

## **A Review of Sterile Fish Production Using Hybrid Crosses or Ploidy Manipulation**

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Sterile fish provide fisheries managers with a tool for controlling population numbers and reproduction of stocked fish. Sterile fish also are useful for stocking sites where hybridization between native and non-native fish is an issue for the perpetuation of native species and locally adapted stocks (Cotter et al. 2000; Budy et al. 2012). Sterile fish have also been used where there are concerns about fish emigrating from where they were stocked (Warrillow et al. 1997). For commercial aquaculture, use of sterile fish can prevent precocious maturation prior to achieving market weight (Brämick et al. 1995).

This review will focus on methods used to produce sterile fish. This includes the production of hybrid fish and triploid production, which involves shocking the egg to keep a set of chromosomes normally excreted from the cell via a polar body shortly after fertilization (Tave 1990). Effects of triploidy on fish behavior and physiology have already been reviewed in an excellent paper by Benfey (1999) and by Maxime (2008). The performance of triploid fish relative to diploids is also a separate issue which will not be reviewed here. For some recent papers on that topic see Tuescher et al. (2003), Withler et al. (1995), Oppendal et al. (2003), Weiss and Zaniboni-Filho (2010) and High and Meyer (2009). Production of sterile mollusks will also not be discussed. As background, the reader should be familiar with the various levels of ploidy, i.e., the number of chromosome sets within a cell. Haploids have 1 set (1N), diploids (2N) have 2 (normal cells), triploids have 3 (3N), and tetraploids have 4 sets (4N). Polyploidy means having a variety of different ploidy levels, e.g., within a population of fish.

### **Sterile Hybrids**

Interspecific hybrids are formed whenever two fish from differing species reproduce. Thousands of hybrid fish species have been reported in the literature, but fewer than 150 of these documented hybrids occur naturally (Argue and Dunham 1999). The low occurrence of natural hybrids can be attributed to differences in spawning behavior, territory or season among species. Also, differences in chromosome number often ensure zero survival among naturally produced hybrids (Argue and Dunham 1999).

The majority of hybrids documented in the literature were produced in a laboratory or hatchery setting. Many of these hybrids were originally produced for research purposes (i.e., to simply see if it is possible to produce a particular cross). Other hybrids were produced with specific goals, such as improved growth (Argue and Dunham 1999), sterility (Chevassus 1983) or disease resistance (Parsons et al. 1986) in mind. Often hybrids have lower survival than non-hybridized fish of the same species (Chevassus 1983). Periods of high hybrid mortality typically occur during three critical time periods: 1) prior to hatch, 2) between hatch and swim-up, and 3) between swim-up and sexual maturation. In salmonids, pre-hatch mortality of hybrids is high but post-hatch survival is comparable to non-hybridized individuals

from the same species (Chevassus 1983). From an aquaculture perspective, high pre-hatch mortality can be accepted because dead eggs are relatively easy to remove and can be removed at a low cost (Chevassus 1983). Hybrids that incur high mortality after swim-up are not desired by aquaculturists because these fish are accepting feed and it is expensive to feed fish when a high percentage are destined to die. Most hybrid salmonid fish are triploid. The triploid process ensures sterility and can significantly improve survival (Chevassus et al. 1983; Arai 1984; Arai 1986; Scheerer et al. 1987; Yamano et al. 1988). For example, Galbreath and Thorgaard (1994) report approximately 7% survival (to age-1) among brown trout *Salmo trutta* males x Atlantic salmon *S. salar* females that were diploid. Triploid fish from the same cross had approximately 35% survival to age-1. However, within Acipenseridae, triploid hybrids of *Acipenser baeri* x (*Huso huso* x *Acipenser ruthensis*) did not survive as well as their diploid counterparts (Fopp-Bayat et al. 2007).

Of the thousands of hybrids documented in the literature, few (~150) have been shown to be fertile (Argue and Dunham 1999). Many have reduced or no fertility. Many hybrids develop gonads that appear normal but egg development is aborted shortly after fertilization. Other hybrids produce abnormal gametes (e.g., sperm without flagella) that are not capable of fertilization. Haploid, diploid, triploid, and tetraploid individuals can result from the production of hybrids (Chevassus 1983). In addition, androgenetic or gynogenetic fish can be produced from such interspecific crosses. This diversity in life forms can be attributed to genetic differences among the species that participate in these crosses. The ploidy of the fish produced is determined in the insemination process. For example, the crossing of grass carp *Ctenopharyngodon idella* with bighead carp *Hypophthalmichthys nobilis* can, within a single lot, lead to the production of diploid, triploid, and tetraploids individuals (Allen and Stanley 1983). Gynogenetic hybrids are often produced when the Amazon molly *Poecilia formosa* hybridizes with other fish from the same genus (Schultz and Kallman 1968). Androgenic, diploid goldfish *Carassius auratus* have been produced using irradiated common carp *Cyprinus carpio* eggs (Bercsényi et al. 1998). From a management perspective, the production of hybrids with an altered ploidy status can allow for easier production of triploid fish or fish that are all the same sex. The majority of hybrid sport fish are diploid, however. As a result, examples of haploid, triploid, or tetraploid hybrids and their production will not be reviewed. When discussing hybrids, the literature typically presents the female species before the male species. This convention is followed below. The direction of the cross is important because differences in survival and fertility are sometimes observed (Argue and Dunham 1999).

Often, the literature documents the existence of a particular cross between species but the fertility of that cross is not known. This lack of information on hybrid fertility is often a product of logistical constraints because it can take several years for a fish to mature. The best way to measure fertility is to try to spawn hybrid fish. Due to the time required for maturation, some studies assume fertility based on gonad development. Hybrid fish often demonstrate little gonad development. This lack of development is not necessarily indicative of sterility. Fish with diminished gonad development may still be fertile. Fertility of hybrid fish is also frequently assumed based on ploidy status. Diploid fish are assumed to be fertile and triploid fish are assumed as sterile. In reality, diploid hybrids may be sterile

due to reproductive barriers such as reduced gonad development or reduced sperm fertility. Tetraploid hybrids can often spawn among each other and are thus fertile. If these tetraploids spawn with either parent species, sterile, triploid fish are often produced.

### *Salmonidae*

The salmonids are a very diverse group of fish and as a result, many possible crosses exist among species. Currently the Utah Division of Wildlife produces two such hybrids: tiger trout (brown trout female x brook trout *Salvelinus fontinalis* male) and splake (lake trout *S. namaycush* x brook trout). Tiger trout grow more slowly than rainbow trout *Oncorhynchus mykiss* (Wagner and Arndt 2001), but they are a popular sport fish in Utah. Diploid tiger trout are naturally sterile whereas diploid splake are not sterile. Regardless, in Utah, both <sup>tiger trout</sup> hybrids are triploid to improve survival (Scheerer et al. 1987).

Table 1 shows basic data from a number of salmonid crosses. The table does not include all salmonid species (e.g., Japanese char *Salvelinus leucomaenis*; Arai 1984, Arai 1986, Yamano et al. 1988). Instead, the table lists a number of common North American species. The table includes information on the sterility of the diploid cross, whether the cross has ever been triploid, and the survival (to swim-up) of each cross. Few of these crosses are naturally produced and much of the data was generated using small laboratory lots. Many of these hybrids have been successfully triploid. Triploid induction success in these hybrids is often variable (50-100%). In most cases, the triploid induction process in these hybrids has not been optimized and instead, a general (i.e., works in many species) triploid process was applied. As a result, triploid induction optimization research is needed for many of the hybrids reported in Table 1. Reports by Suzuki and Fukuda (1971), Blanc and Chevassus (1979), Chevassus et al. (1983), Seeb et al. (1988), Dorson et al. (1991), Gray et al. (1993), and Joyce et al. (1994) were particularly useful in the development of the table.

The data presented in Table 1 provides insight into the production of salmonid hybrids. Less than 50% of the crosses produced were sterile. Generally, sterility (with all of the crosses presented in Table 1) was measured through gonad development or the production sperm that demonstrated motility upon activation. In all instances, however, the crosses had relatively small gonads. Also, the production of motile sperm is not necessarily indicative of fertility. Thus, it is likely that many of these crosses are truly sterile. Few studies attempted to produce F<sub>2</sub> generation fish and such tests, however, would provide an accurate determination of sterility. Also, the table shows that crosses among three major salmonid genera (*Oncorhynchus*, *Salmo*, and *Salvelinus*) are possible. Crosses involving rainbow trout have been widely tested. Interestingly, despite the presence of widespread hybridization between cutthroat trout *O. clarkii* and rainbow trout in the wild, few crosses with cutthroat trout have been tested in the laboratory. The table is not comprehensive and other crosses have been documented. For example, brook trout *Salvelinus fontinalis* naturally hybridizes with bull trout *Salvelinus confluentus* (Leary et al. 1983). Bull trout were not included in the table because few hybridization tests have occurred using this species.

