

## ARTIFICIAL PROPAGATION OF THE WHITE STURGEON, *ACIPENSER TRANSMONTANUS* RICHARDSON

SERGE I. DOROSHOV, WALLIS H. CLARK, Jr., PAUL B. LUTES, RICHARD L. SWALLOW\*, KENNETH E. BEER, ANN B. McGUIRE and MICHAEL D. COCHRAN\*\*

*Aquaculture Program, Animal Science Department, University of California at Davis, Davis, CA 95616 (U.S.A.)*

\* *Department of Biology, Coker College, Hartsville, SC 29550 (U.S.A.)*

\*\* *California Department of Fish and Game, Central Valley Hatchery, P.O. Box 501, Elk Grove, CA 95624 (U.S.A.)*

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### ABSTRACT

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The first successful reproduction of white sturgeon in captivity was conducted from February to May 1980. Ovulation and spermiation were induced in wild-caught broodstock with injections of pituitary glands from sturgeon and common carp. Eggs were incubated and hatched in MacDonald jars. Larvae and fingerlings were reared in large numbers using a variety of intensive and extensive techniques. The procedures and preliminary results of this work are described.

### INTRODUCTION

North American sturgeon populations have exhibited a progressive decline from their historical levels, apparently a result of overfishing and alterations to the animals' spawning habitats. One of these species, the white sturgeon (*Acipenser transmontanus*), is endemic to the north Pacific Coast of America and inhabits the coastal zone, estuaries, and watersheds of the Sacramento River (California), the Columbia River (Oregon), and the Fraser River (British Columbia). Historically, this fish supported a major sturgeon fishery in North America, yet overharvesting at the turn of the century resulted in a drastic decline in its abundance (Craig and Hacker, 1940; Skinner, 1962; Semakula and Larkin, 1968). The Columbia and Fraser rivers presently support small commercial fisheries, whereas only sport fishing is allowed for this animal in the Sacramento River (Kolkhorst et al., 1980). It appears that the only means of ensuring further commercial exploitation of sturgeon is through hatchery production, stock replenishment, and domestication of sturgeon species (Kozin, 1964).

Attempts to propagate sturgeon artificially in North America were made as early as 1875 (Leach, 1920). These first trials were unsuccessful, and further efforts were not made for several decades. Recently, however, interest in culturing sturgeon and paddlefish has been renewed. The artificial propagation of paddlefish is being established in the midwestern United States (Purkett, 1963; Russel, 1973), and recently the first successful attempts to reproduce lake, Atlantic, and shortnose sturgeon have been made (Binkowski and Czeskleba, 1980; Smith et al., 1980; Buckley and Kynard, 1981). A research program has been initiated by the University of California, Davis, on the reproductive biology and physiology of the white sturgeon (*A. transmontanus*) with the goal of developing the technology necessary for stock replenishment operations and commercial growout ventures (McGuire, 1980). The present paper describes the results of our first year's work.

## MATERIALS, METHODS, AND RESULTS

### *Animal collection, maintenance, and preparation*

Adult white sturgeon were collected in the San Francisco Bay and the Sacramento River from December 1979 through May 1980. In the San Francisco Bay, animals were located feeding on herring roe and were caught using sportfishing gear with treble hooks. Animals in the river system were caught using conventional hook and line as they migrated toward their spawning grounds.

After capture, each fish was tagged and brought to shore in a 500-l rectangular tank. Because sturgeon are cartilaginous, all fish handling and moving was done with a canvas stretcher to distribute weight and avoid damaging the internal organs. The fish were transported by land to the Davis facilities in a 1150-l rectangular tank supplied with aerated water. This tank could transport from 4 to 7 fish for the 2-3-h drive, with no mortality encountered. Upon arrival at Davis, the brood fish were held for several days in a 10-m<sup>3</sup> rectangular tank with a water flow rate of 50 l/min and a water temperature of 12-13°C. Fish collected from salt water (salinity range, 20-28 ppt) were transported in a diluted saltwater solution (18 ppt); once at Davis, the fish were further acclimated to fresh water over a 24-h period.

