

Transporting Adult and Larval Gulf Menhaden and Techniques for Spawning in the Laboratory¹

William F. Hettler

*National Marine Fisheries Service, NOAA
Southeast Fisheries Center, Beaufort Laboratory
Beaufort, North Carolina 28516*

ABSTRACT: Fifty-five adult gulf menhaden (*Brevoortia patronus*) were transported from Gulf Breeze, Florida, to Beaufort, North Carolina, in 30 h without mortality. The life-support features during transport included a 600-L cylindrical, unbaffled fish tank, a continuous-duty pump, a filter using crushed oyster shells and activated carbon, tris-buffered seawater, oxygen, and monitors for temperature, oxygen, and pH. Spawning, induced by temperature, photoperiod, and injections of human chorionic gonadotropin and carp pituitary, occurred following transport and occurred nine times from November 1982 through March 1982. Larvae from two spawns were transported to the Gulf of Mexico for shipboard feeding experiments.

Gulf menhaden (*Brevoortia patronus*) constitute the largest fishery by weight in the United States. In view of their importance, laboratory experiments on environmental factors affecting growth and survival of the young stages are needed, but such studies have been hindered because of the unavailability of eggs and larvae. Although Atlantic menhaden (*B. tyrannus*) have been spawned and reared in the laboratory (Hettler 1981), gulf menhaden have not. Proposed studies with larval gulf menhaden, which are found only in the Gulf of Mexico and its estuaries, made it necessary to transport adult fish to our laboratory at Beaufort, North Carolina, for spawning and larval culture.

Gulf menhaden were first brought by truck from Gulf Breeze, Florida, to Beaufort, North Carolina (1,335 km) on 17–18 June 1981, but survival was poor. Improvement of the transport procedure resulted in less than 1% mortality during a second trip on 15–16 October 1981. The improved transport methods, the techniques used for spawning gulf menhaden, and the procedure for transporting larvae for shipboard experiments are described.

Materials and Methods

Collecting — Several hundred adult fish were collected by cast net from Hoffman Bayou near Gulf Breeze on 22 September 1981, and placed in two 1.5-m diameter cylindrical tanks with running seawater at Gulf Breeze for 3 weeks to recover from capture stress. During this period salinities ranged from 28.5 to 31.0 ‰ and temperature decreased from 27.4° to 22.2°C.

Transporting Adults — The life-support system for transporting adult menhaden consisted of a fish tank, tank

lid, pump, filter, and oxygen supply (Fig. 1). All components in contact with the seawater were non-corrosive. The 1.2-m diameter, 1-m deep cylindrical fiberglass fish transport tank had black walls and a white bottom. An epoxy-coated wooden lid with a 30-cm² hinged access port of clear acrylic was bolted to the rim of the fish tank. No surface water baffles were used.

The system was driven by a 115-V ac, magnetic-coupled centrifugal pump powered by a 12-V dc to 115-V ac inverter connected to the truck battery. During operation, the pump and inverter required 25 A from the truck's electrical system; even during night driving, this did not overload the truck's 60-A alternator. The pump functioned to (1) move seawater through a modified 100-L fiberglass rapid sand pressure filter containing a 30-kg layer of crushed oyster shell and a 10-kg layer of activated carbon and to (2) generate a concentric water flow in the fish transport tank. This concentric flow in the cylindrical tank provided a stabilizing gyroscopic motion and furnished a water current against which the menhaden could swim. Proper operation of the pump and filter was monitored from a pressure gauge mounted on the pump outlet and visible from within the truck cab. The filter material and piping restrictions resulted in a back-pressure of 0.35 kg/cm and a flow rate of 50 L/min.

Oxygen was added by using a compressed oxygen tank, pressure regulator, and a single airstone. A sensor was mounted in the transport tank wall to measure the dissolved oxygen and temperature. To prevent a decline in pH during transport because of respired CO₂, tris-buffer (Trizma—8.3, Sigma Chemical Co., St. Louis, Missouri) was added to the seawater at a concentration of 3 g/L and back-titrated to a pH of 8.15 with concentrated HCl (0.5 mL/L), following the procedure of McFarland and Norris (1958). Unbuffered seawater had a pH of 7.75. The use of tris-buffer increased the buffering capacity of the transport water 30-fold compared to untreated seawater.