**Table 1:** Summary of crosses among several salmonid fish species. Included is information regarding the sterility of diploid (2N) individuals of each cross, whether triploid (3N) individuals of each cross have been produced, and the survival (S) of individuals of each cross to the swim-up stage. Terms in the table have been coded: S= sterile, NS = not sterile, Y = yes, N = no, H = high (>25%), M = medium (10-25%), L = low (1-10%), VL = very low (<1%) and 0 = no survival. Question marks indicate where information is lacking. Crosses where no information is presented have not been documented in the literature.

Female Species	Male Species										
	Rainbow	Cutthroat	Brown	Atlantic	Brook	Lake	Sockeye	Chinook	Chum	Pink	Coho
Rainbow Trout	2N = NS 3N = Y S = H	2N = NS 3N = Y S = H	2N = NS 3N = Y S = M	2N = ? 3N = Y S = VL	2N = NS 3N = Y S = M	2N = NS 3N = Y S = M	2N = ? 3N = ? S = L	2N = ? 3N = ? S = 0	2N = ? 3N = Y S = L	2N = ? 3N = Y S = L	2N = ? 3N = Y S = H
Cutthroat Trout	2N = NS 3N = Y S = H	2N = NS 3N = Y S = H	2N = NS 3N = Y S = H	2N = NS 3N = Y S = VL	2N = NS 3N = Y S = M	2N = NS 3N = Y S = M	2N = ? 3N = ? S = L	2N = ? 3N = ? S = 0	2N = ? 3N = Y S = L	2N = ? 3N = Y S = L	2N = ? 3N = Y S = H
Brown Trout	2N = S 3N = Y S = L	2N = NS 3N = Y S = H	2N = NS 3N = Y S = H	2N = NS 3N = N S = L	2N = S 3N = Y S = H	2N = S 3N = Y S = L	2N = S 3N = Y S = L	2N = ? 3N = ? S = 0	2N = ? 3N = N S = L	2N = ? 3N = N S = L	2N = ? 3N = N S = L
Atlantic Salmon	2N = ? 3N = N S = 0	2N = NS 3N = Y S = M	2N = NS 3N = Y S = M	2N = NS 3N = Y S = H	2N = NS 3N = Y S = L	2N = NS 3N = Y S = L	2N = NS 3N = Y S = L	2N = ? 3N = N S = 0			
Brook Trout	2N = ? 3N = N S = 0	2N = NS 3N = N S = 0	2N = S 3N = N S = M	2N = NS 3N = N S = 0	2N = NS 3N = Y S = H	2N = ? 3N = N S = 0	2N = ? 3N = N S = 0	2N = ? 3N = N S = L			
Lake Trout	2N = NV 3N = Y S = M	2N = S 3N = Y S = L	2N = S 3N = Y S = L	2N = NS 3N = Y S = 0	2N = NS 3N = Y S = H						
Sockeye/Kokanee Salmon							2N = NS 3N = Y S = H	2N = NS 3N = ? S = H			
Chinook Salmon	2N = ? 3N = ? S = 0	2N = ? 3N = ? S = 0	2N = ? 3N = ? S = 0	2N = ? 3N = N S = 0	2N = ? 3N = N S = L	2N = ? 3N = N S = L	2N = NS 3N = Y S = H	2N = NS 3N = Y S = H	2N = ? 3N = N S = 0	2N = ? 3N = Y S = H	2N = NS 3N = Y S = M
Chum Salmon	2N = ? 3N = Y S = 0	2N = ? 3N = N S = 0	2N = ? 3N = N S = 0	2N = ? 3N = N S = 0	2N = ? 3N = N S = L	2N = ? 3N = N S = L	2N = NS 3N = ? S = M	2N = ? 3N = Y S = H	2N = NS 3N = Y S = H	2N = NS 3N = ? S = M	2N = ? 3N = Y S = VL
Pink Salmon							2N = NS 3N = ? S = H	2N = NS 3N = Y S = H	2N = NS 3N = ? S = H	2N = NS 3N = Y S = H	2N = NS 3N = Y S = H
Coho Salmon	2N = ? 3N = N S = L	2N = ? 3N = N S = 0	2N = ? 3N = N S = 0	2N = ? 3N = N S = 0	2N = ? 3N = N S = 0	2N = ? 3N = N S = 0	2N = NS 3N = ? S = M	2N = NS 3N = Y S = H	2N = NS 3N = Y S = M	2N = NS 3N = N S = L	2N = NS 3N = Y S = H

## Centrarchidae

Hybridization is widespread among the centrarchids, particularly among the sunfishes. In the wild, hybrids involving green sunfish *Lepomis cyanellus*, bluegill *L. macrochirus*, pumpkinseed *L. gibbosus*, and orangespotted sunfish *L. humilus* are particularly common. The fertility of these hybrids is quite variable. For example, Dawley (1987) found that pumpkinseed x green sunfish hybrids are naturally triploid. Pumpkinseed x bluegill F<sub>1</sub> hybrids, in contrast, were successfully used to produce F<sub>2</sub> and F<sub>3</sub> generation fish (Lagler and Steinmetz 1957). Few *Lepomis* sp. hybrids are completely sterile (Argue and Dunham 1999). Most hybrids within this genus demonstrate some fertility, albeit at a lower level than non-hybridized fish. Often the sex ratio of these hybrids is quite skewed. For example, Ricker (1948) found that redear sunfish *L. microlophus* x bluegill hybrids were only 4% female and that the F<sub>2</sub> generation was all male. Argue and Dunham (1999) state that the fertility of warmouth *L. gulosus* x bluegill, green sunfish x warmouth, green sunfish x redear sunfish, bluegill x green sunfish, warmouth x green sunfish, warmouth x redear sunfish, green sunfish x warmouth, and redear sunfish x green sunfish are high. All other combinations of these species had lower fertility. *Lepomis* is a diverse genus and many hybrid combinations are possible. Argue and Dunham (1999) provides a good review of the fertility of various hybrids within this genus.

Hybridization is also common among other genera within Centrarchidae. White crappie *Pomoxis annularis* and black crappie *P. nigromaculatus* readily hybridize (Smith et al. 1994). Such hybrids are not sterile and can produce F<sub>2</sub> generation fish or can backcross with either white or black crappie (Smith 1992). The fertility of F<sub>2</sub> generation crappie hybrids is relatively low and when managers are concerned about over-population, these fish can be stocked in waters that lack crappie (Hooe and Buck 1991). Hybridization is also common among the black basses. Largemouth bass *Micropterus salmoides* and smallmouth bass *M. dolomieu* readily cross and the resulting hybrids are not sterile (Whitmore and Hellier 1988). Generally, all species within the genus *Micropterus* are capable of hybridizing with one another and the resulting progeny are not sterile (Argue and Dunham 1999).

## Percidae

The most commonly produced perch hybrid is the saugeye (walleye *Sander vitreum* x sauger *Sander canadense*). The fertility of this cross is related to the direction that the hybrid is produced. Johnson et al. (1988) found that if F<sub>1</sub> generation walleye x sauger are crossed with either parent species that only 10% of the eggs hatched. Interestingly, Malison et al. (1990) noted that gonad development in the walleye x sauger cross appeared comparable to similar sized walleye. In contrast, Hearn (1986) found that F<sub>1</sub> sauger x walleye could be successfully spawned with 45% survival to swim-up. The survival of F<sub>1</sub> x sauger was 36% and was 90% for F<sub>1</sub> x walleye (Hearn 1986).

Hybridization has also been reported among other perches. Naturally produced hybrids have been reported among darters from the genus *Etheostoma* (Argue and Dunham 1999). The sterility of these darter hybrids is variable and range from infertile to highly fertile. Crosses involving yellow perch *Perca flavescens* have not been documented in the literature.