Sex and state of gonadal development of each animal were determined by the following procedure. Each fish was removed from the holding tank and placed ventral side up on a canvas stretcher fitted with a hood to accommodate its head (Fig. 1). This stretcher was suspended between two wooden stands ("sawhorses"). To provide continuous water circulation, a tygon tube 1.5 cm in diameter was inserted in the mouth of the fish and fixed with a foam rubber plug, and water was pumped through the tube from a container beneath the stretcher. Using a #10 surgical blade, a midventral incision 2-3 cm long was made just anterior to the pelvic fins. This allowed for easy identification of the sex, determination of the stage of gonadal maturation, and removal

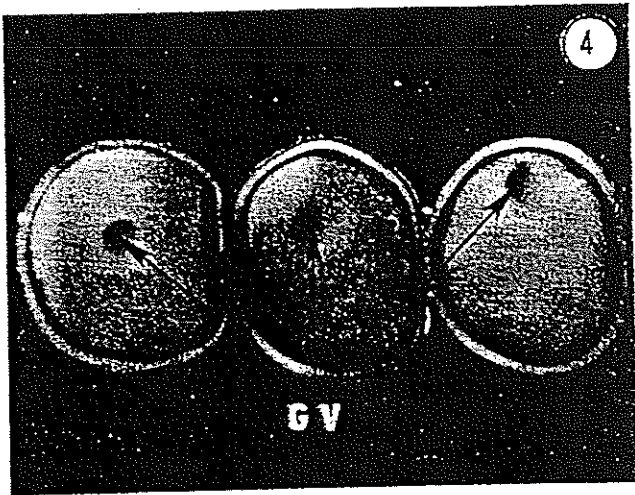
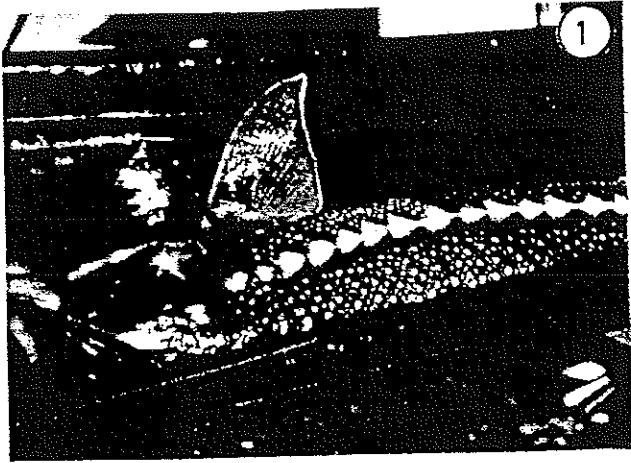


Fig. 1. Canvas stretcher for handling broodstock.

Fig. 2. For pituitary extraction, the frontal skull bone is removed by drilling.

Fig. 3. Location of the exposed pituitary gland (PG) in the white sturgeon, after removal of the brain.

Fig. 4. Three substages of oocyte maturation, showing the location of the germinal vesicle (GV).

Fig. 5. Removal and collection of sturgeon ova.

of oocyte samples from the ovary. The incision was sutured with a continuous stitch using a cutting-edged, half-circle surgical needle and 0.3–0.5-mm polypropylene monofilament (Ethicon ISLX 0.35 metric). The entire procedure lasted approximately 10 min and appeared to cause little stress to the fish.

We collected 9 mature females (3 from the bay; 6 from the river) and 10 males (all from the river) for spawning induction; their individual body weights ranged from 20 to 60 kg. The ages of the fish ranged from 16 to 24 years, as determined by the number of annular rings observed on a 0.4–0.6-mm cross-section of tissue that was removed from the first pectoral fin ray, dried, and microscopically examined. Other fish considered unsuitable for induced spawning were sacrificed and used for pituitary gland extractions. For pituitary extraction, a 2-inch (5-cm) hole was drilled into the dorsal surface of the skull (Fig. 2); the dermal bone and underlying cartilage were removed; and the pituitary gland was carefully exposed immediately beneath the brain (Fig. 3). The gland was removed and either frozen in liquid nitrogen or dehydrated in acetone.

Before proceeding to spawning induction, we used the following procedure, routinely applied to amphibian eggs (Scheutz, 1967), to determine the oocyte maturation stage. Freshly sampled oocytes were measured and placed in boiling water for several minutes to harden the yolk; they were then sectioned with a razor blade and viewed with a dissecting microscope. With this technique, we classified three progressive substages of oocyte maturation within the stage IV oocyte as described by Deflaf and Ginzburg (1954). We assume these substages (Fig. 4) can be correlated with the degree of "readiness" of an individual fish for hormonal induction of ovulation. The substages are defined as:

- IVa: oocytes are round, 3.0–3.5 mm in diameter; germinal vesicle (GV) is centrally positioned;
- IVb: oocytes are round or irregularly shaped, 3.5–3.8 mm in diameter; the GV has moved toward the animal pole;
- IVc: oocytes are ovoid, 3.8–4.0 mm in diameter; the GV lies in the cortical ooplasm of the animal pole.