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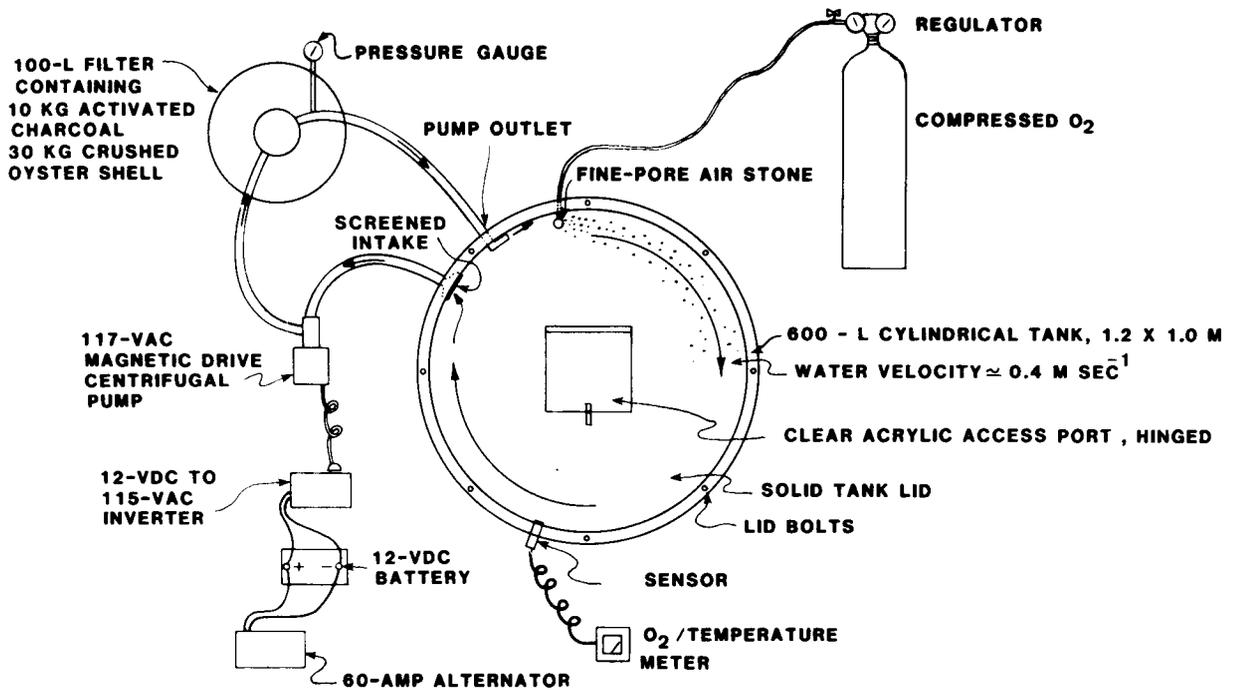


Fig. 1. Diagram of the life support system (top view) used to transport adult gulf menhaden. The system, mounted in a pickup truck, used the vehicle's battery and alternator. The pH was monitored with a portable meter (not shown).

Fifty-five healthy adult gulf menhaden, 17–21 cm (standard length) and 110–185 g, were placed in a transport tank containing 600 L of filtered, oxygenated, and buffered seawater (22°C; salinity 31.2 ‰). Food had been withheld from the fish for 2 days before transport to reduce their metabolic rate and production of feces. Tricaine methanesulfonate (15 mg/L) was used to anesthetize the fish for transfer to the transport tank but no anesthetic was used during transport.

Temperature, oxygen, and pH were monitored during the trip to Beaufort, which took 30 h, including an overnight rest stop. Water temperatures ranged from 17 to 23°C. Dissolved oxygen was maintained between 8 and 14 mg/L. The transport tank was vented to allow CO₂ to escape. On the basis of initial and final pressures of the compressed oxygen tank, it was calculated that less than 75 L of O₂ were required for maintaining this concentration of dissolved oxygen. Final pH of the seawater when the fish were unloaded at Beaufort was 7.80. A 7-mg/L antimicrobial bath (Furan 2, Aquarium Pharmaceuticals, Perkasie, Pennsylvania) was given to the fish for 1 h in the transport tank with the filter turned off at the end of the trip. No fish died during transport.