### *Moronidae*

Hybridization is common within the genus *Morone*. White bass *Morone chrysops* regularly hybridize with white perch *M. americana* and yellow bass *M. mississippiensis* in the wild. The resulting hybrids are not sterile and F<sub>2</sub> generation hybrids and backcrosses between hybrids and the parent species are regularly reported (Waldman and Bailey 1992). The hybrid striped bass (i.e., wiper) is a cross between white bass and striped bass *M. saxatilis*. This hybrid is highly sought after by anglers and is commonly produced in hatcheries. There are two hybrid striped bass crosses; striped bass x white bass (called palmetto bass) and white bass x striped bass (called sunshine bass). Bishop (1967) successfully produced F<sub>2</sub> generation palmetto bass and noted that striped bass x palmetto bass had a high egg hatch rate but that approximately 50% of the resulting fry were deformed. It appears that F<sub>2</sub> generation palmetto bass are sterile and survival of the white bass x palmetto bass cross after hatch is low (<0.02%; Harrell 1984). The sunshine bass has also been reported to be fertile (Kerby et al. 2002); however, little is known about the survival of the F<sub>2</sub> generation and the ability of the sunshine bass to backcross with either parent species.

### *Esocidae*

The most widely produced hybrid from this group is the tiger muskellunge (muskellunge *Esox masquinongy* x northern pike *E. lucius*). The fertility of this hybrid has been questioned (Bartley et al. 2000). Buss and Miller (1967) reported a successful backcross between tiger muskellunge and muskellunge. They also note gonad development in the tiger muskellunge. Other researchers have attempted to produce crosses using tiger muskellunge and found it to be sterile (Argue and Dunham 1999). Most workers have produced tiger muskellunge using the female muskellunge. It is also possible to produce this cross using female northern pike. No tests on the fertility of this cross have been documented in the literature. Buss and Miller (1967) tested all possible crosses among six *Esox* species. They successfully produced F<sub>1</sub> offspring from each cross. Generally, the survival of these offspring was low (<1%). Adults of most of the crosses had gonad development, albeit the gonads of these hybrids were smaller than those of similar sized non-hybrid fish. They did note that F<sub>2</sub> hybrids between chain pickerel *E. niger* and pickerel *E. americanus* had good hatch rates and survival.

### *Catfishes*

Hybridization has been known to naturally occur among catfishes from the genus *Ictalurus*. Most of these hybrids are fertile (Argue and Dunham 1999). The best studied cross from this genus is between channel catfish *I. punctatus* and blue catfish *I. furcatus*. This cross has potential aquaculture value and is typically produced by hand-stripping gametes although natural production of this hybrid has also been documented (Dunham and Argue 2000). The fertility of this hybrid is reduced compared to the parent species. F<sub>1</sub> generation hybrids have smaller testes and ovaries and lower ovulation rates than pure channel catfish or blue catfish (Dunham and Argue 2000). Egg survival decreased with generation and is 5.1% by the F<sub>3</sub> generation (vs. 73.9% for channel catfish control; Dunham and Argue 2000). Crosses

between channel catfish and white catfish *Ameiurus catus* have also been produced and been documented to be fertile (Argue and Dunham 1999). Goudie et al. (1994) produced half-sib groups by fertilizing channel catfish eggs with a mixture of sperm from channel catfish, blue catfish, black bullhead *A. melas* and flathead catfish *Pylodictus olivaris*. The fish were reared as mixed hybrid lot and the paternal species of the progeny was determined at 1, 4 and 8 months of age using either a genetic test or visual identification. The results showed that all species were capable of fertilizing channel catfish eggs. The percentage of fish fathered by channel catfish ranged from 54-70% (depending on lot and sampling time). The percentage of fish fathered by blue catfish was 26-38% and the percentage fathered by either black bullhead or flathead catfish ranged between 0 and 8% (Goudie et al. 1994). This study did not assess sterility among these crosses but the results to show that it is possible to produce F<sub>1</sub> generation fish from each cross. A follow-up study (Zhang and Tiersch 1997) determined the number of chromosomes in each cross. No signs of androgenesis, gynogenesis, polyploidy or aneuploidy was seen in the genomes of the hybrids. The authors believed that the crosses would be able to produce haploid gametes (Zhang and Tiersch 1997). Black bullhead and flathead catfish however, had different sized chromosomes than channel catfish and it was suspected that these crosses could produce aneuploid gametes. Thus, F<sub>1</sub> channel catfish x black bullhead and channel catfish x flathead catfish may be sterile or have reduced fertility (Zhang and Tiersch 1997). Despite the fact that laboratory tests have demonstrated that it is possible to cross black bullhead, flathead catfish, and blue catfish with channel catfish, there have not been tests that have determined whether F<sub>1</sub> hybrids can be produced by crossing the three paternal species with each other. Also, no information regarding hybridization among various bullhead species is present in the literature.

Hybridization appears to be more common among the madtoms. Menzel and Raney (1973) noted the presence of naturally produced *Noturus gyrinus* x *N. miurus* hybrids in Cayuga Lake, New York. Naturally produced hybrids between *N. flavus* and *N. insignis* have been found in West Virginia (Welsh and Cincotta 2004). Most madtoms have similar reproductive behavior (e.g., spawning habitat and temperature preference) and it has been suggested that these behavioral similarities lead to the occurrence of hybrids among these species. The sterility of madtom hybrids is not known.

### *Catostomidae*

Introgression has been frequently observed in suckers in the western United States. The bluehead sucker *Catostomus discobolus* has been found to hybridize with mountain sucker *C. platyrhynchus*, desert sucker *C. clarki* and Rio Grande sucker *C. plebius* (Argue and Dunham 1999). Natural hybridization between mountain suckers and desert suckers has also been documented (Koehn and Rasmussen 1967). The Sonora sucker *C. insignis*, Utah sucker *C. ardens*, and flannelmouth sucker *C. latipinnis* have been known to hybridize with razorback suckers *Xyrauchen texanus* (Argue and Dunham 1999). The Utah sucker can hybridize with the June sucker *Chasmistes liorus* (Smith 1992). The fertility of all of these previously listed catostomid hybrids is not known. McDonald et al. (2008) collected and performed genetic tests on suckers collected throughout the Colorado River basin. Widespread

hybridization was observed among white sucker *C. commersoni* and flannelmouth sucker. It appeared that this hybrid is not sterile (McDonald et al. 2008). Hybridization among white suckers and bluehead suckers was also documented (McDonald et al. 2008). This hybrid was relatively rare and genetic evidence indicates that this hybrid rarely survives past the F<sub>1</sub> generation. No flannelmouth x bluehead hybrids were found but flannelmouth x bluehead x white sucker hybrids were found (McDonald et al. 2008). The white sucker is not native west of the continental divide and it appears that the introduction of this species has facilitated introgression between flannelmouth and bluehead suckers. It appears that these two species cannot reproduce without prior white sucker introgression (McDonald et al. 2008).

### *Cyprinidae*

The cyprinids are a diverse, speciose group. Hybridization and species introgression is very common in this group. It would be impossible to discuss all cyprinids in this review. Thus, this review will focus on hybrids involving species from the Colorado River basin and those of fisheries management interest. Unfortunately as a whole, cyprinids are poorly studied. As a result, information about many species in the region is lacking.

Species introgression is very common among the genus *Gila*. Humpback chub *G. cypha* and bonytail *G. elegans* hybrids are commonly reported in the Colorado River (Holden and Stalnaker 1970). Genetic data from these species seems to indicate that hybrids between these two species are fertile (Holden and Stalnaker 1970). Several species including humpback chub, bonytail chub, Utah chub *G. atraria*, *G. seminuda*, and *G. jordani* appear to readily hybridize with the roundtail chub *G. robusta* (DeMarais and Dowling 1992). Data suggests that hybrids between bonytail, humpback chub, and roundtail chub are fertile and hybridization among these species is an important evolutionary force behind the morphological diversity of these species (DeMarais and Dowling 1992). Despite widespread evidence of introgression among species within the genus *Gila*, no studies have directly tested the fertility of hybrids. Still, the levels of observed introgression indicate that hybrid progeny are likely fertile.

Hybridization also occurs in the genus *Rhinichthys*. Introgression has been observed between longnose dace *R. cataractae* and speckled dace *R. osculus* (Smith 1973). It appears that hybrids between these two species are fertile (Smith 1973). The redbreast shiner *Richardsonius balteatus* has been demonstrated to hybridize with peamouth *Mylocheilus caurinus*, which is a species that is not found in Utah (Aspinwall et al. 1993). It is not known if this species hybridizes with any cyprinid species found within the state.