#### *Induction of ovulation and spermiation*

For ovulation induction, we used injections of pituitaries obtained from white sturgeon as well as pituitaries from the common carp, *Cyprinus carpio* (Stoller Fish Inc., IA). The dosage used for induction of ovulation ranged from 3 to 8 mg of dried gland per kg of body weight (Table I). Females with oocytes in stage IVa received three consecutive injections at 12–24-h intervals at 20, 30, and 50% of the total dosage, respectively. Fish with oocytes in stage IVb received two injections at a 12–24-h interval at 30 and 70% of the total dosage; those in stage IVc received one injection of the total dosage. All injections were intramuscular and consisted of acetone-dried glands homogenized in 1 ml of distilled water.

TABLE I

Induction of ovulation in white sturgeon females

Fish #	1	2	3	4	5	6	7	8	9
Place of capture <sup>1</sup>	SFB	SFB	SFB	SR	SR	SR	SR	SR	SR
Bodyweight (kg)	36.	58.5	22.9	52.2	23.2	22.5	27.9	54.	28.3
Egg maturation stage	IVb	IVb	IVa	IVb	IVc	IVc	IVc	IVb	IVc
Pituitaries injected <sup>2</sup>	S	S	S	S	S	S	S + C	C	C
Dosage (mg/kg)	5.2	3.8	4.2	3.8	8.4	4.9	5.3	4.6	4.9
Response <sup>3</sup>	+	-	-	++	-	++	+	++	+
Fecundity (thousand eggs/kg)	10.4	8.8	7.8	8.8	?	8.1	10.2	10.9	7.6

<sup>1</sup> SFB, San Francisco Bay; SR, Sacramento River.<sup>2</sup> S, sturgeon, C, carp.<sup>3</sup> ++, complete ovulation; +, incomplete ovulation; -, no ovulation.

After injection, females were held individually in 1300-l circular tanks supplied with flowing water (15–10 l/min) at a temperature of 12–15°C. Beginning 15 h after the last injection, the bottom of the holding tank was checked at 5-h intervals for the presence of ova. Once ova were noted, the female was removed from the tank and placed on a stretcher; ovulation was verified, if necessary, by catheterization of the oviducts with a plastic or glass tube, 5-mm inner diameter. Females exhibiting no response within 48 h of the last injection were sacrificed, and their ovaries were removed and examined.

After ovulation, the female was suspended and bled through the tail. A midventral incision approximately 25 cm long was made, and the flowing ova were collected in plastic bowls (Fig. 5). If ovulation was incomplete and a substantial portion of the ripe oocytes remained within the gonads, the ovaries were removed. The eggs were then counted in 3-g subsamples to determine and record the relative amounts of ovulated and nonovulated eggs and the total amount of ripe eggs in the female.

Six of the 9 females injected with either sturgeon or carp pituitaries responded with complete or partial ovulation and produced fertile ova 3.8–4.0 mm in diameter. Three females did not ovulate; all or most of their oocytes remained in the ovaries, were enlarged, and demonstrated a characteristic white splotchy coloration. The strongest response to hormonal induction was observed in females with oocytes in stages IVb and IVc injected at a dosage rate of 4–5 mg/kg body weight. Both sturgeon and carp pituitaries were effective in inducing ovulation. Individual fecundity ranged from 180 000 to 590 000 ripe eggs per fish, with relative fecundity increasing correspondingly with the size of the fish from 7 600 to 10 900 eggs/kg body weight (Table I).

To induce spermiation, males were injected, if necessary, with one dose of either sturgeon or carp pituitaries (0.5–1.0 mg/kg body weight); several males

were induced repeatedly over a 2-week period. As in ovulation, sturgeon and carp pituitaries both were effective in inducing spermiation. Sperm were collected by manual stripping, and the seminal plasma was diluted 1 : 200 with water just prior to fertilization.

