOVA with replication was conducted on the data gathered. The data was compared to a 0.05 acceptance level.

Results

In the exposure experiment, it was found that there was a significant difference ($p<0.05$) among which cryoprotectant was use on the haddock eggs, but there was not a difference in how long the eggs were exposed to the cryoprotectant.

For the cryoprotectant concentration level test, it was determined that the survivability of the haddock eggs depended on the type of cryoprotectant used and at what concentration ($p<0.05$).

Discussion/Further Research

Basic data on the structure and requirements of a cell or tissue must be determined to proceed with any cryobiology techniques. Cryoprotectants are a necessary component to cryopreservation for they protect the cell structure from being damaged during ice crystal formation in sub-zero temperatures. The preliminary results found through these experiments has helped specify the next step of research

that needs to be done in this area with haddock eggs. The cryoprotectant used as well as its concentration can now be narrowed down and pin-pointed to a more specific number by using the range found during these trials. Although the data gathered is valuable, these experiments should be replicated again to strengthen the results found here.

In future research, the eggs that survived the cryoprotectant solution exposure should be incubated and hatched to determine the hatching success after manipulation. This was not conducted during these trials due to limited space availability to hatch the eggs. Also research on which developments stage of the egg is most tolerant to the expose to a cryoprotectant needs to be determined.

Part III - Fish Tagging

We surveyed cultured species tagging methods and discovered a wide array of products. The most applicable tags we found are manufactured by Stoffel Seals and are available from Unisource, a distributorship in Southborough, Mass. They are used primarily for product tagging of aquacultured fish and other perishables. These tags are metal, can be embossed with a logo and can not be removed for reuse.

We experimented with several samples of the tags to determine if they withstood prolonged immersion in salt water. Experiments on their applicability on live fish await the growth of our 1998 year-class haddock to a marketable size. The applicability of the tags on differentiating farm-raised fish from wild-caught fish seems well established. Information on these products follows.

Acknowledgments

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