Common carp are another cyprinid species that has been subject to hybridization research. For example, allotetraploid fish are produced when common carp are bred with crucian carp *Carassius carassius* (Liu et al. 2001). When crossed back with the parent species, the resulting progeny are triploid (Liu et al. 2001). Stanley (1976) hybridized common carp with grass carp. The grass carp x common carp cross produced larvae that died after hatch. Surviving offspring were produced using the reciprocal cross, common carp x grass carp. There was some evidence that the resulting offspring were tetraploid (Stanley 1976). Gynogenetic fish and androgenetic grass carp were also produced (Stanley 1976). Grass

carp have also been crossed with bighead carp and a mixture of diploid, triploid, and tetraploid progeny were produced (Allen and Stanley 1983).

### Other Species

Several other hybrids have been documented in the literature that are of potential management interest in Utah. For example, gizzard shad *Dorosoma cepedianum* can hybridize with threadfin shad *D. petenense* and the resulting  $F_1$  progeny are fertile (Argue and Dunham 1999). In addition, hybridization is common within the genus *Gambusia*. Hybrid progeny from this genus are typically fertile (Argue and Dunham 1999). Hybrids between *Poecilia formosa* and *P. sphenops* were sterile (Schultz and Kallman 1968).

### Ploidy Manipulation

Most sterile fish are produced by creating triploids (e.g., Hussain et al. 1995; Kerby et al. 1995), although viable progeny have been produced by fertilizing eggs from triploid loach (*Misgurnus anguillicaudatus*) with normal haploid spermatozoa (Matsubara et al. 1995). The triploidization process involves shocking the egg as it goes through meiosis, a natural mechanism in which a fertilized egg sheds one set of chromosomes and combines the remaining set with the set provided by the sperm. In normal fertilization, the second set from the female exits the cell, while the male and female sets merge to provide the diploid (2N) nucleus. To create a triploid (3N), the second set, known as the 'second polar body', is forced back into the cell (Figure 1).

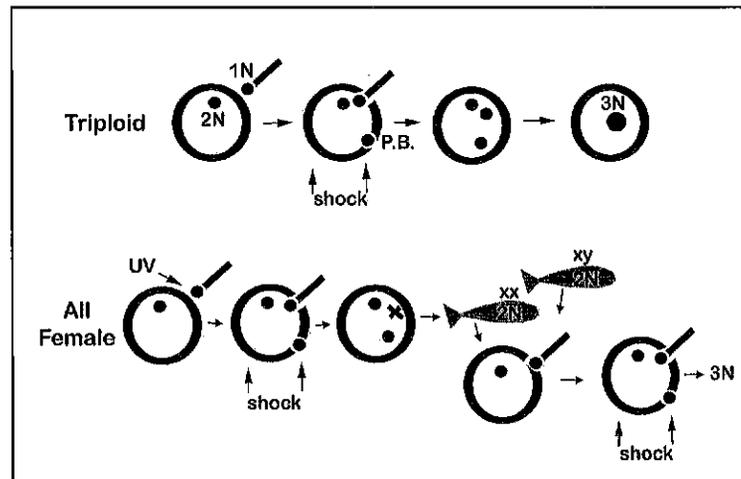


Figure 1. Diagram showing the process for creation of triploid and all-female triploid fish.

Another method to create triploids is to cross a tetraploid with a diploid (Meyers and Hersberger 1991); However, these 'interploidy' crosses have a low level of fertilization success. Tetraploids are required, which require shocks during mitosis (first cleavage) and rearing of these progeny to brood size. Tetraploids tend to be 'subvital', and have poor survival, slow growth, and deformities (Myers et al.

1986). However, Myers and Hershberger (1991) found that the interploid hybrid performance compared favorably with heat-shocked triploids. Arai (2001) noted in Japan's Fisheries Experiment Station, Nagano Prefecture, that only 0.006 to 0.065% of pressure treated eggs survived to be tetraploid; however, the staff there was able to generate nearly 100% triploids crossing the tetraploids with diploid rainbow trout. Tetraploids were also successfully crossed with other tetraploids to continue the lineage. Other observations made at the same facility were 1) occurrence of diploid-tetraploid mosaicism, 2) when crossed with tetraploids, diploid females produced less yield than tetraploid females due to larger sperm head from tetraploids, 3) production of more males than females (about 4:1), 4) better growth of triploids (from tetraploid female x diploid sex-reversed male) than normal diploids and tetraploids (tetraploid female x tetraploid male) in 2-year old fish, and 5) better growth of all-female interploid triploids (tetraploid female x diploid sex-reversed male) than induced all-female triploids produced by the second polar body inhibition after fertilization of eggs from 2-year old fish. Personal experience with tetraploid rainbow trout *Oncorhynchus mykiss* at Egan Hatchery, Bicknell, Utah, indicated that the interploid strategy for producing triploids requires more effort and tank space and fertilization success of interploid crosses was poor. Poor survival of tetraploids has also been observed for walleye (Malison et al. 2001) and yellow perch (Malison et al. 1993), but growth and survival was comparable to diploid controls in a study using heat shock on mud loach *Misgurnus mizolepis* to produce tetraploids (Nam et al. 2001a).

Gynogenetic, i.e., 'all-female' triploids have applications in aquaculture and fisheries management. Since triploid males still have the same behaviors as diploid males despite not being fertile, they pose a risk to native fish populations by attempting to spawn with diploid females. The extent of this problem is not well known. Another application for fisheries management includes use of all-female walleye, which grow faster than males; the females would reach a harvestable size sooner and reach a trophy size, especially if triploid (Malison et al. 1998). For fish species in which the female tends to grow larger, producing gynogens makes economic sense as well. E.g. in Japan, Arai (2001) reported that all-female triploid rainbow trout x amago salmon *O. masou ishikawae* and rainbow trout x Japanese char are used commercially. All-female diploids are of interest for some applications, such as for the paddlefish and sturgeon caviar industry (Mims et al. 1997).

To create all-female triploids, sperm is irradiated with ultraviolet light to inactivate the DNA, but can still initiate fertilization. For irradiation, the sperm is diluted 0.5 ml in 2 ml extender solution and spread to 13 mm thick; a magnetic stirrer slowly mixes during the 4 min exposure (bulb is 6 cm above sperm)(Chourrout 1982). See Palti et al. (1997) for a similar irradiation protocol. After 'fertilization' the egg is shocked to keep the second polar body, resulting in a diploid cell in which all the DNA is from the female (Figure 1). The egg may also be shocked during the first cleavage to create the diploid nucleus (Tave 1990). These diploids must then be reared to brood stock and their eggs submitted to the triploidy process to get all-female triploids. All-female (gynogens) triploids may be beneficial in cases such as that described by Warrillow et al. (1997), where male triploids still behave like fertile males and can emigrate or interfere with reproduction of native species and stocks. All-male triploids may be purposefully be used for this activity to deliberately interfere with spawning of target species such as

brook trout, which tend to overpopulate and stunt. Androgenic triploids are created by UV irradiation of the egg, followed by fertilization. The sperm's DNA is doubled by shocking the egg at first cleavage (mitosis) creating a 2N egg with all-male DNA. As with the females, these fish need to be reared to brood stock, and their sperm used to create triploid progeny using the traditional shock methods. However, half of these will still produce some female offspring, and half will be 'supermales' that produce males when crossed with normal diploids (Tave 1990). Since the early 20<sup>th</sup> century, several fish and amphibian species have been manipulated to produce gynogens, androgens, and triploids (see review by Ihssen et al. 1990).

Various shock methods have been employed to induce retention of the second polar body. These include cold shock (Svärdson 1945; Valenti 1975), heat shock (Swarup 1959), and pressure (Streisinger et al. 1981; Chourrout 1984; Chourrout et al. 1986). Cold shocks are used more frequently for warmwater species and heat shocks are more effective for cold water species (Ihssen et al. 1990). Various recipes that manipulate variables such as post-fertilization time, shock duration, shock temperature have been tested and are summarized in Table 2 (Salmonidae) and Table 3 (non-salmonids).