At the laboratory, menhaden were transferred individually after being anesthetized to a cylindrical vinyl-lined pool, 4.5 m in diameter, containing 20,000 L of seawater. Unfiltered, ambient temperature seawater flowed

into the pool at 40 L/min. Temperature decreased from 21° to 14°C during the 2-week period between arrival at the laboratory and transfer of fish to a temperature-controlled tank. Fish were fed dry salmon starter mash, sifted through a 2-mm sieve and floated on the water surface, about 5 times a day, which they filtered from the water column. One fish died before the fish were moved for spawning.

Spawning — Fifty-four gulf menhaden were moved on 30 October to a cylindrical fiberglass tank (1.8 m in diameter, 2,000 L capacity) and conditioned for spawning. Water temperature was raised and maintained at 20°C; salinity ranged between 26 and 35 ‰, and the photoperiod was set at 8L:16D. Seawater continually flowed through the tank at 10 L/min. A concentric water flow of 10 cm/s provided an orienting current into which the menhaden could swim. The fish were continued on the salmon starter diet.

Nine attempts to spawn gulf menhaden from November 1981 through March 1982 were successful; the number of eggs recovered ranged from 10,000 to 300,000. Voluntary spawning was induced by using methods similar to those described by Hettler (1981) for Atlantic menhaden. About 12 to 15 fish were injected with hormones for each spawning. Because menhaden cannot be sexed easily by external characters, fish sex was determined after the initial spawning of "virgin" fish by attempting to strip remaining gametes manually and noting whether each fish produced

eggs or milt. Once the sex was determined, fish were fin-clipped (males right pectoral fin, females left pectoral fin) for subsequent identification as male or female. For each spawning, about two females were used for each male. During the spawning season, each fish was used at least twice. To induce spawning, both males and females received a first injection of 250 IU of human chorionic gonadotropin 24 h before a second injection of 15 mg of carp pituitary. Both injections were intraperitoneal. The commercial acetone-dried carp pituitary was finely ground in a glass-Teflon homogenizer (No. A 21172, Thomas Co., Philadelphia, Pennsylvania), mixed in a carrier solution of 1% NaCl in distilled water, and injected with a 22-gauge needle. In previous studies, the pituitary had not been finely ground and had to be injected with a 16-gauge needle, which caused a much larger puncture wound (Hettler 1981).

Spawning always occurred 10–14 h after the pituitary injection and therefore scheduling of experiments requiring eggs for larvae production was easily coordinated. Fertilized eggs were removed from the egg-collecting tank (Hettler 1981) and hatched, and the larvae were reared in 100-L fiberglass tanks containing algae-fed rotifers, *Brachionus plicatilis* (Hettler 1981; Hettler and Powell 1981).

Transporting Larvae — Menhaden larvae were used in experiments concerned with age and growth both in our laboratory and aboard a research ship operating in the Gulf of Mexico. To supply larvae for two cruises, groups of 200–300 larvae, 1 to 3 weeks after hatching, were placed in plastic bags containing 8 L of filtered seawater, sufficient algae (*Nannochloris*) to turn the water pale green, and a rotifer concentration of 50/mL. The air in the bags was displaced with pure oxygen, the bags were sealed with rubber bands and placed in insulated tropical fish shipping boxes for the 2-day trip to the research vessel. The lids were periodically removed to furnish light for feeding during transport. Twenty-four bags of larvae were shipped. Survival of each group of larvae was never less than 50% by the time the larvae were used at sea, which was 4 to 7 days after they were first placed in the bags. Additional food was added daily to the bags containing larvae from a 12-L carboy of concentrated rotifers. For the experiments, menhaden larvae were placed in water samples from different depths and locations in the gulf, given time to feed, and then sacrificed for stomach content analysis.