### *Egg fertilization and incubation*

Eggs were fertilized in vitro following the procedure described by Ginzburg et al., (1963). The diluted sperm were added to bowls containing eggs, and after 5 min, the sperm-egg mixture was washed repeatedly with water. Immediately after being rinsed, the fertilized eggs were treated with a suspension of river silt for 15–30 min to inhibit adhesiveness of the egg jelly coat and to prevent clumping and suffocation of the eggs during incubation. Nonadhesive eggs were placed into MacDonald incubators at a density of 400–800 g of eggs (approximately 30 000–60 000 eggs) per 18-l jar. The eggs were incubated at a water flow rate of 4–7 l/min and a water temperature of 14–16°C.

The eggs of *Acipenser transmontanus* clearly demonstrated holoblastic cleavage (Beer and Doroshov, in preparation) similar to that of other acipenserid and anuran amphibian eggs. Typically, the eggs cleaved synchronously into equal-sized small (micromere) and large (macromere) blastomeres located at the animal and vegetal poles of the eggs. Nearly every batch of eggs, however, contained a few abnormal eggs with asynchronous cleavage in which development was arrested at the early stages, before or during gastrulation.

Fertilization success, which we defined as reaching the 8th cleavage stage, ranged from 20 to 95% in different females. Hatching began in all batches on the 7th day of embryonic development and continued for 20–35 h (Fig. 6). Little or no mortality occurred during incubation; thus, hatching success closely paralleled fertilization rate. As they emerged, larvae were collected in nylon mesh nets placed under the water outflow from the incubation jars. These larvae appeared similar to those of other sturgeon species; they had underdeveloped major organs (eyes, mouth, fins, digestive system, etc.) and a large yolk sac, and they ranged in mean body length from 10 to 10.8 mm (Fig. 7).

### *Larval and early fingerling rearing*

The newly hatched larvae were placed into 600-l circular tank at a density of 10 000–15 000 per tank, a water flow rate of 15 l/min, and a temperature of 16–18°C. Live food (*Moina* sp., collected in ponds) was offered to these fish from the fifth day posthatch and for the subsequent 2 weeks. External feeding was observed to begin at days 8–11 posthatch, at which point the mean length of the larvae had reached 17–19 mm, the yolk was depleted, and the melanine plug was discharged from the spiral valve. For the remainder of the rearing period, food consisted of live *Tubifex* and adult brine shrimp, *Artemia salina*, at a feeding rate of approximately 30% of the fingerlings' bio-

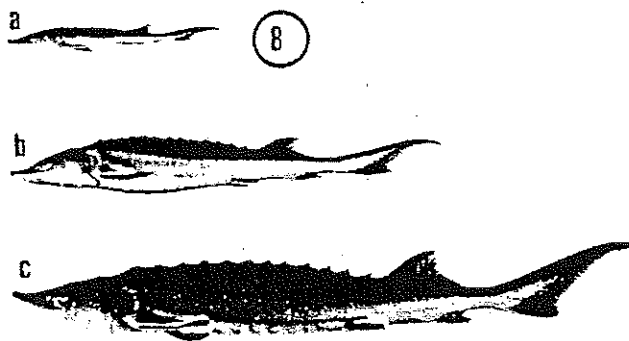
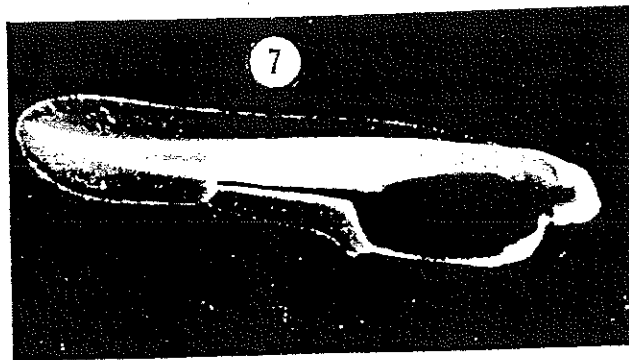


Fig. 6. Sturgeon larvae hatching in MacDonald incubators.

Fig. 7. A newly emerged white sturgeon larva.

Fig. 8. Sturgeon fingerlings approximately 5 months posthatch, under different feeding regimes. (a) 3 g body weight, raised on a limited supply of live food. (b) 28 g body weight, raised on ad libitum feeding of live food and ground fish. (c) 70 g body weight, raised on artificial diets.

mass per day. Survival rates at 40 and 60 days were 75 and 60%, respectively, and corresponding mean weights were 1 and 3 g.