Heat shocks for species in the Salmonidae family tended to be more effective at temperatures  $\geq 26^{\circ}\text{C}$ , with higher temperatures leading to greater mortality. Longer durations, e.g., 20 min versus 10 min, led to higher triploidy percentages (Guoxiong et al. 1989; Teskeredžić et al. 1993). For warmwater species, the development of the egg is much faster, even accounting for temperature differences. As seen in Table 8, the degree-minutes to optimal shock timing are 10-75% of that required for Salmonidae. Interestingly, the white sturgeon (*Acipenser transmontanus*) had a similar to slightly higher degree-minutes relative to Salmonidae. Since both Salmonidae and Acipenseridae are known to be relatively primitive fish families (Bond 1979), this suggests that there is a potential relationship between evolutionary phylogeny and egg development rate. This would suggest that timing of shocks for other primitive fishes such as bowfin, gar, and paddlefish would also be similar (e.g., 100-200  $^{\circ}\text{C}\cdot\text{min}$ ). A study on diploid gynogen production in paddlefish suggested that even longer (288-300  $^{\circ}\text{C}\cdot\text{min}$ ) development time is needed prior to applying the thermal shock (Mims et al. 1997). As Table 8 indicates, there is wide variation in the degree-minutes for optimal shock delivery among species studied to date, ranging from 14  $^{\circ}\text{C}\cdot\text{min}$  for Thai silver barb *Puntius gonionotus* (Koedprang and Na-Nakorn 2000) to 240  $^{\circ}\text{C}\cdot\text{min}$  for white sturgeon. For species yet to be studied, the data suggest finding the results for the nearest taxonomic relatives and testing ranges around the parameter values for that species.

For cold shocks, duration appears to have an impact on triploidy. E.g., durations of 45-60 min led to higher percent triploidy in common carp (Basavaraju et al. 2002) than a 10 min duration (Ojima and Makino 1978). In turbot *Scophthalmus maximus*, triploidy increased with durations ranging between 5 and 40 min (Piferrer et al. 2000; Table 3). White crappie *Pomoxis annularis* triploidy rates were higher after 60 min than 45 min at  $5^{\circ}\text{C}$  (Baldwin et al. 1990). Prolonged exposure however, can lead to egg mortality; e.g., Chrisman et al. (1983) noted channel catfish *Ictalurus punctatus* eggs died after exposure of eggs for 2-3 h to  $5^{\circ}\text{C}$ .

Similarly various pressure treatments have been evaluated for a number of species (see Table 4 for Salmonidae, Table 5 for non-salmonids). Pressure treatments can be applied

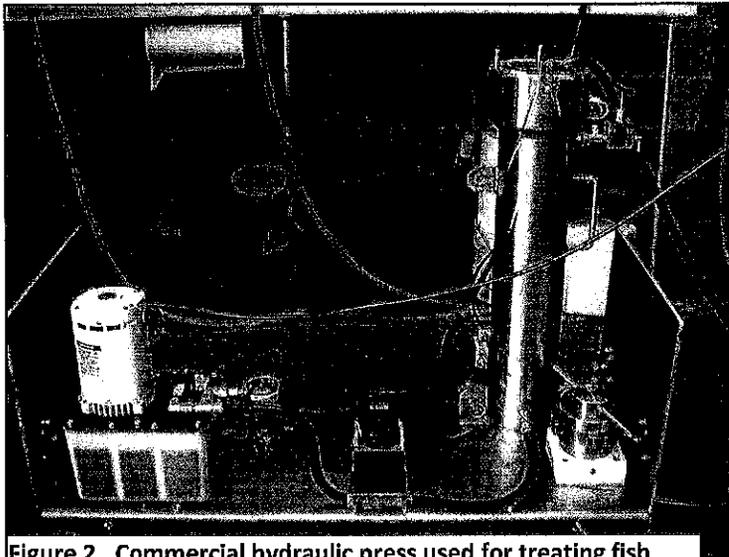


Figure 2. Commercial hydraulic press used for treating fish eggs. Note foot pedal between hydraulic pump and pressure cylinder on the right. The cylinder piston cap is shown on the bottom right.

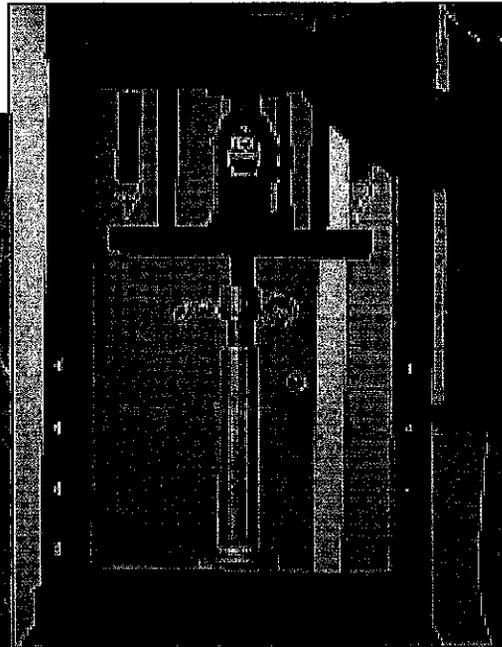


Figure 3. Hand operated press with cylinder with trout eggs.

using hollow cylinders (in which eggs are placed) to which pressure is applied via a hydraulic pump (Fig. 3) or press. Benfey et al. (1988) found that the larger apparatus was only 70% as effective as the smaller French pressure cell when used with chum salmon *Oncorhynchus kisutch*. Increases in pressure, with duration and time post-fertilization constant, led to increases in mortality (Cassani and Caton 1986; Garret et al. 1992; Peruzzi and Chatain 2000). However, in studies where pressure duration was increased but time post-fertilization and pressure were constant, mortality did not necessarily correlate with duration of pressure treatment (Onozato 1984; Cassani and Caton 1986; Wills et al. 1994; Malison et al. 2001). In studies by Chourrout (1984) and Garcia-Abiado et al. (2001), survival relative to controls increased with the triploidy percentage. However, when 100% triploidy was achieved, pressure duration was negatively correlated with survival (Chourrout 1984). In studies with species from Salmonidae, pressures of at least 6,000-7,000 psi were needed to achieve 100% triploidy (Chourrout 1984), though later studies indicated higher pressures (8,000-10,000 psi) were more consistently effective (Teskeredžić et al. 1993; Guoxiong et al. 1989; Kozfkay et al. 2005). The optimal timing of the pressure shock for Salmonidae species (about 20-40 min after fertilization; Table 9) is similar to the timing for thermal shocks, indicating a similar mechanism for retaining the second polar body. Pressure shock parameters that led to 100% triploidy are summarized in Table 9 for all the papers reviewed.

Gynogen production data (i.e., diploid yield) is relevant to the triploidy discussion since the recipes used to create diploid gynogens operate on the same 'polar-body-retention' principle. Therefore the same parameters (time post fertilization, duration, etc.) used to optimize the yield should also be applicable to

triploid production. A summary of the gynogen literature is presented in Table 6 for Salmonidae and Table 7 for non-salmonids.

**Table 2.** Comparison of studies of Salmonidae species comparing the triploid yield resulting from various levels of time post-fertilization, duration of shock, and shock temperature. The incubation temperature and study references are also provided.

Species Shock	Time post-fertilization (min)	Duration (min:sec)	Triploid (%)	Incubation temperature (°C)	Reference
Rainbow trout					
Heat: 34 C	10	1:00	16	10	Thorgaard et al. 1981
Heat: 35 C	10	1:00	24		
Heat: 36 C	10	1:00	42		
Heat: 37 C	10	1:00	45		
Heat: 28 C	40	10:00	70.0	10	Lincoln and Scott 1984
Heat: 28 C	40	10 :00	90.0		Lou and Purdom 1984
Heat: 26 C	20	20	97	10	Wagner and Arndt 2001
Heat: 27 C	20	20	100		
Heat: 28 C	20	20	97		
Steelhead					Guoxiong et al. 1989
Heat: 26 C	20	1:15	13.0	7.5	"
	20	2:30	23.0		"
	20	5:00	47.8		"
	20	10:00	60.8		"
	20	20:00	100.0		"
Heat: 28 C	20	1:15	19.0		"
	20	2:30	33.0		"
	20	5:00	50.0		"
	20	10:00	65.0		"
	20	20:00	90.0		"
Heat: 30 C	20	1:15	0.0		"
	20	2:30	23.0		"
	20	5:00	45.0		"
	20	10:00	67.8		"
	20	20:00	0.0		"
Heat:32 C	20	1:15	9.0		"
	20	2:30	20.0		"
	20	≥5:00	lethal		"
Heat: 34 C	20	1:15	0.0		"
	20	2:50	100.0		"
Heat: 36 C	20	1:15	30.0		"
Brook trout Cold: -1.5 C	1 min	120	0	10	Lemoine and Smith 1980