Discussion

Menhaden can be difficult to collect and transport for laboratory studies without significant mortalities. Survival of fish from the June transport attempt was only 13% 2 weeks after arrival at the laboratory. However, 2 weeks after the October trip, only one fish of 55 had died. The success of the transporting process used in October is attributed to four major factors.

First, the fish were not transported immediately after capture as they had been in June. A period of 3 weeks in

captivity before transporting ensured that most of the fish injured during capture had either recovered or died. This initial period of captivity also preconditioned the fish to tolerate confinement in the relatively small transport tank. All individuals appeared to exhibit normal schooling behavior in the transport tank and showed no signs of unusual stress as they did during the first attempt.

Second, a time of year for transporting was selected that coincided with cool average air temperatures of about 20°C, gulf menhaden availability in the estuary, and gonad maturation. During the first transport attempt, water temperature in the transport tank was influenced by air temperatures up to 35°C, and by direct solar radiation, and approached 30°C. Although no fish died in the transport tank during the first attempt, heat stress probably contributed to the eventual poor survival. For future transporting, the fish tank, filter, and water hoses will be insulated to reduce heat exchange. If transporting during summer or winter periods were necessary, temperature control would be required. However, adult gulf menhaden inhabit estuaries until October where they are easily captured before they emigrate to offshore waters to spawn. Adult menhaden are also available in gulf estuaries in late spring and summer, but their gonads are then in a resting stage.

Third, the transport tank shape (circular instead of rectangular) was appropriate for adult menhaden. Pelagic, obligatory schooling menhaden do not survive well in the laboratory or during transport in square or rectangular tanks (personal observation). The cylindrical tank provided the largest volume of water with the smallest area of wall surface without angular corners against which menhaden could impinge and thus suffer damage. In adult menhaden the delicate lateral line system is concentrated entirely in the head and is easily injured by impact. The circular shape of the tanks also permitted the pump discharge to develop a concentric water flow in the tank which oriented the fish into a uniform swimming pattern instead of random movements. The importance of water flow and circular tanks for menhaden survival was also reported by Hope (1982). An added benefit of the circular tank was that the gyroscopic motion of the water maintained by continuous pumping eliminated the need for internal compartments or surface baffles, which could have damaged the menhaden. Further, the gyroscopic stabilization of the water made the truck easier to drive. The transport tank walls were colored black because white walls attract menhaden and cause head injuries (Hettler 1981; Hope 1982).

Fourth, the life-support system provided filtered, oxygenated water of the proper pH. The crushed oyster shell and tris-buffer maintained pH at normal seawater levels. Previous studies have documented the value of buffering seawater in closed systems (McFarland and Norris 1958; Bower et al. 1981). The crushed shells provided mechanical filtration and served as a substrate for bacterial filtration. The activated carbon served to adsorb organics, particularly mucous, which fish secrete when handled. No foaming of the water or foul odor was observed.

Gulf menhaden requirements for gamete maturation, ovulation, and fertilization in the laboratory were not known, but since gulf menhaden are cognates of Atlantic menhaden, they were assumed to have similar thermal and hormonal prerequisites for spawning. Gulf menhaden spawn between mid-October and late March, with a spawning peak in December (Christmas and Waller 1975); spawning at this time of year suggests that a photoperiod of short day length is appropriate. In contrast, Atlantic menhaden are known to spawn year around and photoperiod may be unimportant. Gulf menhaden eggs, as noted in plankton collections, are associated with a mean water temperature of 19°C and a salinity of 34 ‰ (Christmas and Waller 1975). Since spawning in the laboratory was accomplished on each attempt, I assume that the proper temperature, salinity, photoperiod, feeding, and tank size prerequisites were provided for gamete maturation and fertilization. Because menhaden are filter feeders and feed vigorously on dry food before and after spawning, I suspect that cannibalism of their own eggs took place — which may explain some of the variation noted in the number of eggs recovered. However, the egg-collector system (Hettler 1981) provided adequate numbers of eggs for rearing of larvae for laboratory and shipboard experiments.

The system described for transporting and the techniques for spawning menhaden should provide fishery biologists with information for larval production at locations distant from the parental habitat. It should no longer be necessary to depend upon artificial fertilization at the capture site to obtain eggs and larvae (Hettler 1968).

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