We introduced a small sample (2 000) of 25-day-old larvae into a 0.04-ha earthen pond fertilized with 5 kg N-P-K (16-16-16) fertilizer and inoculated with *Moina* sp. 4 days after stocking. Two days later, a dense bloom of *Moina* sp. was apparent; this bloom lasted several days and was followed by a small concentration of *Daphnia*. Pond rearing continued for 40 days, during which the water temperature fluctuated from 14 to 23°C; the result was a 70% survival rate and a mean fingerling weight of 1.9 g.

#### *Growout of juveniles*

During the first growing season, our main goal was to obtain preliminary information on the response of sturgeon fingerlings to various rearing and feeding schemes. We monitored the growth rate of one group of animals fed a diet of live and frozen natural feeds and of a second group of animals fed an artificial diet with a high protein content (Oregon Moist Pellets, OMP). The first group

of fish consisted of 70-day-old fingerlings with a mean individual weight of 5.3 g. These fish were reared at stocking rates of 50–100 fish per 250-l circular tank, a water flow rate of 4 l/min, and a water temperature that fluctuated between 19 and 26°C. For the first month, these fish were fed live and frozen brine shrimp at 20–30% body weight per day; in the second month, they received a diet of ground, frozen herring at 15% body weight per day. At the end of the 76-day rearing period, these fish were 5 months old; their mean body weight (in different tanks) ranged from 22 to 35 g (average 28.5 g), their survival from 69 to 100% (average 80.5%), and their mean daily gain in body weight from 3.7 to 8.2% (average 5.8%). The gross feed conversion efficiency in this group varied considerably during the rearing period and among different progenies; in general, it ranged from 10 to 30%.

We attempted to wean the second group of fingerlings to pelleted diets developed for salmon and trout. Six thousand fingerlings, aged 30–40 days post-hatch and with a mean body weight of 0.8 g, were transferred to standard trout-rearing troughs. They were fed by Allen automatic feeders and held for 80 days at a water temperature of 18–23°C. Their initial food was an OMP starter diet of 0.2–0.6-mm pellet diameter. Approximately 2 weeks later, pellet size was increased to 0.8 mm and after another 2 weeks, to 1.5 mm diameter. These diets were presented at a rate of 8 feedings per hour, 24 h a day. A limited amount of ground grass shrimp (*Palaemon* sp., 200–400 g per trough) also was fed daily or every other day to the fingerlings to provide a transition between feeds and to maintain the fish that refused to eat the artificial diet. The animals that made the transition to OMP were noticeably larger and had higher body-to-head ratios; they were selected continuously and transferred to 600-l circular tanks indoors, where they were fed solely the artificial feed with pellet diameters of both 1.5 and 3.1 mm. From these tanks, they were moved to outdoor raceways and fed 3.1-mm diameter pellets. During the 80-day rearing period, the total of fingerlings weaned and transferred to the artificial diet was approximately 20% of the initial population. These animals suffered no mortality and reached a mean weight of 70.1 g (Fig. 8). Those fingerlings that remained in the troughs and did not accept OMP grew slowly (their final body weight reached only 2–3 g at the end of the 80 days), and their mortality rates were 15–20% per month. At 15 months posthatch, the fish weaned to artificial diets are currently being held in outdoor raceways and tanks as potential captive broodstock and have reached a mean weight of 1150 g.

## DISCUSSION

The artificial reproduction of acipenserids presents a variety of problems, most of which are related to their unusual reproductive physiology. One such problem is the lack of consistent success in spawning females of apparently similar ovarian states, i.e., late stage IV. As described earlier, the method of sturgeon ovarian staging is based on morphological observations of individual oocyte variables including diameter, shape, and germinal vesicle location.

Spawning of animals classified using these variables is only partly successful. To increase the success rate, the criteria presently used need to be refined or new criteria sought.

A physiological approach may prove effective. The process of final ovarian maturation and ovulation is complex and highly specific, especially at the endocrinological level. There are several likely candidates that might be monitored and examined for correlation with ovarian maturation. Strong possibilities include levels of systemic and ovarian hormones such as gonadotropin and sex steroids. Another approach would be to determine the degree of correlation between free amino acid levels in the blood and ovarian maturation. This approach is based on the assumption that the degree and kind of ovarian protein synthesis, for which the amino acids act as precursors, are directly related to the ovarian maturation state.