Heat: 27 C	10	10	33	10	Wagner et al. 2007
Heat: 28 C	10	7,10	57,72		
Heat: 28 C	15	7	72		
Heat: 29 C	10	10	85		
Heat: 29.4 C	18	7	97, 61		
Heat: 29.4 C	18	5	50		
Coho salmon Heat:24 C	20	10	0	10	Teskeredžić et al. 1993
	20	20	60		
	40	10	0		
	40	20	20		
	60	10	0		
	60	20	0		
	80	10	0		
	80	20	0		
Heat: 26 C	20	10	15	10	"
	20	20	95		
	40	10	15		
	40	20	20		
	60	10	0		
	60	20	10		
	80	10	0		
	80	20	0		
Heat: 28 C	20	10	50	10	"
	20	20	100		
	40	10	25		
	40	20	25		
	60	10	0		
	60	20	10		
	80	10	0		
	80	20	0		
Chinook salmon Heat: 26.5 C	20	10	40	4	Denton 1987
	10	10		14	
Heat: 28.0 C	5	10	92	5.7	"
	15	10	78	14	
Heat:29.5 C	5	5	25	7	"
	15	5	40	14	

**Table 3.** Comparison of the results of various studies of non-salmonid species comparing the triploid yield from various levels of time post-fertilization, duration of shock, and temperature. The acclimation temperature is provided in the 'Time post-fertilization' column above the time values for the referenced study.

Species Shock	Time post-fertilization (min)	Duration (min)	Triploid (%)	Reference
Walleye	(11 C)			Malison et al. 2001
Heat: 28 C	1,2,5	25	0,0,0	"
Heat: 29 C	1,2,5	25	0,0,8	"
Heat: 30 C	1,1,2,5	25	0,17,19,8	"
	2, 5	10	0, 7	"
Heat: 31 C	1,1,2,5	25	50,33,25,35	"
Heat: 32 C	1	25	Lethal	"
	2, 5	10	18, 18	"
Heat: 34 C	2, 5	10	lethal	"
Yellow perch	(11 C)			Malison et al. 1993
Heat: 24 C	2,5	25	7,33	"
Heat:26 C	2,5	10	0, 11	"
		25	50, 22	"
Heat:28 C	1,3	25	77, 100	"
	2,5	10	53, 67	"
	2,5	25	53, 67	"
Heat:29 C	1,3	25	72, 100	"
Heat:30 C	1,3	25	90, 100	"
	2,5	10	100, 93	"
Heat:31 C	1,3	25	100,100	"
Heat:32 C	1,3	25	lethal	"
Saugeye	(10.2 C)			Garcia-Abiado et al. 2001
Cold: 1.2 C	5	120	0-58	
		150	10-100	
		180	0-90	
Heat: 31 C	5	15	81.6, 77.8,	Garcia-Abiado et al. 2001
		20	80.0	
Heat: 32 or 33 C			lethal	Garcia-Abiado et al. 2001
Threespine stickleback				
Cold: 0 C	3	90-180	56	Swarup 1959
Heat: 33.5-40 C	10	5	50	Swarup 1959
Sterlet	Up to 1 <sup>st</sup>			
Heat: 34 C	cleavage	3	52	Vasetskii 1967
Common carp				
Cold: 0 C	10	10	some	Ojima and Makino 1978
Cold: 0-2 C	(20 C)			Gervai et al. 1980
	5	45	100	
Heat: 40 C	(26-28 C)			Basavaraju et al. 2002

	1 2 3	1:30 1:30 1:30	96-100 96-96 100-100	
Powan Cold: 0 C	10	13 h	3	Svärdson 1945
Mud Loach Cold: 2 C	(25 C) 5	60	96	Nam et al. 2001b
Plaice x <i>Platichthys flesus</i> Cold: 0 C	15	2-5 h	100	Purdom 1972
Turbot Cold: 0 C Cold: 2 C Cold: 4 C	(13-14 C) 5:00 5:00 5:00	5, 10, 20, 40 5, 10, 20, 40 5, 10, 20, 40	8, 12, 87, 84 0, 8, 49, 71 0, 2, 13, 50	Piferrer et al. 2000 " "
European sea bass Cold: 1 C  Cold: 1 C	(13 C) 4 5 6 7 5	20 20 20 20 10, 15 20, 25	51 61 38 37 85, 100 100,100	Peruzzi and Chatain 2000 "
Cold: 0 C	1, 2 3 4 5 6 7 8,9,10	5 5 5 5 5 5	39,27 41 66 88 59 48 5, 11, 9	Felip et al. 1997
Cold: 2 C	3,5,7	5	31,48,31	"
Grass Carp Cold: 5 C Heat: 38 C Heat: 40 C Heat: 42 C Heat: 42 C Heat: 42 C Heat: 40 C Heat: 42 C	(22-24.8C) 5:00 3:30-4:30 4:00 5:30 4:30 4:30 4:30 4:25 4:00 4:00 4:00 4:13 4:00 5:00 4:00	6:00 1:00 1:00 1:00 1:00 1:20 1:20 1:00 1:00 1:00 1:00 1:00 1:00 2:00 1:00 1:00	18 0 50.0 33.3 16.6 16.6 11.1 0 100 0 100 66.7 8 0-100	Cassani and Caton 1986 " " " " " " " " " " " " " " "
Rohu Heat: 40 C	(13.5 C) 7:00	2:00	24	Reddy et al. 1990

Thai silver barb Cold: 2 C	(28 C) 0:30	10:00	64-100	Koedprang and Na-Nakorn 2000
White crappie Heat: 36 C Heat: 38 C Heat: 40 C Cold: 5 C	(21-23 C) 5:00 5:00 5:00 5:00	5:00 3:00, 5:00 3:00, 5:00 45:00 60:00	0-10 8,4 4,0 0-24 92,72	Baldwin et al. 1990 " " "
Tilapia sp. Cold: 11 C Cold: 10 C Heat :38 C Heat: 39.5 C Heat: 40 C	(32) 15:00 5:00 15:00 3:00 5:00	60 ND 1 h 4:00 30:00	75 100 10 <100 64-100	Valenti 1975 Lutz 1998 " Byamungu et al. 2001 Penman et al. 1987
Heat: 40-41 C Heat: 40-41 C Heat: 40-41 C Heat: 42 C	(24-25C) 5-10 (28-31 C) 4-6 (30-31 C) 7-10 3:00	4:00-5:00 4:00-5:00 4:00-5:00 3:00-4:00	75 80 0 100	El Gamal et al. 1999 " " Byamungu et al. 2001
Cold: 12-13 C	5:00	5:00	96	"
Channel catfish Cold: 5 C	(27 C) 5:00 5:00	60:00 120:00,180:00	100 lethal	Chrisman et al. 1983
European catfish Heat: 40.5 C	(20 C) 7:00 8:00 9:00	1:00 1:00 1:00	83 27 63	Linhart and Flajshans 1995
Pejerrey Cold:0.5-1.0 C	(18.5-19 C) 6 6 9 12	40 80 40 40	62 100 0 0	Strüssmann et al. 1993
White sturgeon Heat: 32 C Heat: 34 C Cold: 3 C	(16 C) 12 12 12 (16 C) 15 15 15 15 (16 C) 12	2:00 3:30 5:00 1 2 3 5	90 100 97 44 100 71 80 1	Van Eenennaam et al. 1996 " "
				"

	12	30	35	
	12	60	57,90	
	12	180	42	

**Table 4.** Comparison of the results of various pressure treatment studies of Salmonidae species comparing the triploid yield and survival to hatch after pressure shocks at various times post-fertilization, pressures, and durations.

Species Pressure	Time post-fertilization (min)	Pressure Duration (min)	Survival relative to control (%)	Triploid (%)	Reference
Rainbow trout					Chourrout 1984
6,000 psi	10	2	17	0	"
6,000 psi	10	4	10	83	"
6,000 psi	10	6	42	62	"
6,000 psi	10	8	71	100	"
7,000 psi	40	3	98	100	"
7,000 psi	40	5	67	100	"
7,000 psi	40	7	39	100	"
8,000 psi	40	10	79	90	Lou and Purdom 1984
Coho salmon (10 C)					Teskeredžić et al. 1993
9,000 psi	20	4	98	90	
	40	4	98	90	
	60	4	79	0	
	80	4	65	0	
10,000 psi	20	4	80	100	"
	40	4	74	100	
	60	4	64	0	
	80	4	15	0	
11,000 psi	20	4	76	90	"
	40	4	62	100	
	60	4	60	60	
	80	4	15	0	
12,000 psi	20	4	30	100	"
	40	4	18	100	
	60	4	7	50	
	80	4	0	0	
Steelhead					Guoxiong et al. 1989
8,000 psi	20	2,4,6	78,82,77	12,0,15	"
9,000 psi	20	2,4,6	79,80,58	5,5,35	"
10,000 psi	20	2,4,6	57,50,57	0,40,85	"
11,000 psi	20	2,4,6	26,22,27	12,85,100	"
12,000 psi	20	2,4,6	19,30,38	70,95,80	"
Atlantic salmon					Johnstone et al. 1989
NO at 5 atm	0	30, 60	80, 66	28,50	"
NO at 11 atm	0	15, 30,60	70,81,62	78,98,100	"
NO at 11 atm	30	60	49	25	"
Freon at 1 atm	0	30,60	71,68	36,64	"
Freon at 3 atm	0	15,30	27,12	75,100	"
Cyclopropane,	0	15,30	86, 83	0,0	"

1atm					
Halothane 0.2 atm	0	10,20	56,42	8,22	"
Halothane 0.2 atm	10	20	73	0	"
Halothane 0.2 atm	20	30	75	8	"
Halothane 0.056-0.124 atm	0	30,60	75 to 91	0	"
Ethane 0.11 atm	0	15, 30	78, 72	2,6	"
Lake trout					Kozfkay et al. 2005
9,000 psi	32 (300°C-min)	5	56	100	
9,500 psi	21 (200°C-min)	5	53	100	"
9,500 psi	32	5	79	100	"
9,500 psi	43 (400°C-min)	5	53	100	"
Arctic grayling	(4 C)				Loopstra and Hansen 2010
8,500 psi	44	3	81	100	
	44	5	78	100	
	44	7	86	100	
9,000 psi	44	5	80	100	"
	62	5	63	ND	
9,500 psi	25	5	44	100	"
	44	5	73	100	
	62	5	70	100	
10,000 psi	44	5	75	100	"

**Table 5.** Comparison of the results of various pressure treatment studies of non-salmonid species comparing the triploid yield and survival to hatch after pressure shocks at various times post-fertilization, pressures, and durations.

Species Pressure	Time post-fertilization (min)	Pressure Duration (min)	Survival relative to control (%)	Triploid (%)	Reference
Saugeye 9,000 psi	(10.2 C)				
	3:57	12	37	85	Garcia-Abiado et al. 2001
	3:58	12	48	100	"
	4:00	12	88	100	"
	4:01	12	31	100	"
	4:04	12	17	100	"
	4:06	12	21	100	"
	4:10	12	73	100	"
	4:13	12	48	100	"
	4:14	12	27	100	"
	4:15	12	20	88	"
	4:19	12	65	50	"
	4:20	12	32	78	"
	4:30	12	82, 38, 87	98, 100, 70	"
	4:34	12	17	57	"
4:55	12	33, 70	63, 60	"	
5:00	12	54	84	"	
Largemouth bass	(22 C)				Garret et al. 1992
	4,000 psi	5:00	3	>100	20.0
	5,000 psi	5:00	3	>100	100.0
	6,000 psi	5:00	3	83	71.4
	6,500 psi	5:00	3	75	100.0
	8,000 psi	5:00	1	60	100.0
	8,300 psi	5:00	1	58	100.0
	8,007 psi	5:00	1	98	100.0
<i>Lepomis</i> sp. hybrid	(22-27 C)				Wills et al. 1994
	6,000 psi	2,3,4	2	118,109,44	83,80,79
		2,3,4	3	90,3,3	100,84,100
		2,3,4	4	20,1,1	100,100,75
	7,000 psi	2,3,4	2	81,39,324	83,83,97
		2,3,4	3	92,38,94	90,86,100
		2,3,4	4	112,142, 49	100,93,93
	8,000 psi	2,3,4	2	38,10,18	100,100,100
		2,3,4	3	25,9,101	97,100,100
	2,3,4	4	34,82,49	100,100,100	
Grass carp	(22-24.8 C)				
	3,000 psi	4	5	86	0
	4,000 psi	4	5	100	33
					Cassani and Caton 1986
					"

4,000 psi	4	7	3	50	"
5,000 psi	4	5	100	83	"
5,000 psi	4	1	100	0	"
6,000 psi	4	5	14	67-100	"
6,000 psi	4	4	73	69	"
6,000 psi	4	4	76	83	"
6,000 psi	4	3	15	100	"
6,000 psi	4	1	100	67	"
7,000 psi	4	3	24	83	"
7,000 psi	4	3	95	100	"
7,000 psi	4	2	65	100	"
7,000 psi	4	1	94	100	"
8,000 psi	4	2	49	99	"
8,000 psi	4	1	68	100	"
9,000 psi	4	1	42	100	"
7,000-8,000 psi	4	1-2	22-100	98	"
Bighead carp 7,348 psi	2 4 5 7	1.5 1.5 1.5 1.5		50 100 78 35	Aldridge et al. 1990
White x striped bass 6,000 psi	(16.5 C) 4		2 70	20	Curtis et al. 1987
7,000 psi	4		2 100	0	"
Striped x white bass 7,000 psi			5 58	0	"
7,000 psi			3 20	20	"
7,000 psi			5 84	9	"
8,000 psi			4 63	53	"
8,000 psi			5 100	30	"
8,000 psi			5 77	0	"
8,000 psi			3 54	46	"
8,000 psi			4 29	100	"
Walleye 7,000 psi	(11 C) 2 5		3,6 91,82 86,73	0,0 0,6	Malison et al. 2001
7,000 psi	4		15,30 98,63	94,73	"
9,000 psi	2 5		3,6 54,36 77,45	13,7 7,28	"
9,000 psi	4		15,30 81,70	72,100	"
Yellow Perch 7,000 psi	(11 C) 5		8,12 96,95	0,13	Malison et al. 1993
9,000 psi	5		8,12 89,84	0,54	"
11,000 psi	5		8,12 63,66	37,50	"

**Table 6.** Summary of research into fish gynogens produced by temperature shock treatment. Some parameters and corresponding gynogen yield have multiple values on the same line to make the table shorter for easier comparison.

Species Shock	Time post-fertilization (min)	Duration (min:sec)	Diploid gynogens (%)	Incubation temperature (°C)	Reference
Rainbow trout Heat: 26 C	115 C-min	20:00	75.0	variable	Palti et al. 1997
Heat: 29 C	99 C-min 288 C-min 384 C-min	10:00 10:00 10:00	48.6 53.3 43.4	variable	"
Heat: 29 C	508 C-min 345 C-min 327 C-min 273 C-min	10:00 10:00 10:00 10:00	45.2, 19.4 74.7 34.1, 5.2 5.6, 1.4	variable	"
Heat: 31.5 C	95 C-min 135 C-min 146 C-min 202 C-min 270 C-min 292 C-min 404 C-min	5 5 5 5 5 5 5	24.6,15.3 3.0 9.9 6.9 17.6 1.6 21.3, 10.9	variable	"
Chinook salmon Heat: 25 C	8 16 24	5,10,15,20 5,10,15,20 5,10,15,20	20-43 18-41 9-46	10 C	Levanduski et al. 1990
Heat:27 C	8 16 24	5,10,15,20 5,10,15,20 5,10,15,20	14-50 12-49 7-32	10	"
Heat:29 C	8 16 24	5,10,15,20 5,10,15,20 5,10,15,20	4-51 4-55 6-50	10	"
Pink salmon Heat: 26 C	10 10 10 10	15 20 25 30	31 42 28 27		Smoker et al. 1995
Heat: 28 C	10 10 10 10	10 12 14 16	25 31 24 20		"
Heat: 30 C	10 10 10	6 8 10	20 16 2		"
Paddlefish					Mims et al. 1997