In the well-established Russian hatcheries, hormonal spawning induction is based exclusively on hypophyztion of broodstock with sturgeon pituitary; the dosage used is approximately 2.5 mg of acetone-dried gland per kg of body weight (Milstein, 1972). Sturgeon pituitaries (1.6–1.8 mg/kg) were also used recently to induce ovulation in the Atlantic sturgeon (Smith et al., 1980). This exclusive use of sturgeon pituitary is based on early findings that sturgeon respond only to homoplastic injections (Gerbilsky, 1972) and on the widespread availability of sturgeon pituitary in the U.S.S.R. Although purified gonadotropin from sturgeon is significantly different from that of common carp (Burzawa-Gerard et al., 1975), the carp gonadotropin still compares favorably with sturgeon gonadotropin in inducing *in vitro* maturation of amphibian and sturgeon oocytes (Apekin, 1975; Burzawa-Gerard et al., 1976). Our results demonstrate that normal ovulation and spermiation are easily induced in sturgeon using carp pituitary glands at higher dosages (1.5 to 2 ×) than those required for sturgeon pituitary. This successful use of carp pituitary is crucial for sturgeon hatchery development outside the U.S.S.R. in countries where sturgeon pituitaries are difficult to obtain. Carp pituitary, although proven effective, is expensive to use. This situation has precipitated research in our laboratory to identify the potency of various gonadotropins, corticosteroids, and progesterone derivatives and the action of synthetic LH-RH neurohormone on sturgeon egg maturation and ovulation.

Another technique requiring improvement is the removal of ovulated eggs from the females. At ovulation, the ova are released into the coelomic cavity. Sturgeon oviducts (Mullerian ducts) are tubes, 50–70 cm long, with a funnel opening at the middle portion of the coelom and a valve in the mid-portion of the duct. During normal oviposition, ova are discharged as the presumed result of a combination of coelomic pressure and muscular contraction. Present hatchery technology requires the removal of ova via a long abdominal incision (15–20 cm), which results in death of the female. This sacrifice precludes the maintenance of genetically identified broodstock and reduces an already small natural reproductive population. As a result, effective cesarean or cannulation techniques should be sought.

Other problems encountered occur during in vitro fertilization of the eggs. Unlike the eggs of teleosts, sturgeon eggs are highly susceptible to polyspermic fertilization (Ginzburg, 1968), which results in abnormal egg cleavage and mortality in early developmental stages. To reduce the chances for polyspermy, the collected sperm are diluted with water immediately before fertilization. At present we are using a dilution of 1 : 200 (seminal plasma to water), as used in Russian hatcheries. This technique, however, does not account for variation in milt quality. The most desirable number of sperm per egg for optimal in vitro fertilization is unknown, and data on such levels are being gathered.

Once fertilized, the zygotes become strongly adhesive for substrate attachment in natural spawning grounds. In intensive incubation techniques, this adhesiveness leads to egg clumping, which results in embryo suffocation. To circumvent this adhesiveness, fertilized eggs are hand washed with river silt, a labor-intensive procedure routinely used in Russian hatcheries. Attempts to mechanize this procedure are being made.

Additional deficiencies in sturgeon culture are the techniques used for fry and fingerling growout. In the intensive rearing techniques used in Russian hatcheries, fry are raised in tanks and fed live *Enchytreus* and *Daphnia*, both cultured in the hatchery. Growth of such fish is poor after 2–3 weeks of feeding, resulting in the production of undersized fingerlings with various nutritional abnormalities. Several Russian attempts to use artificial diets have apparently failed. Alternatively, pond rearing results in higher growth rates but is costly and inefficient in its use of land and labor.

In our experiments, fish weaned to high-protein, artificial diets (Oregon Moist Pellets) appeared to grow far better than fish fed with live or frozen natural feeds. The growth rate obtained was similar to the growth rate of the hybrid *Huso huso* × *Acipenser ruthenus* grown experimentally in Japan (Suzuki and Nishi, 1977). Weaning onto artificial diets, however, results in considerable mortality apparently related to nonrecognition of the food particles by the majority of fish initially fed live food. Similar difficulties were experienced recently in work with the Atlantic sturgeon (Smith et al., 1980). Although further research is urgently needed on the nutrition, digestive physiology, and feeding behavior of sturgeon, higher survival during weaning appears possible using improved feeding techniques and presenting artificial diets at the early larval stages.

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