Heat: 28 C	2-22	4	0	18	
Heat:30 C	1-22	1	≤4	18	"
Heat: 34 C	1-22	1	≤2	18	"
Heat: 35 C	2-8	2	0	18	"
	10-14, 22	2	≤4		
	16, 18, 20	2	11, 18, 14		
	2-22	4	0		
Heat: 40 C	2-22	2	0*	18	"
	2-22	4	0		
Stinging catfish Cold: 2 C	3:00	5:00	54	28	Gheyas et al. 2001
	3:00	10:00	98		
	3:00	15:00	98		
	3:00	20:00	98		
	3:00	25-30	lethal		
Cold: 2 C	3:00	10:00	98	28	"
	5:00	10:00	99		
	7:00	10:00	98		
	9:00	10:00	39		
	11-15	10:00	<2		
Cold: 4 C	3:00	10:00	1	28	"
	3:00	15:00	28		
	3:00	20:00	66		
	3:00	25:00	70		
	3:00	30:00	21		
	3:00	35-40	lethal		
Cold: 6 C	3:00	10:00	0	28	"
	3:00	15:00	0		
	3:00	20:00	5		
	3:00	25:00	19		
	3:00	30:00	16		
	3:00	35-50	lethal		
Cold: 8 C	3:00	15:00	0	28	"
	3:00	20-50	lethal		
African catfish Cold: 3 C	1	40	28	28	Volckaert et al. 1994
	2	40	6		
	3	40	2		
	4	40	62		
	5	40	60		
	6	40	56		
	7,8,10	40	44,20,17		
Cold: 5 C	1	40	17	28	"
	2	40	6		
	3	40	46		
	4	40	80		

	5 6 7,8,10	40 40 40	41 53 21, 4, 0		
Cold: 7 C	1 2 3 4 5 6 7,8,10	40 40 40 40 40 40 40	4 12 44 41 27 20 3, 1, 0	28	"
Heat: 39 C	1,2 3,4 5,6,8,10	2 2 2	13,17 12,13 4-7	28	"
Heat: 40 C	1,2 3,4 5,6,8,10	2 2 2	42,26 4,1 0	28	"
Heat:41 C	1,2 3,4 5,6,8,10	2 2 2	26,42 46,25 0-3	28	"
Muskellunge Heat: 26 C	20	10,15,20,2 5,30	1-3	12	Lin and Dabrowski 1996
Heat: 28 C	20	6, 8,10, 15, 20	0.6-3.0	12	"
Heat: 30 C	20	2, 4, 6, 8, 10	0.3,2.1 5-6	12	"
White sturgeon Heat: 34 C	15	1 2 3 5	0 100 98 0	16	Van Eenannaam et al. 1996
Heat: 32 C	12	2:00 3:30 5:00 15:00 30:00 60,180	0 100 0 0 100 0	16	"
Cold: 3 C	12	60	100	16	"

**Table 7.** Summary of fish research on gynogens produced by pressure treatment. The percentage of diploid gynogens indicates the percentage that retained the second polar body after treatment. Some parameters and corresponding gynogen yield have multiple values on the same line to make the table shorter for easier comparison.

Species Pressure (pounds per square inch)	Time post- fertilization (min)	Pressure duration (min)	Survival relative to control (%)	Gynogen 2N (%)	Reference
Rainbow trout 9,000	2,904 C-min 2,730 to 3,456 C-min (10 C)	3 3	25.0, 28.8 <11.4	variable	Palti et al. 1997
7,112	5	6	85	0	Onozato 1984
8,534	5	6	66	38	"
9,245	5	4,5	84,69	20,44	"
	5	6,7	89,86	100	
	5	8,10	99,79	100	
9,956	5	6	93	100	"
11,379	5	6	88	92	"
Cherry salmon 9,245	(10 C) 5 15 30 60-240	6 6 6 6	76 82 56 ≤50	69 64 58 0	"
African catfish 5,983	(28 C) 1,2 3,4 5,6 7,8,10	1.5 1.5 1.5 1.5		14,27 18,78 42,3 ≤3	Volckaert et al. 1994
7,977	1,2 3,4 5,6 7,8,10	1.5 1.5 1.5 1.5		38,43 45,81 16,35 ≤28	"
9,971	1,2 3,4 5,6 7,8,10	1.5 1.5 1.5 1.5		54,33 16,50 14,3 ≤7	"
European sea bass 8,000	(13 C) 4,5 6 7	2 2 2		50,50 80, 68 47	Peruzzi and Chatain 2000
8,500	6	2	63	100	"
9,000	6	2	47	100	"
9,500	6	2	40	100	"
Tilapia 8,000	(28 C) 9,17 25,35	2 2		15,14 3,6	Peruzzi et al. 1993

**Table 8.** Summary of parameters that provided 100% triploid fish after heat or cold shock treatment.

Species Shock	Time post-fertilization (min)	Duration (min:sec)	Triploid (%)	Degree-min post-fertilization (°C-min)	Reference
Rainbow trout Heat 27 C	20	20	100	200	Wagner and Arndt 2001
Steelhead Heat: 26 C	20	20:00	100.0	150	Guoxiong et al. 1989
Heat:34 C	20	2:50	100.0	150	"
Coho salmon Heat:28 C	20	20:00	100.0	200	Teskeredžić et al. 1993
Yellow Perch Heat: 28-31 C	3	25	100.0	33	Malison et al. 1993
Heat: 30 C	2	10	100.0	33	"
Heat: 31 C	1	25	100.0	11	"
Saugeye Cold: 1.2 C	5	150	10 to 100	51	Garcia-Abiado et al. 2001
Common Carp Cold:0-2 C	5	45	100	100	Gervai et al. 1980
Heat: 40 C	1-3	1:30	96-100	26-84	Basavaraju et al. 2002
Grass Carp Heat: 40-42 C	4:00-4:13	1	100	88-99	Cassani and Caton 1986
Thai silver barb Cold: 2 C	0:30	10	64-100	14	Koedprang and Na-Nakorn 2000
Plaice x <i>Platichthys flesus</i> Cold: 0 C	15	120-300	100		Purdom 1972
European sea bass Cold:1 C	5	15-25	100	65	Peruzzi and Chatain 2000
Tilapia sp. Cold:10 C	5	ND	100	ND	Lutz 1998
Heat: 40 C	5	30	64-100	ND	Penman et al. 1987
Heat: 42 C	3	3-4	100	ND	Byamungu et al. 2001
Channel catfish Cold: 5 C	5	60	100	135	Chrisman et al. 1983
Pejerrey Cold:0.5-1.0 C	6	80	100	111-114	Strüssmann et al. 1993
White sturgeon Heat: 32 C	12	3:30	100	192	Van Eenennaam et al. 1996
Heat: 34 C	15	2:00	100	240	"
Cold: 3 C	12	60	100	192	"



**Table 9.** Summary of parameters that provided 100% triploid fish after pressure treatment.

Species Pressure(psi)	Time post- fertilization (min)	Pressure Duration (min)	Survival relative to control (%)	Triploid (%)	Reference
Rainbow trout 6,000	10	8	71	100	Chourrout 1984
7,000	40	3-7	39-98	100	"
9,245	5	6-10	79-99	100*	Onozato 1984
9,956	5	6	93	100	"
Steelhead 11,000	20	6	27	100	Guoxiong et al. 1989
Coho salmon 10,000	20-40	4	80	100	Teskeredžić et al. 1993
11,000	40	4	62	100	"
12,000	20-40	4	18-30	100	"
Atlantic salmon NO at 162	0	60	66	100	Johnstone et al. 1989
Freon at 44	0	30	12	100	"
Brook trout 9,500	35	5	42	100	Wagner et al. 2007
Lake trout 9,000-9,500	21-43	5	53-79	100	Kozfkay et al. 2005
Arctic grayling 8,500-10,000	44-62	3-7	44-86	100	Loopstra and Hansen 2010
Saugeye hybrid 9,000	3:58-4:14	12	27-88	100	Garcia-Abiado et al. 2001
9,000	4:30	12	38	100	"
Largemouth bass 5,000-6,500	5	3	75->100	71-100	Garret et al. 1992
8,000-8,300	5	1	58-98	100	"
<i>Lepomis</i> hybrids 6,000	2-4	3-4	1-90	75-100	Wills et al. 1994
7,000	4	3	94	100	"
7,000	2	4	112	100	"
8,000	2-4	2-4	9-101	97-100	"
Grass carp 6,000	4	3	15	100	Cassani and Caton 1986
7,000	4	1-3	65-95	100	"
8,000-9,000	4	1	42-68	100	"
Bighead carp 7,348	4	1:30		100	Aldridge et al. 1990
Striped x white bass 8,000	7	4	29	100	Curtis et al. 1987
Walleye 9,000	4	30	70	100	Malison et al. 2001

European sea bass 8,500-9,500	6	2	63	100	Peruzzi and Chatain 2000
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\*diploid gynogen percentage

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